2013

NORM
NORM-VET

Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway

UNIVERSITETSSYKEHUSET NORD-NORGE
DAVVI-NORGGA UNIVERSITEHTABUOHCCEVIESSU

Veterinærinstituttet
Norwegian Veterinary Institute

folkehelseinstituttet

2013

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ISSN: 1502-2307 (print) / 1890-9965 (electronic)

Any use of data from NORM/NORM-VET 2013 should include specific reference to this report.

Suggested citation: ***NORM/NORM-VET 2013. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2014. ISSN:1502-2307 (print) / 1890-9965 (electronic).***

This report is available at www.vetinst.no and www.antibiotikaresistens.no

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I. INTRODUCTION

Antimicrobial resistance is an emerging problem worldwide. It reduces the effectiveness of antimicrobial treatment of infectious diseases in humans and animals thereby leading to increased morbidity and mortality, as well as higher costs. It is well established that there is a strong association between the usage of antimicrobial agents and the occurrence of resistance. The selective pressure exerted following use of antimicrobial agents is a key issue in the epidemiology of resistance. Moreover, resistance can be disseminated through the spread of resistant pathogenic bacteria themselves or by horizontal transfer of resistance genes from one type of bacteria to another. Such transfer is not limited to closely related bacteria; it can also take place between bacteria of different evolutionary origins and/or ecological niches. Thus, antimicrobial drug usage and resistance in one ecological compartment can have consequences for the occurrence of resistance in another compartment. When addressing antimicrobial resistance – the occurrences, causes, consequences and preventive measures – a holistic approach is needed, encompassing both data on usage and resistance in human and veterinary medicine, as well as microbes in food production.

Several countries have implemented monitoring programmes for antimicrobial resistance and usage of antimicrobial agents in recent years. Some programmes focus primarily on human usage and resistance in human pathogens, whereas others also include data concerning veterinary medicine and food production. The EU supported this broad approach in 1998 through the Copenhagen recommendations and at later follow-up conferences. The World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Animal Health Organization (OIE) have through several expert consultations emphasised the importance of monitoring antimicrobial drug usage and resistance in both human and veterinary medicine and published several reports and recommendations in this regard.

In response to the growing concern about antimicrobial resistance, the Norwegian Ministry of Health and Social Affairs issued a national action plan against antimicrobial resistance in March 2000. Again, the importance of monitoring both the human and veterinary sectors, including food production, was emphasised. The action plan recognised the need for ongoing surveillance as a fundamental component of the strategy for containment of antimicrobial resistance. The NORM and NORM-VET programmes were consequently established in order to provide and present microbiologically and epidemiologically valid data on the occurrence and distribution of antimicrobial resistance over time. The national action plan formally expired by the end of 2004. However, a conference organized in September 2004 by the Norwegian Institute of Public Health and supported by the Norwegian government, issued a report, which forms the basis for containment of antimicrobial resistance in the years to come. The need for continued surveillance of both resistance and drug usage was emphasised. An integrated national strategy for prevention of infections in the health service and antibiotic resistance (2008 – 2012) was issued in the summer of 2008.

The NORM surveillance programme for antimicrobial resistance in human pathogens was established in 1999 and is coordinated by the Department of Microbiology and Infection Control at the University Hospital of North Norway in Tromsø. The NORM-VET monitoring programme for antimicrobial resistance in the veterinary and food production sectors was established in 2000 and is coordinated by the Norwegian Zoonosis Centre at the Norwegian Veterinary Institute. The usage of antimicrobial agents is based on reporting of wholesalers' data for humans and animals, which was made mandatory from January 1st 2002, as well as human prescription data reported to the Norwegian Institute of Public Health. Data on the usage of feed additives, i.e. coccidiostatic growth promoters, are collated at the Norwegian Food Safety Authority.

This report, which is the fourteenth annual joint report from NORM and NORM-VET, presents data for 2013. In addition to resistance data, the NORM/NORM-VET reports present data on the usage of antimicrobial agents in humans and animals in Norway. The present report, together with earlier reports and reports from the years to come, form a basis for the detection, interpretation and evaluation of trends regarding usage of antimicrobial agents and occurrence of resistance in Norway. The NORM and NORM-VET programmes are valuable tools for setting policies, assessing risks and evaluating interventions.

The editors would like to thank all those who contributed to data collection and the writing of this report, for excellent work.

Tromsø / Oslo, September 2014

II. SAMMENDRAG

Dette er den fjortende felles rapporten fra Norsk overvåkingssystem for antibiotikaresistens hos mikrober (NORM) og Norsk overvåkingsprogram for antibiotikaresistens i mikrober fra fôr, dyr og næringsmidler (NORM-VET). Rapporten presenterer data over forekomst av antibiotikaresistens og forbruk av antibiotika til mennesker og dyr i 2013. Data fra relevante prosjekter som ikke er med i de kontinuerlige overvåkingsprogrammene, presenteres også.

Både NORM og NORM-VET programmene er deler av Regjeringens tiltaksplan mot antibiotikaresistens som ble offentliggjort i 2000. NORM koordineres av Avdeling for mikrobiologi og smittevern, Universitetssykehuset Nord-Norge i Tromsø. NORM-VET koordineres av Zoonosesenteret ved Veterinærinstituttet i Oslo. Programmene har et godt samarbeid og utgir en felles årsrapport.

Forbruk av antibiotika til dyr

Forbruket av antibiotika i norsk husdyrproduksjon og akvakultur er lavt. Totalsalget av veterinære antibiotika til terapeutisk bruk på landdyr i 2013 var 6229 kg. Fra 1995 til 2013 er salget av veterinære antibiotika til landdyr redusert med 35 %. For preparater som nesten utelukkende benyttes til produksjonsdyr (landdyr) er reduksjonen i denne perioden på 38 %, mens salget av veterinære antibakterielle preparater som kun brukes til kjæledyr, har økt med 18 % (fra 467 til 553 kg).

Forbruksmønsteret til produksjonsdyr har utviklet seg i gunstig retning siden 1995 idet andelen av rene penicillinpreparater har økt betraktelig parallelt med at bruk av kombinasjonspreparater med penicillin og dihydro-streptomycin har gått ned. Siden det første penicillin-preparatet til smådyr kom på markedet i Norge (1994) har bruk av veterinære penicillinpreparater, i kg, til smådyr økt fra 1 % til 64 % av totalsalget av slike preparater markedsført kun til kjæledyr.

Nedgangen i antibiotikaforbruket til produksjonsdyr (landdyr) og endringene i forskrivningsmønsteret skyldes først og fremst at husdyrnæringen i andre halvdel av 1990-tallet gjennomførte systematiske kampanjer for å redusere forbruket av antibiotika til dyr samt for riktig bruk av antibiotika.

Totalsalget av veterinære antibiotika til terapeutisk bruk hos oppdrettsfisk i Norge var i 2013 på 972 kg aktiv substans, hvorav 69 % var kinoloner. Forbruket av antibiotika i oppdrettsnæringen er redusert med 99 % siden 1987. Denne betydelige reduksjonen kan tilskrives innføringen av effektive vaksiner til laks og ørret samt andre infeksjonsforebyggende tiltak, herunder bedrede miljøforhold.

Avoparcin ble brukt som antibakteriell vekstfremmer i norsk broiler- og kalkunproduksjon fra 1986 inntil det ble forbudt i 1995. Samme år innførte husdyrnæringene et selvpålagt forbud mot bruk av alle antibakterielle vekstfremmere. Koksidiostatika som fôrtilsetningsstoff brukes fremdeles i norsk fjørfeproduksjon. Forbruksmønstret for koksidiostatika er endret fra monensin til narasin etter 1996. Narasin har i de senere årene utgjort hovedparten av forbruket. Salgstallene, i kg aktiv substans, er mer enn fordoblet siden forbudet mot bruk av antibakterielle vekstfremmere ble innført, noe som kan forklares ved økt produksjon av broilere.

Forbruk av antibiotika hos mennesker

I 2013 var humant forbruk av antibiotika til systemisk bruk 20,0 DDD/1000 innbyggere/dag. Dette var en reduksjon i forhold til 2012. Det samlede forbruket har vært forholdsvis stabilt gjennom mange år, men det har skjedd en gradvis økning og en forskyvning mellom de ulike antibakterielle undergrupper. Salget av smalspektret penicillin ble redusert i 2013. Det urinveisantiseptiske middelet metenamin har de seneste årene økt kraftig, og i 2013 utgjorde metenamin 18 % av totalt salg målt i DDD.

I 2013 utgjorde penicillinene 41 % av det totale antibiotikaforbruket i Norge målt i DDD. Bruken av bredspektrede penicilliner holdt seg stabil mens bruk av penicillinase-stabile penicilliner ble redusert, i motsetning til økningen observert i tidligere år. Tetracykliner utgjorde 18 %, mens makrolider og linkosamider utgjorde 10 % av totalt salg i 2013. Salget av cefalosporiner, monobaktamer og karbapenemer utgjorde kun 3 % av totalsalget. Over år har forbruket av kinoloner økt. Det utgjorde kun 4 % av totalforbruket i 2013, men bruken er mer enn doblet på 10 år.

Rundt 85 % av all DDD selges på resept i allmennpraksis. Bruken av antibakterielle midler varierer mellom kjønn, alder og bosted. I 2013 utgjorde salget til sykehus 7 % av totalt antibiotikasalg. I sykehus brukes penicilliner i stor grad (46 % av antibiotikasalget målt i DDD til sykehus). Tilsvarende i allmennpraksis er 40 %. Den nest viktigste gruppen i 2011 var; på sykehus: cefalosporiner (19 %), og i allmennpraksis: tetracykliner (20 %).

Resistens hos kliniske isolater fra dyr

Kliniske isolater av *Staphylococcus pseudintermedius* (n=201) fra hund ble undersøkt. Forekomsten av antibiotikaresistens var svært høy, og kun 11,4 % av isolatene var følsomme for alle undersøkte antibiotika. 23,9 % av isolatene var resistente mot ett antibiotikum (hovedsakelig penicillin), mens 27,9 % var resistente mot to, 15,4 % mot tre og 21,4 % mot fire eller flere antibiotika. Ett isolat var meticillinresistent.

Resistens hos indikatorbakterier fra dyr

Forekomsten av ervervet antibiotikaresistens blant bakterier som er en del av den normale tarmfloraen, kan være indikator på selektivt antibiotikapress i ulike populasjoner.

I 2013 ble det resistenstestet *Escherichia coli* fra avføringsprøver fra hund (179 isolater), eggproduserende hønseflokker (186 isolater), kalkunflokker (109 isolater), samt fra kalkunkjøtt (154 isolater). Forekomsten av resistente *E. coli* var moderat hos hund med 83 % av isolatene følsomme for alle de testede antibiotika. Fra høns var forekomsten moderat til høy med 79,6 % følsomme isolater. Forekomsten av resistente *E. coli* i kalkun og kalkunkjøtt, var høy med hhv. 74,3 og 66,9 % følsomme isolater.

Fra hund ble resistens mot ett antibiotikum påvist hos 5,6 % av isolatene, og det vanligste var resistens mot ampicillin. I tillegg var 3,9 % resistente mot to, 1,7 % mot tre og 5,6 % mot fire eller flere antibiotika. ESBL-produserende *E. coli* ble påvist hos 2,1 % av hundene.

I flokkene med eggproduserende høner var 11,3 % av isolatene resistente mot ett antibiotikum, og 9,1 % av

isolatene var resistente mot to eller flere antibiotika. Resistens mot sulfametoxazole var den resistensformen som var mest vanlig. ESBL-produserende *E. coli* ble ikke påvist fra høns.

Resistens mot ett antibiotikum ble påvist hos 13,8 % av isolatene fra kalkun og hos 16,9 % av isolatene fra kalkunkjøtt, og det vanligste var resistens mot ampicillin. I tillegg var hhv. 11,9 % og 16,2 % av isolatene resistente mot to eller flere antibiotika. ESBL-produserende *E. coli* ble påvist fra 1,5 % av kalkunflokkene og fra 2,6 % av kalkunfiletene. Alle isolatene hadde bla_{CMY-2} genet.

Ved bruk av selektiv metodikk, ble det påvist kinolonresistente *E. coli* i 49,4 % av kalkunkjøttet. Ett av isolatene hadde MIC-verdier som antyder tilstedeværelse av plasmidmediert kinolonresistensgener, mens de resterende sannsynligvis er resistente pga. kromosomale mutasjoner. Forekomst av kinolonresistente bakterier i matkjeden er bekymringsfullt, da det kan ha innvirkning på resistensutvikling i bakteriepopulasjonen hos mennesker.

Enterococcus faecalis fra 89 eggproduserende hønseflokker og 33 kalkunflokker, samt *E. faecium* fra 103 eggproduserende hønseflokker og 95 kalkunflokker ble resistenstestet. Det var en høy forekomst av resistens hos *Enterococcus* spp. fra høns, og en svært til ekstremt høy forekomst hos *Enterococcus* spp. fra kalkun. Til sammen var 58,4 % av *E. faecalis* isolatene og 61,2 % av *E. faecium* isolatene fra høns følsomme for alle de testede antibiotika, mens det fra kalkun var hhv. 45,4 % følsomme *E. faecalis* og 28,4 % følsomme *E. faecium*.

Fra hønene var 36,0 % av *E. faecalis* og 32,0 % av *E. faecium* resistente mot ett antibiotikum, og dette var hovedsakelig hhv. tetracyklin og narasin. I tillegg var 5,6 % av *E. faecalis* isolatene og 6,8 % av *E. faecium* isolatene resistente mot to eller flere antibiotika. Tilsvarende for kalkun var 21,2 % av *E. faecalis* og 52,6 % av *E. faecium* resistente mot ett antibiotikum, og dette var hovedsakelig hhv. tetracyklin og narasin. Hele 21,2 % av *E. faecalis* og 19,0 % av *E. faecium* var resistente mot to eller flere antibiotika.

Det er en høy frekvens av tetracyklinresistens hos *E. faecalis* fra høner og kalkun til tross for ubetydelig bruk av oxytetracyklin til fjørfe. Tilsvarende er det en moderat til høy frekvens av resistens mot erytromycin hos *Enterococcus* spp. fra høner og kalkun. Erytromycin har aldri blitt benyttet til fjørfe i Norge. Imidlertid kan resistensen være ervervet pga. tidligere bruk av spiramycin da kryssresistens mellom erytromycin og spiramycin er vanlig. Spiramycin var godkjent til bruk hos fjørfe i Norge inntil 1998.

Som tidligere år var forekomsten av resistens mot narasin moderat hos *E. faecalis* og høy hos *E. faecium* fra kalkun. Dette kan ikke forklares ved bruk av narasin som koksidiostatikum da det til kalkun hovedsaklig benyttes monensin, og det er så vidt vi vet ikke kryssresistens mellom monensin og narasin.

Ved bruk av selektiv metodikk ble det påvist VRE fra 12,2 % av kalkunflokkene. Ingen av prøvene fra de eggleggende hønseflokkene var positive for VRE. Alle de *vanA* positive kalkunisolatene ble identifisert som *E. faecium*. Dette er en økning fra tidligere resultater, men kan være et resultat av endring i prøvetakingsmetodikk fra avføringsprøver til sokkeprøver.

Resistens hos zoonotiske bakterier og andre enteropatogene bakterier

Zoonose-bakterier isolert fra dyr

I 2013 ble det resistenstestet 15 isolater av *Salmonella* spp. fra norske dyr. To isolater, hhv. *S*. Kedougou fra hund og *S*. Virchow fra gris, viste resistens mot fluorokinoloner. Multiresistent monofasisk *S*. Typhimurium ble isolert fra en grisebesetning. Isolatet var resistent mot tetracyklin, ampicillin, sulfametoxazol og streptomycin.

Forekomsten av resistens hos *Campylobacter jejuni* fra norske broilere er lav. Hele 87 (90,6 %) av de 96 undersøkte isolatene var følsomme for alle de testede antibiotika. Forekomsten av resistens mot de ulike antibiotika har gjennom årene vært relativt stabil hos *C. jejuni* fra fjørfe.

Kliniske isolater av enteropatogene bakterier

For kliniske *Salmonella* isolater fra mennesker sett under ett var forekomsten av multiresistens (MDR) på knappe 10 % og forekomsten av ESBL holdt seg under 2 %. Når det gjelder blodkulturisolater (totalt 73 isolater), var forekomsten av MDR høyest for *S*. Typhi og *Salmonella* spp. (alle andre enn *S*. Typhi, Paratyphi, Typhimurium og Enteritidis). For øvrig var det gjennomgående at forekomsten av resistens i *S.* Typhimurium-gruppen (inkludert *S. enterica* serovar 4,[5],12:i:-) er høyere enn for andre *Salmonella* serovarer for flere antibiotika, samt at resistensforekomsten er økende for tetracyklin og ampicillin i denne gruppen. Dette gjelder både for innenlandssmittede og pasienter som er smittet i utlandet.

Når det gjelder *Campylobacter* ser det ut til å være en tendens til at isolater ervervet ved innenlandssmitte har begynt å nærme seg resistensnivået for utenlandssmittede når det gjelder kinoloner og tetracyklin.

De fleste tilfeller av *Shigella*-infeksjoner i Norge kan knyttes til smittekilder i utlandet. Antibiotikaresistens var følgelig utbredt hos *Shigella*-isolater, spesielt hos *S. flexneri,* i likhet med det som rapporteres fra andre land. MDR hos *S. flexneri* ligger på ca. 70-80 % og er hos *S. sonnei* 30-40 %. ESBL hos *Shigella* er foreløpig ikke svært hyppig med en forekomst på knappe 5 %. Det synes dessuten å være en tendens til økende resistens mot kinoloner hos *Shigella*.

Antibiotikaresistens hos *Yersinia enterocolitica* ligger stabilt lavt, bortsett fra for ampicillin som *Yersinia* er naturlig resistent mot.

Resistens hos kliniske isolater fra mennesker

Forekomsten av antibiotikaresistente kliniske bakterie-isolater fra mennesker var, som i de foregående år, meget lav i 2013. Det ble påvist fire tilfeller av meticillin-resistente *Staphylococcus aureus* (MRSA) blant de 1155 blodkulturisolater (0,3 %) som ble inkludert i NORM-protokollen. Dette samsvarer godt med tall fra laboratorienes datasystemer som rapporterte at 16 av 1651 (1,0 %) *S. aureus* fra blodkultur og spinalvæske var MRSA. Andelen er på samme nivå som i 2010 (1,0 %), 2011 (0,5 %) og 2012 (1,0 %). Meldesystemet for infeksjons-sykdommer (MSIS) registrerte 659 tilfeller av MRSA-infeksjon i 2013 mot 563 i 2011 og 575 i 2012. De fleste tilfellene var pasienter med sårinfeksjoner og abscesser. MRSA utgjør fortsatt en svært liten andel av *S. aureus* isolater fra sårprøver (10 av 867, 1,2 %) hvilket er på samme nivå som i 2011 (1,3 %) og i 2012 (0,7 %). MSIS registrerte videre 823 tilfeller av MRSA

kolonisering i 2013 mot 635 i 2012. Det totale antallet MRSA-meldinger økte dermed fra 1210 i 2012 til 1482 i 2013 (+22 %). Resultatene fra overvåkingen viser at det totale antallet personer med påvist infeksjon eller kolonisering med MRSA fortsetter å øke, men at antallet med alvorlige infeksjoner foreløpig er stabilt på et lavt nivå. Forekomsten av fusidinresistens blant *S. aureus*-isolater fra sårprøver holder seg stabil og utgjør 9,0 % sammenliknet med 9,5 % i 2012.

Blodkulturisolater av *E. coli* og *Klebsiella* spp. var som tidligere stort sett følsomme for bredspektrede antibiotika. Forekomsten av resistens og nedsatt følsomhet for gentamicin hos *E. coli* var 5,8 % sammenliknet med 5,9 % i 2012. Forekomsten av resistens og nedsatt følsomhet for ciprofloxacin i *E. coli* fortsatte å øke til 12,3 % sammenliknet med 9,1 % i 2011 og 11,7 % i 2012. Det er en klar samvariasjon mellom forbruket av fluorokinoloner og nedsatt følsomhet for denne antibiotikagruppen. *Klebsiella* spp. hadde lavere forekomst av resistens mot fluorokinoloner enn *E. coli*.

Produksjon av bredspektrede betalaktamaser (ESBL) er blitt et utbredt problem i mange land, og forekomsten har også vært økende i Norge. Til sammen 80 av 1612 *E. coli* (5,0 %) og 18 av 652 *Klebsiella* spp. (2,8 %) fra blodkultur ble rapportert som ESBL-positive. Forekomsten er stabil fra 2012 (5,5 % i *E. coli* og 2,3 % i *Klebsiella* spp.). De fleste isolatene kunne verifiseres som ESBL positive ved molekylære analyser, og det er derfor grunn til å følge utviklingen med spesiell oppmerksomhet. Andelen av ESBL-positive isolater var fortsatt høyere blant *E. coli* fra blodkulturer (5,0 %) enn fra urinprøver (2,1 %). To *K. pneumoniae* blodkulturisolater hadde nedsatt følsomhet for meropenem og inneholdt henholdsvis KPC og OXA-48 karbapenemasegener. Karbapenemaseproduserende Enterobacteriaceae, *Pseudomonas aeruginosa* og *Acinetobacter* spp. har vært underlagt meldeplikt til MSIS siden juli 2012.

Blant *Haemophilus influenzae*-isolater fra systemiske infeksjoner (n=79) var 15,2 % betalaktamase-positive og 19,0 % var resistente mot cefuroxim som uttrykk for kromosomal betalaktamresistens. I alt 56,5 % av *Neisseria meningitidis*-isolater hadde nedsatt følsomhet for penicillin G, men alle var fortsatt følsomme for andre relevante antibiotika. *Neisseria gonorrhoeae* (n=225) viste nedsatt følsomhet for penicillin G (98,2 %) og azithromycin (56,6 %). Hele 73,8 % var resistente mot ciprofloxacin, og fire isolater var også resistente mot ceftriaxon.

Det ble påvist fire enterokokkisolater fra blodkultur med klinisk signifikant vankomycinresistens i 2013 (1 VanB *E. faecalis*, 2 VanB *E. faecium* og 1 VanA *E. faecium*). Forekomsten av nedsatt følsomhet for ampicillin i *E. faecium* ligger fortsatt på 80-90 %, og høygradig gentamicinresistens ble påvist i 23,6 % av *E. faecalis* og 46,8 % av *E. faecium*. Nesten alle *E. faecium*-isolater med høygradig gentamicinresistens (79/81) hadde samtidig nedsatt følsomhet for ampicillin. Det ble ikke påvist linezolidresistente enterokokker i 2013.

Streptococcus pneumoniae fra blodkultur og spinalvæske var generelt følsomme for alle undersøkte antibiotika, men 3,0 % (18/608) av isolatene hadde nedsatt følsomhet for penicillin G. Dette er en reduksjon fra 6,3 % i 2012. Ett enkelt isolat var penicillinresistent og hadde samtidig nedsatt følsomhet for cefalosporiner. Forekomsten av makrolidresistens blant systemiske pneumokokkisolater gikk ned fra 6,0 % i 2012 til 3,6 % i 2013.

Streptococcus pyogenes (beta-hemolytiske streptokokker gruppe A) fra blodkultur hadde lavere forekomst av erytromycinresistens (1,9 %) enn isolater fra halsprøver (5,4 %) og sår (4,1 %). Alle isolatene var følsomme for penicillin G.

I alt 401 tilfeller av tuberkulose ble meldt til MSIS i 2013. Det ble utført resistensbestemmelse av 318 isolater av *Mycobacterium tuberculosis*. Seks isolater (1,9 %) fra pasienter smittet i henholdsvis Afrika (n=2), Asia (n=2) og Europa utenfor Norge (n=2) ble klassifisert som multiresistente.

Det ble utført resistensbestemmelse av 176 *Candida* blodkulturisolater av åtte ulike species. De vanligste artene var *C. albicans* (n=113), *C. glabrata* (n=26), *C. tropicals* (n=18) og *C. parapsilosis* (n=10). Alle *C. albicans* og *C. tropicalis* var følsomme for amphotericin B, fluconazol, voriconazol og anidulafungin. Som forventet ble det påvist høy forekomst av resistens mot fluconazol blant *C. glabrata*. Amfotericin B var aktivt mot alle gjærsoppisolater bortsett fra to *C. kruzei*. Resultatene er i samsvar med tidligere studier fra Norge.

Konklusjon

Antibiotikaresistens er fortsatt et begrenset problem i Norge når det gjelder bakterier fra både mennesker og dyr. Det lave forbruket av antibiotika og det fordelaktige forbruksmønsteret må opprettholdes for å bevare den gunstige situasjonen. Resultatene som presenteres i denne rapporten, viser at norske strategier for antibiotikabruk og antibiotikaresistens hittil har vært vellykkede både i husdyrholdet og i helsevesenet. Faren for økende resistensproblemer er imidlertid til stede i form av økt antibiotikaforbruk i Norge og import av resistente bakterier fra andre land. Det er derfor nødvendig med fortsatt aktiv innsats for å sikre at vi også i fremtiden kan gi effektiv antibiotikabehandling til dem som trenger det. NORM/NORM-VET-rapporten er et viktig bidrag til arbeidet med å forebygge utvikling og spredning av antibiotikaresistens.

III. SUMMARY

This is the 14th joint report from the NORM surveillance programme for antimicrobial resistance in human pathogens and the NORM-VET monitoring programme for antimicrobial resistance in animal pathogens and the food production sectors. The report presents data on the occurrence of antimicrobial resistance and the usage of antimicrobial agents in humans and animals for the year 2013. The NORM and NORM-VET programmes are part of the Norwegian Government's Action Plan against Antimicrobial Resistance issued in 2000. NORM is coordinated by the Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø. NORM-VET is coordinated by the Norwegian Zoonosis Centre, Norwegian Veterinary Institute, Oslo. Successful collaboration has been established between NORM and NORM-VET and a joint report is issued annually.

Usage of antimicrobial agents in animals

The usage of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in food producing animals in Norway is low compared to other countries. In 2013, the total sales of antimicrobial VMPs for terrestrial animals were 6,229 kg. The annual sales, in kg active substance, of antimicrobial VMPs approved for use in terrestrial animals decreased by approximately 35% from 1995 to 2013. The reduction in use is solely accounted for by a reduction in the use in food producing animals (38% reduction) while for antimicrobial VMPs marketed for companion animals an increase of 18% in sales is observed. The sales patterns of antimicrobial VMPs for terrestrial food producing animals have gradually become more favourable as the proportion of penicillin use has increased; the proportion accounted for by pure penicillin preparations rose gradually from 25% of total sales in 1995 to 49% in 2013. In this period the sales of aminoglycosides decreased from 27% to 10% of total sales; this is due to a reduction in the use of combination preparations of penicillin and dihydro-streptomycin in food producing animals. The reduced sales of antimicrobial VMPs in terrestrial animals as well as the favourable prescribing patterns are mainly explained by a campaign on prudent use of antimicrobials conducted by the Norwegian husbandry organisations and the Norwegian Medicine Authority during the second part of the 1990s. Furthermore, a target set by the Norwegian husbandry organisations to reduce the sales by 25% with 1995 as the reference year is thought to have had a major impact on this decrease.

In 2013, the total sales of antimicrobial agents for therapeutic use in farmed fish were 972 kg of active substance of which quinolones accounted for 69%. The sales of antimicrobial VMPs in Norwegian aquaculture declined by approximately 99% from 1987 to 1996 and have thereafter remained relatively constant. This reduction is mainly attributed to the introduction of effective vaccines in salmonids. In 2013, the total sales of ionophore coccidiostat feed additives, in kilograms of active substance, were more than twice the amounts used prior to the withdrawal of the antimicrobial growth promoters in 1995. This is explained by increased production of broilers. While monensin was the most frequently used ionophore in poultry in 1995, the usage of coccidiostats has since then been dominated by narasin.

Usage of antimicrobial agents in humans

In 2013, the overall sales of antibacterials for systemic use in humans were 20.0 Defined Daily Doses (DDD)/1,000 inhabitants/day, this was a reduction compared to 2012. The total consumption has been relatively stable over many years, although there has been a gradual increase in consumption and a shift among the various subgroups. The sales of narrow spectrum penicillin have dropped. The use of the urinary antiseptic agent methenamine still increases and, in 2013, it accounted for 18% of total sales, measured in DDDs.

In 2013, penicillins accounted for 41% of the total antibiotic human use. Since 2005, an annual increase in the use of penicillins with extended spectrum and beta-lactamase resistant penicillins has been observed. In 2013, however, the use of penicillins with extended spectrum was stable while the use of beta-lactamase resistant penicillins was reduced. Tetracyclines accounted for 18% of total consumption in 2013 while the consumption of macrolides and lincosamides accounted for 10%. Sales of cephalosporins, monobactams and carbapenems constitute 3% of total sales. Over years, there has been a marked increase in quinolone use. This group accounted for only 4% of total consumption in 2013, but sales have more than doubled in 10 years.

Around 85% of all DDDs are sold through prescriptions in ambulatory care. The use of antibacterials varies according to gender, age and area of residence. In 2013, sales to hospitals accounted for 7% of total antibiotic sales. Penicillins accounted for around 46% of the sales to hospitals and for 40% in ambulatory care. The other main group in hospitals was cephalosporins (19%), and in ambulatory care tetracyclines (20%).

Resistance in animal clinical isolates

Clinical isolates of *Staphylococcus pseudintermedius* (n=201) from dogs were included in the survey. The prevalence of antimicrobial resistance was extremely high. In total, only 11.4% of the isolates were susceptible to all antimicrobial agents included. Altogether, 23.9 % of the isolates were resistant to one antimicrobial agent (predominantly penicillin), 27.9% to two, 15.4% to three and 21.4% to four or more antimicrobial agents. One isolate was confirmed to be methicillin resistant.

Resistance in indicator bacteria from animals

The prevalence of antimicrobial resistance among certain bacteria of the normal enteric microflora can serve as an indicator for the selective antimicrobial pressure in various populations.

In 2013, *Escherichia coli* isolated from faecal samples from dogs (179 isolates), layer flocks (186 isolates), turkey flocks (109 isolates) and turkey meat samples (154 isolates) were included. The prevalence of resistant *E. coli* was moderate in dogs, moderate to high in layers, and high in turkeys and in turkey meat. Of the dog and layer isolates, 83.0% and 79.6%, respectively, were susceptible to all antimicrobial agents included. Of the turkey and turkey meat isolates, 74.3% and 66.9% of the isolates were susceptible to all antimicrobials included in the test panel, respectively.

In dogs, 5.6% of the isolates were resistant to one antimicrobial agent and this was predominantly ampicillin.

In addition, 3.9% were resistant to two, 1.7% to three and 5.6% to four or more antimicrobial agents. ESBL producing *E. coli* were detected in 2.1% of the dogs.
In layer flocks, 11.3% of the isolates were resistant to one antimicrobial agent. Sulfamethoxazole was the most frequently identified resistance determinant in layer flocks. This is in contrast to previous results for poultry breeder flocks where ampicillin resistance was more commonly found, and may be explained by a larger use of penicillins in poultry breeder flocks than in layer flocks. In total, 9.1% of the isolates were resistant to two or more antimicrobial agents. In contrast to the findings in the broiler production lines, ESBL producing *E. coli* were not detected in any of the 204 samples, indicating a prevalence in layer flocks below 1.8%.
Altogether, 13.8% of the isolates from turkey flocks and 16.9% of the isolates from turkey meat were resistant to one antimicrobial agent, predominantly ampicillin. In total, 11.9% and 16.2% of the turkey and turkey meat isolates were resistant to two or more antimicrobial agents, respectively. ESBL producing *E. coli* were detected in 1.5% of turkey flocks and in 2.6% of meat samples. All isolates had a beta-lactam resistance profile corresponding to an AmpC phenotype. PCR and sequencing showed that all isolates contained the bla_{CMY-2} gene.
By use of a selective method, *E. coli* resistant to quinolones were found in 49.4% of the turkey meat samples. One of the isolates had a MIC profile indicating the possible presence of a plasmid mediated quinolone resistance gene (PMQR), whereas resistance in the other isolates was probably mediated by mutations in the QRDR. The presence of quinolone resistant bacteria in food production animals is of concern. Resistant bacteria in the food chain may have an impact on resistance development in human bacterial populations and it should be an overall goal to keep the level of resistant bacteria in production animals and through the meat processing chain at the lowest possible level.
Enterococcus faecalis from 89 layer flocks and 33 turkey flocks, and *E. faecium* from 103 layer flocks and 95 turkey flocks were included. There was a high occurrence of resistance among *Enterococcus* spp. from layer flocks, and a very to extremely high occurrence of resistance among *Enterococcus* spp. from turkey flocks. In total 58.4% of the *E. faecalis* and 61.2% of the *E. faecium* from layer flocks were susceptible to all antimicrobial agents. From turkey flocks, 45.4% of the *E. faecalis* and 28.4% of the *E. faecium* were susceptible to all antimicrobial agents. In total, 36.0% of the *E. faecalis* and 32.0% of the *E. faecium* were resistant to one antimicrobial agent and this was mainly tetracycline and narasin, respectively. In addition, 5.6% of the *E. faecalis* and 6.8% of the *E. faecium* from layer flocks were resistant to two or more antimicrobial agents. In turkey flocks, 21.2% of the *E. faecalis* and 52.6% of the *E. faecium* were resistant to one antimicrobial agent. As for layer flocks this was mainly tetracycline resistance in *E. faecalis* isolates and narasin resistance in *E. faecium* isolates. In total, 21.2% of the *E. faecalis* and 19.0% of the *E. faecium* from turkey flocks were resistant to two or more antimicrobial agents. Surprisingly there was a high frequency of tetracycline resistance among *E. faecalis* in layers and turkeys despite insignificant use of oxytetracycline for clinical purposes in Norwegian poultry production. Such high frequency is also seen for broilers. Equivalently, there was a moderate to high frequency of resistance to erythromycin among *Enterococcus* spp. from layers and turkeys. Erythromycin has never been used in poultry in Norway. However, resistance may have been acquired by former use of spiramycin as cross-resistance between erythromycin and spiramycin is common. Spiramycin was licensed for use in poultry until 1998 when it was withdrawn due to limited sales.
The prevalence of resistance to narasin was moderate among *E. faecalis* isolates and high among *E. faecium* isolates from turkey, and this is consistent with previous results from turkey. This cannot be explained by use of this substance as the dominating coccidiostat used in turkey production is monensin. To our knowledge there is no cross-resistance between monensin and narasin.
By use of selective methods, VRE were found in 12.2% of the turkey flocks. All *vanA* positive isolates were identified as *E. faecium*. This is an increase from 2007, but may be a result of improved sampling methods using boot swabs (per flock) instead of faecal samples (from groups of turkey). Boot swab sampling mirrors the prevalence in the broiler house and not the actual prevalence in the live birds. None of the layer flocks were positive, indicating a VRE prevalence in Norwegian layer flocks below 1.8%.

Resistance in zoonotic and non-zoonotic enteropathogenic bacteria

In 2013, a total of 15 isolates of *Salmonella* spp. from animals were susceptibility tested. Two isolates, one *S.* Kedougou from dog and one *S.* Virchow from pig showed resistance to fluoroquinolones. The emerging multidrug resistant (MDR) monophasic *S.* Typhimurium resistant to tetracycline, ampicillin, sulfamethoxazole and streptomycin, was isolated from one pig herd.
The prevalence of antimicrobial resistance among *C. jejuni* isolates from Norwegian broilers is low. A total of 90.6% of the 96 isolates included were susceptible to all antimicrobial agents tested. Over the years, the prevalence of resistance to various antimicrobials in *Campylobacter jejuni* from Norwegian broilers has been rather stable.

The frequency of multidrug resistance in human clinical isolates of all *Salmonella* isolates was just below 10% and the frequency of ESBL stays below 2%. Among the 73 blood culture isolates of *Salmonella*, the highest frequency of MDR was found in *S.* Typhi and *Salmonella* serovars other than *S.* Typhi, *S.* Paratyphi, *S.* Typhimurium-group and *S.* Enteritidis. Antimicrobial resistance in the *S.* Typhimurium-group (including *S. enterica* serovar 4,[5],12:i-) seems to be more prevalent than for other serovars, and resistance to ampicillin and tetracycline is still increasing in this group. This applies to domestically acquired strains as well as to strains acquired abroad.
For *Campylobacter*, domestically acquired isolates tend to approach the prevalence of quinolone and tetracycline resistance seen in isolates acquired abroad.
Most cases of shigellosis are acquired abroad, and there is widespread resistance, especially in *S. flexneri*, as reported from other countries. There may be a trend of increasing resistance to quinolones. The frequency of MDR is approximately 70-80% in *S. flexneri* and 30-40% in *S. sonnei.* The ESBL prevalence in *Shigella* is just below 5%. Antimicrobial resistance in *Yersinia enterocolitica* remains low, except for the intrinsic resistance against ampicillin.

Resistance in human clinical isolates

The prevalence of resistance in human clinical isolates was still low in Norway in 2013. Only four methicillin resistant *Staphylococcus aureus* (MRSA) blood culture isolates were detected among the 1,155 strains included in the NORM protocol (0.3%). During 2013 the total number of systemic *S. aureus* isolates from blood cultures and cerebrospinal fluids was 1,651, including 16 MRSA strains (1.0%). This prevalence is at the same level as in 2011 (0.5%) and 2012 (1.0%). The Norwegian Surveillance System for Communicable Diseases (MSIS) registered 659 cases of MRSA infections in 2013 compared to 563 in 2011 and 575 in 2012. The majority of MRSA cases were reported to be wound infections and/or abscesses. Conversely, the prevalence of MRSA among non-invasive *S. aureus* isolates is still very low at 1.2% (10/867) which is at the same level as 1.3% in 2011 and 0.7% in 2012. Furthermore, MSIS registered 823 cases of MRSA colonisation in 2013 compared to 635 in 2012. The total number of MRSA notifications thus increased from 1,210 in 2012 to 1,482 in 2013 (+ 22%). The results indicate an increasing number of MRSA infections and colonisations, while the prevalence of invasive disease has until now remained stable at a low level. The prevalence of fusidic acid resistant *S. aureus* wound isolates has stabilised around 9-10%.

E. coli and *Klebsiella* spp. blood culture isolates were generally susceptible to broad-spectrum antimicrobials. The prevalence of gentamicin non-susceptibility in *E. coli* was 5.8% in 2013 compared to 5.9% in 2012. The increase in the prevalence of *E. coli* non-susceptibility to fluoroquinolones continued and reached 12.3% in 2013 compared to 9.1% in 2011 and 11.7% in 2012. There is a clear correlation between the total usage of fluoro-quinolones and non-susceptibility to these agents. The prevalence of resistance to fluoroquinolones is lower in *Klebsiella* spp. isolates than in *E. coli*.

Extended spectrum beta-lactamases (ESBL) have emerged as a significant clinical problem in many countries, including Norway. A total of 80/1,612 (5.0%) *E. coli* and 18/652 (2.8%) *Klebsiella* spp. blood culture isolates were reported with this phenotype in 2013. The prevalence is essentially unchanged from 2012 (5.5% for *E. coli* and 2.3% in *Klebsiella* spp.). As most of these isolates were verified by molecular methods, the trend should be closely monitored. The proportion of ESBL positive isolates is higher among *E. coli* from blood cultures (5.0%) than among urinary tract isolates (2.1%). Two *K. pneumoniae* blood culture isolates displayed reduced susceptibility to meropenem and contained KPC and OXA-48 determinants, respectively. Carbapenemase producing Entero-bacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp. have been notifiable to MSIS since July 2012.

Among *Haemophilus influenzae* isolates from systemic infections (n=79), 15.2% displayed beta-lactamase production and 19.0% were resistant to cefuroxime, thus indicating chromosomal resistance to beta-lactam antibiotics. In total, 56.5% of *Neisseria meningitidis* isolates (n=23) were intermediately susceptible to penicillin G by current breakpoints, but all remained susceptible to other relevant antibiotics. *Neisseria gonorrhoeae* isolates (n=225) demonstrated non-susceptibility to penicillin G (98.2%) and azithromycin (58.6%), as well as resistance to ciprofloxacin (73.8%) and even ceftriaxone in four cases (1.8%).

Four enterococcal blood culture isolates with clinically significant vancomycin resistance were detected in 2013 (1 VanB *E. faecalis*, 2 VanB *E. faecium* and 1 VanA *E. faecium*). The prevalence of non-susceptibility to ampicillin in *E. faecium* has stabilised around 80-90%, and high-level gentamicin resistance (HLGR) was detected in 23.6% of *E. faecalis* and 46.8% of *E. faecium*. Almost all HLGR *E. faecium* (79/81) isolates were also non-susceptible to ampicillin. Enterococcal resistance to linezolid was not detected in 2013.

Streptococcus pneumoniae from blood cultures and cerebrospinal fluids were generally susceptible to relevant antimicrobials, however 3.0% (18/608) of the isolates displayed reduced susceptibility to penicillin G which is a decrease from 6.3% in 2012. A single isolate was resistant to penicillin G and at the same time showed reduced susceptibility to cephalosporins. The prevalence of macrolide resistance among pneumococcal blood culture isolates decreased from 6.0% in 2012 to 3.6% in 2013.

Streptococcus pyogenes (group A streptococcus) isolates from blood cultures had lower rates of erythromycin resistance (1.9%) than isolates from throat (5.4%) and wound (4.1%) samples). All isolates were susceptible to penicillin G.

A total of 401 cases of tuberculosis were reported to MSIS in 2013. Susceptibility testing was performed on 318 *Mycobacterium tuberculosis* isolates. Six isolates (1.9%) originating from Africa (n=2), Asia (n=2) and Europe outside Norway (n=2), were classified as multidrug resistant (MDR).

Susceptibility testing was performed on 176 blood culture isolates *Candida spp.* of eight different species. The most common species were *C. albicans* (n=113), *C. glabrata* (n=26), *C. tropicals* (n=18) and *C. parapsilosis* (n=10). All *C. albicans* and *C. tropicalis* isolates were susceptible to amphotericin B, fluconazole, voriconazole and anidulafungin. As expected, a high prevalence of resistance to fluconazole was detected in *C. glabrata*. Amphotericin B was active against all yeasts except two *C. kruzei* isolates. The results are in accordance with previous studies from Norway.

Conclusion

Antimicrobial resistance is still a limited problem among clinically important microbes in Norway. The relatively low usage of antimicrobial agents as well as appropriate patterns of use must be maintained to preserve this rather favourable situation. The data presented in this report show that Norwegian antimicrobial policies in food production and health care have been successful. However, the situation may rapidly change if the use of antimicrobial agents in Norway increases or resistant clones are imported from abroad. A continued effort is needed to prevent the development and spread of antimicrobial resistance and thereby ensure the effectiveness of antimicrobials when such treatment is needed. The NORM/NORM-VET report is a vital component in the work aimed at preventing the development and spread of antimicrobial resistance in Norway.

IV. POPULATION STATISTICS

Population statistics for human and animal populations are presented in order to facilitate comparison of Norwegian data with corresponding figures from other countries. The data are collected by Norwegian authorities as shown in the various tables below.

TABLE 1. Human population in Norway as of January 1st, 2014.
Data provided by Statistics Norway.

Age group	All	Males	Females
0 to 6 years	437,990	224,782	213,208
7 to 15 years	555,871	284,160	271,711
16 to 44 years	2,007,750	1,031,362	976,388
45 to 66 years	1,408,723	718,204	690,519
67 to 79 years	477,962	227,968	249,994
80 years and older	220,760	80,958	139,802
All age groups	5,109,056	2,567,434	2,541,622

TABLE 2. Livestock population in Norway in 2013.
Data provided by the Register of Production Subsidies as of 31 July, 2013.

	Number* of	
Animal category	Herds	Animals
Cattle	15,000	849,000
Dairy cows only**	8,700	204,000
Suckling cow only**	4.200	66,000
Combined production (cow)**	800	32,200
Goat	1,300	64,100
Dairy goat**	320	33,000
Sheep	14,200	2,253,000
Breeding sheep > 1 year**	14,100	872,000
Swine	2,200	835,000
Breeding animal > 6 months**	1,200	55,000
Fattening pigs for slaughter**	2,000	457,000
Laying hen flocks > 250 birds	570	4,130,000
Broilers	690	-
Turkey, ducks, geese for slaughter (flock > 250 birds)	53	372,000

* Numbers > 100 rounded to the nearest ten, numbers > 1000 rounded to the nearest hundred.
** Included in above total.

TABLE 3. Production volume of the most important species in Norwegian aquaculture during the time period 1992-2013. *Data provided by the Norwegian Directorate of Fisheries updated by 06.06.2013.*

Year	Atlantic salmon (ton)	Rainbow trout (ton)	Cod (ton)	Arctic char (ton[2])	Halibut (ton[2])	Blue mussels (ton)	Scallops[1] (ton)	Oysters (ton)
1992	141,000	-	-	-	-	-	-	-
1993	170,000	-	-	-	-	-	-	-
1994	204,686	14,571	569	262	63	542	-	-
1995	261,522	14,704	284	273	134	388	-	-
1996	297,557	22,966	191	221	138	184	-	-
1997	332,581	33,295	304	350	113	502	-	-
1998	361,879	48,431	203	200	291	309	-	-
1999	425,154	48,692	147	498	451	662	67	41
2000	440,061	48,778	169	129	548	851	38	8
2001	435,119	71,764	864	318	377	920	22	3
2002	462,495	83,560	1,258	319	424	2,557	5	2
2003	509,544	68,931	2,185	272	426	1,829	1	2
2004	563,915	63,401	3,165	365	648	3,747	46	3
2005	586,512	58,875	7,409	352	1,197	4,885	3	2
2006	629,888	62,702	11,087	897	1,185	3,714	4	1
2007	744,222	77,381	11,104	394	2,308	3,165	6	4
2008	737,694	85,176	18,052	468	1,587	2,035	4	3
2009	862,908	73,990	20,924	421	1,568	1,649	7.7	3.8
2010	939,575	54,451	21,240	492	1,610	1,930	10.3	2.1
2011	1,064,86	58,472	15,273	276	2,767	1,743	13	2
2012	1,241,48	70,364	10,033	309	1,741	1,967	21	2
2013[3]	1,165,95	72,497	3,770[3]	281	1, 385	2,328	23	5

[1]From the wild population. [2]After 2001 in numbers of 1,000 individuals. [3] Preliminary numbers.

Import of live animals

Import of live animals (excluding fish and companion animals) to Norway in 2013 was limited to 30 cattle, 12 sheep and 20,611 day old chicks.

V. USAGE OF ANTIMICROBIAL AGENTS

USAGE IN ANIMALS

Kari Grave

Therapeutic usage of veterinary antimicrobial agents

Total sales (6,229 kg active substance) in Norway of antimicrobial veterinary medicinal products (VMPs) for therapeutic use split into sales for use in food producing animals and companion animals in the period 1995-2013 are shown in Figure 1. The data are based on sales from drug wholesalers to Norwegian pharmacies and from feed mills to fish farmers (see Appendix 1) of veterinary antimicrobial agents for therapeutic use and includes pharmaceutical formulations approved for food animals, including horses, and companion animals. Thus, the figures represent national sales data for veterinary antimicrobial agents (see Appendix 1 for inclusion criteria).

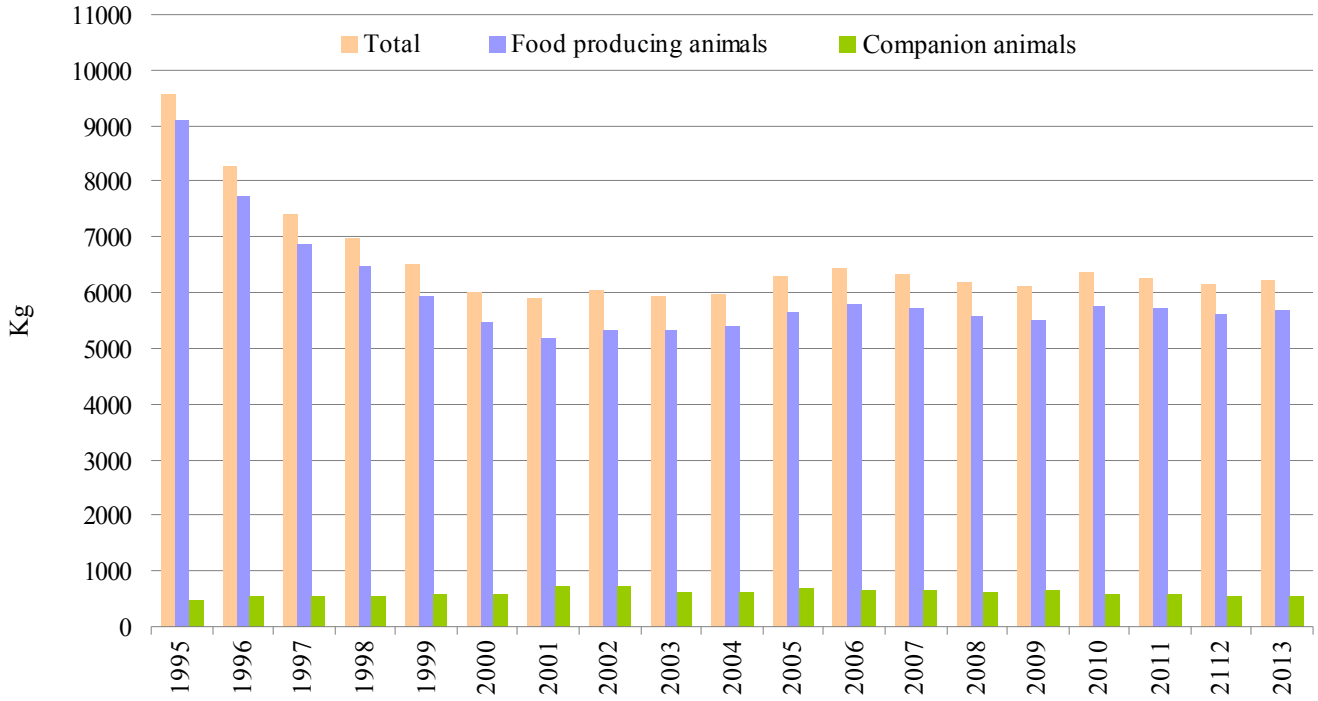

FIGURE 1. Total sales, in kilograms active substance, and estimated sales for food producing animals (terrestrial animals) and companion animals of antimicrobial veterinary medicinal products (VMP) for therapeutic in Norway for the years 1995-2013 (farmed fish not included).

In the period 1995-2013 the total sales of antimicrobial VMPs for use in terrestrial animals decreased by 35%. Of antimicrobial VMPs used almost solely for food production animals the reduction was 38%, while for products used in companion animal only an increase of 18% was observed (Figure 1).

An increase in the sales of pure penicillin VMPs for food producing terrestrial animals is observed for the period 1995-2013 from 25% to 49% of total sales and this is accounted for by products used in food producing and companion animals (Figures 2-3). In this period the sales of aminoglycosides as combination preparations decreased from 27% to 10% of the total sales; this is due to a reduction in the use of combination preparations of penicillin and dihydro-streptomycin in food producing animals (Figure 2).

The observed peak in the sales of sulfonamides in companion animals in 2001-2002 is probably due to use in sheep of a trimethoprim-sulfonamide VMP marketed for companion animals because of a withdrawal in 2001 of a product used for mastitis in sheep (Figure 3).

The sales of the antimicrobial VMPs defined by the World Health Organization (WHO) with highest priority for human medicine i.e. fluoroquinolones and macrolides are negligible (Figures 2-3). Note that there are no cephalosporin VMPs marketed in Norway for food producing animals.

The reduced sales of antimicrobial VMPs in food producing animals as well as the favourable prescribing patterns are mainly explained by a campaign on prudent use of antimicrobials conducted by the Norwegian husbandry organisations and the Norwegian Medicine Authority during the second part of the 1990s. Furthermore, a target set by the Norwegian husbandry organisations to reduce the sales by 25% with 1995 as the reference year is thought to have had a major impact on this decrease.

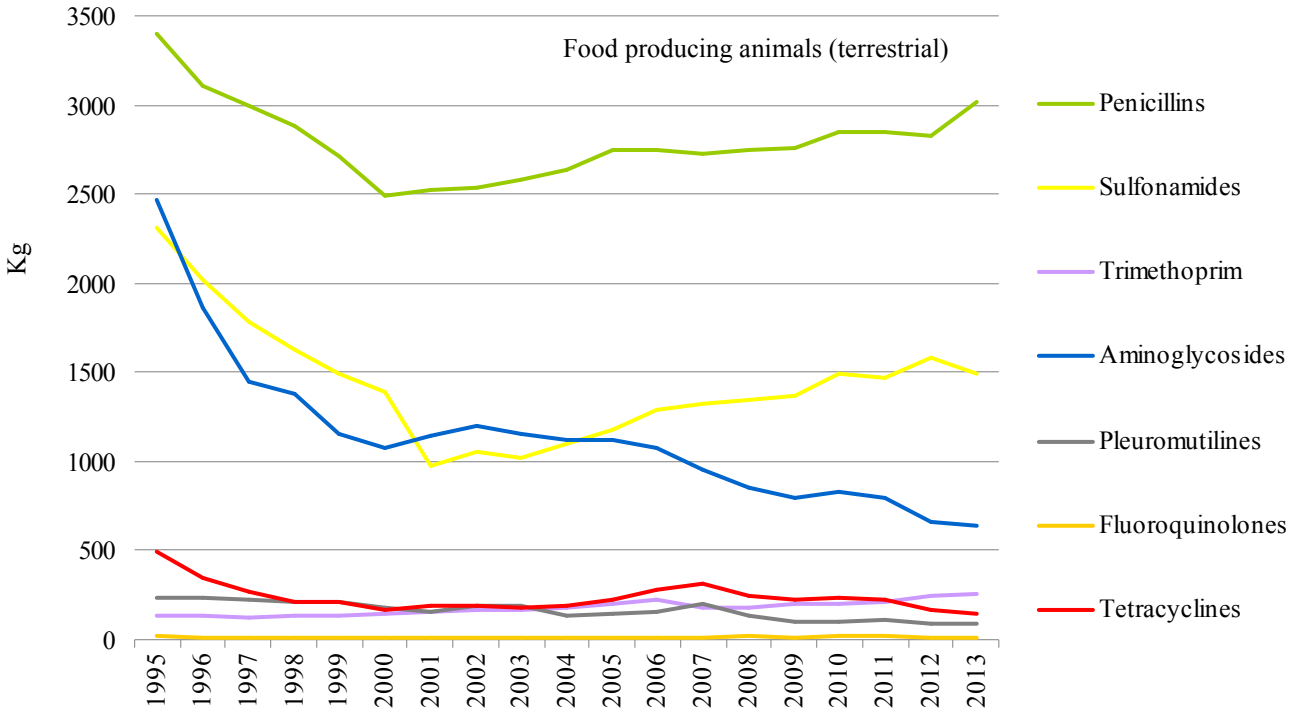

FIGURE 2. Sales in Norway (kilograms active substance) of antimicrobial veterinary medicinal products (VMP) mainly for therapeutic used in food producing animals for the years 1995-2013 (farmed fish not included). In addition, minor amounts of amphenicols (range 19-27 kg) were sold in 2008 - 2013 and of macrolides (range 0.2-15 kg) during 1995-2013.

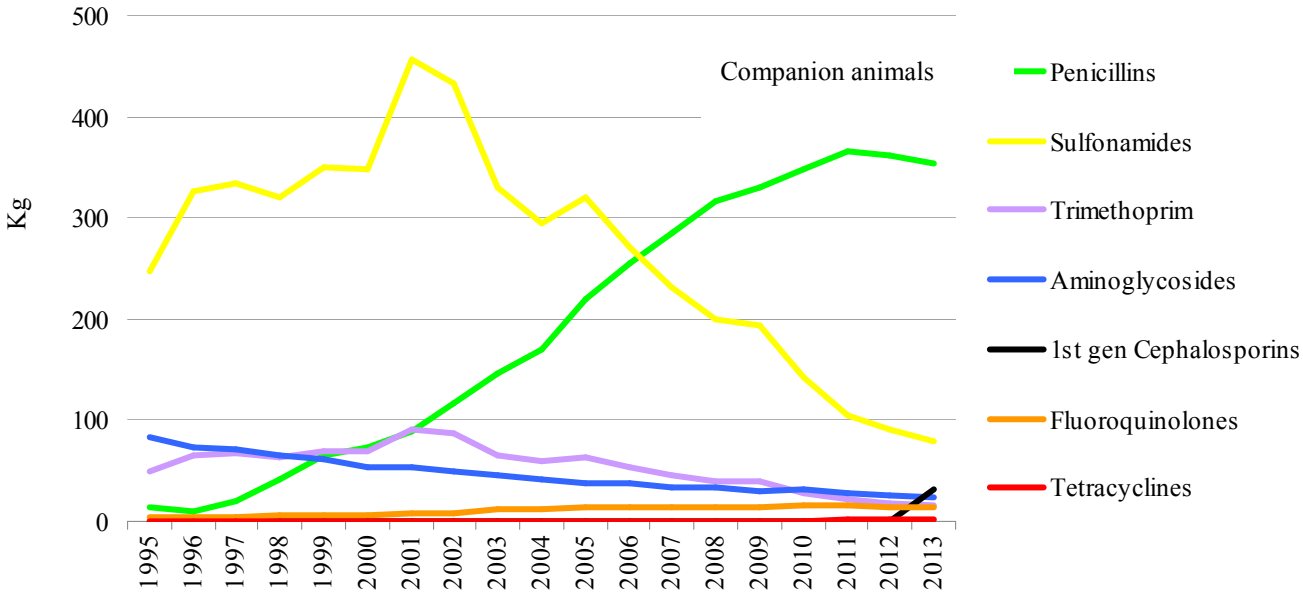

FIGURE 3. Sales in Norway, in kilograms of active substance, of antimicrobial veterinary medicinal products (VMP) marketed for therapeutic use in companion animals for the years 1995-2013. In addition, minor amounts of a 3rd generation cephalosporin (0.9-1.1 kg) were sold annually during 2008-2013 and of macrolides (0.4-5kg) from 1998-2005.

An increase of 18% in the sales, in kg active substance, from 465 to 553 kg of antimicrobial VMPs marketed for companion animals from 1995-2013 is observed (Figure 3). This increase is mainly accounted for by penicillins, and in 2013 approximately 87% of the penicillin products sold for companion animals was as a combination of amoxicillin and clavulanic acid (Figure 4).

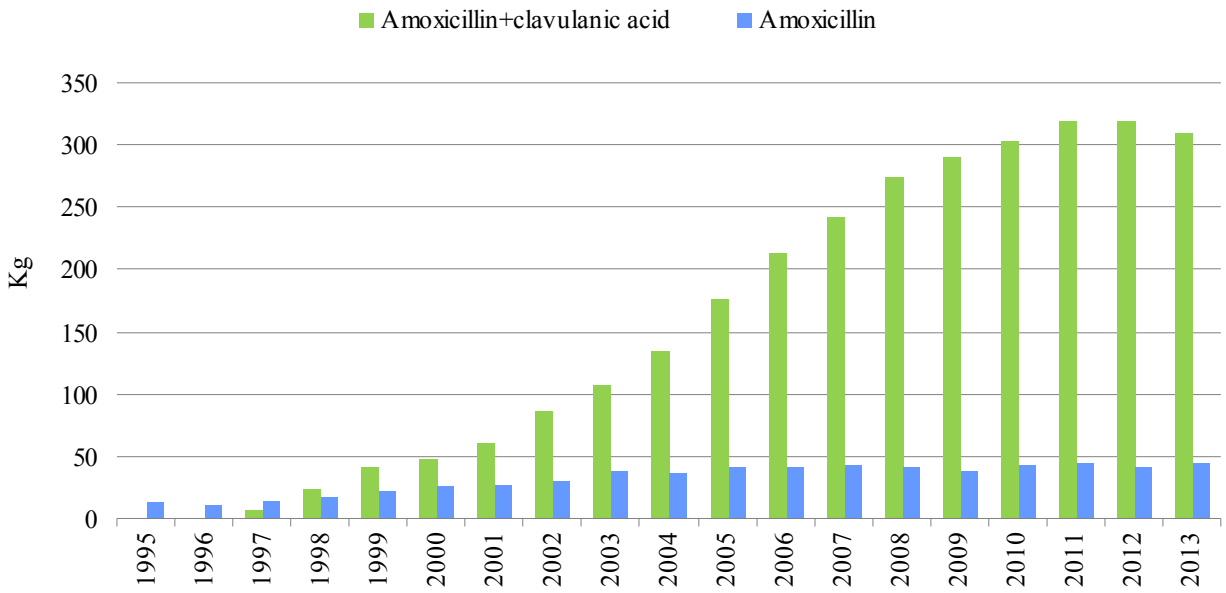

FIGURE 4. Sales, in kg of active substance, of penicillin veterinary medicinal products for companion animals 1995-2013.

The annual sales of antimicrobial VMPs for use in farmed fish peaked in 1987 when the sales amounted to 48 tonnes (Figure 5). In 2013, the sales of antimicrobial VMPs for use in farmed fish was 972 kg active substance, of which 69% were quinolones (Table 4); this implies that the sales have declined by approximately 99% from 1987.

The significant decrease in the usage of antimicrobial agents in Norwegian aquaculture from 1987 is mainly attributed to the introduction of effective vaccines against bacterial diseases in Atlantic salmon and rainbow trout and to some extent also to improved health management.

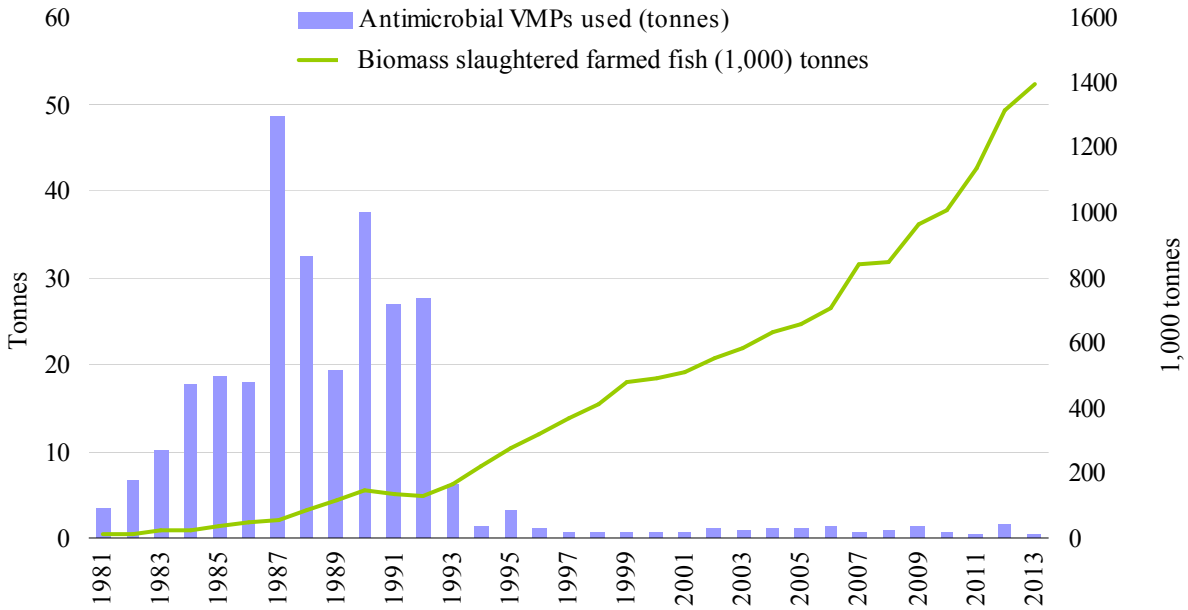

FIGURE 5. Total sales, in kilograms of active substance, of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in farmed fish in Norway in the period 1981-2013 versus produced biomass (slaughtered) farmed fish.

TABLE 4. Total sales, in kilograms of active substance, of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in farmed fish in Norway in the period 2003-2013.

Group of substances/active substance		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Tetracyclines:	Oxytetracycline	45	9	8	0	19	23	40	10	1	1	0
Amphenicols:	Florfenicol	154	111	202	302	139	166	303	275	336	191	300
Quinolones:	Flumequine	60	4	28	7	18	1	1	0	0	0	0
	Oxolinic acid	546	1,035	977	1,119	406	681	926	308	212	1,399	672
Combinations:	Spectinomycin + lincomycin (2+1)	0	0	0	50	66	70	43	57	0	0	0
Total		**805**	**1,159**	**1,215**	**1,478**	**648**	**941**	**1,313**	**649**	**549**	**1,591**	**972**

Antimicrobial and coccidiostatic feed additives

Data on the sales of various substances and categories of feed additives (Table 5) were obtained through annual reports from the Norwegian Food Safety Authority.
The glycopeptide avoparcin was licensed in Norway as growth promoter in broilers and turkeys in 1986. In 1995 the food animal production industry voluntarily abandoned the use of all antimicrobial growth promoters (AGPs), including avoparcin. These measures resulted in an immediate decline in the use of AGPs (Figur 6). No antimicrobial growth promoters have been used in animals in Norway since 1997.

TABLE 5. Total sales, in kilograms of active substance, of coccidiostats as feed additives in Norway 2003-2013. Data were obtained through annual reports from the Norwegian Food Safety Authority.

Active substance	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Lasalocid	108	173	37	13	17	16	63	0	0	0	0
Monensin	717	817	852	889	919	897	885	805	1,060	1,080	1,174
Salinomycin	0	0	0	0	0	0	0	0	0	0	0
Narasin	5,067	5,270	5,318	5,615	7,065	9,212	8,621	9,080	9,394	10,378	12,345
Total ionophore coccidiostats	**5,892**	**6,260**	**6,207**	**6,517**	**8,001**	**10,125**	**9,569**	**9,885**	**10,454**	**11,458**	**13,519**
Amprolium/etopabat	42	0.8	0	0	0	0	0	0	0	0	0
Total others	**42**	**0.8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

The total sales of ionophore coccidiostats (kilograms of active substance) have been doubled since the withdrawal of antimicrobial growth promoters in 1995 and have since then almost totally been dominated by narasin (Table 5, Figur 6). The sales of ionophore coccidiostats are highly correlated to the number of slaughtered chicken produced in this period.

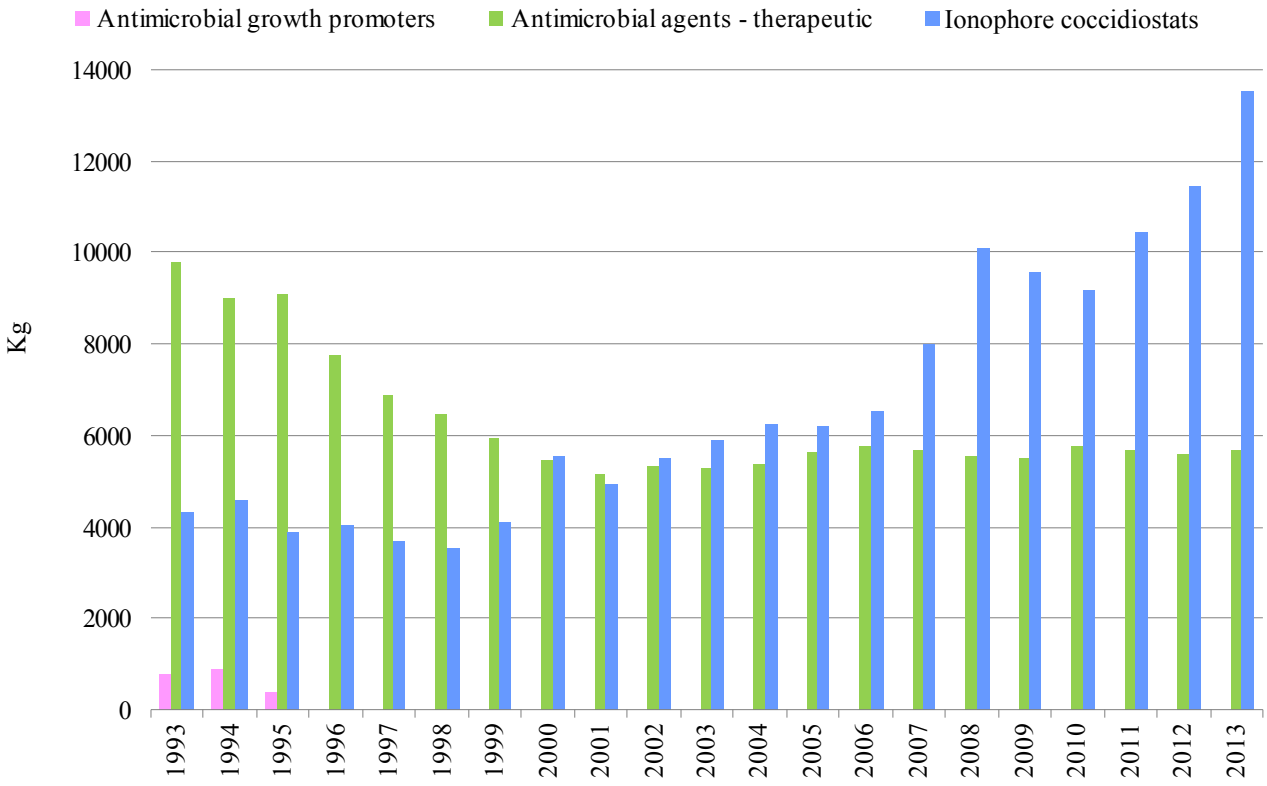

FIGURE 6. Sales, in kilograms of active substance, of antimicrobial veterinary medicinal products (VMPs) for terrestrial food producing animals and of antimicrobial growth promoters and ionophore coccidiostats in Norway during 1993-2013.

USAGE IN HUMANS

Hege Salvesen Blix

In 2013, the overall sales of antibacterials for systemic use in humans were 20.0 Defined Daily Doses (DDD)/1,000 inhabitants/day. The use of antibiotics decreased in 2013 compared to 2011 and 2012, when a *Mycoplasma pneumoniae* epidemic caused higher prescription rates for macrolide and tetracycline antibiotics. Increased sales of antibacterials in the first decade of this century have mainly been caused by penicillins and increased use of methenamine. When methenamine is excluded, the level of antibiotic use in 2013 was 16.3 DDD/1,000 inhabitants/day (Table 6, Figure 7).

TABLE 6. Human usage of antibacterial agents in Norway 2006-2013 by ATC groups. The usage is presented as DDD (Defined Daily Doses)/1,000 inhabitants/day and in % change 2012-2013. The methodology for collection of data on human usage of antimicrobial agents is presented in Appendix 2.

ATC	Groups of substances	2006	2007	2008	2009	2010	2011	2012	2013	Change (%) 2012-2013
J01A	Tetracyclines	3.24	3.32	3.22	3.09	3.12	3.47	3.87	3.53	- 8
J01B	Amphenicols	0.002	0.001	0.001	0.002	0.001	0.0005	0.0002	0.0002	-
J01CA	Penicillins with extended spectrum	2.74	2.93	3.09	3.15	3.19	3.21	3.34	3.33	-
J01CE	Beta-lactamase sensitive penicillins	4.63	4.70	4.71	4.47	4.44	4.47	4.3	4.1	- 5
J01CF	Beta-lactamase resistant penicillins	0.66	0.72	0.77	0.80	0.82	0.88	0.90	0.78	- 13
J01CR	Combination of penicillins	0.01	0.02	0.02	0.02	0.03	0.03	0.04	0.05	+ 35
J01D	Cephalosporins, monobactams, carbapenems	0.60	0.60	0.60	0.58	0.55	0.56	0.55	0.52	- 5
J01E	Sulfonamides and trimethoprim	1.04	1.02	0.98	0.94	0.87	0.87	0.87	0.85	- 2
J01F	Macrolides, lincosamides and streptogramins	2.24	2.30	2.13	1.89	2.01	2.31	2.26	1.93	- 14
J01G	Aminoglycosides	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	-
J01M	Quinolones	0.62	0.67	0.70	0.71	0.73	0.75	0.75	0.71	- 5
J01X*	Other antibacterials	3.18	3.30	3.48	3.65	3.84	3.93	4.04	4.12	+ 2
	Total exclusive of methenamine	**16.3**	**16.9**	**16.8**	**16.2**	**16.3**	**17.2**	**17.4**	**16.3**	**- 6**
	Total all antimicrobial agents	**19.0**	**19.7**	**19.8**	**19.4**	**19.7**	**20.6**	**21.0**	**20.0**	**- 5**

*J01X includes glycopeptides, colistin, fusidic acid, metronidazol (i.v.), nitrofurantoin, linezolid and methenamine. Of total J01X, methenamine constitutes 3.7 DDD/1,000 inhabitants/day in 2013.

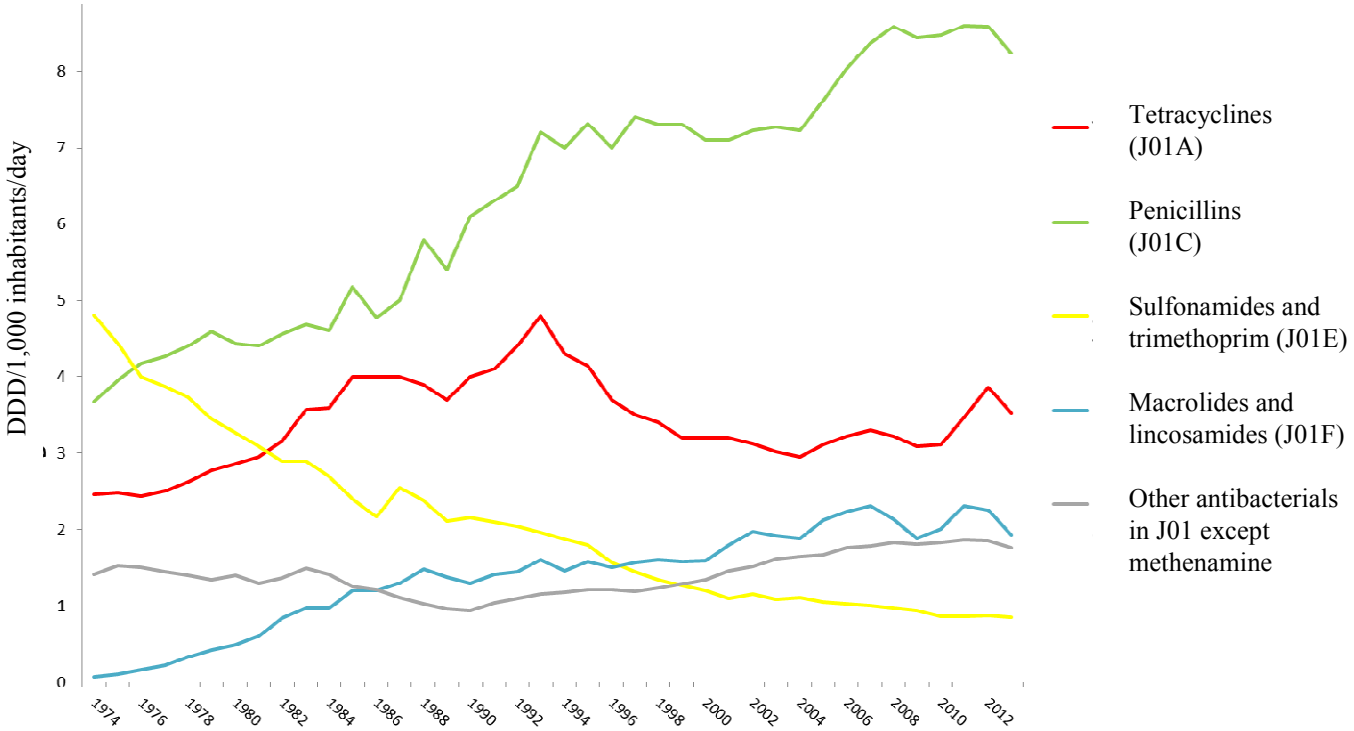

FIGURE 7. Sales of tetracyclines (J01A); penicillins (J01C); sulfonamides and trimethoprim (J01E); macrolides, lincosamides and streptogramines (J01F); and other antibacterials in Norway 1974-2013. Other types of antibacterials include all other antibacterials in ATC group J01, except methenamine (J01XX05).

Antimicrobial total usage in humans and animals, measured in weight of active substance

In 2013, the overall sales in Norway of antibacterials for use in humans, terrestrial animals and farmed fish measured in weight of active substance were 56.4 tonnes (Figure 8). Humans accounted for 87%, terrestrial animals for 11 % and aquaculture for only 2% of the total use. The increase of 13 % (in tonnes) from 2005 is caused by increased use in humans. When excluding methenamine, the increase was 5 % (from 41.0 tonnes in 2005 to 42.8 tonnes in 2013). During these years the use in terrestrial animals has been stable.

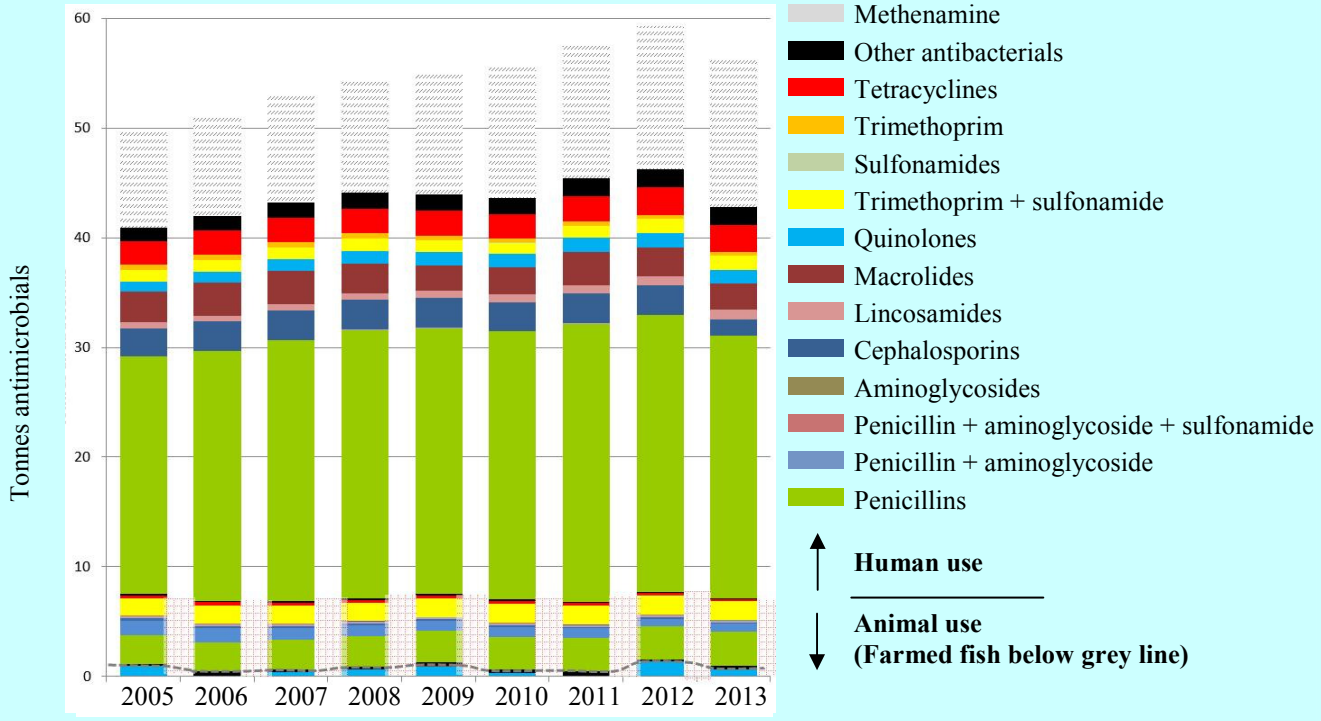

FIGURE 8. Sales, in tonnes of active substance, of human and veterinary antibacterials, for the years 2005-2013. Use in farmed fish is included and appears below the grey line.

According to Table 7, oral formulations are dominating in human medicine while for veterinary medicine the dominating formulations are the parenteral ones. The oral formulations represent 84 % of the total weight followed by parenteral formulations which constitute 15 % of the total weight. Use of other formulations e.g. for eye, ear and skin is limited.

TABLE 7. Sales, in kg of active substance, of human and veterinary antibacterials according to formulation in 2013.

Formulation	Humans	Terrestrial animals	Aquaculture
Dermal	104	3	
Oral	43,910	2,291	972
Parenteral	5,090	3,388	
Eye / ear	34	12	
Intramammary		395	
Others	58	119	
Total	49,195	6,208*	972

* There is a minor discrepancy of 36 kg between the total sales (excluding dermal and eye/ear formulations) between Table 7 and Figure 1 on page 14; this is due to differences in rounding of calculations.

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In 2013, the penicillins (ATC group J01C) accounted for 41% of the total antibacterial use in Norway (Figure 9). Over the years there has been a shift towards use of more broadspectered penicillins. The beta-lactamase sensitive penicillin-group (J01CE) is the largest of the penicillin subgroups. Penicillins with extended spectre (J01CA) now represent 40% of the penicillin group compared to around 30% a decade ago (2003) (Figures 9-10). This is mainly due to increasing use of amoxicillin and pivmecillinam. Pivmecilinam is used for urinary tract infections, at the expense of sulfonamides and trimethoprim, which have decreased over the years (Figure 7).
Total use of the group J01F macrolides, lincosamides and streptogramins has followed a wavy pattern over the years, although the relative frequencies within the group have remained relatively unchanged over the years (Figures 7 and 11). The shifts in use could be explained to some degree by the recurrent epidemics of *M. pneumoniae* in Norway, occurring with four- to six-years intervals.
In the latest years, sales of cephalosporins, monobactams and carbapenems have been stable and this group represents 3% of the total sales of antibacterials (Figure 9). The internal subgroup pattern has changed over time (Figure 12). Today, 1st and 3rd generation cephalosporins constitute 48% and 29% of ATC group J01D, respectively.
In 2013, the use of quinolones decreased for the first time since the fluoroquinolones came to the market in Norway. The quinolones represent only a small fraction (4%) of total antibacterial sales, but the sales have more than doubled since 2000 (Figure 9). Ciprofloxacin is the main substance accounting for 96% of the quinolone group in 2013.
The increase of ATC group J01X is mainly due to the urinary prophylactic agent methenamine, accounting for 18% of total antibacterial use (Figure 9).

The usage of antibacterials varies among the 19 Norwegian counties; the county using the least is using around 70% (in DDDs) of the county using the most (Figure 13). There is a trend of the same high-use and low-use counties over the years. The same pattern is seen when looking at number of prescriptions/1,000 inhabitants.

Antibacterials are prescription-only drugs in Norway. Around 85% of the total human sales of antibacterials are used outside institutions (hospitals and nursing homes). Physicians are the main prescribers to humans, but dentists prescribe 5% (measured in DDDs) of antibiotics (J01) to humans in ambulatory care. The prescription of antibiotics by dentists has increased by 60% (measured in DDDs) from 2004-2013. In 2013, dentists most often prescribed phenoxymethylpenicillin (76% of all antibiotic DDDs prescribed by dentists) followed by amoxicillin (10%) and clindamycin (6%).

For ambulatory care, the most important antibiotic groups in 2013 were penicillins (J01C: 40% of DDDs), tetracyclins (J01A: 20%) and macrolides and lincosamides (J01F: 10%). The three most commonly used antibiotics for outpatients in 2013 were phenoxymethylpenicillin, pivmecillinam and doxycycline. These three substances accounted for 44% of all prescriptions and 51% of all DDDs prescribed when excluding methenamine.

Females use more antibiotics than males; 28% of the females purchased at least one antibiotic course in 2013 compared to 19% of the males. The gender pattern is similar in all regions of the country (Figure 14). The highest use is found among young children, young women and the elderly (Figure 15). Among those who use antibacterials, the elderly use more, both with regard to amount (measured in DDDs) and to number of prescriptions. For those above 70 years, 2-3 prescriptions are dispensed every year compared to 1-2 for younger persons. Since the dosages for young children are much lower than in adults, the number of DDDs per user will be lower than in adults (Figure 16).

In 2013, the antibacterial sales (in DDDs) to hospitals represented around 7% of total sales of antibacterials for human use in the country. The therapy pattern of antibacterials in hospitals does not change much from one year to another (Figure 17).
Penicillins (J01C) represent 46% of the use measured in DDDs in hospitals (J01CE 18%, J01CA 15%, J01CF 10% and J01CR 3%). The second largest group is the cephalosporins; 19% of all DDDs, the dominant subgroup being 3rd generation cephalosporins (J01DD, 9%). In 2013, seven substances accounted for 52% of DDDs used, these being benzylpenicillin, cloxacillin, cefotaxime, ampicillin, ciprofloxacin, cefalotin and pivmecillinam. Three single substances accounted for 30% of all antibacterial use in hospitals; benzylpenicillin (14%), cloxacillin (8%) and cefotaxime (8%). Six selected groups mainly used in hospitals are shown in Figure 18. Since 2006, there has been a stable increase in the use of carbapenems and piperacillin with enzyme inhibitor. The use of 3rd generation cephalosporins decreased in 2013 and the use of 2nd generation cephalosporins has been decreasing over many years.

The use of antimycotics for systemic use has been increasing in Norway (Figure 19). Hospital use represents 23% of total use measured in DDDs. Fluconazole is the most commonly used agent. In July 2013, a warning regarding the use of oral ketoconazole was issued due to increased risk of liver damage. This resulted in decreased use of ketoconazole in ambulatory care. In hospitals, 62 % of the DDDs are parenteral use, and there has been an increased use of amphothericin B.

The National Guidelines for antibiotic use in ambulatory care and nursing homes were updated in 2013 and National Guidelines for hospital use were first published in 2013. The Antibiotic Center for Primary Care (ASP) was established in 2006 and a National Centre for Antibiotic Use in Hospitals was established in 2011. These centres are responsible for the continuous updating of national treatment guidelines, and this will hopefully have a positive impact on therapy traditions and antibacterial prescribing patterns in Norway.

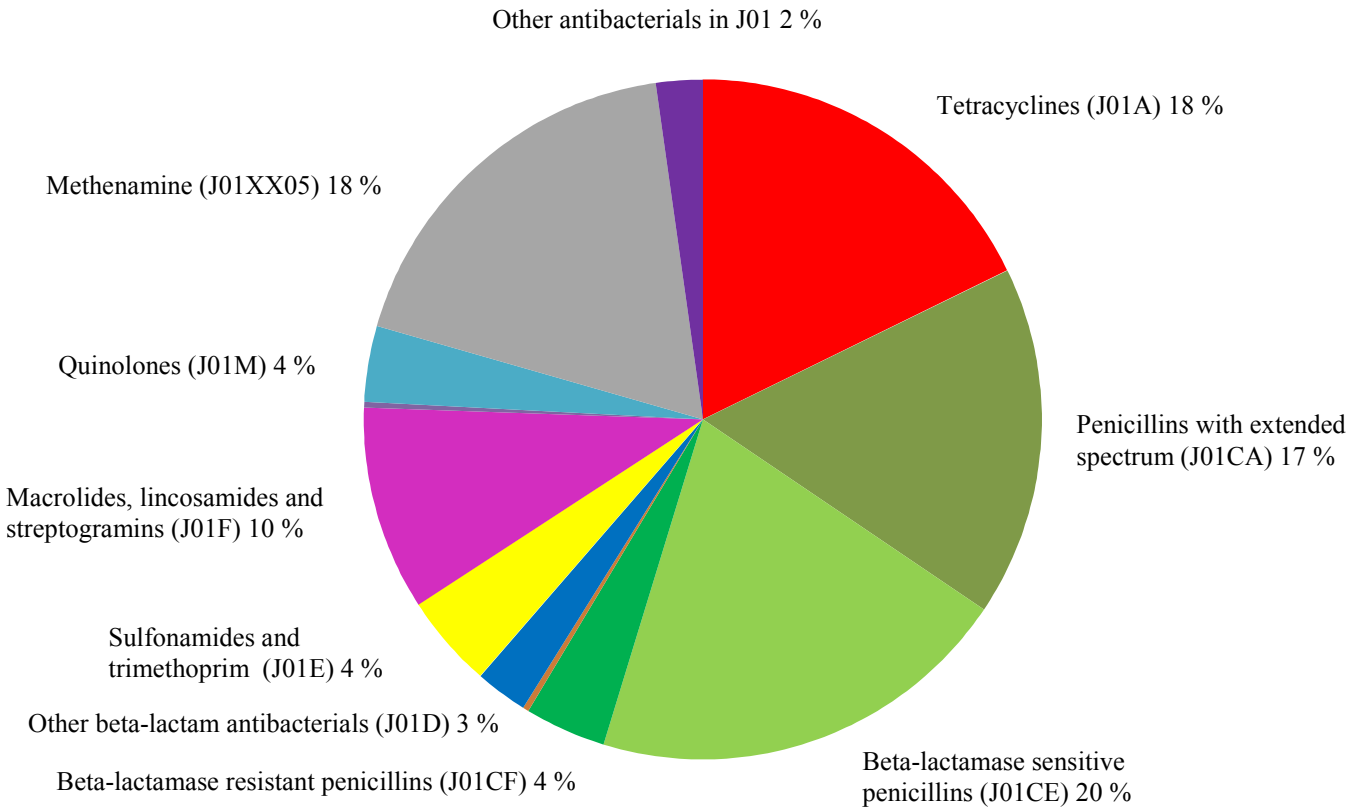

FIGURE 9. Relative amount of antibacterial agents for systemic use in 2013 in Defined Daily Doses (DDD) (total sales).

TABLE 8. Human usage of single antibacterial agents for systemic use in Norway 2008-2013. Sales are given in DDD/1,000 inhabitants/day. The methodology for collection of data on human usage of antibacterial agents is presented in Appendix 2.

ATC group	ATC code	Substance	2008	2009	2010	2011	2012	2013
J01A - Tetracyclines	J01A A02	Doxycycline	1.9	1.78	1.83	2.09	2.36	2.02
	J01A A04	Lymecycline	0.52	0.54	0.59	0.76	0.90	1.0
	J01A A06*	Oxytetracycline	0.17	0.16	0.15	0.03		
	J01A A07	Tetracycline	0.62	0.60	0.54	0.58	0.62	0.54
	J01A A08*	Minocycline	0.0002	0.0003	0.001	0.002	0.006	0.009
	J01A A12	Tigecycline	0.0004	0.0005	0.0004	0.0002	0.0002	0.0003
J01B - Amphenicols	J01B A01	Chloramphenicol	0.001	0.002	0.0007	0.0005	0.0002	0.0002
J01CA - Penicillins with extended spectrum	J01C A01	Ampicillin	0.10	0.11	0.09	0.09	0.09	0.10
	J01C A04	Amoxicillin	1.34	1.31	1.34	1.39	1.45	1.40
	J01C A08	Pivmecillinam	1.65	1.72	1.75	1.73	1.78	1.83
	J01C A11*	Mecillinam	0.008	0.008	0.008	0.008	0.008	0.008
J01CE - Beta-lactamase sensitive penicillins	J01C E01	Benzylpenicillin	0.24	0.28	0.22	0.24	0.24	0.22
	J01C E02	Phenoxymethyl-penicillin	4.46	4.19	4.22	4.23	4.07	3.85
	J01C E08*	Benzathine benzylpenicillin	0.0001	0.0002	0.0002	0.0001	0.0002	0.0002
J01CF - Beta-lactamase resistant penicillins	J01C F01	Dicloxacillin	0.64	0.67	0.70	0.74	0.76	0.57
	J01C F02	Cloxacillin	0.13	0.13	0.12	0.14	0.14	0.21
	J01C F05*	Flucloxacillin	0.0005	0.0007	0.0005	0.0003	0.0005	0.001
J01CR - Combination of penicillins, incl. beta-lactamase inhibitors	J01C R02*	Amoxicillin and enzyme inhibitor	0.0012	0.003	0.003	0.002	0.004	0.007
	J01C R05	Piperacillin and enzyme inhibitor	0.02	0.02	0.02	0.03	0.03	0.04

ATC group	ATC code	Substance	2008	2009	2010	2011	2012	2013
J01DB - First gen. cephalosporins	J01D B01	Cefalexin	0.23	0.21	0.20	0.19	0.18	0.17
	J01D B03	Cefalotin	0.07	0.07	0.07	0.08	0.08	0.08
J01DC - Second gen. cephalosporins	J01D C02	Cefuroxime	0.11	0.10	0.09	0.09	0.08	0.07
J01DD - Third gen. cephalosporins	J01D D01	Cefotaxime	0.10	0.11	0.11	0.12	0.12	0.12
	J01D D02	Ceftazidime	0.01	0.01	0.01	0.01	0.01	0.01
	J01D D04	Ceftriaxone	0.02	0.02	0.02	0.03	0.03	0.03
J01DF - Monobactams	J01D F01	Aztreonam	0.0007	0.0006	0.0006	0.0005	0.0007	0.001
J01DH - Carbapenems	J01D H02	Meropenem	0.04	0.04	0.04	0.04	0.05	0.05
	J01D H03	Ertapenem	0.001	0.002	0.002	0.002	0.002	0.002
	J01D H51	Imipenem and enzyme inhibitor	0.003	0.002	0.002	0.002	0.002	0.002
J01E - Sulfonamides and trimethoprim	J01E A01	Trimethoprim	0.64	0.60	0.56	0.55	0.51	0.48
	J01E E01	Sulfamethoxazole and trimethoprim	0.34	0.33	0.31	0.32	0.36	0.37
J01F - Macrolides, lincosamides and streptogramins	J01F A01	Erythromycin	1.08	0.92	0.94	1.18	1.06	0.85
	J01F A02	Spiramycin	0.01	0.01	0.01	0.01	0.01	0.01
	J01F A09	Clarithromycin	0.37	0.31	0.34	0.37	0.39	0.30
	J01F A10	Azithromycin	0.38	0.37	0.41	0.44	0.48	0.41
	J01F F01	Clindamycin	0.28	0.28	0.31	0.32	0.33	0.37
J01G - Aminoglycosides	J01GA01*	Streptomycin	0.0003	0.0002	0.0002	0.0002	0.0001	0.0001
	J01G B01	Tobramycin	0.03	0.03	0.03	0.03	0.03	0.03
	J01G B03	Gentamicin	0.04	0.04	0.04	0.05	0.05	0.05
	J01G B06*	Amikacin	0.0007	0.0008	0.0009	0.001	0.001	0.001
J01M - Quinolones	J01M A01	Ofloxacin	0.04	0.03	0.03	0.03	0.02	0.02
	J01M A02	Ciprofloxacin	0.66	0.67	0.70	0.71	0.72	0.69
	J01MA12*	Levofloxacin	0.0008	0.004	0.003	0.002	0.002	0.001
	J01MA14*	Moxifloxacin	0.001	0.001	0.004	0.006	0.004	0.005
J01X - Other antibacterials	J01X A01	Vancomycin	0.01	0.01	0.01	0.01	0.01	0.01
	J01X A02	Teicoplanin	0.001	0.001	0.001	0.001	0.001	0.001
	J01X B01	Colistin	0.004	0.005	0.004	0.004	0.004	0.005
	J01X C01	Fusidic acid	0.006	0.005	0.004	0.005	0.005	0.004
	J01X D01	Metronidazole	0.07	0.07	0.07	0.07	0.07	0.06
	J01X E01	Nitrofurantoin	0.36	0.36	0.37	0.39	0.37	0.36
	J01X X05	Methenamine	3.02	3.19	3.37	3.44	3.57	3.67
	J01XX08	Linezolid	0.007	0.008	0.009	0.01	0.01	0.007
	J01XX09	Daptomycin	0.000	0.000	0.0001	0.0004	0.0009	0.001
Antibiotics in other ATC groups	J04AB02	Rifampicin	0.003	0.004	0.004	0.004	0.005	0.004
	J04A**	Rifampicin	0.067	0.087	0.086	0.082	0.086	0.082
	A07AA09	Vancomycin	0.001	0.001	0.001	0.001	0.002	0.002
	A07AA11	Rifaximin		0.001	0.001	0.002	0.004	0.007
	A07AA12	Fidaxomicin					0.0001	0.0003
	P01AB01	Metronidazole	0.22	0.22	0.23	0.24	0.23	0.24
	D06AX09/ R01AX06*	Mupirocin in kg ointment/cream (2%)	3.9	5.1	4.5	4.6	7.3	8.6

*Drugs not licensed in the Norwegian market in 2013. ** Given as the amount DDD/1,000 inhabitants/day of rifampicin in plain and combination products.

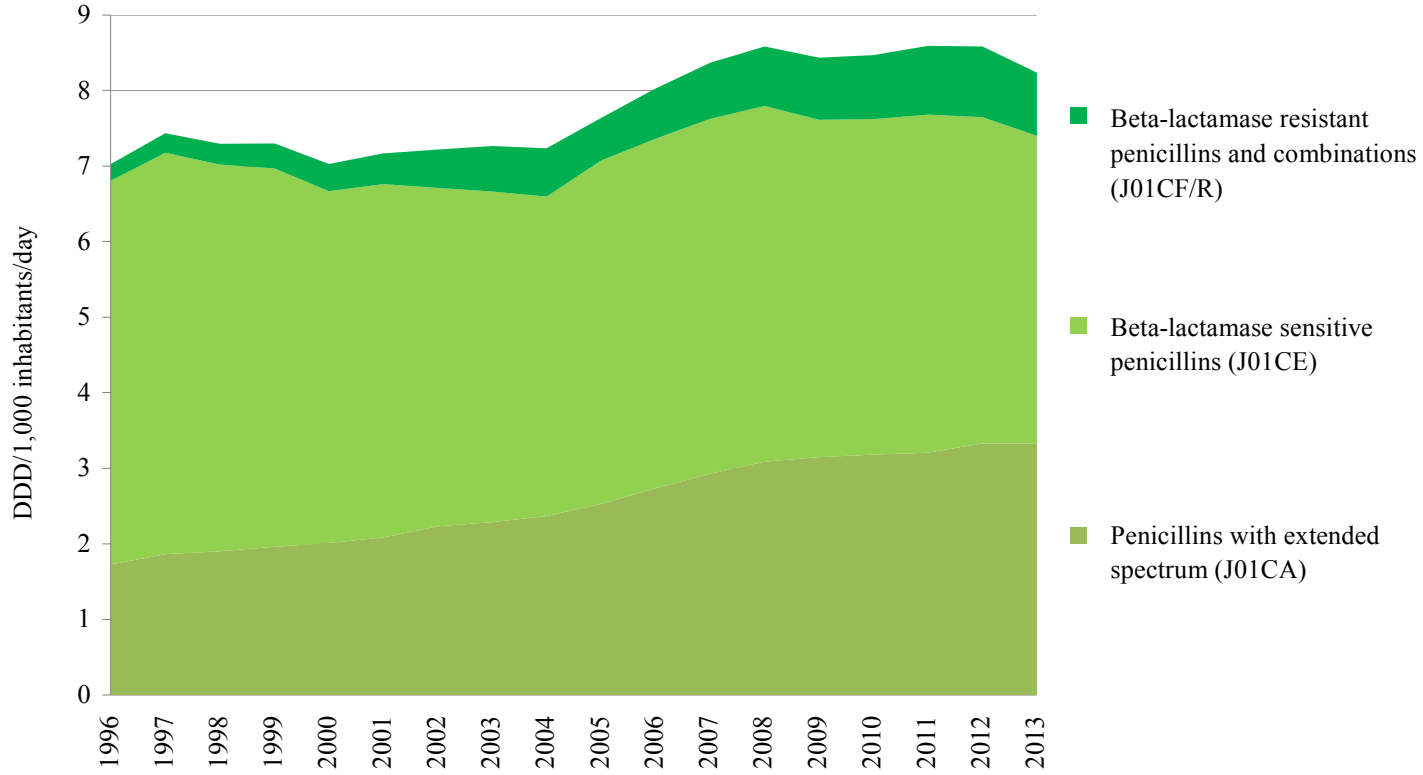

FIGURE 10. Sales of penicillins (J01C) in Norway 1996-2013 and changes within groups of penicillins.

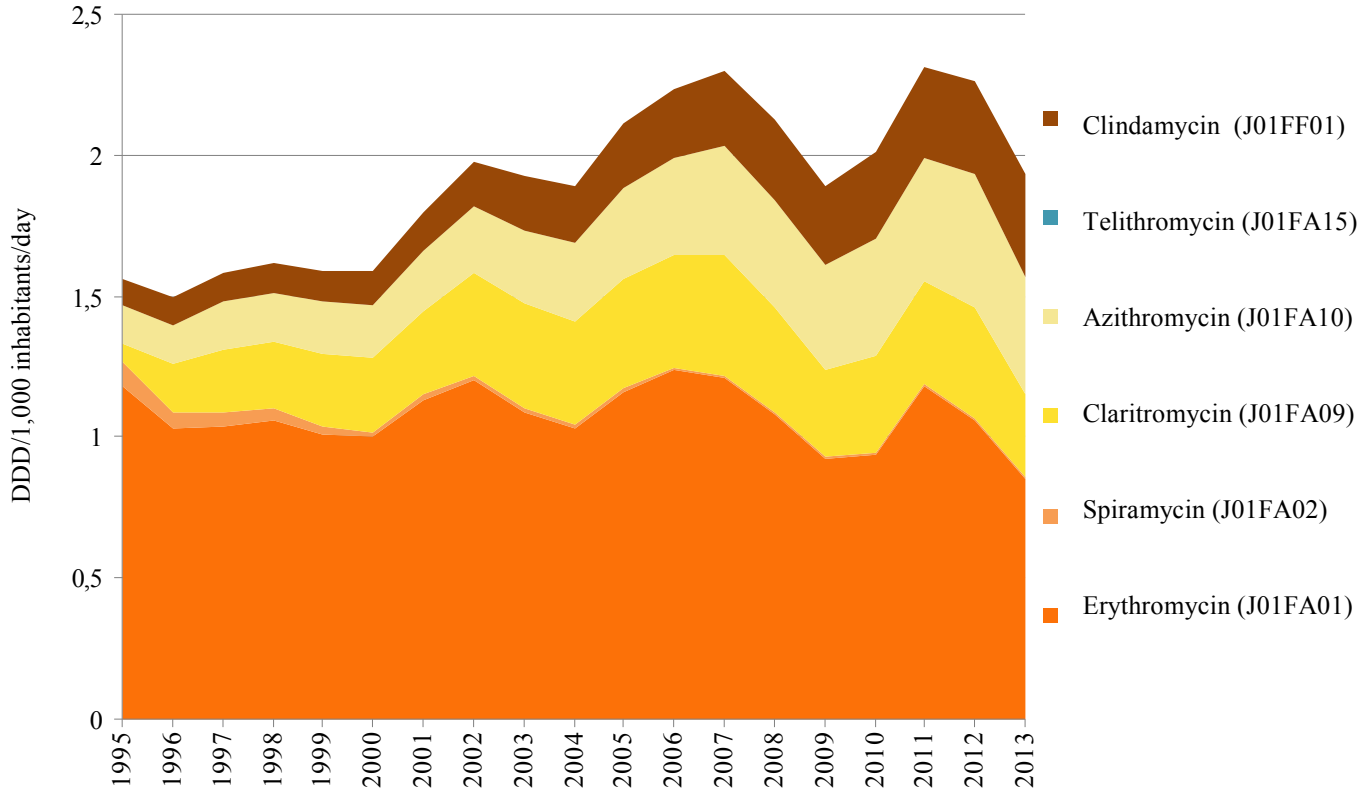

FIGURE 11. Sales of macrolides, lincosamides and streptogramins (J01F) in Norway 1996-2013.

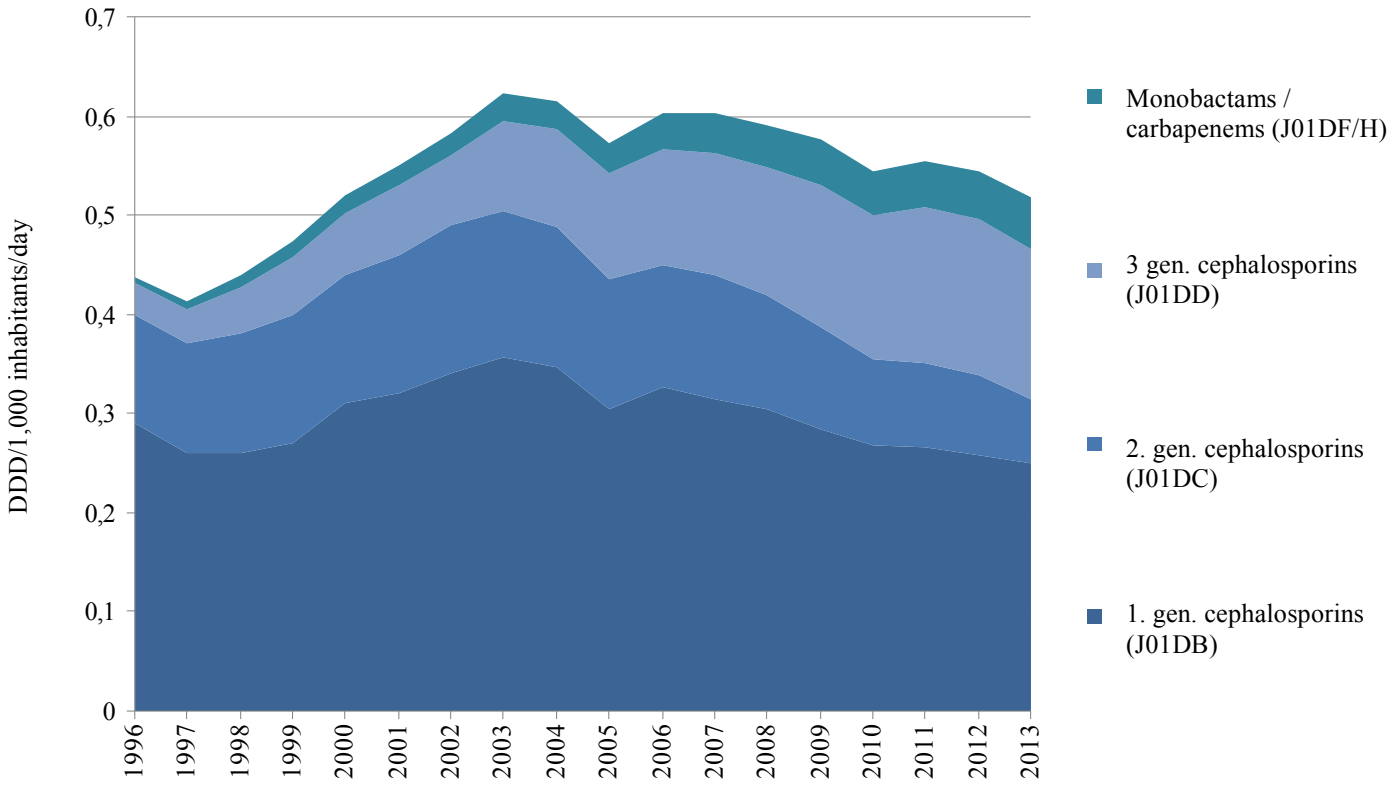

FIGURE 12. Sales of cephalosporins, monobactams and carbapenems (J01D) in Norway 1996-2013 and changes within generations of cephalosporins and monobactams/carbapenems.

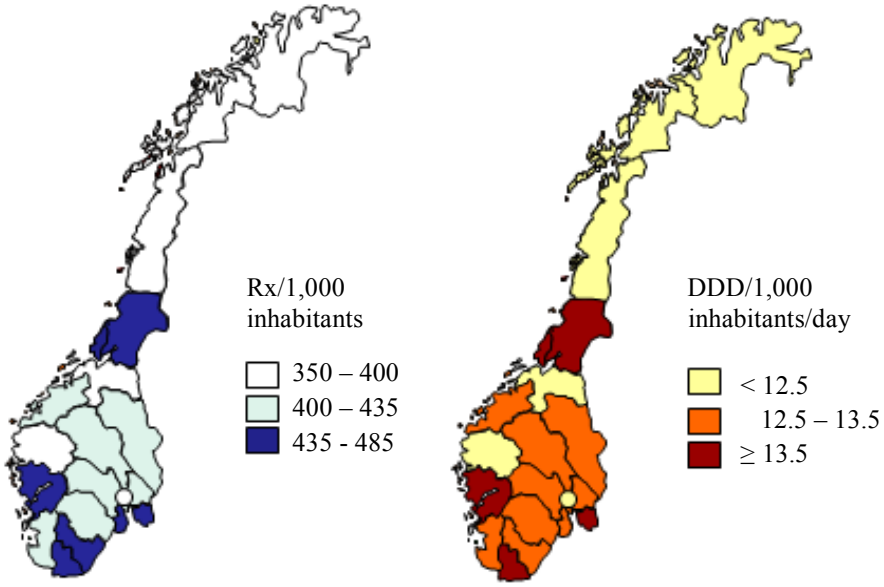

FIGURE 13. Sales of antibacterial agents for systemic use (ATC group J01, excl. methenamine) in outpatients in the different counties of Norway in 2013. Measured as number of prescriptions (Rx)/1,000 inhabitants and number of DDD/1,000 inhabitants/day. Data from the Norwegian Prescription Database (excl. health institutions).

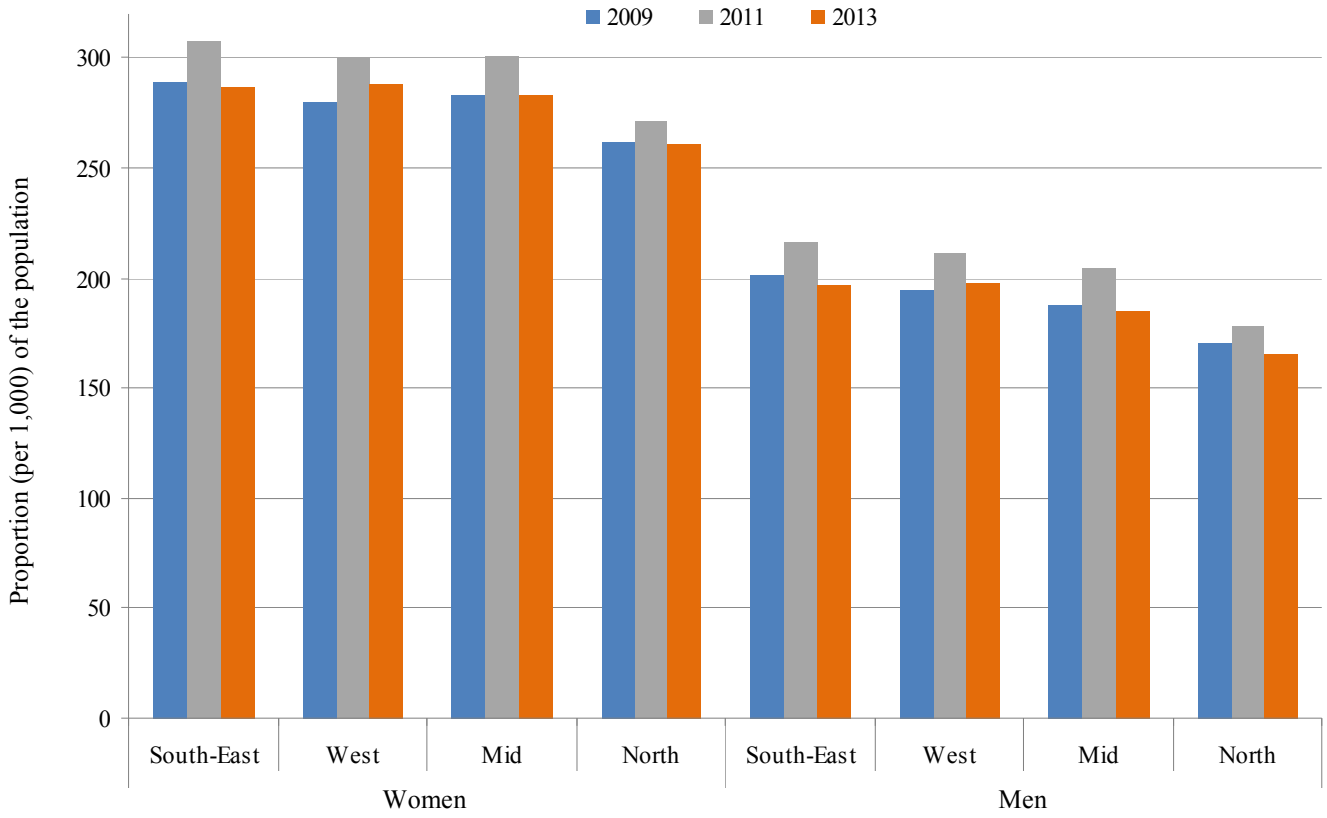

FIGURE 14. One year prevalence of systemic antibacterial use in ambulatory care by gender and health region in Norway for the years 2009, 2011 and 2013. Antibacterials for systemic use include ATC group J01, vancomycin (A07AA09), rifaximin (A07AA11), fidaxomicin (A07AA12) and metronidazole (P01AB01).

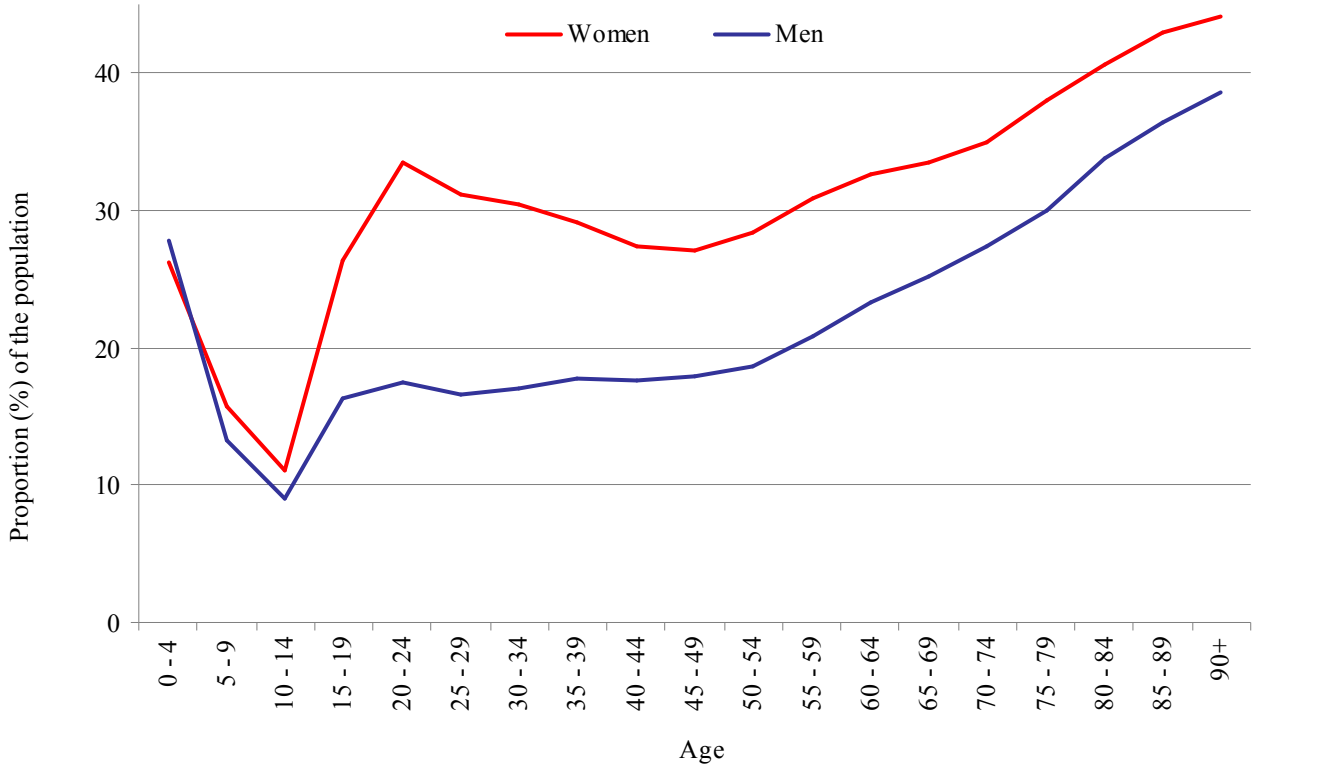

FIGURE 15. Proportion (%) of the population having dispensed at least one prescription of antibacterials (one year prevalence) in ambulatory care by gender and age in Norway, 2013. Antibacterials included are antibacterials for systemic use (ATC group J01), oral vancomycin (A07AA09), rifaximin (A07AA11), fidaxomicin (A07AA12) and oral metronidazole (P01AB01). Prevalence in age groups above 65+ is adjusted according to persons from these age groups living outside institutions.

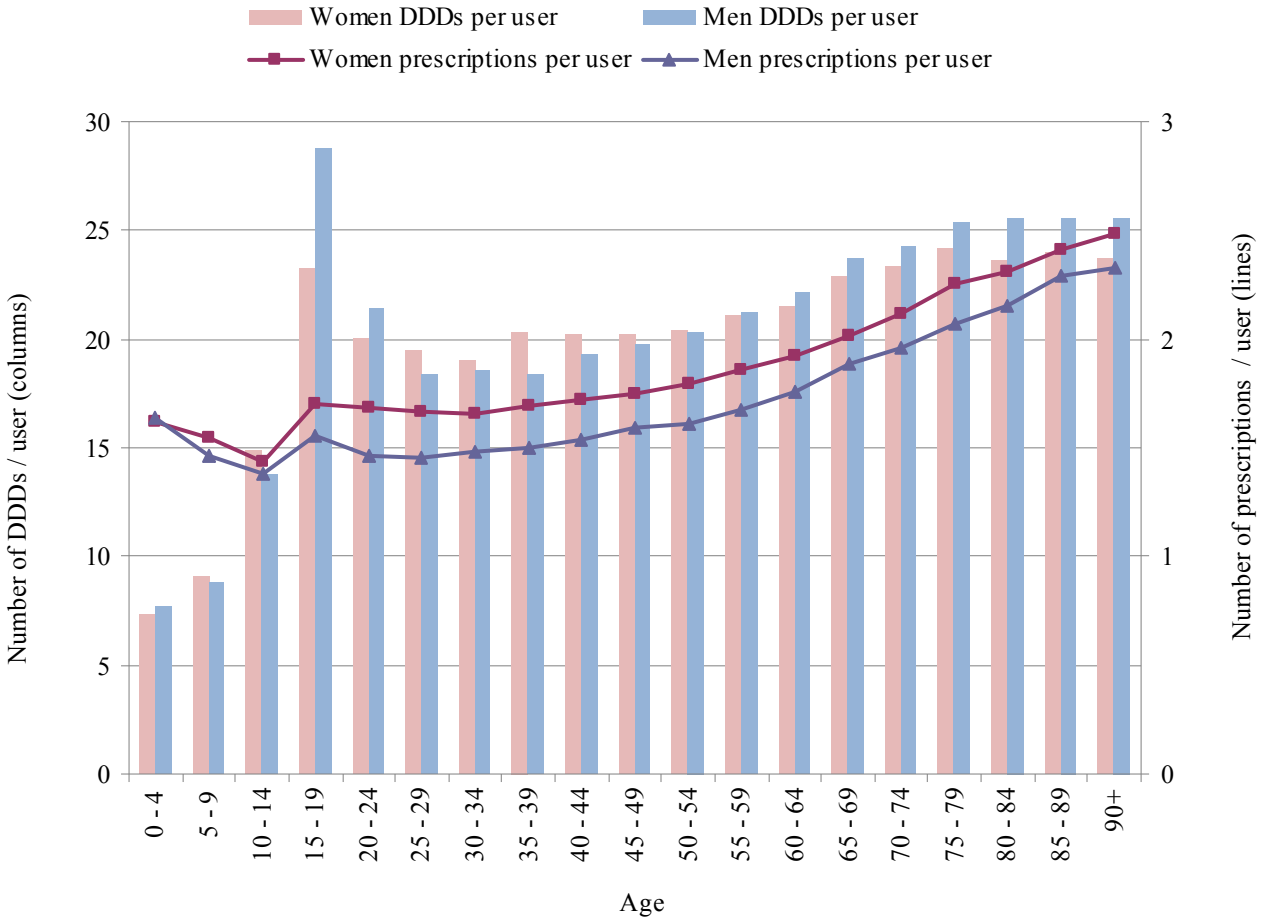

FIGURE 16. Mean number of prescriptions per person and mean number of DDDs per person among users of antibacterials in ambulatory care by gender and age in Norway, 2013. Antibacterials included are antibacterials for systemic use (ATC group J01, excl. methenamine).

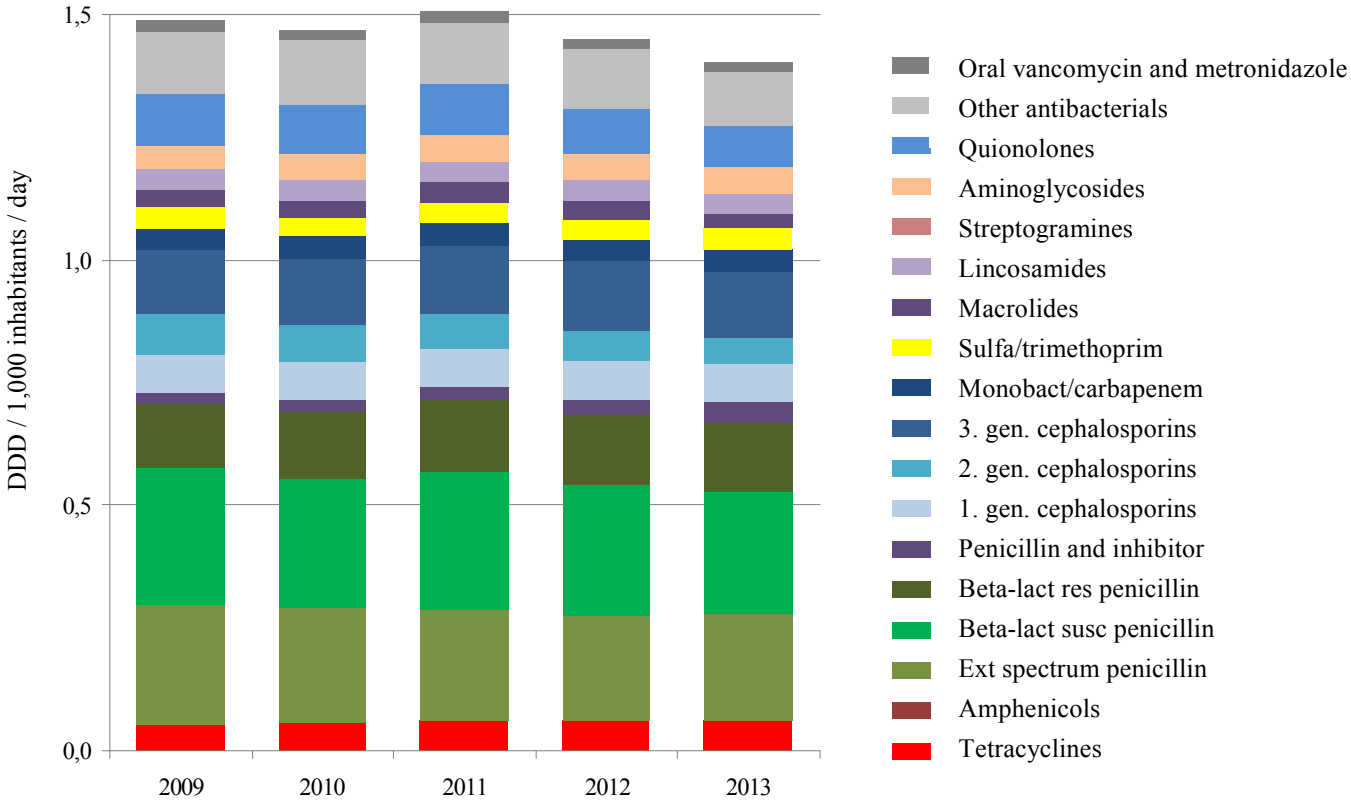

FIGURE 17. Proportions of antibacterial agents for systemic use in Norwegian hospitals 2009-2013, measured in DDD/1,000 inhabitants/day.

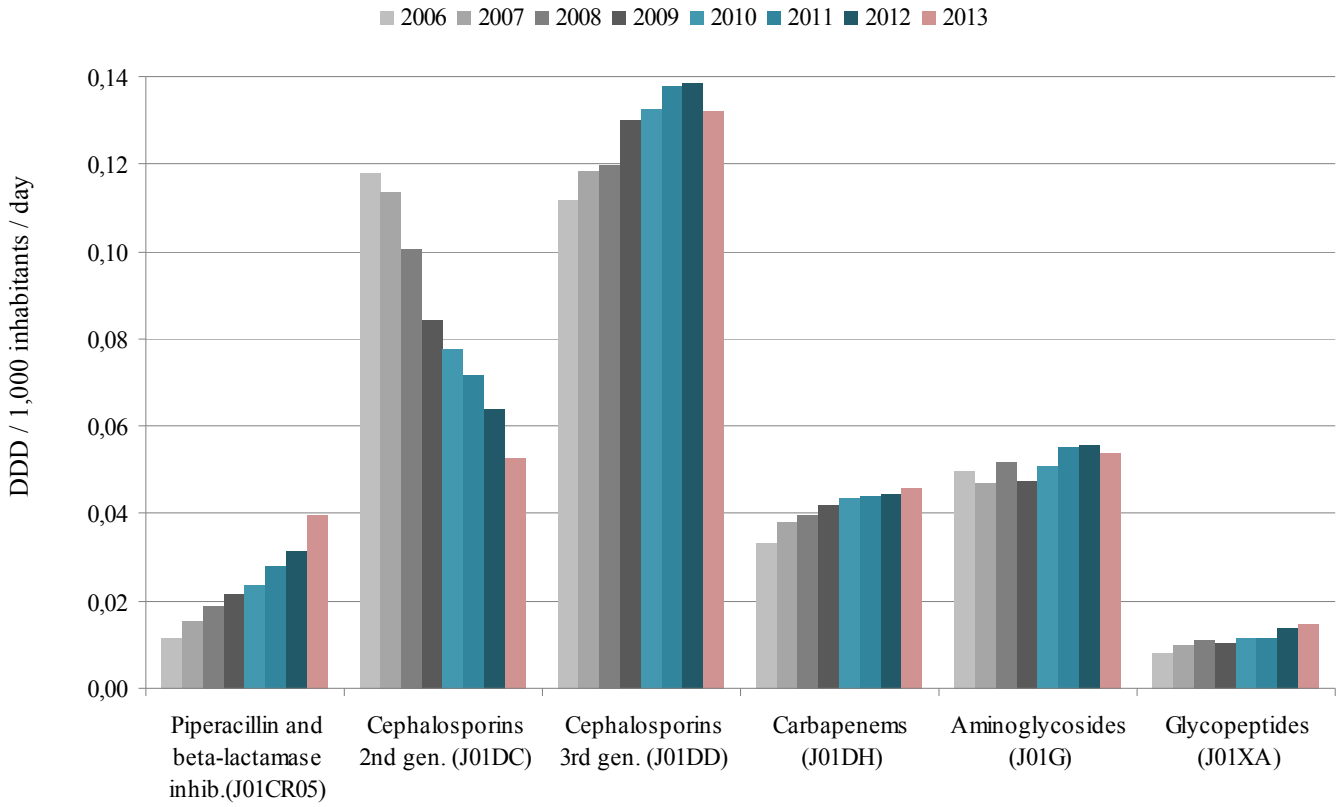

FIGURE 18. Proportions of selected antibacterial agents for systemic use in Norwegian hospitals 2006-2013, measured in DDD/1,000 inhabitants/day.

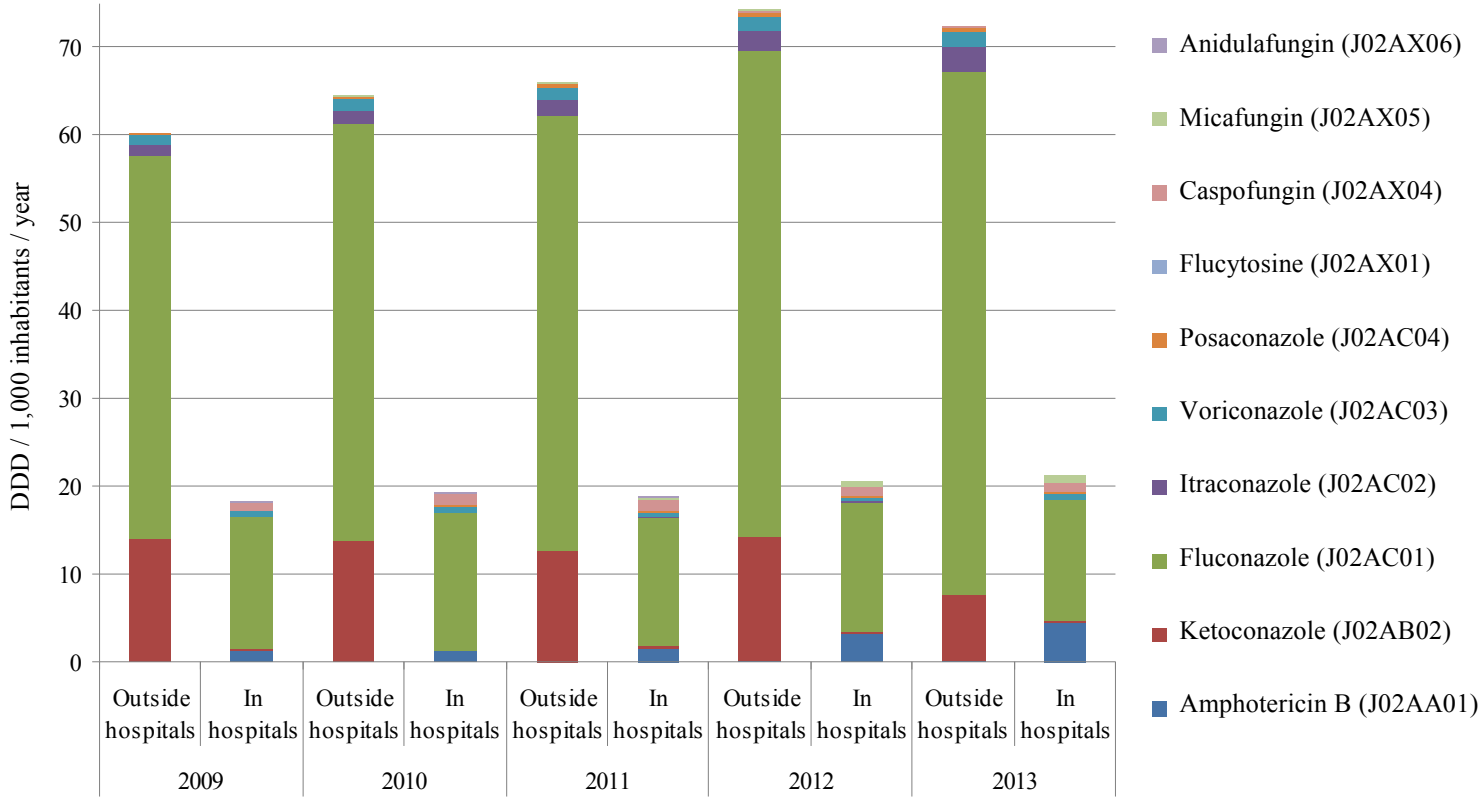

FIGURE 19. Proportions of antimycotics for systemic use in Norway for ambulatory care and hospitals 2009-2013, measured in DDD/1,000 inhabitants/year.

VI. OCCURRENCE OF ANTIMICROBIAL RESISTANCE

A. ANIMAL CLINICAL ISOLATES

Madelaine Norström, Jannice Schau Slettemeås, Marianne Sunde, Anne Margrete Urdahl

According to the NORM-VET plan, the clinical isolates included in 2013 were *Staphylococcus pseudintermedius* from infections in dogs. Sampling, laboratory methods and data processing are described in Appendix 3.

Staphylococcus pseudintermedius from dog

A total of 201 isolates of *Staphylococcus pseudintermedius* from clinical submissions were subjected to NORM-VET. The results are presented in Table 9 and in the text.

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TABLE 9. Antimicrobial resistance in isolates of *Staphylococcus pseudintermedius* from dogs (n=201) in 2013.

	Resistance (%)		Distribution (%) of MIC values (mg/L)*													
Substance	%	[95% CI]	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256
Penicillin G**	82.1	[76.1-87.1]	17.4	1.5	9.5	13.9	10.4	13.4	15.4	12.9	5.5					
Oxacillin	0.5	[0.0-2.7]			0.5	65.7	25.9	6.0	1.5	0.5						
Cefalothin	0.0	[0.0-1.8]		27.4	68.2	2.5	2.0									
Gentamicin	2.5	[0.8-5.7]				0.5	91.5	4.5	1.0	0.5	1.5	0.5				
Kanamycin	22.4	[16.8-28.8]				0.5	20.9	50.7	4.5	0.5	0.5	0.5		21.9		
Ciprofloxacin	3.5	[1.4-7.0]		7.0	41.3	42.8	4.5	1.0	0.5	0.5	2.5					
Trimethoprim	5.5	[2.8-9.6]						2.0	73.0	17.0	2.5	0.5	0.5	4.5		
Clindamycin	18.0	[12.9-23.9]				82.1	3.5	2.5	0.5	0.5	1.0	0.5	0.5	9.0		
Erythromycin	20.9	[15.5-27.2]				43.3	33.3	2.5	1.0		0.5		0.5	18.9		
Chloramphenicol	5.5	[2.8-9.6]							47.3	46.3	1.0			5.5		
Tetracycline	34.9	[28.3-41.8]					62.7	2.5	1.0		1.0	3.5	28.4	1.0		
Fusidic acid	57.7	[50.6-64.6]			9.0	22.4	10.9	6.0	4.5	14.9	31.3	1.0				

*Bold vertical lines denote epidemiological cut-off values for resistance. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested for are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

**Resistance to penicillin G was based on beta-lactamase production. All isolates with a positive beta-lactamase test had MIC values > 0.125 mg/L, and all beta-lactamase negative isolates had MIC values ≤ 0.125 mg/L.

***Of 165 isolates sensitive to clindamycin, five were classified as resistant based on inducible clindamycin resistance.

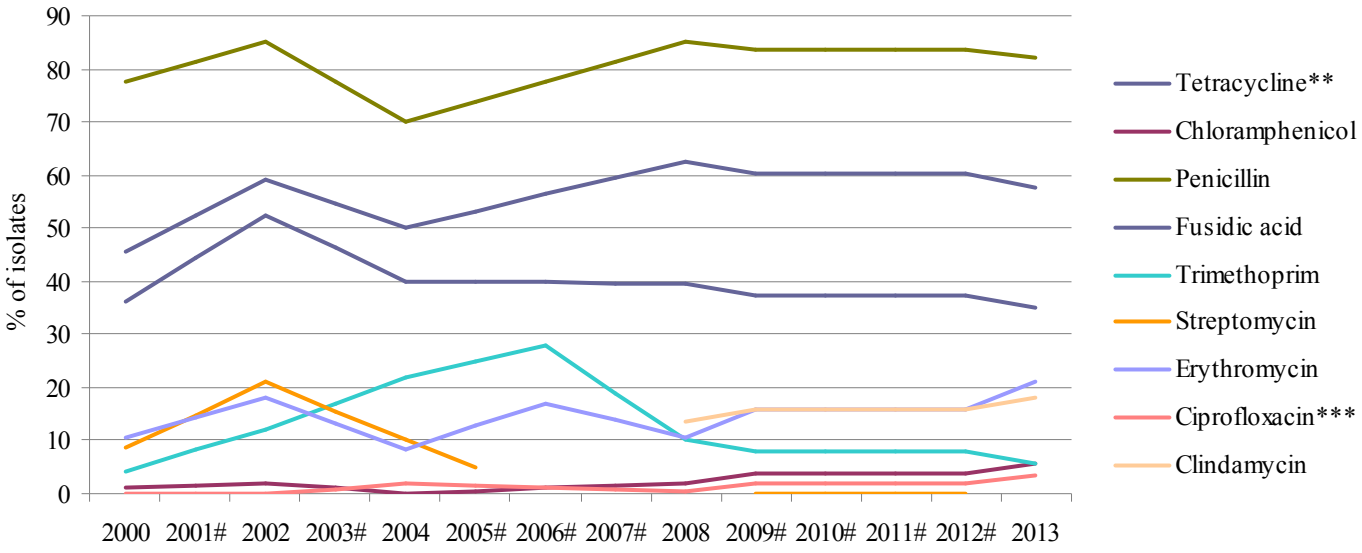

FIGURE 20. Prevalence of resistance to various antimicrobials in beta-haemolysin-producing *Staphylococcus* spp. from skin and ear infections in dogs 2000-2013. #Interpolated results (not monitored these years). The cut-off values in NORM-VET 2013 were applied. ** Oxytetracycline instead of tetracycline before 2008. ***Enrofloxacin instead of ciprofloxacin in 2004.

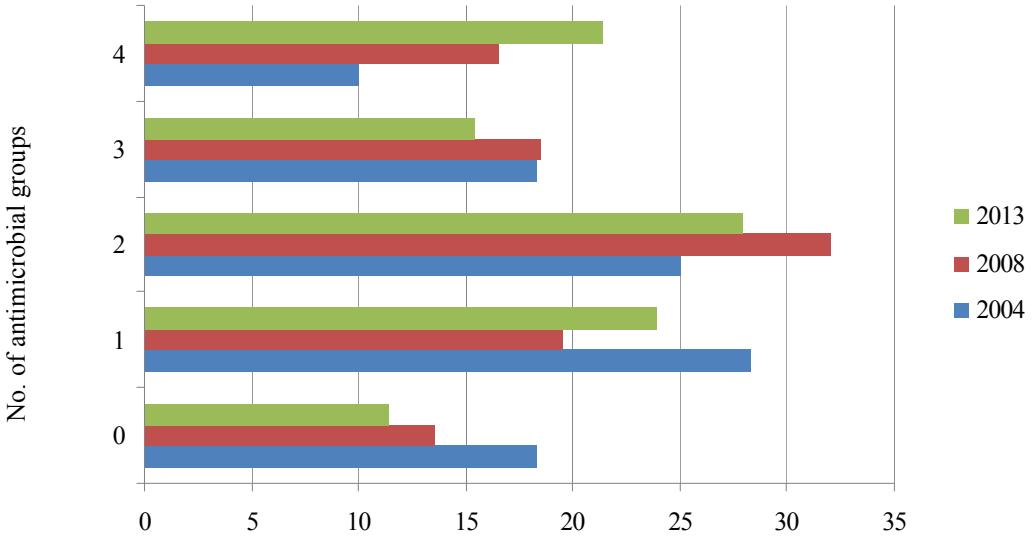

FIGURE 21. Antimicrobial resistance profiles for beta-haemolysin-producing *Staphylococcus* spp. from skin and ear infections in dogs 2004, 2008 and 2013. Proportion of isolates susceptible to all antimicrobials included and proportion of isolates resistant to one, two, three and four or more antimicrobials

RESULTS AND COMMENTS

The occurrence of resistance among *Staphylococcus pseudintermedius* was extremely high. In total, 11.4% of the isolates were susceptible to all antimicrobial agents included. Altogether, 23.9 % of the isolates were resistant to one antimicrobial agent (predominantly penicillin), 27.9% to two (predominantly penicillin and fucidic acid), 15.4% to three (predominantly penicillin, fucidic acid and tetracycline), and 21.4% to four or more antimicrobial agents. A single isolate with oxacillin MIC > 2 mg/L was confirmed to be methicillin resistant (*mecA* detected by PCR). This isolate was additionally resistant to chloramphenicol, erythromycin, clindamycin and kanamycin.

Comparison to previous years is difficult as species determination of *Staphylococcus* spp. (including *Staphylococcus pseudintermedius*) has only been routinely performed since 2008. However, as many as 92.5% of the 2008 isolates were identified as *Staphylococcus pseudintermedius*, and with the current knowledge that *Staphylococcus pseudintermedius* is the most common cause of bacterial skin infections in dogs, a valid assumption is that the majority of previously characterised isolates has been *Staphylococcus pseudintermedius*. Compared to previous years, the overall trend then shows that resistance to penicillin, fusidic acid and tetracycline remains high (Figure 20). However, it should be noted that the prevalences were slightly lower in 2004. This may be explained by differences in sampling procedure as the 2004 isolates were collected from first time skin infections only (NORM/NORM-VET 2004). More importantly, as shown in Figure 21, there has been an increase in the proportion of isolates resistant to one, two, three and four or more antimicrobials in the years 2004, 2008 and 2013. See also separate presentation on page 30.

Antimicrobial resistance in bacteria from companion animals

Monitoring of antimicrobial resistance in relevant bacterial species from companion animals is of importance. The companion animals live in close contact with their owners and handlers, and zoonotic transmission of microorganisms, including resistant bacteria, cannot be excluded. NORM-VET has since the start of the programme in year 2000 included bacteria (staphylococci and *E. coli*) from dogs and horses a few times. In 2013, *Staphylococcus pseudintermedius* isolates from dogs with skin and ear infections were susceptibility tested. In addition, faecal samples from dogs were screened for extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* by use of a selective method. Susceptibility testing of *E. coli* based on random selection of isolates from the same material was performed in parallel. Below is an overview of the results obtained and comparison to earlier result from the NORM-VET programme.

Screening for ESBL producing *E. coli*

A total of 191 faecal samples from dogs were screened for *E. coli* resistant to 3rd generation cephalosporins. The samples originated from healthy dogs sampled at five animal clinics geographically spread throughout Norway. ESBL producing *E. coli* were obtained from four samples (2.1%, 95% CI: 0.6-5.3%). Further characterisation by the use of molecular techniques confirmed the species and the presence of an ESBL encoding gene in all four isolates. In 2008, we performed a similar investigation including faecal samples from healthy dogs from the same geographical areas and using the same laboratory method. One ESBL positive *E. coli* isolate was then found (0.6%, 95% CI: 0.0-3.4). The finding of four isolates in 2013 may indicate an increase of ESBL positive *E. coli* in healthy dogs. However, the observed change in occurrence is not statistically significant and further monitoring is needed to follow the situation.

Resistance trends in *Staphylococcus pseudintermedius*

S. pseudintermedius is considered the most important coagulase positive staphylococcal species in dogs and has been included in previous studies by NORM-VET in 2004[1], 2008[2] and in some of the earliest years of the programme. Relatively high prevalences of resistance have previously been reported, especially to penicillin, fusidic acid and tetracycline. The results obtained in 2013 show similar tendencies. However, for the first time in the NORM-VET programme one of the included isolates was defined as a methicillin resistant *S. pseudintermedius* (MRSP). The emergence of MRSP among dogs represents a relatively new phenomenon with a considerable increase in Europe from the first reports in 2005-2006. The first finding of MRSP from a dog in Norway dates back to 2008. MRSP in dogs represent a therapeutic challenge and the zoonotic potential of *S. pseudintermedius* should not be underestimated.

Concluding remarks

The findings from the 2013 NORM-VET programme may possibly indicate an increase in occurrence of resistance in important bacterial species associated with companion animals. The findings emphasise a need for continued monitoring in years to come in order to confirm and follow these trends.

References:

1. NORM/NORM-VET 2004. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2005. ISSN:1502-2307 (print) / 1890-9965 (electronic).
2. NORM/NORM-VET 2008. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2009. ISSN:1502-2307 (print) / 1890-9965 (electronic).

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B. INDICATOR BACTERIA FROM ANIMALS AND FOOD

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The prevalence of acquired antimicrobial resistance among certain bacteria of the normal enteric microflora can be used as an indicator of the selective pressure from use of antimicrobial agents in various populations. These bacteria may form a reservoir of transferable resistance genes from which antimicrobial resistance can be spread to other bacteria, including those responsible for infections in animals or humans. Thus, monitoring of resistance among indicator bacteria of the normal enteric microflora from healthy animals, as well as from feed and food, is important to get an overview of the resistance situation, detect trends and evaluate the effects of interventions.

In NORM-VET, *Escherichia coli* and *Enterococcus* spp. are used as indicator bacteria. In 2013, faecal samples from dogs and boot swabs from layer flocks and from turkey, as well as *E. coli* from turkey fillets, were included.

The substances included in the test panels might not always be those used in veterinary medicine, but are included because of their importance for human health. Some of the cut-off values defining resistance applied in NORM-VET have been changed over the years. To facilitate comparisons in this report, data on prevalence of resistance presented in earlier reports have been recalculated using the cut-off values applied in 2013. Sampling, laboratory methods and data processing are described in Appendix 3.

Escherichia coli from dogs, layers, turkey and turkey meat

E. coli isolates were obtained from 179/191 dog faecal samples (93.7%) collected at five different veterinary clinics. Samples from a total of 204 layer flocks and 131 turkey flocks were examined and *E. coli* isolates were obtained from 186 samples (91.2%) and 109 samples (83.2%), respectively. From turkey meat, *E. coli* isolates were obtained from 154 out of 156 samples (98.7%). One isolate per positive sample was susceptibility tested. The results are presented in Tables 10-11, and in the text.

TABLE 10. Antimicrobial resistance in isolates of *Escherichia coli* (n=179) from faecal samples from dogs in 2013.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*															
			0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	2.8	[0.9-6.4]							90.5	6.7				1.7	0.6		0.6	
Chloramphenicol	0.6	[0.0-3.1]								1.1	68.2	29.6	0.6			0.6		
Florfenicol	0.0	[0.0-2.0]									45.3	54.2	0.6					
Ampicillin	10.6	[6.5-16.1]							4.6	62.0	21.8	1.1	1.1	0.6			8.9	
Cefotaxime	0.6	[0.0-3.1]			38.0	58.7	2.8				0.6							
Ceftazidime	1.7	[0.3-4.8]					71.5	26.8	1.7									
Sulfamethoxazole	2.2	[0.6-5.6]										13.4	31.3	38.5	8.4	1.7		0.6
Trimethoprim	6.1	[3.1-10.7]				4.5	24.6	59.8	5.0				0.6	5.6				
Gentamicin	0.0	[0.0-2.0]						80.0	19.6	0.6								
Streptomycin	8.4	[4.8-13.4]								0.6	49.2	40.8	1.1	0.6	1.7	3.9	1.7	0.6
Kanamycin	2.2	[0.6-5.6]										97.8	0.6	1.7				
Ciprofloxacin	1.7	[0.3-4.8]	1.1	39.7	57.5	0.6	1.1											
Nalidixic acid	1.2	[0.1-4.0]							1.7	62.6	33.5	1.1		0.6		0.6		
Colistin	0.6	[0.0-3.1]						82.7	14.0	2.8	0.6							

*Bold vertical lines denote epidemiological cut-off values for resistance. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC-value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

TABLE 11. Antimicrobial resistance in isolates of *Escherichia coli* from layers (n=186), turkey (n=109) and turkey meat (n=154) in 2013.

Substance	Sample	Resistance (%)	[95% CI]	Distribution (%) of MIC values (mg/L)*															
				0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Tetracycline	Layers	7.0	[3.8-11.7]							82.3	9.1	0.5	1.1		4.3	2.2	0.5			
	Turkey	12.8	[7.2-20.6]							81.7	5.5				2.8	7.3	0.9	1.8		
	Meat	17.5	[11.8-24.4]							76.0	6.5				5.8	5.8	3.9	1.9		
Chloramphenicol	Layers	0.5	[0.0-3.0]								12.4	69.9	17.2		0.5					
	Turkey	0.9	[0.0-5.0]								11.9	78.0	9.2				0.9			
	Meat	2.6	[0.7-6.5]								10.4	79.2	7.8				2.6			
Florfenicol	Layers	0.0	[0.0-2.0]									67.2	28.0	4.8						
	Turkey	0.0	[0.0-3.3]									73.4	25.7	0.9						
	Meat	0.0	[0.0-2.4]									70.1	29.2	0.6						
Ampicillin	Layers	9.2	[5.4-14.2]							14.0	62.9	13.4	0.5		0.5		2.2	6.5		
	Turkey	12.8	[7.2-20.6]							12.8	58.7	13.8	1.8				5.5	7.3		
	Meat	23.4	[16.9-30.9]							4.5	62.3	9.1	0.6				2.6	20.8		
Cefotaxime	Layers	0.0	[0.0-2.0]		5.9	53.8	37.1	3.2												
	Turkey	0.0	[0.0-3.3]			54.1	43.1	2.8												
	Meat	0.0	[0.0-2.4]		1.3	44.2	53.2	1.3												
Ceftazidime	Layers	0.5	[0.0-3.0]					87.1	12.4	0.5										
	Turkey	0.0	[0.0-3.3]					64.2	35.8											
	Meat	0.0	[0.0-2.4]					74.0	26.0											
Sulfamethoxazole	Layers	11.3	[7.1-16.7]										22.0	41.4	22.6	2.7			0.5	10.8
	Turkey	9.2	[4.5-16.2]										35.8	41.3	11.9	1.8	0.9			8.3
	Meat	5.2	[2.3-10.0]										26.0	48.1	17.5	3.2				5.2
Trimethoprim	Layers	5.9	[3.0-10.3]				9.7	47.8	33.9	2.7					5.9					
	Turkey	3.7	[1.0-9.1]				4.6	45.9	42.2	3.7					3.7					
	Meat	3.2	[1.1-7.4]				10.4	45.5	39.0	1.9					3.2					
Gentamicin	Layers	2.1	[0.6-5.4]					0.5	65.6	30.1	1.6			1.6	0.5					
	Turkey	2.7	[0.6-7.8]						74.3	19.3	3.7		0.9	1.8						
	Meat	0.6	[0.0-3.6]					1.3	72.1	22.7	3.2				0.6					
Streptomycin	Layers	4.3	[1.8-8.3]								1.6	28.0	62.4	3.8	1.1	2.2	0.5	0.5		
	Turkey	4.6	[1.5-10.4]								0.9	48.6	42.2	3.7	2.8	0.9	0.9			
	Meat	5.2	[2.3-10.0]								1.3	36.4	55.8	1.3		1.3	2.6	1.3		
Kanamycin	Layers	2.2	[0.6-5.4]										97.8	2.2						
	Turkey	0.9	[0.0-5.0]										99.1	0.9						
	Meat	1.9	[0.4-5.6]										98.1	1.3	0.6					
Ciprofloxacin	Layers	0.5	[0.0-3.0]	7.0	59.1	33.3		0.5												
	Turkey	0.9	[0.0-5.0]	0.9	61.5	36.7		0.9												
	Meat	1.2	[0.2-4.6]	0.6	61.7	36.4			0.6		0.6									
Nalidixic acid	Layers	0.5	[0.0-3.0]							9.1	63.4	25.8	1.1			0.5				
	Turkey	0.9	[0.0-5.0]							6.4	70.6	22.0				0.9				
	Meat	1.2	[0.2-4.6]							3.9	66.2	27.9	0.6				0.6	0.6		
Colistin	Layers	0.5	[0.0-3.0]						73.1	19.9	6.5	0.5								
	Turkey	0.9	[0.0-5.0]						86.2	10.1	2.8	0.9								
	Meat	0.0	[0.0-2.4]						71.4	22.7	5.8									

*Bold vertical lines denote epidemiological cut-off values for resistance. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

DOGS

The data indicate a moderate occurrence of resistance among *E. coli* from faecal samples of dogs. In total, 83.0% of the isolates were susceptible to all antimicrobial agents included. Altogether, 5.6% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin), 3.9% to two, 1.7% to three and 5.6% to four or more antimicrobial agents (Figure 23).

Moderate occurrence of resistance among *E. coli* from dogs was also the findings in the two previous studies by NORM-VET (NORM-VET 2004 and NORM-VET 2008). Similar to the results from 2008, the most commonly found resistance was to ampicillin (Figure 22). This may be due to the fact that penicillins are the most commonly used antimicrobial product in companion animals (Figure 3). The prevalence of resistance to sulfamethoxazole has decreased from 9% in 2004 and 10% in 2008 to only 2.2% in 2013. In the same time periode, the use of sulfonamides has decreased similarly (Figure 3).

The findings of quinolone resistant and multiresistant *E. coli* from dogs are similar to the results in 2004 and 2008.

In the same time periode, the usage of fluoroquinolones for companion animals has been stable and limited (Figure 3).

By using a non-selective method, one isolate was resistant to third generation cephalosporins and the presence of an ESBL encoding gene was confirmed (see data on selective screening below).

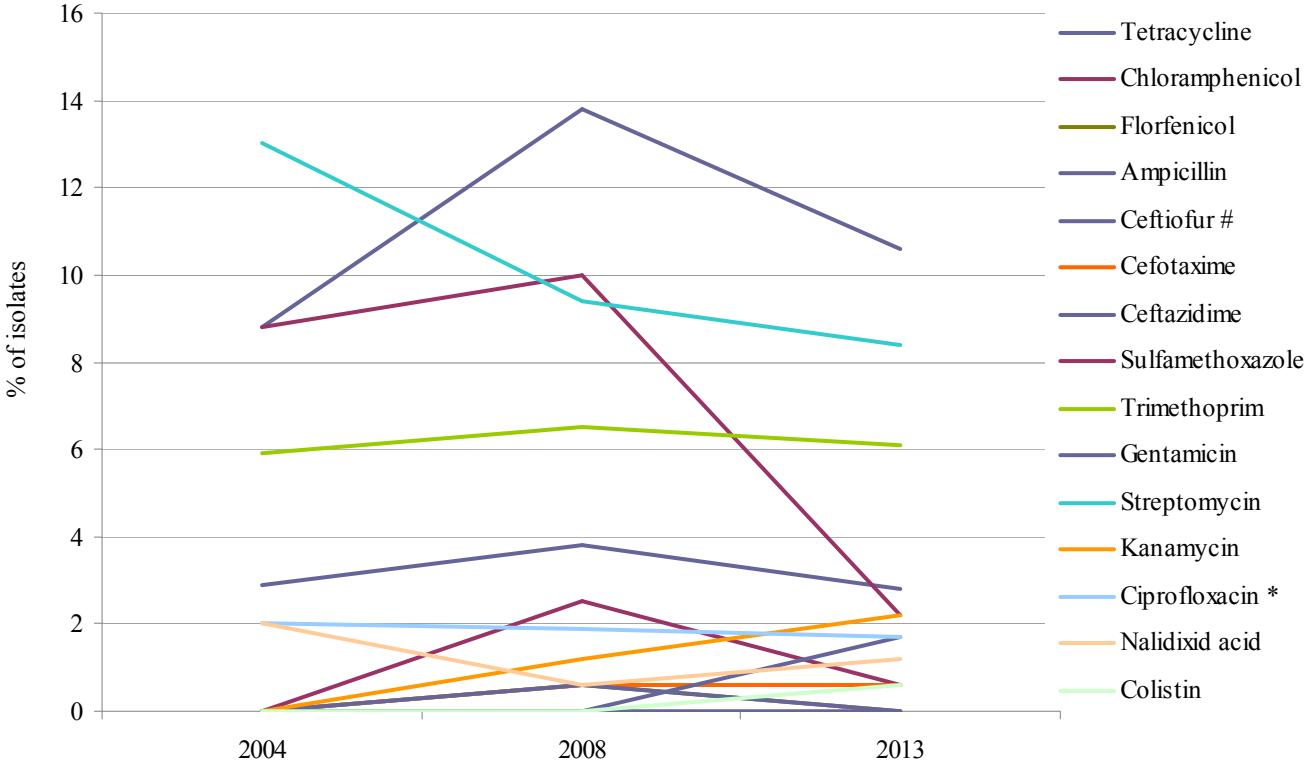

FIGURE 22. Prevalence of resistance to various antimicrobials in *E. coli* isolates from dogs 2004, 2008 and 2013. The cut-off values in NORM-VET 2013 were applied. #Ceftiofur instead of cefotaxime in 2004. *Enrofloxacin instead of ciprofloxacin in 2004.

LAYER FLOCKS

The data indicate a moderate to high occurrence of resistance among *E. coli* from layers flocks. In total, 79.6% of the isolates were susceptible to all antimicrobial agents included. Altogether, 11.3% of the isolates were resistant to one antimicrobial agent (predominantly sulfamethoxazole and ampicillin), 1.6% to two, 1.6% to three and 5.9% to four or more antimicrobial agents. Resistance to sulfamethoxazole was the most frequently identified resistance determinant, followed by resistance to ampicillin, tetracycline and trimethoprim.

This is the first year samples from layer flocks have been investigated and comparisons to previous results are therefore difficult. Sulfamethoxazole was the most frequently identified resistance determinant in layer flocks, followed by ampicillin. This is in contrast to the findings in poultry breeder flocks investigated in NORM-VET 2012 where ampicillin resistance was most commonly identified (15.0%), followed by resistance to sulfamethoxazole. This difference may be explained by a higher use of penicillins in poultry breeder flocks than in layer flocks due to retention periods for eggs.

TURKEY FLOCKS

The data indicate a high occurrence of resistance among *E. coli* from turkey flocks. In total, 74.3% of the isolates were susceptible to all antimicrobial agents included. Altogether, 13.8% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin), 3.7% to two (mainly tetracycline and ampicillin), 3.7% to three and 4.6% to four antimicrobial agents. Resistance to tetracycline and ampicillin were the most frequently identified resistance determinant, followed by resistance to sulfamethoxazole, streptomycin and trimethoprim.

Compared to the results from 2007, the resistance to ampicillin seems to have decreased from 15.1% to 12.8%. This decrease is, however, not significant.

TURKEY MEAT

The data indicate a high occurrence of resistance among *E. coli* from turkey meat samples. In total, 66.9% of the isolates were susceptible to all antimicrobial agents included. Altogether, 16.9% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin), 7.1% to two (mainly tetracycline and ampicillin), 5.8% to three and 3.2% to four or more antimicrobial agents.

In contrast to the results from turkey flocks, the resistance to ampicillin seems to have increased from 13.4% in 2007 to 23.4% in 2013. Though this was a non-significant result and further monitoring is needed to follow the situation.

When comparing the observed prevalences of resistance between *E. coli* isolates from layer and turkey flocks and turkey meat, the prevalences were quite similar. However it was a higher proportion of *E. coli* resistant to two or more substances among the turkeys and turkey meat (Figure 23). There was a higher proportion of susceptible isolates originating from dogs compared with the poultry.

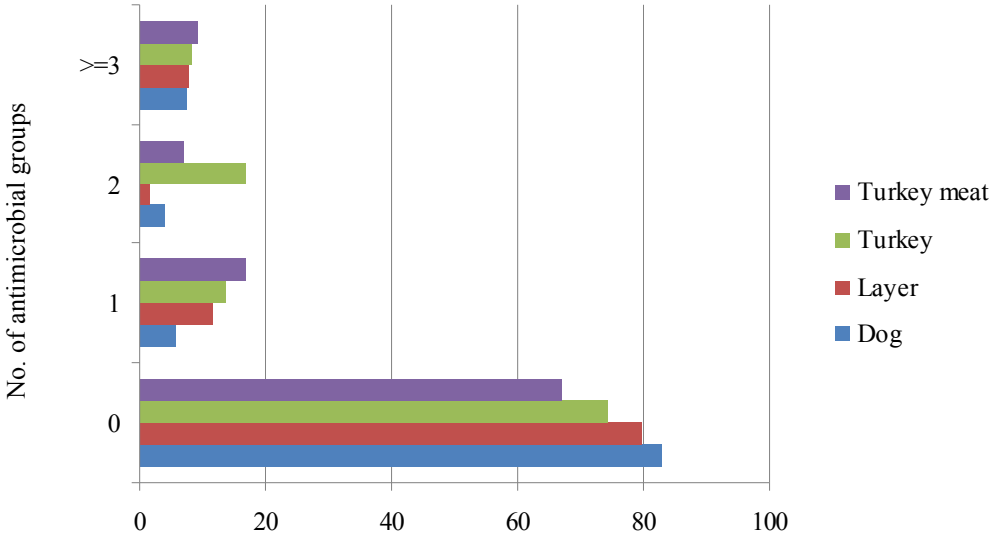

FIGURE 23. Antimicrobial resistance profile for *E. coli* from faecal samples from dogs, layers, turkeys and turkey meat.

Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from dogs, layers, turkey and turkey meat

Acquired resistance to cephalosporins among Gram-negative bacteria has called on special attention in recent years. Production of extended-spectrum beta-lactamases (ESBLs) or transferable AmpC are major mechanisms behind such resistance. Among production animals, poultry seem to be associated with the highest prevalences of *Escherichia coli* and *Salmonella* producing ESBLs/AmpC.

In 2013, screening for ESBL producing *E. coli* was performed on samples from dogs, layer flocks, turkey flocks and from turkey meat using a selective method. A total of 191 faeceal samples from dogs, 204 boot swab samples from layer flocks, 131 boot swab samples from turkey flocks and 156 turkey meat samples were screened for the presence of ESBL producing *E. coli*.

Sampling, laboratory methods and data processing are described in Appendix 3. The data are presented in the following text.

RESULTS AND COMMENTS

DOGS

ESBL producing *E. coli* were detected in four of the 191 dogs (2.1%, 95% CI: 0.6-5.3%). From two of the isolates, the $bla_{\text{CMY-2}}$ gene was detected. The other two isolates carried $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-1}}$ in combination with $bla_{\text{TEM-1}}$, respectively. By the non-selective method described above, only one isolate was resistant to third generation cephalosporins and the $bla_{\text{CTX-M-1}}$ and $bla_{\text{TEM-1}}$ genes were identified.

One of the isolates with $bla_{\text{CMY-2}}$ was additionally resistant to sulfamethoxazole, while the one with $bla_{\text{CTX-M-1}}$ and $bla_{\text{TEM-1}}$ was additionally resistant to streptomycin, sulfamethoxazole and trimethoprim.

The findings of ESBL in dogs are also described in a separate presentation on page 30.

LAYER FLOCKS

ESBL producing *E. coli* were not detected in any of the 204 samples, indicating a prevalence below 1.8%. This is in contrast to previous results from the broiler production. Like many other countries, broiler production in Norway has a high prevalence of *E. coli* resistant to third generation cephalosporins with 43% [95% CI: 36.7-49.2] positive broiler flocks (NORM-VET 2011), 7.3% [95% CI: 3.8-12.4%] positive poultry breeder flocks, and 32.2% [95% CI: 25.9-39.1%] positive broiler meat samples (NORM-VET 2012). All isolates with an AmpC phenotype carried the $bla_{\text{CMY-2}}$ gene. There is no selection pressure from cephalosporin usage in Norway, and no commercial preparations for livestock containing cephalosporins are available on the market. The poultry production in Norway is, however, dependent on import of breeding animals and these animals are a likely source of resistant bacteria. The difference seen between layers and broiler production may be explained by differences in import and breeding lines.

TURKEY FLOCKS AND TURKEY MEAT

ESBL producing *E. coli* were detected in two of the 131 flock samples (1.5%, 95% CI: 0.2-5.4%), and in four of the 156 meat samples (2.6%, 95% CI: 0.7-6.4%). All isolates had a beta-lactam resistance profile corresponding to an AmpC phenotype. PCR and sequencing showed that all isolates contained the $bla_{\text{CMY-2}}$ gene.

Selective methods for isolation of ESBL producing *E. coli* have not previously been performed on turkey samples and comparisons to previous years are therefore difficult. However, resistance to third generation cephalosporins was neither detected by non-selective methods, in this year, nor in 2007.

There is no selection pressure from cephalosporin usage in turkey, and no commercial preparations for livestock containing cephalosporins are available on the market. As for the broiler production, a likely source of these resistant bacteria may be import of breeding animals (parent stock).

High prevalence of quinolone resistant *E. coli* in Norwegian turkey production

Resistance to critically important antimicrobial agents in bacteria from food producing animals has called on special attention in recent years. Quinolones is a class of antimicrobial agents defined by WHO as critically important for human health. Resistance to quinolones in bacteria is usually caused by mutations in the quinolone resistance determining region (QRDR) involving the genes *gyrA*, *gyrB*, *ParC* and *ParE*[1]. In addition, plasmid mediated quinolone resistance (PMQR) genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, *aac(6')-1b-cr*) are responsible for low-level resistance to quinolones. In Norwegian livestock production the usage of quinolones is nearly negligible and resistance to this class of antimicrobial agents among bacteria from production animals is usually a rare finding[2]. However, in 2009 we surprisingly found that 8% of the investigated broiler flocks were positive for *E. coli* expressing resistance to quinolones[3]. This may indicate that poultry could be associated with a high prevalence of quinolone resistant bacteria. Inclusion of isolates was based on random selection of isolates after growth on agar with no antimicrobials. In 2013, a selective method for detection of quinolone resistant *E. coli* was applied for the first time on meat (fillet) from turkey produced in Norway.

Results and discussion

By use of a selective method *E. coli* resistant to quinolones were found in 79 of 156 meat samples investigated (49.4%, 95% CI: 41.3-57.5). Further susceptibility testing of the isolates using Etest® (bioMérieux Inc., Durham, North Carolina, USA) with ciprofloxacin and nalidixic acid, and Liofilchem® MIC Test Strip (Liofilchem® s.r.l., Roseto degli Abruzzi, Italy) with nalidixic acid (1/3 of the isolates), showed that all isolates, except one, had MICs to nalidixic acid > 256 mg/L. The MICs for ciprofloxacin were for all isolates above the cut-off value used by NORM-VET (EUCAST 20.02.2014); > 0.06 mg/L. One of the isolates had an MIC profile corresponding to the possible presence of a plasmid mediated quinolone resistance gene (PMQR), whereas resistance in the other isolates was probably mediated by mutations in the QRDR.

The presence of bacteria resistant to quinolones in food production animals is of concern. Resistant bacteria in the food chain may have an impact on resistance development in human bacterial populations and it should be an overall goal to keep the level of resistant bacteria in production animals and through the meat processing chain at the lowest possible level. Further studies are needed in order to fully understand the role of resistant bacteria in food and their impact on the resistance epidemiology in humans.

References:

1. Fàbrega A, Madurga S, Giralt E, Vila J. (2009). Mechanism of action of and resistance to quinolones. Microb Biotechnol. 2, 40-61.
2. NORM/NORM-VET 2012. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2013. ISSN:1502-2307 (print) / 1890-9965 (electronic).
3. NORM/NORM-VET 2009. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2010. ISSN:1502-2307 (print) / 1890-9965 (electronic).

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Enterococcus spp. from layers and turkey

Samples from a total of 204 layer flocks and 131 turkey flocks were examined. *E. faecalis* was identified in 89 of the layer flocks (43.6%) and 33 of the turkey flocks (25.2%), while *E. faecium* was identified in 103 of the layer flocks (50.5%) and 95 of the turkey flocks (72.5%) One isolate per positive sample was susceptibility tested. Sampling, laboratory methods and data processing are described in Appendix 3. The results are presented in Tables 12-13 and in the text.

TABLE 12. Antimicrobial resistance in *E. faecalis* from layers (n=89) and turkey (n=33) in 2013.

		Resistance (%)		Distribution (%) of MIC values (mg/L)*														
Substance	Sample	[95% CI]		0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥ 2048
Tetracycline	Layers	31.5	[22.0-42.2]			48.3	19.1		1.1	2.2	4.5	5.6	19.1					
	Turkey	45.5	[28.1-63.6]			36.4	18.2				6.1	27.3	12.1					
Chloramphenicol	Layers	0.0	[0.0-4.1]				1.1	10.1	84.3	4.5								
	Turkey	3.0	[0.1-15.8]					3.0	90.9	3.0			3.0					
Ampicillin	Layers	1.1	[0.0-6.1]		2.2	38.2	55.1	3.4		1.1								
	Turkey	0.0	[0.0-10.6]		6.1	51.5	39.4	3.0										
Erythromycin	Layers	10.1	[4.7-18.3]			11.2	34.8	33.7	10.1	1.1	6.7	2.2						
	Turkey	18.2	[7.0-35.5]			21.2	18.2	24.2	18.2	3.0	6.1			9.1				
Streptomycin	Layers	0.0	[0.0-4.1]									2.2	58.4	37.1	2.2			
	Turkey	3.0	[0.1-15.8]									3.0	21.2	72.7				3.0
Gentamicin	Layers	0.0	[0.0-4.1]						2.2	50.6	41.6	5.6						
	Turkey	0.0	[0.0-10.6]						3.0	69.7	27.3							
Kanamycin	Layers	0.0	[0.0-4.1]									27.0	53.9	18.0	1.1			
	Turkey	0.0	[0.0-10.6]									24.2	66.7	9.1				
Vancomycin	Layers	0.0	[0.0-4.1]				91.0	7.9	1.1									
	Turkey	0.0	[0.0-10.6]				84.8	15.2										
Bacitracin#	Layers	3.3	[0.7-9.5]				5.6	2.2	4.5	56.2	28.1		2.2	1.1				
	Turkey	18.2	[7.0-35.5]					6.1	9.1	57.6	9.1		3.0	12.1	3.0			
Linezolid	Layers	0.0	[0.0-4.1]			11.2	71.9	16.9										
	Turkey	0.0	[0.0-10.6]			9.1	72.7	18.2										
Virginiamycin	Layers	0.0	[0.0-4.1]			4.5	3.4	9.0	10.1	60.7	12.4							
	Turkey	0.0	[0.0-10.6]					12.1	18.2	39.4	30.3							
Narasin	Layers	1.1	[0.0-6.1]	39.3	55.1	4.5			1.1									
	Turkey	12.1	[3.4-28.2]	3.0	27.3	12.1	3.0	42.4	9.1	3.0								

*Bold vertical lines denote microbiological cut-off values for resistance. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested. # Measured in U/ml.

TABLE 13. Antimicrobial resistance in *E. faecium* from layers (n=103) and turkey (95) in 2013.

		Resistance (%)		Distribution (%) of MIC values (mg/L)*														
Substance	Sample	[95% CI]		0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥ 2048
Tetracycline	Layers	7.8	[3.4-14.7]			88.3	3.9			1.0	1.0	4.9	1.0					
	Turkey	19.0	[11.6-28.2]			75.8	3.2	1.1	1.1	1.1	2.1	4.2	11.6					
Chloramphenicol	Layers	0.0	[0.0-3.5]					33.0	63.1	3.9								
	Turkey	0.0	[0.0-3.8]			1.1		16.8	75.8	4.2	2.1							
Ampicillin	Layers	1.0	[0.0-5.3]		26.2	34.0	13.6	18.4	6.8	1.0								
	Turkey	4.2	[1.2-10.4]		25.3	18.9	16.8	15.8	18.9	4.2								
Erythromycin	Layers	29.1	[20.6-37.9]			18.4	38.8	7.8	5.8	9.7	12.6	3.9		2.9				
	Turkey	12.7	[6.7-21.0]			29.5	43.2	10.5	4.2	4.2	5.3	2.1		1.1				
Streptomycin	Layers	0.0	[0.0-3.5]							1.0	1.9	35.9	55.3	5.8				
	Turkey	1.1	[0.0-5.7]								2.1	22.1	72.6	2.1	1.1			
Gentamicin	Layers	0.0	[0.0-3.5]					2.9	35.9	54.4	5.8	1.0						
	Turkey	0.0	[0.0-3.8]					4.2	38.9	53.7	3.2							
Kanamycin	Layers	0.0	[0.0-3.5]									6.8	53.4	33.0	6.8			
	Turkey	0.0	[0.0-3.8]								1.1	13.7	42.1	30.5	11.6	1.1		
Vancomycin	Layers	0.0	[0.0-3.5]				98.1	1.9										
	Turkey	0.0	[0.0-3.8]				98.9	1.1										
Bacitracin#	Layers	2.9	[0.6-8.3]				58.3	8.7	5.8	6.8	17.5			1.0	1.9			
	Turkey	14.6	[8.3-23.5]				44.2	4.2	4.2	9.5	21.1	2.1	3.2	4.2	7.4			
Linezolid	Layers	0.0	[0.0-3.5]			14.6	57.3	28.2										
	Turkey	0.0	[0.0-3.8]			4.2	33.7	61.1	1.1									
Virginiamycin	Layers	5.8	[2.2-12.2]			27.2	28.2	38.8		2.9	2.9							
	Turkey	1.1	[0.0-5.7]			22.1	13.7	58.9	4.2		1.1							
Narasin	Layers	0.0	[0.0-3.5]	15.5	53.4	28.2		1.0	1.9									
	Turkey	41.1	[31.1-51.6]	1.1	5.3	4.2	3.2	5.3	40.0	41.1								

*Bold vertical lines denote microbiological cut-off values for resistance. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested. # Measured in U/ml

RESULTS AND COMMENTS

E. faecalis is known to be inherently resistant to the streptogramin virginiamycin, while *E. faecium* is known to be susceptible to this antimicrobial agent. Virginiamycin is therefore not included in the following comments. The resistance profiles of the two bacterial species are rather different and therefore presented separately.

LAYER FLOCKS

The data indicate a high occurrence of resistance among *Enterococcus* spp. from layer flocks. *E. faecalis*: In total 58.4% of the isolates were susceptible to all antimicrobial agents included in the test panel. Resistance to one (mainly tetracycline) and two (mainly tetracycline and bacitracin) antimicrobial agents was detected in 36.0% and 5.6% of the isolates, respectively. *E. faecium*: In total 61.2% of the isolates were susceptible to all antimicrobial agents included in the test panel. Resistance to one (mainly erythromycin) and two (mainly erythromycin and tetracycline) antimicrobial agents was detected in 32.0% and 5.8% of the isolates, respectively. In addition, 1.0% of the isolates were resistant to three antimicrobial agents.

This is the first year samples from layer flocks have been investigated. Previous results among *E. faecalis* and *E. faecium* in broilers show that resistance rates to tetracycline, chloramphenicol, bacitracin, streptomycin, erythromycin and narasin found among isolates from layer flocks are lower than in broiler flocks. Some of these observed differences are not statistically significant, though the results for chloramphenicol, streptomycin and bacitracin in *E. faecalis*, and erythromycin, bacitracin and naracin in *E. faecium* are significant. Surprisingly, there is a high frequency of tetracycline resistance among *E. faecalis* (31.4%) despite insignificant use of oxytetracycline for clinical purposes in Norwegian poultry production. High frequencies are also seen for broilers and turkey. Equivalently, there is a high frequency of resistance to erythromycin among *E. faecium*. Erythromycin has never been used in poultry in Norway. However, resistance may have been acquired by former use of spiramycin as cross-resistance between erythromycin and spiramycin is common. Spiramycin was licensed for use in poultry until it was withdrawn in 1998 due to limited sales.

Coccidiostats are routinely used in Norwegian broiler production, and since 1996 such use has been dominated by the ionophore narasin. Layer flocks, however, are routinely vaccinated for coccidiosis and narasin is not used. This probably explains the difference in narasin resistance seen between isolates from broilers and layers, as the selection pressure exerted by the use of narasin in broiler production is considered the reason why narasin resistance is frequently observed among enterococci from broilers, *E. faecium* in particular.

No vancomycin resistant *E. faecium* or *E. faecalis* isolates were detected neither by random selection nor by selective methods (see next page).

TURKEY FLOCKS

The data indicate a very high to extremely high occurrence of resistance among *Enterococcus* spp. from turkey flocks. *E. faecalis*: In total 45.4% of the isolates were susceptible to all antimicrobial agents included in the test panel. Resistance to one (mainly tetracycline) and two antimicrobial agents was detected in 21.2% of the isolates, respectively. Resistance to three antimicrobial agents was detected in 12.1% of the isolates. *E. faecium*: In total 28.4% of the isolates were susceptible to all antimicrobial agents included in the test panel. Resistance to one (mainly narasin) and two (mainly narasin and bacitracin) antimicrobial agents was detected in 52.6% and 15.8% of the isolates, respectively. In addition, 3.2% of the isolates were resistant to three antimicrobial agents.

The prevalence of resistance to narasin was moderate. Compared to the results in NORM-VET 2007, there has been a decrease in narasin resistance. However, this can be explained by a change in the cut-off value. Narasin resistance cannot be explained by use of this substance as the dominating coccidiostat used in turkey production is monensin, and to our knowledge there is no cross-resistance between monensin and narasin. Resistance to bacitracin still persists among enterococci from turkey, although a non-significant decrease from previous results was observed. Bacitracin was formerly used as a growth promoter, but the use was negligible during the 1990s. No use of bacitracin has been recorded in animal production in Norway after 1997. Since the surveillance in 2007, it may seem like there has been a small decrease of resistance in *E. faecium* for several of the antimicrobial agents. However, the observed differences were non-significant, and more data are needed in the future to see if this is an upcoming trend.

No vancomycin resistant *E. faecium* or *E. faecalis* isolates were detected by random selection. See next page for results by selective isolation methods. Avoparcin, which induces cross-resistance to vancomycin, was routinely used as a growth promoter in Norwegian broiler and turkey production from 1986 until it was banned in 1995. Studies have shown that this use has selected for an extensive reservoir of vancomycin resistant enterococci (VRE) in Norwegian poultry production. The reservoir has persisted after the ban was implemented (see data on selective screening for VRE below).

Vancomycin resistant *Enterococcus* spp. (VRE) from layers and turkey

Samples from a total of 204 layer flocks and 131 turkey flocks were examined for the presence of VRE. The results are presented in text and in Table 14. Laboratory methods and data processing are described in Appendix 3.

TABLE 14. Antimicrobial resistance in vancomycin resistant *Enterococcus faecium* from turkey (n=16) in 2013.

		Distribution (%) of MIC values (mg/L)													
Substance	Resistance (n)	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥ 2048
Tetracycline	0		15	1											
Chloramphenicol	0				11	5									
Ampicillin	2	4			1	9	2								
Erythromycin	0		13	3											
Streptomycin	0						1	7	7	1					
Gentamicin	0				2	9	4	1							
Kanamycin	0								3	10	2	1			
Vancomycin	16											16			
Bacitracin[#]	0			12		1		3							
Linezolid	0		8	5	3										
Virginiamycin	0		5	1	10										
Narasin	2		1			13	2								

Bold vertical lines denote microbiological cut-off values for resistance. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested. [#] Measured in U/ml.

RESULTS AND COMMENTS

None of the samples from layers were positive for VRE, indicating a prevalence of VRE in Norwegian layer flocks below 1.8%. A total of 16 (12.2%, 95% CI: 7.1-19.1%) of the turkey samples were positive for VRE. All *vanA* positive isolates were identified as *E. faecium*. Two of the isolates showed additional resistance to narasin, and two isolates to ampicillin.

Compared to the previous survey in NORM-VET 2007, the number of VRE positive samples has apparently increased from 8.6% to 12.2%. However, this change was non-significant and may be a result of improved sampling method using boot swabs (per flock) instead of faecal samples (from groups of turkey). Boot swab sampling mirrors the prevalence in the broiler house and not the actual prevalence in the live birds.

C. ZOONOTIC AND NON-ZOONOTIC ENTEROPATHOGENIC BACTERIA

Ina Haagensen, Madelaine Norström, Jannice Schau Slettemeås, Anne Margrete Urdahl, Astrid Louise Wester

Zoonotic and non-zoonotic enteropathogenic bacteria represent a considerable public health problem. Furthermore, the occurrence of acquired antimicrobial resistance in such bacteria represents a major public health concern. Therefore, it is of great importance to monitor the occurrence of antimicrobial resistance in zoonotic and other enteropathogenic bacteria at relevant stages in the farm-to-fork continuum. In Norway, *Salmonella* isolates from control programmes concerning feed samples, animals and food products, as well as diagnostic samples from animals are monitored for antimicrobial resistance. Additionally in 2013, antimicrobial resistance in isolates of *Campylobacter jejuni* from broiler was included.
Regarding human isolates, all *Salmonella*, *Shigella* and *Yersinia enterocolitica* isolates are included, as well as a representative number of *Campylobacter* isolates. Sampling, laboratory methods and data processing are described in Appendix 4.

The NORM results on human isolates of enteropathogenic *Enterobacteriaceae* are interpreted according to the clinical breakpoints given by The European Committee on Antimicrobial Susceptibility Testing (EUCAST). In case of missing clinical breakpoints, epidemiological cut-off values (ECOFFs) were used based on zone-distribution judgments or, as for *Campylobacter*, on ECOFFs given by EUCAST. Multi-drug resistance (MDR) was defined as resistance to three or more antimicrobial categories according to the 2011 ECDC/CDC joint definitions.

SALMONELLA SPP.

Salmonella from animals

The situation regarding occurrence of *Salmonella* spp. in food production animals in Norway is very good as such animal populations are considered virtually free from *Salmonella* spp. To document and maintain this favourable situation, Norway runs an extensive surveillance programme that covers both live animals (cattle, pigs and poultry) and meat samples. The *Salmonella* isolates examined in NORM-VET include those that are detected in this programme, as well as those detected by clinical submissions to the Norwegian Veterinary Institute. The data are presented in Table 15 and in the text.

TABLE 15. Antimicrobial resistance in *Salmonella* spp. (n=15) from animals (pig=2, dog=5, cat=1, poultry=2, wild birds=5); *S.* Typhimurium (n=7) and other *Salmonella* spp. (n=8) in 2013.

Substance	Resistance (n)	Distribution (n) of MIC values (mg/L)*																	
		0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	1							13	1						1				2
Chloramphenicol	0								5	9	1								
Florfenicol	0									14		1							
Ampicillin	1							13	1							1			
Cefotaxime	0			7		7	1												
Ceftazidime	0						6	9											
Sulfamethoxazole	2										3	5	5						2
Trimethoprim	0				2	10	3												
Gentamicin	0						15												
Streptomycin	1										9	5				1			
Kanamycin	0										15								
Ciprofloxacin	2		1	12	1		1												
Nalidixic acid	1									11	3					1			
Colistin	0						15												

*Bold vertical lines denote epidemiological cut-off values for resistance. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested for are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

In 2013, a total of 15 *Salmonella* spp. isolates from animals were susceptibility tested. The seven isolates of *S.* Typhimurium included one each from a cat and a pig herd, and five from wild birds. The remaining isolates belonged to three different serovars; *S.* Kedougou from dogs (n=5) and a poultry flock (n=1), *S.* Virchow from one pig herd and *S.* Panama from one poultry flock.

Two isolates, one *S.* Kedougou from dog and one *S.* Virchow from pig, showed resistance to fluoroquinolones. The emerging multi-resistant (resistant to tetracycline, ampicillin, sulfamethoxazole and streptomycin) monophasic *S.* Typhimurium was isolated from one pig herd.

Salmonella from human clinical specimens

In 2013, the National Reference Laboratory (NRL) for Enteropathogenic Bacteria at the Norwegian Institute of Public Health (NIPH) performed antimicrobial susceptibility testing on a total of 1,424 unique *Salmonella* isolates from human infections. As indicated in Table 16, 18.8% was reported as acquired in Norway, 73.5% was acquired abroad, whereas the place of acquisition was unknown for 7.7%. Travel abroad is considered a risk factor for obtaining bacteria carrying antimicrobial resistance. To enable evaluations with this in mind, figures of the proportions of place of acquisition from 2010 to 2013 have been introduced when presenting the results for the different *Salmonella* serovars.

TABLE 16. Distribution of human isolates of *Salmonella* serovars (n=1,424) in 2013 according to place of acquisition.

	Place of acquistion		
	Norway	Abroad	Unknown
S. Typhimurium including *S. enterica* serovar 4,[5],12:i- (n=237)	72	145	20
S. Enteritidis (n=633)	47	539	47
S. Typhi (n=11)	0	10	1
S. Paratyphi (n=16)	0	16	0
Other *Salmonella* (n=527)	149	336	42
Total (n=1,424)	**268 (18.8%)**	**1,046 (73.5%)**	**110 (7.7%)**

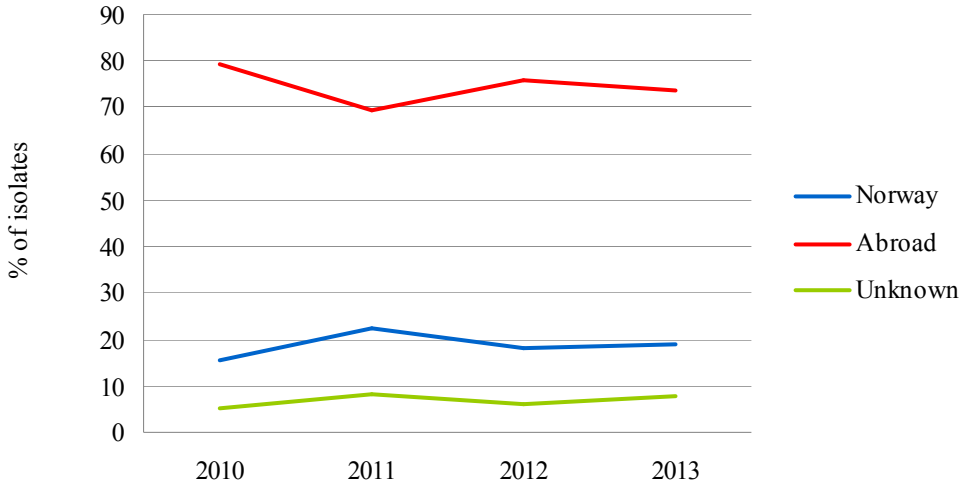


FIGURE 24. Place of acquistion for all isolates of *Salmonella* from 2010 to 2013.

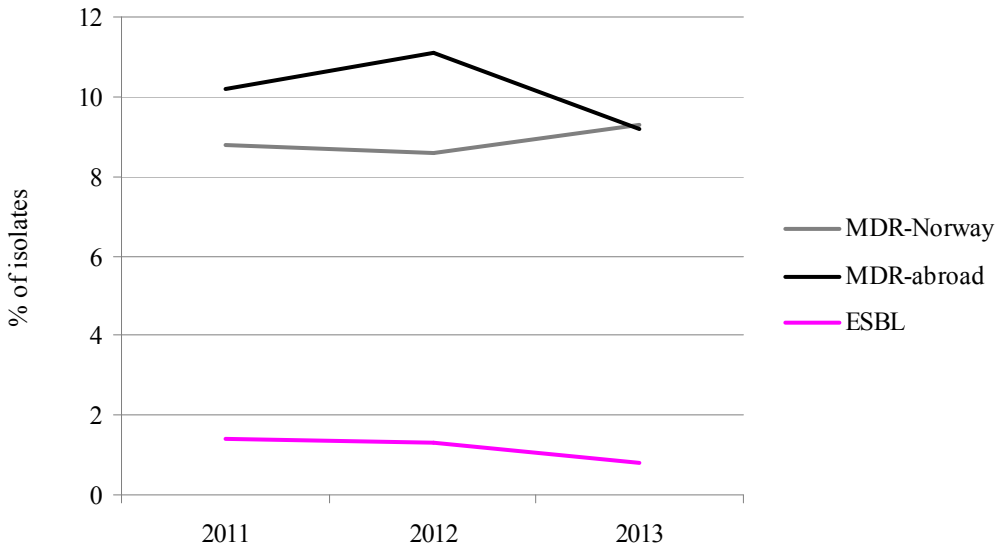


FIGURE 25. Percentage of all *Salmonella* isolates in 2010-2013 that were resistant to ≥ 3 groups of antimicrobials (MDR) and carrying ESBL of either $ESBL_A$ or $ESBL_M$ type. All ESBL-carrying isolates were acquired abroad, but for one; for which information on place of acquisition lacked. None of the differences shown are statistically significant.

ANITMICROBIAL RESISTANCE IN BLOOD CULTURE ISOLATES OF SALMONELLA

A total of 73 strains were isolated from blood culture, representing 0.6% of all blood culture isolates when skin contaminants are excluded. There were three *S.* Typhimurium and its monophasic variant, 30 *S.* Enteritidis, eight *S.* Typhi, twelve *S.* Paratyphi, and 20 other *Salmonella* isolates (Figure 26) representing fourteen different serovars, of which *S.* Brandenburg, *S.* Kedogou, *S.* Panama, *S.* Saintpaul, *S.* Stanley and *S.* Wirchow were all represented by two isolates, whereas seven other serovars were only represented by one isolate each. Although the numbers are small, it seems that the most frequent serovar in blood culture, *S.* Enteritidis, is fairly sensitive to all groups of antimicrobials, in spite of having been acquired abroad in more than half of the cases (16 out of 30; all continents represented). It should be emphasised that low-level ciprofloxacin resistance is underestimated because of the "non-systemic breakpoints" used. However, when calculating MDR, low-level resistance (against nalidixic acid) and/or high-level resistance (against ciprofloxacin) counted as quinolone resistance. On the contrary, the frequency of multidrug resistance (MDR) in *S.* Typhi is alarmingly high (Figure 27), probably reflecting the frequency of resistance in the countries where the isolates were acquired (four from the Indian subcontinent, one from Egypt, one from Tanzania and the rest from Asia not further specified, or unknown). Additionally, *Salmonella* was isolated from sample materials indicating serious infection in six patients: *S.* Typhi from the gall bladder, *S.* Enteritids from synovial fluid, aspiration and biopsy material not further specified *S.* Oranienburg from an abscess, and *S.* London from an unspecified aspiration material.

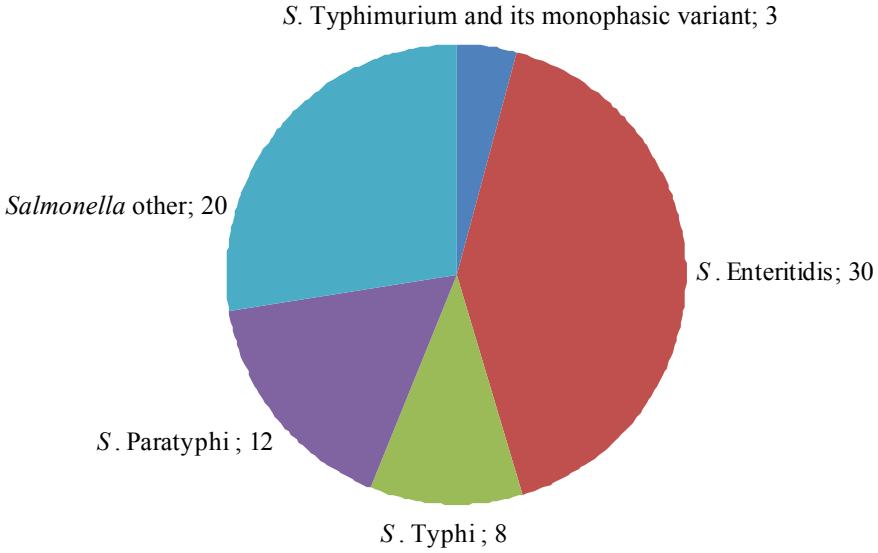


FIGURE 26. Distribution of blood culture isolates of different *Salmonella* serovars (n=73) in 2013.

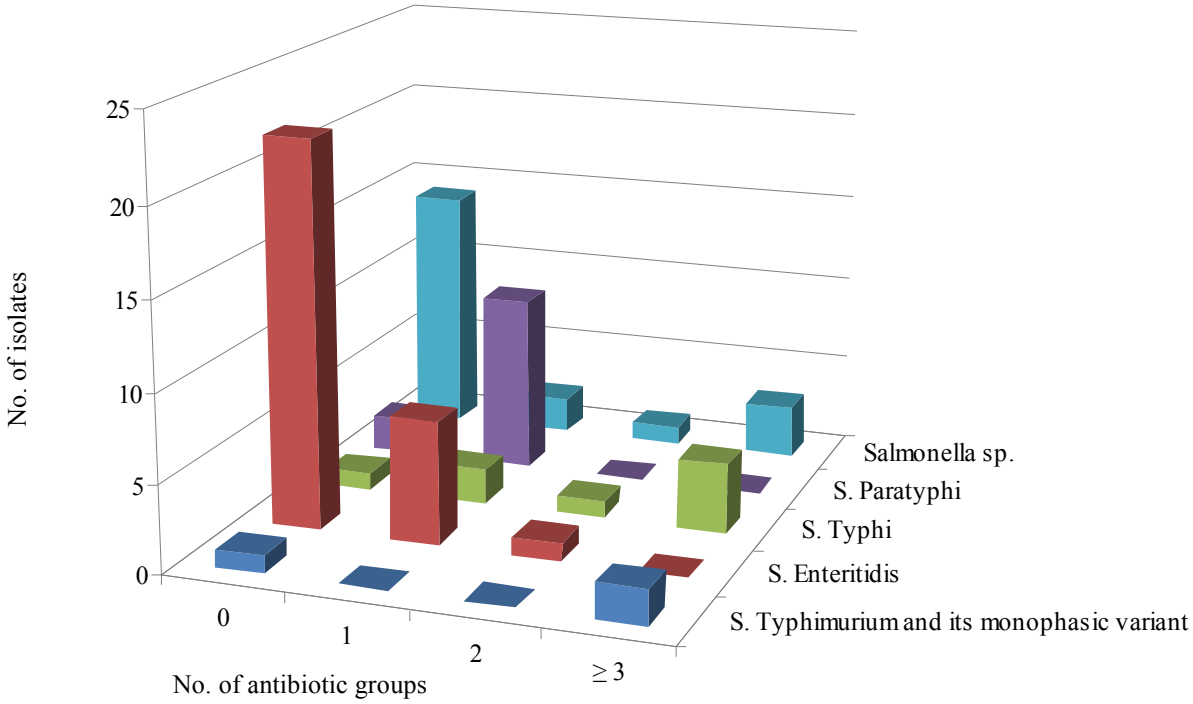


FIGURE 27. Antimicrobial resistance in *Salmonella* isolated from blood culture 2013 (n=73) with the number of isolates resistant to none, one, two, or three or more antimicrobial groups, respectively.

RESISTANCE IN SALMONELLA IRRESPECTIVE OF SAMPLE MATERIAL

The numbers of *S.* Typhi and *S.* Paratyphi isolates remain low. For 2013 the total number of isolates were eleven and 16, respectively. As many as 40% of the *S.* Typhi isolates were MDR (resistance to ≥ 3 antimicrobial groups; ampicillin 60%, trimethoprim-sulfamethoxazole 60% and chloramphenicol 40%), whereas none of the *S.* Paratyphi strains were MDR. A total of eleven strains carried extended spectrum beta-lactamases (ESBL). Five of these were *S.* Typhimurium or its monophasic variant (four $ESBL_A$ and one $ESBL_M$), six were *Salmonella* of other serovars with either $ESBL_A$ (two *S.* Infantis, one *S.* Kentucky, one *S.* Bovismorbificans and one *S. enterica subsp. enterica* 4,5,12:i:?) or $ESBL_M$ (one *S.* Stanley).

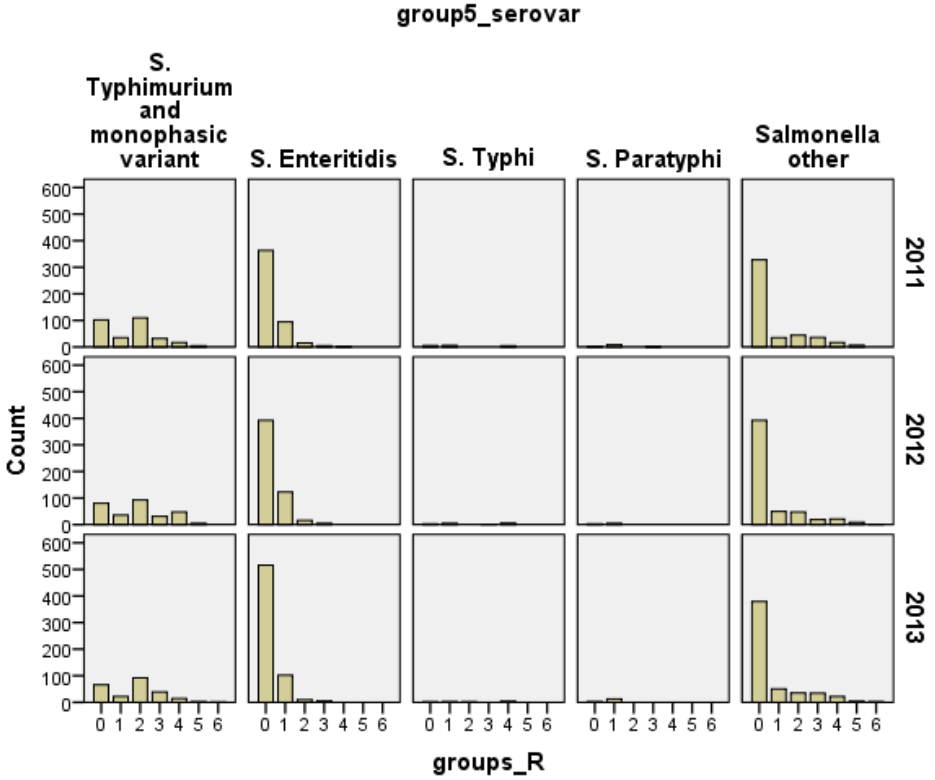


FIGURE 28. Distribution of number of antimicrobials that *Salmonella* isolates were resistant to; by serovar groups and by year.

The dominating serovars were *S.* Typhimurium (n=119) and its monophasic variant (n=118), with 237 isolates (16.6%) of all *Salmonella* isolates, and *S.* Enteritidis with 633 (44.5%) of the isolates. From 2010 on, phage typing has not been performed, and thus results on *S.* Typhimurium definite phage type (DT) 104 are not available. DT 104 is of special concern, however, because of carriage of a specific MDR pattern, namely resistance to ampicillin, (chloramphenicol), streptomycin, sulfonamides, and tetracycline, thus the term A(C)SSuT resistance profile. However, we do not systematically test for resistance against all these antimicrobials any more but consider giving priority to perform PCR detecting DT104-associated genes.

The results of the antimicrobial susceptibility testing for 2013 isolates are presented in Tables 17-20, in Figures 29-35, and in the text.

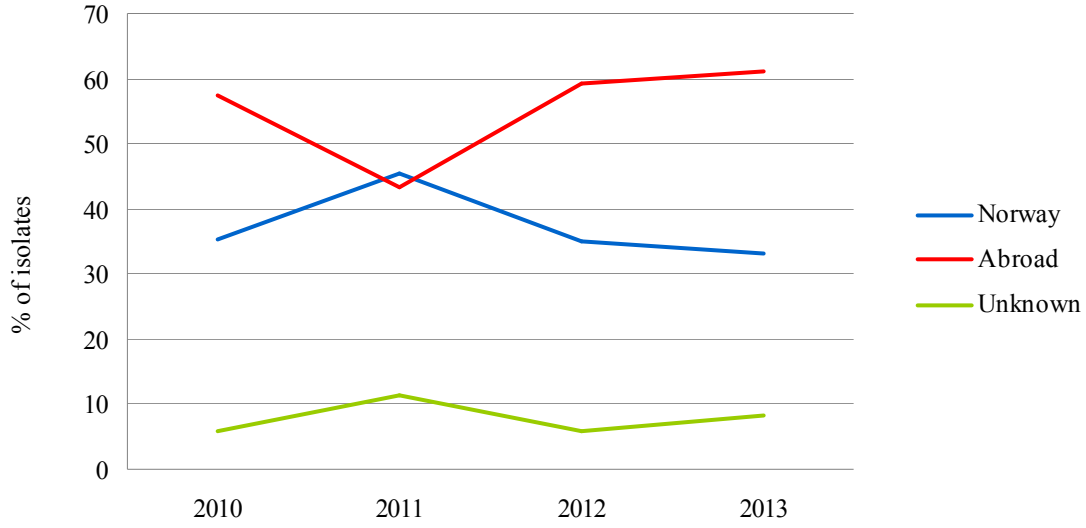


FIGURE 29. Place of acquisition of *S.* Typhimurium group isolates 2010-2013.

TABLE 17. Human isolates of domestically acquired *Salmonella* Typhimurium-group (n=72) during 2013, including domestically acquired *S. enterica* serovar 4,[5],12:i:- (n=25). Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin [1]	≤ 8	> 8	50.0	-	50.0
Chloramphenicol [1]	≤ 8	> 8	84.7	-	15.3
Tetracycline [2]	≥ 13	< 13	48.6	-	51.4
Nalidixic acid [2]	≥ 19	< 19	88.9	-	11.1
Ciprofloxacin [1, 3]	≤ 0.5	> 1	100.0	0.0	0.0
Trimethoprim-sulfamethoxazole[1,*]	≤ 2	> 4	95.8	0.0	4.2

[1] EUCAST clinical breakpoints for Enterobacteriaceae 2014, version 4.0. [2] Epidemiological cut-off values based on zone distribution evaluations. [3]Low-level resistance against ciprofloxacin is underestimated by using these breakpoints; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

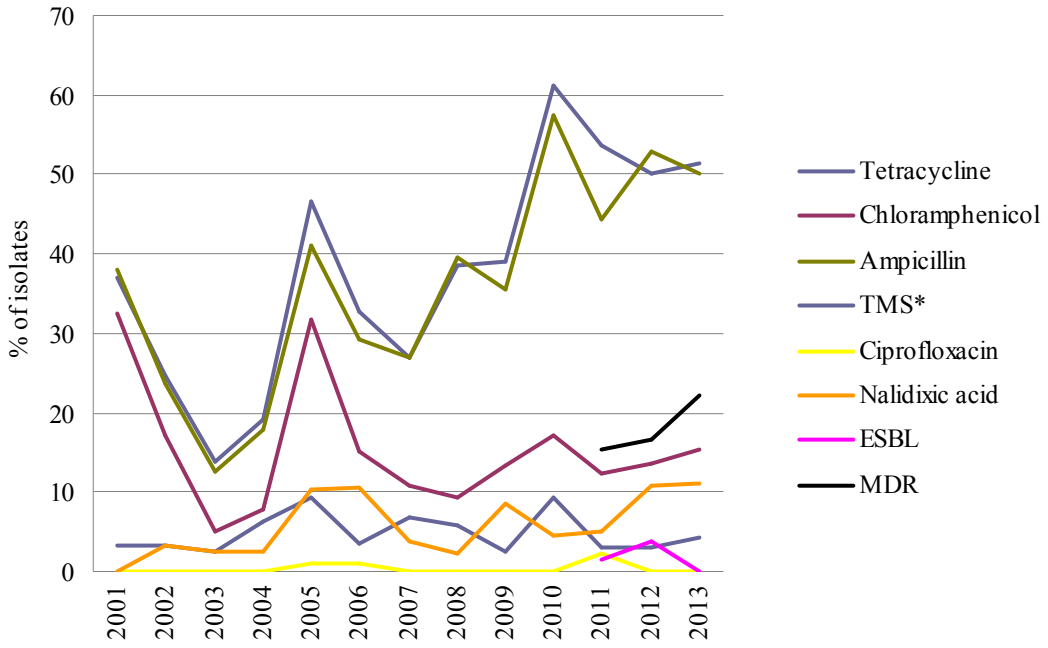


FIGURE 30. Percentage of resistance to various antimicrobial agents in *Salmonella* Typhimurium-group (including *S. enterica* serovar 4,[5],12:i:- from humans infected in Norway 2001-2013. *TMS=Trimethoprim-sulfamethoxazole.

TABLE 18. Human isolates of *Salmonella* Typhimurium-group acquired abroad during 2013 (n= 145), including *S. enterica* serovar 4,[5],12:i:- (n= 84). Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin [1]	≤ 8	> 8	31.0	-	69.0
Chloramphenicol [1]	≤ 8	> 8	85.5	-	14.5
Tetracycline [2]	≥ 13	< 13	23.4	-	76.6
Nalidixic acid [2]	≥ 19	< 19	84.1	-	15.9
Ciprofloxacin [1, 3]	≤ 0.5	> 1	98.6	0.7	0.7
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	88.9	0.0	11.1

[1] EUCAST clinical breakpoints for Enterobacteriaceae 2014. [2] Epidemiological cut-off values based on zone-distribution evaluations. [3]Low-level resistance against ciprofloxacin is underestimated by using these breakpoints; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

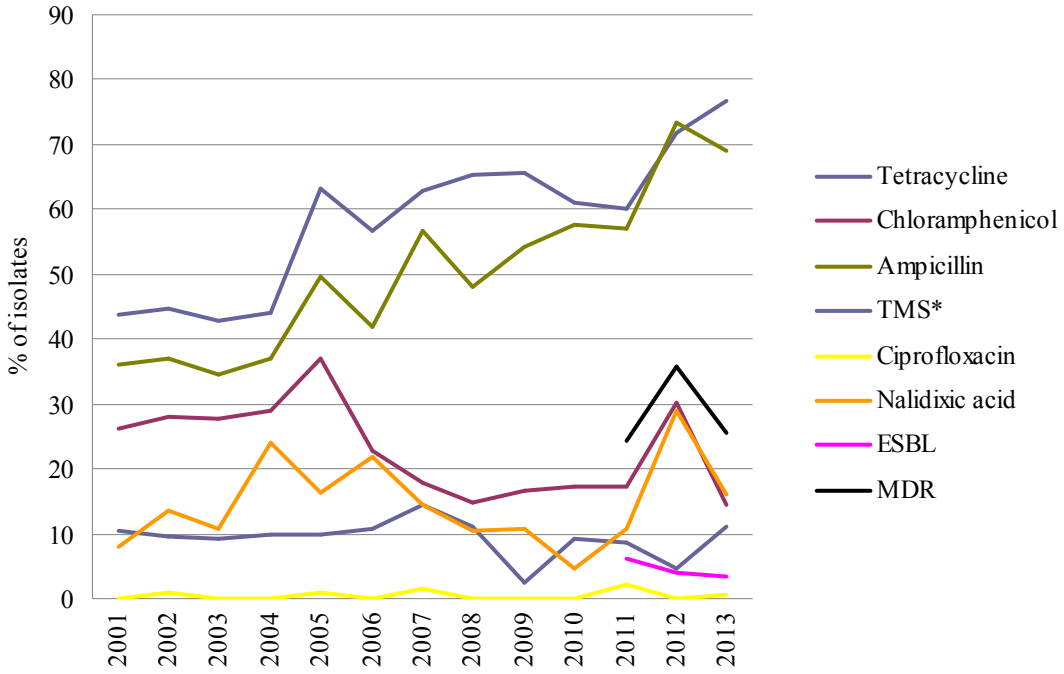


FIGURE 31. Percentage of resistance to various antimicrobial agents in human *Salmonella* Typhimurium-group (including *S. enterica* serovar 4,[5],12:i:- from humans infected outside Norway 2001-2013. *TMS=Trimethoprim-sulfamethoxazole.

TABLE 19. Human isolates of *Salmonella* Enteritidis (n=633[#]), acquired during 2013, irrespective of place of acquisition. Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin[1]	≤ 8	> 8	95.9	-	4.1
Chloramphenicol [1]	≤ 8	> 8	99.8	-	0.2
Tetracycline [2]	≥ 13	< 13	98.4	-	1.6
Nalidixic acid [2]	≥ 19	< 19	84.5	-	15.5
Ciprofloxacin [1, 3]	≤ 0.5	> 1	100.0	0.0	0.0
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	98.4	0.0	1.6

Place of infection; Norway (n=47), abroad (n=539), unknown (n=47). [1]EUCAST clinical breakpoints for Enterobacteriaceae 2014, version 4.0. [2] Epidemiological cut-off values based on zone-distribution evaluations. [3]Low-level resistance against ciprofloxacin is underestimated by using these breakpoints; EUCAST emphasises evidence of poor clinical response in systemic infections. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

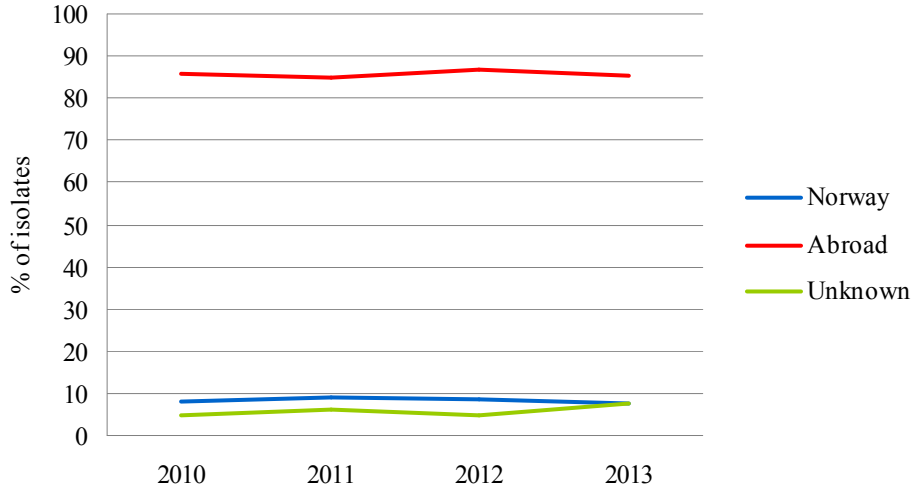


FIGURE 32. Place of acquisition of *S.* Enteritidis 2010-2013.

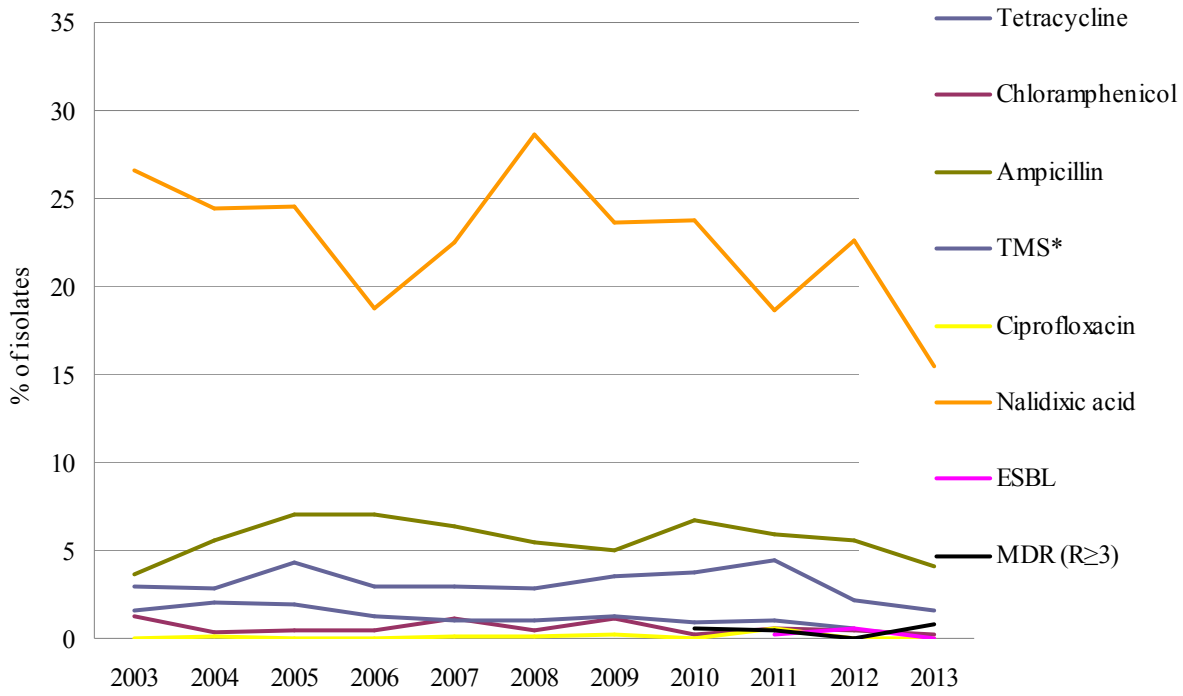


FIGURE 33. Percentage of resistance to various antimicrobial agents in *S.* Enteritidis from humans in 2003-2013. *TMS=Trimethoprim-sulfamethoxazole.

TABLE 20. Human isolates of *Salmonella* spp. (including *S.* Paratyphi B variant Java, but excluding *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi) (n=508[#]), acquired during 2013, irrespective of place of acquisition. Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin [1]	≤ 8	> 8	85.8	-	14.2
Chloramphenicol [1]	≤ 8	> 8	94.7	-	5.3
Tetracycline [2]	≥ 13	< 13	82.9	-	17.1
Nalidixic acid [2]	≥ 19	< 19	81.0	-	19.0
Ciprofloxacin [1, 3]	≤ 0.5	>1	95.8	0.7	3.4
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	91.1	0.0	8.9

Place of infection; Norway (n=142), abroad (n=324), unknown (n=42). [1] EUCAST clinical breakpoints for Enterobacteriaceae 2014, version 4.0. [2]Epidemiological cut-off values based on zone-distribution evaluations. [3]Low-level resistance against ciprofloxacin is underestimated by using these breakpoints; EUCAST emphasises evidence of poor clinical response in systemic infections. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

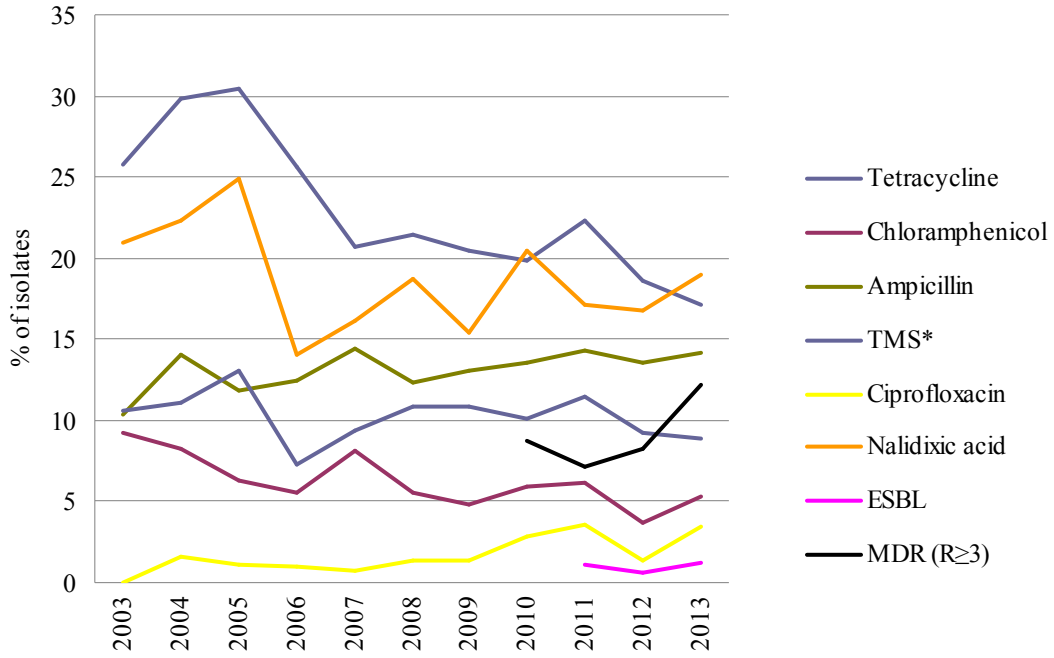


FIGURE 34. Percentage of resistance to various antimicrobial agents in *Salmonella* spp. (including *S.* Paratyphi B variant Java; but excluding *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi) from humans in 2003-2013.
*TMS=Trimethoprim-sulfamethoxazole.

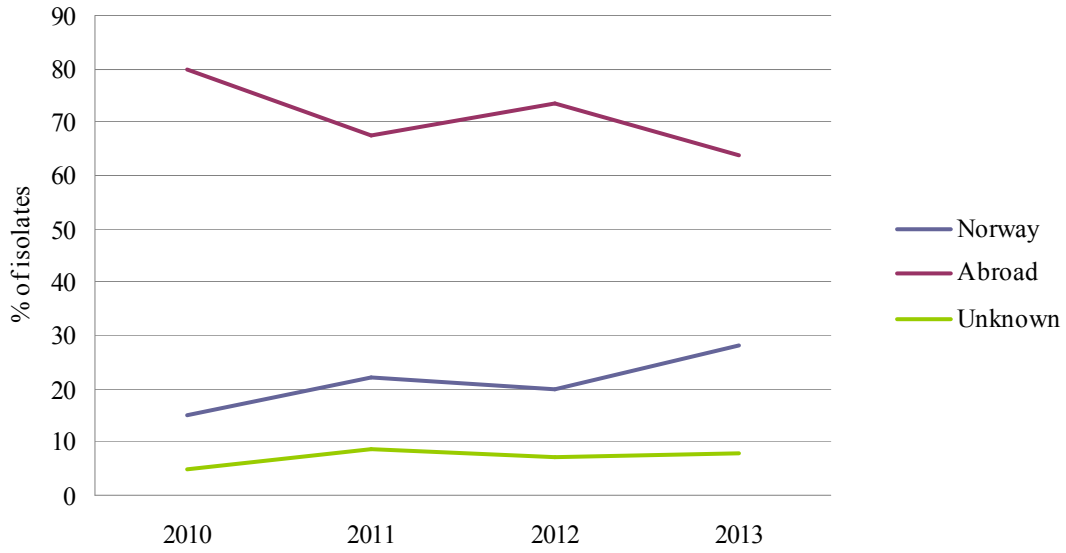


FIGURE 35. Place of acquisition of other *Salmonella* sp. 2010-2013.

FURTHER RESULTS AND COMMENTS

The overall tendencies were that the general level of resistance was highest for the *Salmonella* Typhimurium-group, and that the proportion of strains resistant to ampicillin and tetracycline within the *S.* Typhimurium-group continues to increase. As demonstrated in Figures 30 and 31, this trend is apparent for both domestically acquired strains and strains acquired abroad. The rates of isolates resistant to ampicillin and tetracycline have practically doubled over the last decade and are now around 50% for domestically acquired isolates and 65-70% in those acquired abroad. Several countries report an increase in MDR *S. enterica* serovar 4,[5],12:i-. In Norway, the number of isolates assigned to this serovar has increased steadily from 59 strains in 2008 to 118 in 2013. The corresponding proportions of this serovar within the *Salmonella* Typhimurium-group were around 20% in 2008 and 2009, and nearly 50% in 2013. Somewhat surprisingly, MDR was less frequent among *S. enterica* serovar 4,[5],12:i- (19.5%) than in *S.* Typhimurium (28.6%). Antimicrobial resistance in *S.* Enteritidis isolates seems fairy stable (Figure 33), possibly due, in part, to a stable proportion of infections acquired abroad (Figure 32). There is still a very low level of resistance to ciprofloxacin. However, the breakpoints used underestimate low-level ciprofloxacin resistance, probably relevant in systemic *Salmonella* infections.

With regard to *Salmonella* spp. (including *S.* Paratyphi B variant Java, but excluding *S.* Typhimurium-group, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi) most infections were acquired abroad and antimicrobial resistance was moderate and fairly stable, but for a possible increase in MDR (Table 20 and Figure 34). Also in this group, resistance to ciprofloxacin was still well below 5%, although the same reservations have to be made considering low-level ciprofloxacin resistant strains in systemic infections.

CAMPYLOBACTER SPP.

Campylobacter spp. from broiler

The isolates of *Campylobacter jejuni* in broilers originate from caecal samples collected by the "Norwegian Action Plan against *Campylobacter* spp. in Broiler Meat Production". All broiler flocks slaughtered before 50 days of age are tested for the presence of *Campylobacter* spp. In 2013, one *C. jejuni* isolate per positive flock was submitted for susceptibility testing. A total of 96 isolates were susceptibility tested. The results are presented in Table 21 and Figure 36, and in the text.

TABLE 21. Antimicrobial resistance in *Campylobacter jejuni* (n=96) from broiler in 2013.

	Resistance		Distribution (n) of MIC values (mg/L)*												
Substance	(%)	[95% CI]	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
Tetracycline	3.1	[0.7-8.9]		41.7	35.4	19.8				1.0		2.1			
Erythromycin	0.0	[0.0-3.8]				91.7	8.3								
Streptomycin	2.1	[0.3-7.3]					35.4	56.3	6.3					2.1	
Gentamicin	0.0	[0.0-3.8]			10.4	79.2	10.4								
Ciprofloxacin	5.2	[1.7-11.7]	2.1	50.0	31.3	11.5				1.0	4.2				
Nalidixic acid	5.2	[1.7-11.7]						1.0	47.9	41.7	4.2			5.2	

*Bold vertical lines denote microbiological cut-off values. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

The prevalence of antimicrobial resistance among *C. jejuni* isolates from Norwegian broilers is low. A total of 90.6% of the included isolates were susceptible to all antimicrobial agents tested. Resistance to one or two antimicrobial agent was detected in 3.1 and 6.2% of the isolates, respectively.

The therapeutic use of antimicrobial agents in broilers is relatively low and the products applicable for such use contain either amoxicillin or phenoxymethylpenicillin. Nalidixic acid is not used in poultry. The prevalence of resistance to ciprofloxacin seems to have increased over the last years from 1.0% in 2007, to 4.2% in 2011, to 5.2% in 2013. However, these are nonsignificant changes and further monitoring is needed to see if this is an upcoming trend. An increase would be of concern as this is a critically important antimicrobial with highest priority in human medicine as defined by the WHO. The prevalence of resistance to other antimicrobial agents among *Campylobacter jejuni* isolates from Norwegian broilers has remained rather stable (Figure 36).

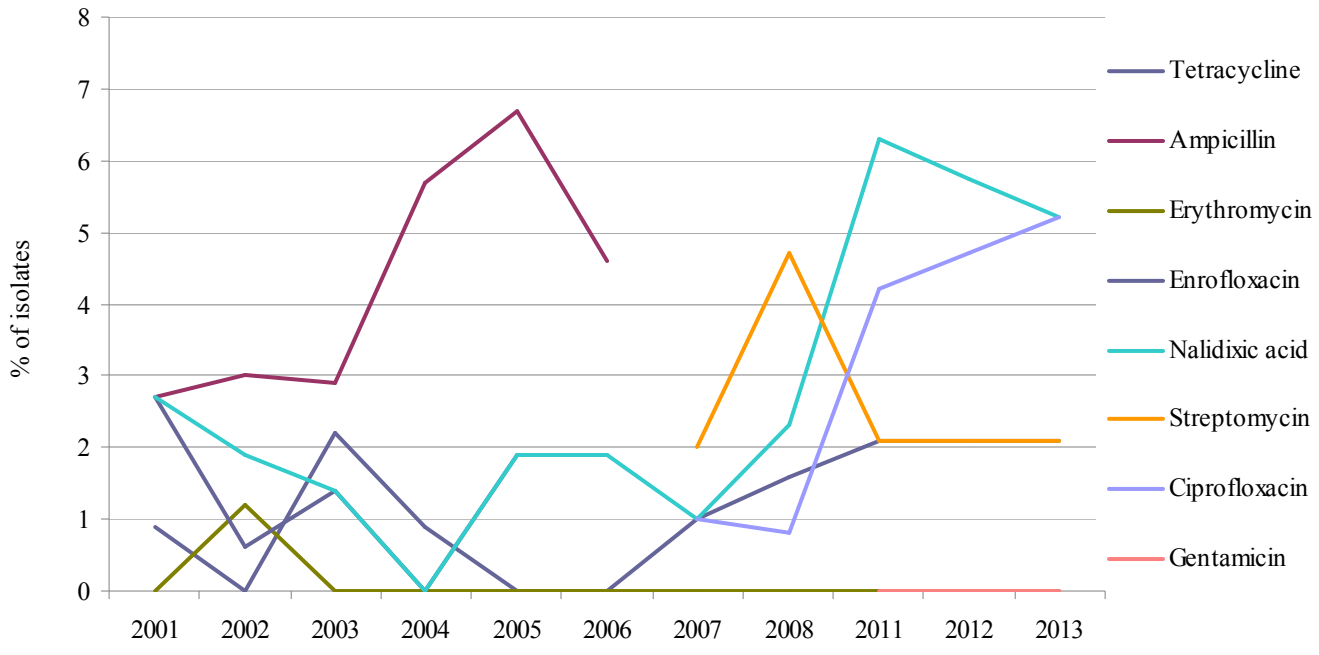


FIGURE 36. Prevalence of resistance to various antimicrobials in *Campylobacter jejuni* from Norwegian broilers 2001-2013. The cut-off values for resistance defined in NORM-VET 2006 were applied for the data generated before 2007.

Campylobacter spp. from human clinical cases

Of the 3,291 human campylobacteriosis cases registered in Norway in 2013, 53.6% were reported as acquired abroad. Based on epidemiological data from patients, the vast majority of cases were judged as sporadic. However, only a fraction of the isolates are forwarded to the NRL. Consequently, quality assured species diagnoses, complete AMR data and molecular epidemiology data on *Campylobacter* isolates are lacking due to resource limitations. Outbreaks with less clear epidemiological links may therefore very well have been overlooked, and the antimicrobial susceptibility testing results presented may therefore be underestimated or overestimated.

Susceptibility testing was performed on a total of 264 isolates of *C. jejuni* (88 from patients infected in Norway, 159 from patients infected abroad and 17 from patients where the place of acquisition of infection was unknown), and on 16 *C. coli* isolates. EUCAST clinical breakpoints and epidemiological cut-off values have been used. The results for *C. jejuni* are presented in Tables 22-23, Figures 37-40, and in the text.

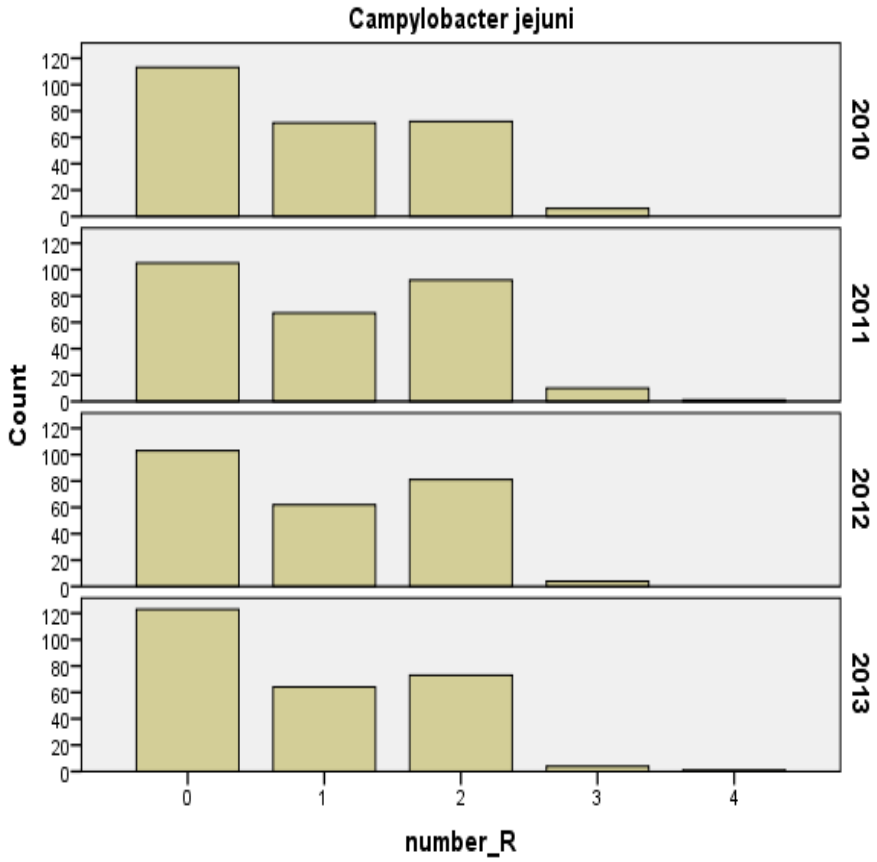


FIGURE 37. Distribution of number of antimicrobials that *Campylobacter jejuni* isolates were resistant to; by year.

TABLE 22. *Campylobacter jejuni* isolates from patients infected in Norway in 2013 (n=88). Distribution (%) of antimicrobial susceptibility categories.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline [1]	≤ 2	> 2	90.9	-	9.1
Erythromycin [1]	≤ 4	> 4	100.0	-	0.0
Gentamicin [2]	≤ 2	> 2	98.9	-	1.1
Nalidixic acid [2]	≤ 16	> 16	87.5	-	12.5
Ciprofloxacin [1]	≤ 0.5	> 0.5	89.8	-	10.2

[1] Clinical breakpoints according to EUCAST 2014 version 4.0. [2] Epidemiological cut-off values according to EUCAST web-pages by July 2014.

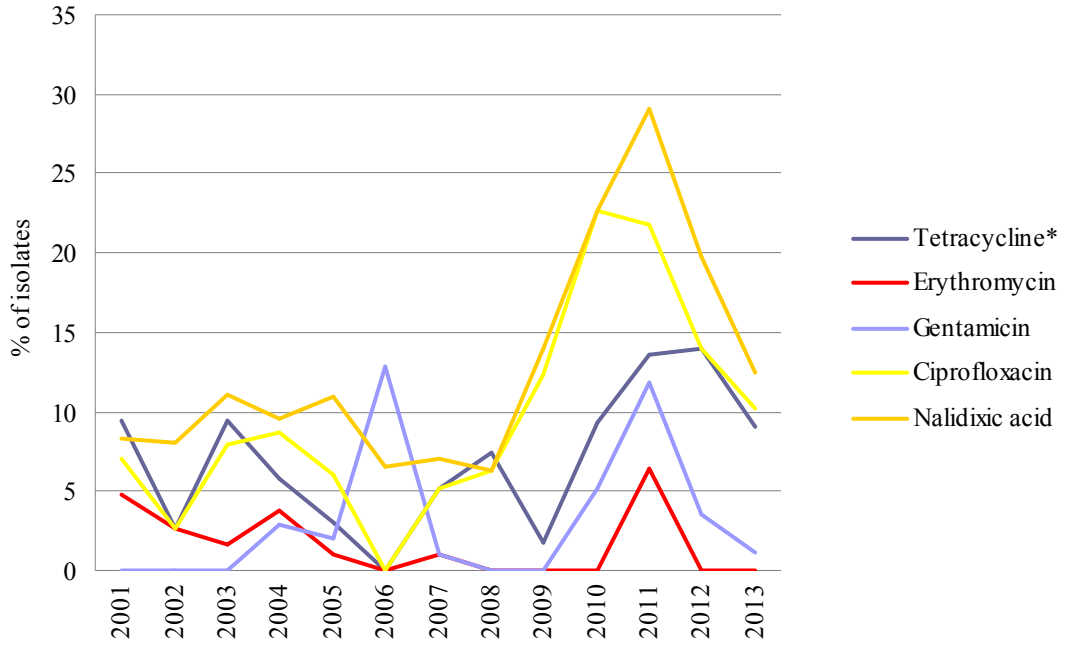


FIGURE 38. Prevalence of resistance in *Campylobacter jejuni* isolated from humans infected in Norway 2001-2013 to various antimicrobials. *Doxycycline before 2006.

TABLE 23. *Campylobacter jejuni* isolates from patients infected outside Norway in 2013 (n=159). Distribution (%) of antimicrobial susceptibility categories.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline[1]	≤ 2	> 2	43.4	-	56.6
Erythromycin[1]	≤ 4	> 4	97.5	-	2.5
Gentamicin[2]	≤ 2	> 2	98.1	-	1.9
Nalidixic acid[2]	≤ 16	> 16	40.3	-	59.7
Ciprofloxacin[1]	≤ 0.5	> 0.5	38.4	-	61.6

[1] Clinical breakpoints according to EUCAST 2014 version 4.0. [2] Epidemiological cut-off values according to EUCAST web-pages by July 2014.

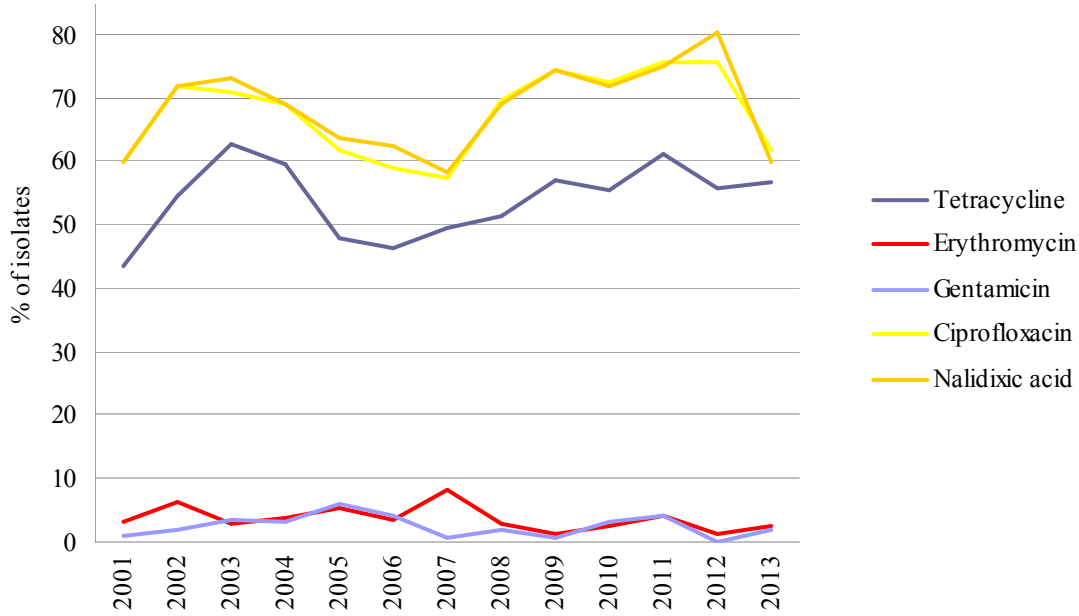


FIGURE 39. Prevalence of resistance to various antimicrobial agents in *Campylobacter jejuni* from humans infected outside Norway 2001-2013. *Doxycycline before 2006.

The data clearly show that resistance was more widespread among *C. jejuni* isolates recovered from patients infected abroad than in patients infected in Norway. Only 25.8% of isolates from infections acquired abroad were susceptible to all antimicrobial agents tested compared to 78.4% of the isolates from patients infected in Norway ($p<0.001$). The main differences between the two groups were seen for quinolones (ciprofloxacin and nalidixic acid) and tetracycline with statistically different levels of resistance between those isolates acquired abroad compared to those acquired in Norway ($p<0.001$ for both antimicrobial groups).

For strains acquired abroad, resistance against all antimicrobials seems relatively stable, and for strains acquired in Norway the sharp increase seen for all antimicrobials in 2012 data is now counteracted. The reason for these changes is unknown. They started before the transition from E-test to MIC test strips in January 2012 (consider histograms for MIC values below). Consequently some unacknowledged changes may have taken place, either methodological or true clonal shifts. However, there might be a tendency towards more resistance against quinolones and tetracycline in isolates acquired in Norway, possibly approaching the level seen in isolates acquired abroad.

Thirteen of the 16 *C. coli* isolates were acquired abroad, and ten of the latter thirteen were resistant to at least one of the antimicrobial agents tested.

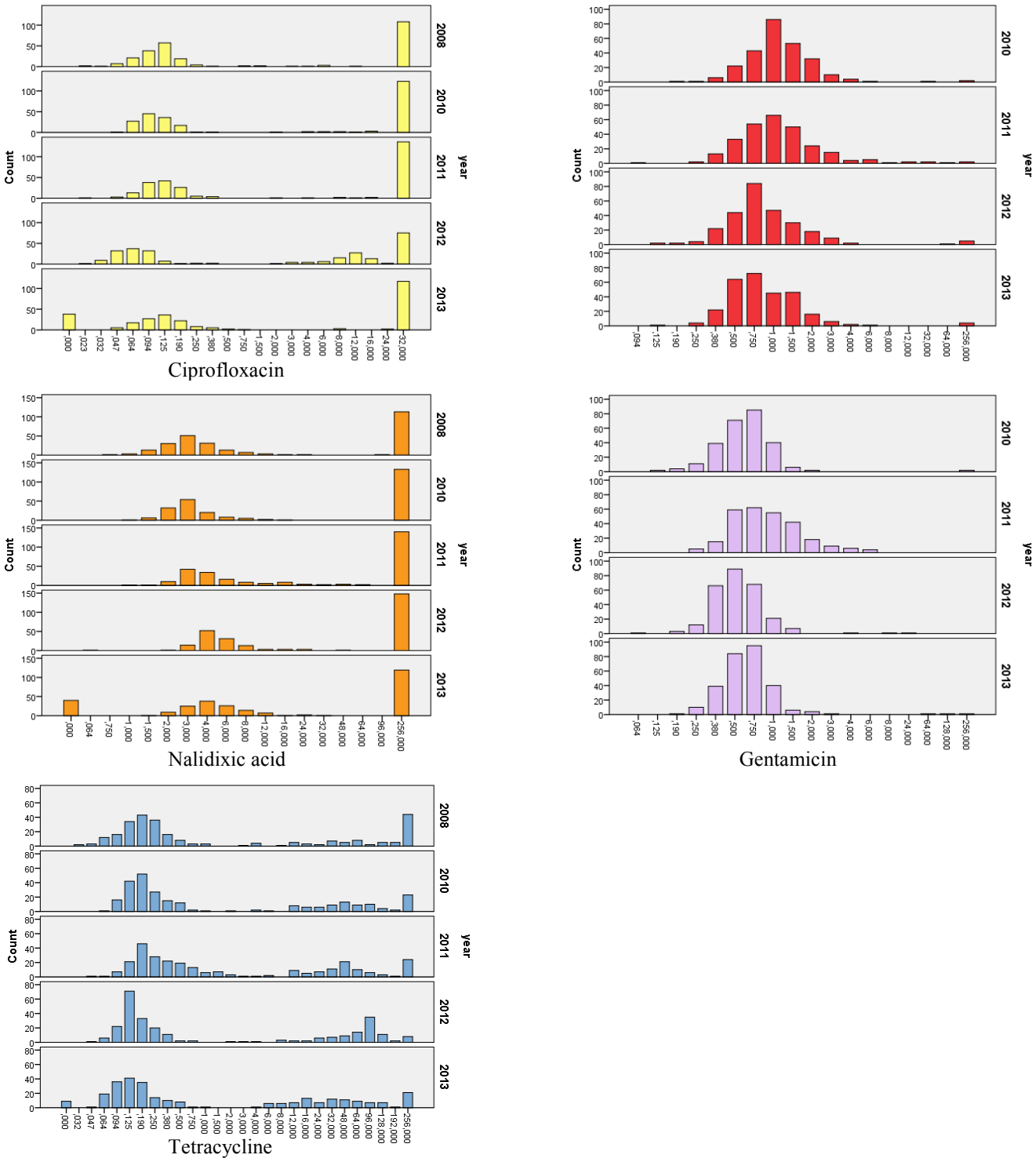


FIGURE 40. Distribution of MIC values for *Campylobacter jejuni* included in NORM 2008-2013. The transition from E-test (AB BIODISK, Solna, Sweden) to MIC test strip (Liofilchem, Roseto degli Abruzzi, Italy) was done 1st of January 2012.

Yersinia enterocolitica from human clinical cases

A total of 47 strains of pathogenic *Yersinia enterocolitica* were analysed in 2013. Thirty-eight belonged to serogroup 3 (18 of these were acquired in Norway, 11 abroad and nine with unknown place of acquisition). Seven strains belonged to serogroup 9, of which five were acquired in Norway and one strains was acquired abroad. A single *Y. enterocolitica* biotype 1B strain was acquired abroad, whereas a biotype 2 strain was acquired in Norway. All *Y. enterocolitica* isolates were tested for drug susceptibility, however the results for cefpodoxim are omitted because there is uncertainty about cut-off values for detecting ESBLs in *Yersininia*. The results are presented in Table 24 and Figures 41-42.

The crude number of isolates was considered low, and judgements should consequently be even more conservative regarding AMR results for *Y. enterocolitica* than for the other enteropathogenic bacteria.

TABLE 24. *Yersinia enterocolitica* serogroups O:3, O:9 and biotype 2 and 1B from human cases in 2013 (n=47). Distributions (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mm) *		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≥ 14	< 14	4.3	-	95.7
Chloramphenicol	≥ 17	< 17	87.2	-	12.8
Tetracycline	≥ 14	< 14	91.5	-	8.5
Nalidixic acid	≥ 19	< 19	100	-	0.0
Ciprofloxacin	≥ 22	< 22	100.0	-	0.0
Trimethoprim-sulfamethoxazole*	≥ 16	< 16	89.4	-	10.6

* As of July 2014 EUCAST recommendations for clinical or epidemiological cut-off values for *Yersinia enterocolitica* are unavailable. The cut-off values used are therefore based on evaluations of the distribution of zone diameters for each antimicrobial. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Almost all isolates of pathogenic *Yersinia enterocolitica* expressed intrinsic resistance to ampicillin. In accordance with the data on zone diameters published from the EUCAST reference database, a small number of strains lacked this attribute. This was also in agreement with a study screening for *blaA* genes *(Sharma S. et al. FEMS Microbiol Lett 2006;257:319-327)*. The prevalence of resistance to other antimicrobial agents appeared stable during the years 2001-2013. However, when EUCAST establishes clinical breakpoints or epidemiological cut-off values for *Y. enterocolitica*, it may be possible to judge with more weight on this matter.

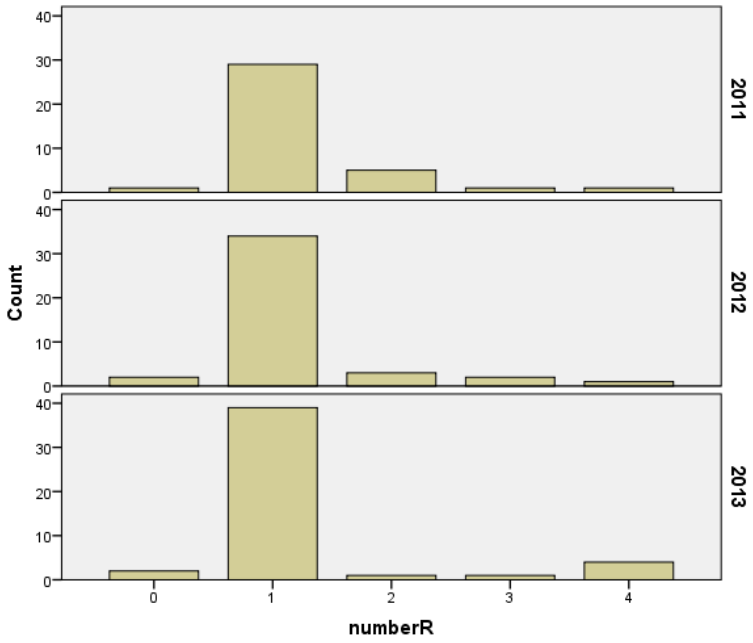


FIGURE 41. Distribution of number of antimicrobials that *Y. enterocolitica* isolates were resistant to; by year

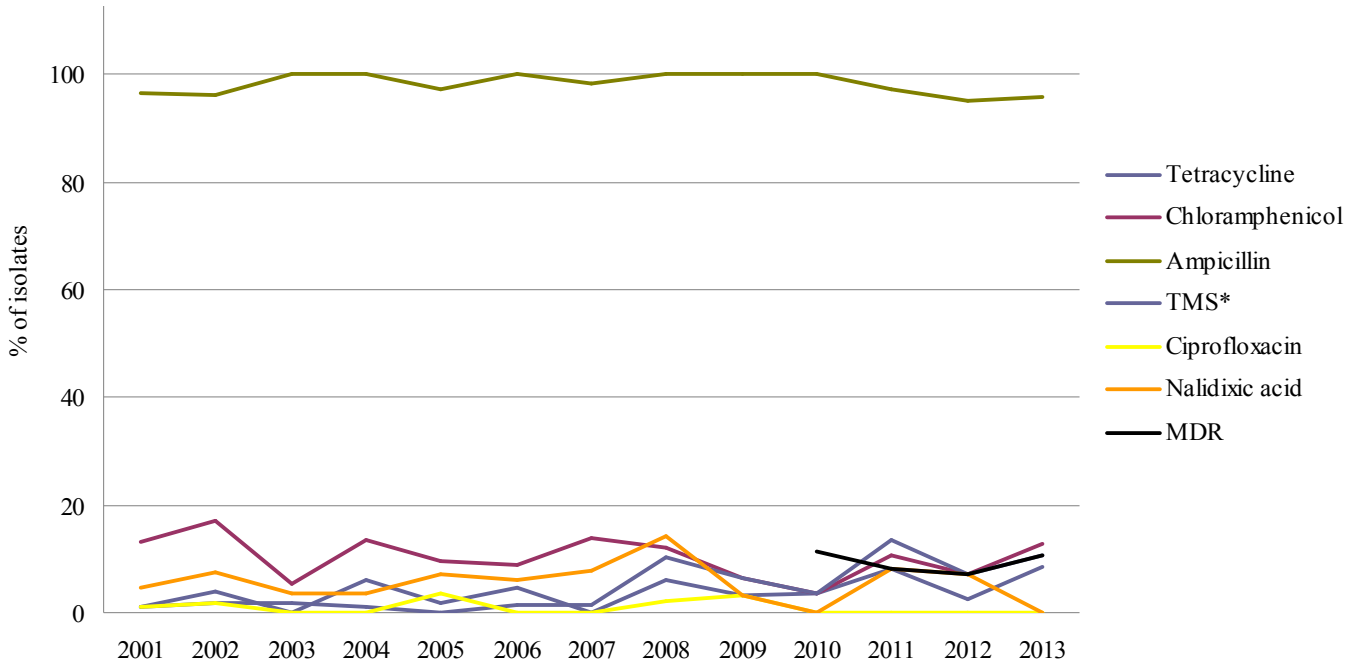


FIGURE 42. Prevalence of resistance to various antimicrobials in *Yersinia enterocolitica* from humans in Norway 2001-2013. *TMS=Trimethoprim-sulfamethoxazole.

Shigella spp. from human clinical cases

In 2013, thirteen (15.9%) of the 82 unique isolates *of Shigella* were domestically acquired. The domestically acquired strains were considered secondary to imported cases. Thus, the prevalence of resistance presented in this report predominantly relates to isolates originating from other countries. The species distribution of the 82 *Shigella* isolates that were tested for drug susceptibility was as follows: *S. sonnei* 52 (63.4%); *S. flexneri* 20 (24.4%); *S. boydii 6* (7.3%); and *S. dysenteriae* 4 (4.9%, serotype 2 and 4). The number of antimicrobial agents that *Shigella* isolates were resistant to are shown in Figure 43. Multi-resistance was defined as resistance to three or more antimicrobial categories. The results for *S. sonnei* and *S. flexneri* are presented in Table 25 and Figure 44, and in Table 26 and Figure 45, respectively.

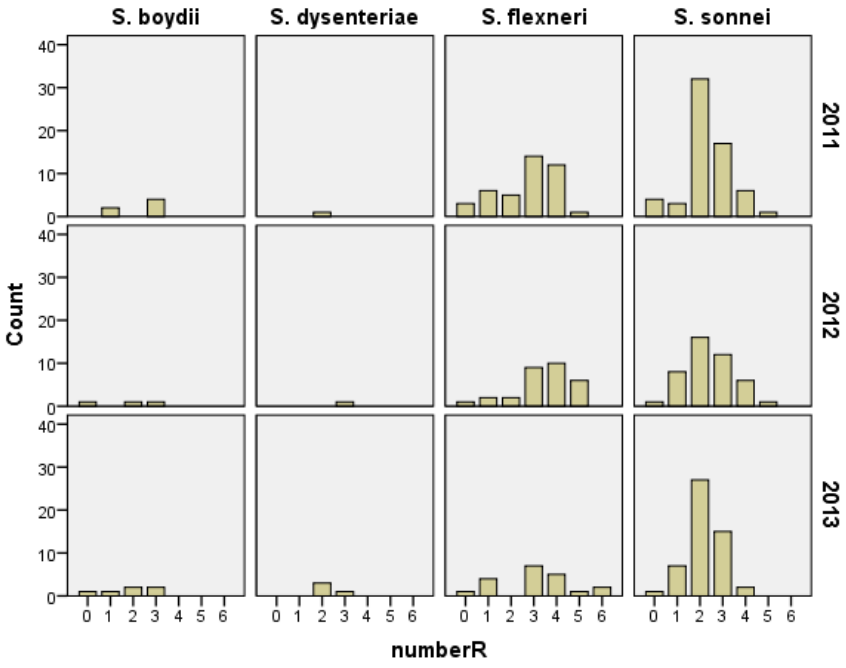


FIGURE 43. Distribution of number of antimirobials that *Shigella* isolates were resistant to; by species and by year.

TABLE 25. *Shigella sonnei* isolates from human cases in 2013 (n=52). Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin [1]	≤ 8	> 8	86.5	-	13.5
Chloramphenicol [1]	≤ 8	> 8	100.0	-	0.0
Tetracycline [2]	≥ 14	< 14	25.0	-	75.0
Nalidixic acid [2]	≥ 19	< 19	57.7	-	42.3
Ciprofloxacin [1]	≤ 0.5	> 1	80.8	0.0	19.2
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	11.5	3.9	84.6

[1] EUCAST breakpoints for Enterobacteriaceae 2013, version 3.1. [2] Epidemiological cut-off values based on zone-distribution evaluations. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 26. *Shigella flexneri* isolates from human cases in 2013 (n=20). Distribution (%) of antimicrobial susceptibility categories. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin [1]	≤ 8	> 8	40.0	-	60.0
Chloramphenicol [1]	≤ 8	> 8	35.0	-	65.0
Tetracycline [2]	≥ 14	< 14	25.0	-	75.0
Nalidixic acid [2]	≥ 19	< 19	75.0	-	25.0
Ciprofloxacin [1]	≤ 0.5	> 1	85.0	5.0	10.0
Trimethoprim-sulfamethoxazole [1,*]	≤ 2	> 4	25.0	0.0	75.0

[1] EUCAST clinical breakpoint for Enterobacteriaceae 2013 version 3.1. [2] Epidemiological cut-off values based on zone-distribution evaluations. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

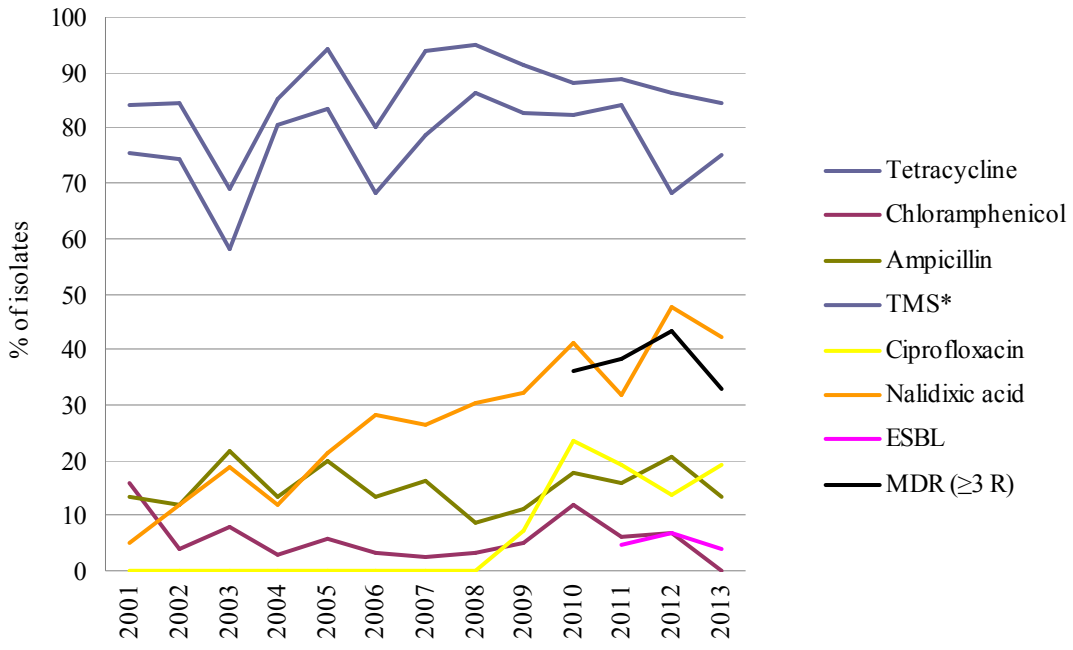


FIGURE 44. Prevalence of resistance to various antimicrobial agents in *Shigella sonnei* from humans in Norway 2001-2013. *TMS=Trimethoprim-sulfamethoxazole

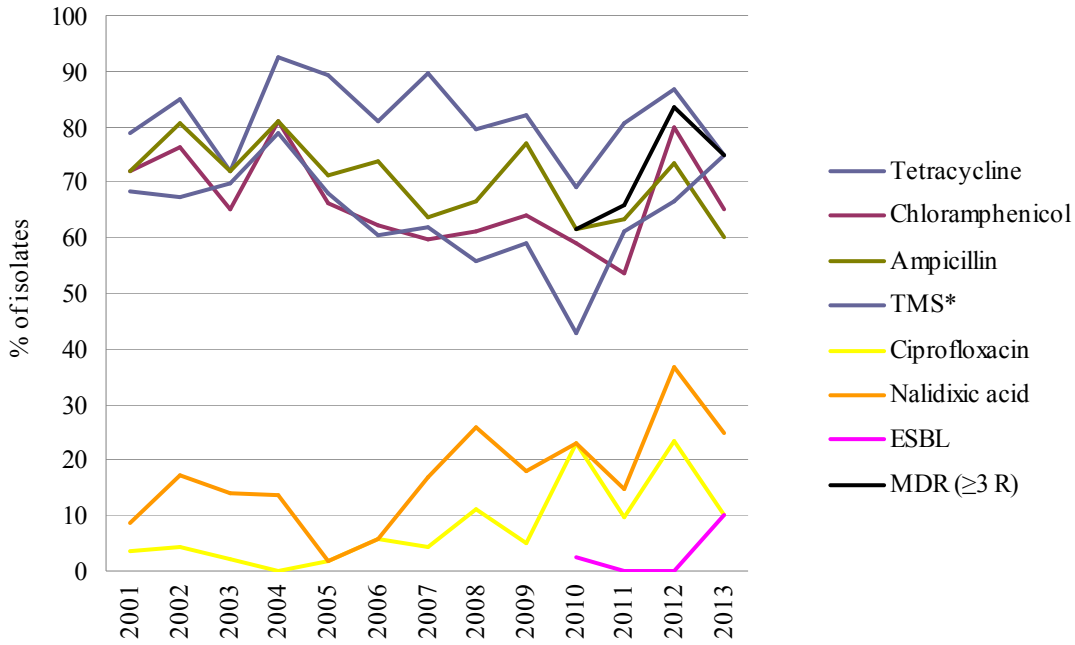


FIGURE 45. Prevalence of resistance to various antimicrobial agents in *Shigella flexneri* from humans in Norway 2001-2013. *TMS=Trimethoprim-sulfamethoxazole.

RESULTS AND COMMENTS

The resistance patterns for *S. sonnei* have been fairly stable during the period 2001-2013. Resistance to nalidixic acid, however, has increased since 2001, and resistance to ciprofloxacin has apparently increased since 2008/2009. A similar development appears for resistance to nalidixic acid and ciprofloxacin in *S. flexneri* isolates. The increase in resistance against other agents that was suggested last year seems to have been counteracted to some extent for unknown reasons. The proportion of multidrug-resistance in *S. sonnei* and *S. flexneri* was 32.7% and 75.0% respectively. Two of the six *S. boydii* isolates displayed MDR, as well as one of the four *S. dysenteriae* strains.

Four strains had reduced susceptibility to cefpodoxime. Three of these strains were phenotypically characterised as $ESBL_A$ producers with inhibitory effect of clavulanic acid (one *S. sonnei* and two *S. flexneri*), whereas one *S. sonnei* strain was an AmpC-producer.

D. HUMAN CLINICAL ISOLATES

Cecilie Torp Andersen, Trude Margrete Arnesen, Dominique Caugant, Petter Elstrøm, Kjersti Wik Larssen, Gunnar Skov Simonsen, Dagfinn Skaare, Martin Steinbakk, Gaute Syversen, Didrik Vestrheim, Frode Width-Gran

Distribution of bacterial species in blood cultures

Surveillance of antimicrobial resistance is usually based on prevalences of reduced susceptibility and resistance to certain combinations of antimicrobials and bacterial species in clinical samples. The NORM surveillance programme generally follows this approach. However, there is a serious limitation to the model because a transition in the distribution from susceptible bacterial species to inherently more resistant ones will represent de facto emergence of resistance which is not easily detected. In order to complement the surveillance of individual species, NORM collects data on all positive blood cultures from the laboratory information systems of the participating institutions. A patient with a given microbial isolate was excluded from registration with a new isolate of the same species within a month from the first entry. This rule was applied irrespectively of changes in the organism´s susceptibility pattern. Isolates of a different species were included in the surveillance. It proved difficult to systematically evaluate the clinical significance of species which are commonly part of the normal skin flora. In Table 27, proportions are therefore estimated for all isolates and for all isolates excluding species considered to be common skin contaminants such as coagulase negative staphylococci, *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp. and *Propioni-bacterium* spp. This does not imply that such isolates cannot cause infections, but only that it was not possible to enforce a standardised protocol for inclusion of the clinically significant isolates. Similarly, all isolates were registered as individual findings although polymicrobial bloodstream infections are regularly detected in some patients. Limitations in the data extraction procedure prohibited in-depth analysis of these parameters.

TABLE 27. Number of blood culture isolates in 2013, proportion of all isolates, and proportion of isolates excluding possible skin contaminants (coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp.) 2009-2013. The table is based on data from the information systems of all laboratories in Norway.

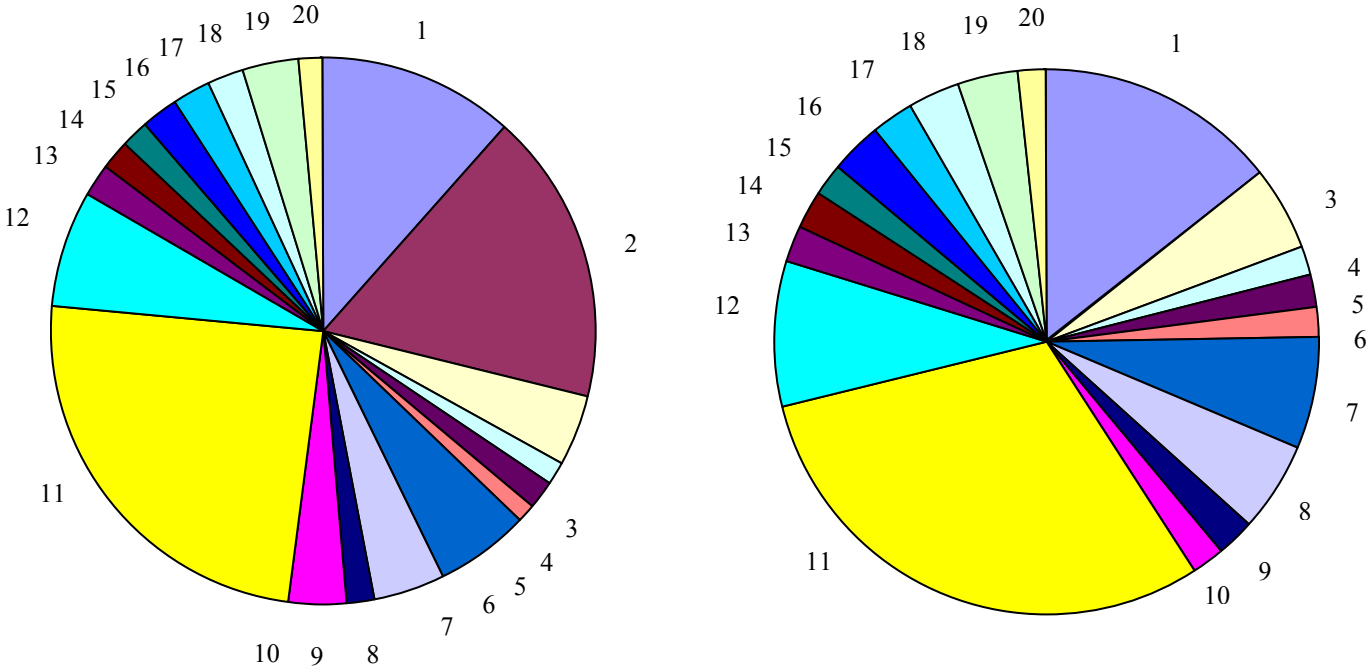
Species	No. of isolates 2013	% of all isolates					% of all isolates excluding skin flora				
		2009	2010	2011	2012	2013	2009	2010	2011	2012	2013
Staphylococcus aureus	1,629	10.6	11.4	11.0	11.3	11.5	13.9	14.5	14.2	15.0	14.3
Coagulase negative staphylococci	2,464	22.3	19.3	20.6	22.5	17.4	-	-	-	-	-
Streptococcus pneumoniae	588	6.2	5.7	5.3	4.2	4.2	8.2	7.3	6.8	5.6	5.2
Streptococcus pyogenes	177	1.3	1.3	1.4	1.0	1.3	1.7	1.6	1.8	1.3	1.6
Streptococcus agalactiae	240	1.4	1.5	1.6	1.6	1.7	1.8	1.9	2.1	2.1	2.1
Beta-haemolytic streptococci group C and G	171	1.1	1.3	1.2	1.2	1.2	1.4	1.7	1.6	1.6	1.5
Viridans- and non-haemolytic streptococci	770	3.5	4.7	4.1	3.8	5.5	4.7	5.9	5.2	5.1	6.8
Enterococcus faecalis	584	4.6	4.6	4.1	4.0	4.1	6.0	5.9	5.2	5.3	5.1
Enterococcus faecium	247	1.3	1.7	1.8	1.5	1.8	1.7	2.1	2.3	2.0	2.2
Other Gram positive aerobic and facultative bacteria	463	2.7	2.8	2.9	3.1	3.3	1.5	1.4	1.6	1.9	2.0
Escherichia coli	3,458	23.0	23.4	24.0	23.9	24.4	30.2	29.6	30.9	31.4	30.4
Klebsiella spp.	958	6.5	6.8	6.1	6.5	6.8	8.6	8.7	7.9	8.6	8.4
Enterobacter spp.	275	1.9	1.6	1.8	1.9	1.9	2.5	2.1	2.3	2.5	2.4
Proteus spp.	240	1.5	1.7	1.7	1.3	1.7	2.0	2.2	2.2	1.8	2.1
Other *Enterobacteriaceae*	330	1.9	2.3	2.2	2.0	2.3	2.6	2.9	2.9	2.7	2.9
Pseudomonas spp.	234	1.9	1.8	1.5	1.7	1.7	2.5	2.2	2.0	2.2	2.1
Other Gram negative aerobic and facultative bacteria	292	1.9	2.3	2.2	2.0	2.1	2.5	2.9	2.8	2.6	2.6
Bacteroides spp.	336	2.2	2.0	2.2	2.3	2.4	2.9	2.6	2.9	3.0	3.0
Other anaerobic bacteria	452	2.3	2.3	2.8	2.8	3.2	2.8	2.6	3.4	3.3	3.5
Yeasts	205	1.9	1.5	1.5	1.4	1.5	2.5	1.9	1.9	2.0	1.8
Total	**14,113**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

As seen in Table 27 and Figure 46, aerobic and facultative Gram-positive and Gram-negative bacteria represented 52.0% and 40.9% of all isolates, respectively. The predominance of Gram-positives among all isolates was at the same level as in previous years. The most common Gram-positive species were coagulase negative staphylococci which represented 17.4% of all isolates. This was a significant decline from 22.5% in 2012, but these fluctuations may result from inconsistent reporting from the laboratories. The difference between aerobic Gram-positives and Gram-negatives was reversed when species of the skin flora (coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propioni-bacterium* spp.) were excluded with 40.8% aerobic Gram-positives and 50.9% aerobic Gram-negatives.

Among aerobic Gram-positives, the prevalence of *S. pneumoniae* has steadily declined from 12.1% in 2005 to 5.2% in 2013 (skin contaminants are excluded), following the introduction of the conjugate pneumococcal vaccine in the national childhood immunisation programme. The proportions accounted for by viridans- and non-hamolytic streptococci increased to 6.8% of isolates (skin contaminants excluded). Further surveillance will determine whether this trend will continue.

E. coli (30.4%) and other *Enterobacteriaceae* (15.8%) accounted for the vast majority of aerobic Gram-negative isolates, but the proportions have remained relatively unchanged over the years. *Pseudomonas* spp. (2.1%) has been fairly stable after a peak in 2005 (2.8%), all figures excluding skin flora.

Anaerobic bacteria and yeasts were less prevalent. Anaerobes accounted for 5.6% (6.5% excluding skin flora) which is a slight increase from 2012 (5.1%; 6.3% excluding skin flora). Yeasts accounted for 1.5% (1.8% excluding skin flora) which is unchanged from earlier years. The major pathogens among anaerobes were members of *Bacteroides* spp. (2.4%/3.0%) and among yeasts *Candida albicans* (0.9%/1.1%). However, a multitude of other species was also represented.



1. *Staphylococcus aureus*
2. Coagulase negative staphylococci
3. *Streptococcus pneumoniae*
4. *Streptococcus pyogenes*
5. *Streptococcus agalactiae*
6. Betahaemolytic streptococci group C and G
7. Non-haemolytic and viridans streptococci
8. *Enterococcus faecalis*
9. *Enterococcus faecium*
10. Other Gram-positive bacteria
11. *Escherichia coli*
12. *Klebsiella* spp.
13. *Enterobacter* spp.
14. *Proteus* spp.
15. Other *Enterobacteriaceae*
16. *Pseudomonas* spp.
17. Other Gram-negative bacteria
18. *Bacteroides* spp.
19. Other anaerobic bacteria
20. Yeasts

FIGURE 46. Distribution of all blood culture isolates (left, n=14,113) and blood culture isolates excluding common skin contaminants (right, n=11,359) such as coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp. Data for 2013 were retrieved from the information systems of all Norwegian laboratories.

Escherichia coli in blood cultures

TABLE 28. *Escherichia coli* blood culture isolates in 2013 (n=1,612). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	57.3	-	42.7
Piperacillin-tazobactam	≤ 8	> 16	94.1	3.8	2.1
Cefuroxime	≤ 8	> 8	92.6	-	7.4
Cefotaxime	≤ 1	> 2	94.5	0.1	5.4
Ceftazidime	≤ 1	> 4	94.4	1.2	4.4
Cefepime	≤ 1	> 4	95.3	1.1	3.6
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	94.2	0.2	5.6
Ciprofloxacin	≤ 0.5	> 1	87.7	0.5	11.8
Tigecycline	≤ 1	> 2	99.5	0.4	0.1
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	73.9	0.7	25.4
ESBL	Negative	Positive	95.0	-	5.0

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The NORM results are interpreted according to the breakpoints of the Norwegian Working Group for Antibiotics (NWGA) at the time of analysis. Norwegian breakpoints for Enterobacteriaceae correspond to EUCAST breakpoints. The *E. coli* wild type was recategorised as susceptible to ampicillin from 2012 and to cefuroxime from 2014, which is in line with EUCAST.

The vast majority of isolates remained fully susceptible to broad-spectrum antimicrobial agents such as cefotaxime (94.5%), ceftazidime (94.4%), cefepime (95.3%), gentamicin (94.2%), piperacillin-tazobactam (94.1%), tigecycline (99.5%) and meropenem (100.0%) (Table 28). There were no significant changes in the prevalence of susceptibility to any of these agents from 2012 to 2013.

The increasing prevalence of non-susceptibility to gentamicin since 2004 remained unchanged with 0.2% I and 5.6% R in 2013 compared to 0.2% I and 5.7% R in 2012 (Figure 47). The prevalence of gentamicin resistance is approximately five times higher than a decade ago. A high proportion of gentamicin non-susceptible isolates (33/94, 35.1%) also produced ESBL enzymes. They were retrieved from 18 different laboratories across the country. The prevalence at individual laboratories (0-13.5%) varied widely due to relatively small numbers. When aggregated by region there were no major geographical differences (South/East 5.2%, West 6.4%, Middle 7.5% and North 3.4%).

The prevalence of non-suscpetibility to ciprofloxacin was 12.3% (0.5% I and 11.8% R) in 2013 compared to 11.7% in 2012 (0.4% I and 11.3% R). This is the highest rate of quinolone resistance ever recorded in NORM. The steadily increasing proportion of non-susceptibility to ciprofloxacin in *E. coli* blood culture isolates corresponds to the situation in almost all other European countries. The temporal association between ciprofloxacin non-susceptibility and ciprofloxacin usage is depicted in Figure 48. A similar association between quinolone use and resistance in systemic *E. coli* isolates is also reported internationally. The resistance rates for ampicillin (42.7% in 2013, 41.9% in 2012) and trimethoprim-sulfamethoxazole (25.4% in 2013, 26.9% in 2012) are relatively stable.

In 2013, detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefotaxime and ceftazidime. All isolates with reduced susceptibility to ceftazidime and/or cefotaxime were further characterised by combination MIC gradient tests. A total of 80 isolates (5.0%) were reported as ESBL positive which is a significant increase from 3.1% in 2010 and 3.3% in 2011, but a minor decrease from 5.5% in 2012 (Figure 50). The isolates originated from 18 different laboratories in all parts of the country. Estimates at laboratory level (0-9.5%) are uncertain due to small numbers. When aggregated at regional level there were only minor geographical differences in the prevalence of ESBL (South/East 4.4%, West 5.8%, Middle 5.4% and North 5.7%). Almost all ESBL isolates were non-susceptible to ampicillin (80/80), cefuroxime (79/80) and cefotaxime (80/80), and most of them were also non-susceptible to ceftazidime (69/80) and cefepime (63/80). Many isolates were intermediately (20/80) or even fully susceptible (53/80) to piperacillin-tazobactam. Most displayed some level of co-resistance to ciprofloxacin (60/80), gentamicin (33/80) and/or trimethoprim-sulfamethoxazole (59/80). All were fully susceptible to meropenem, and only a single isolate displayed reduced susceptibility to tigecycline. Twenty-two additional isolates were reported as non-susceptible to cefotaxime (n=8) and/or ceftazidime (n=22) without being confirmed as ESBL producers.

Seventy-nine *E. coli* ESBL isolates were molecularly characterised which revealed a predominance of CTX-M groups 1 (n=52) and 9 (n=22). The remaining five isolates harboured derepressed chromosomally encoded AmpC (n=2) or plasmid encoded CMY (n=2) enzymes.

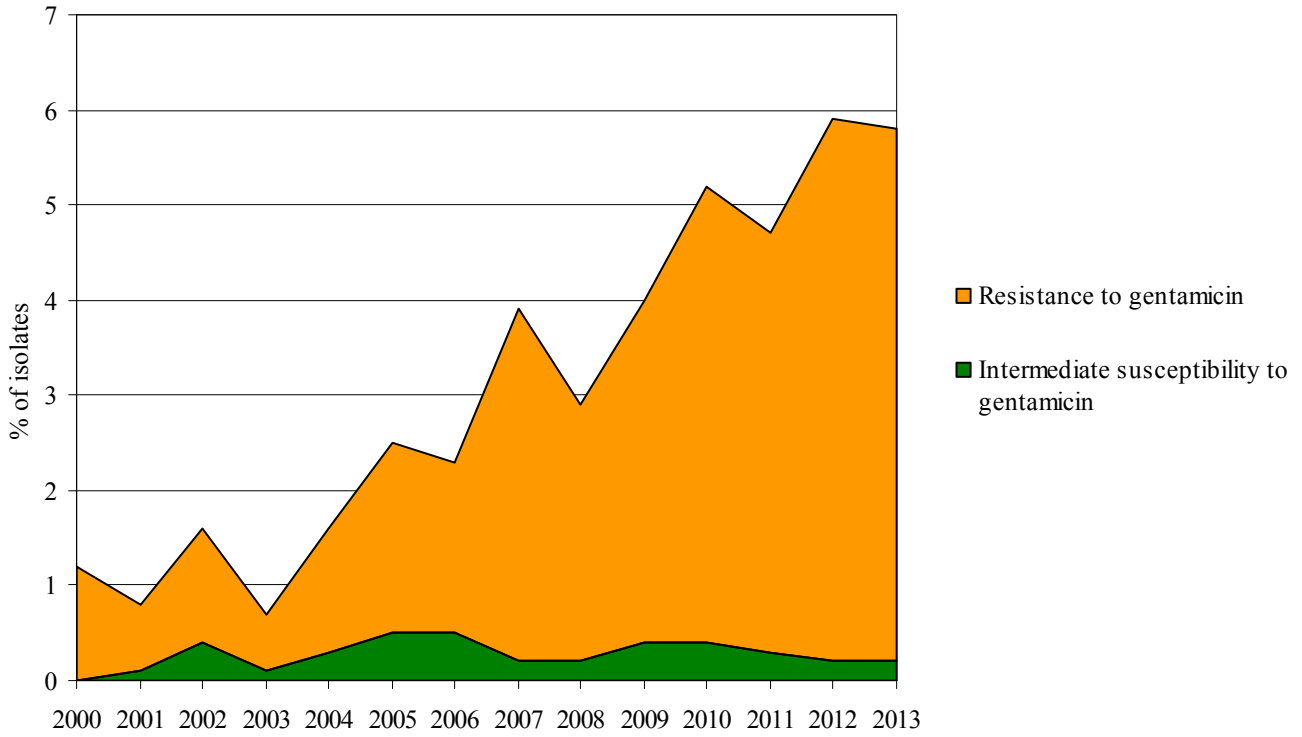


FIGURE 47. Prevalence of intermediate susceptibility and resistance to gentamicin in *Escherichia coli* blood culture isolates 2000-2013.

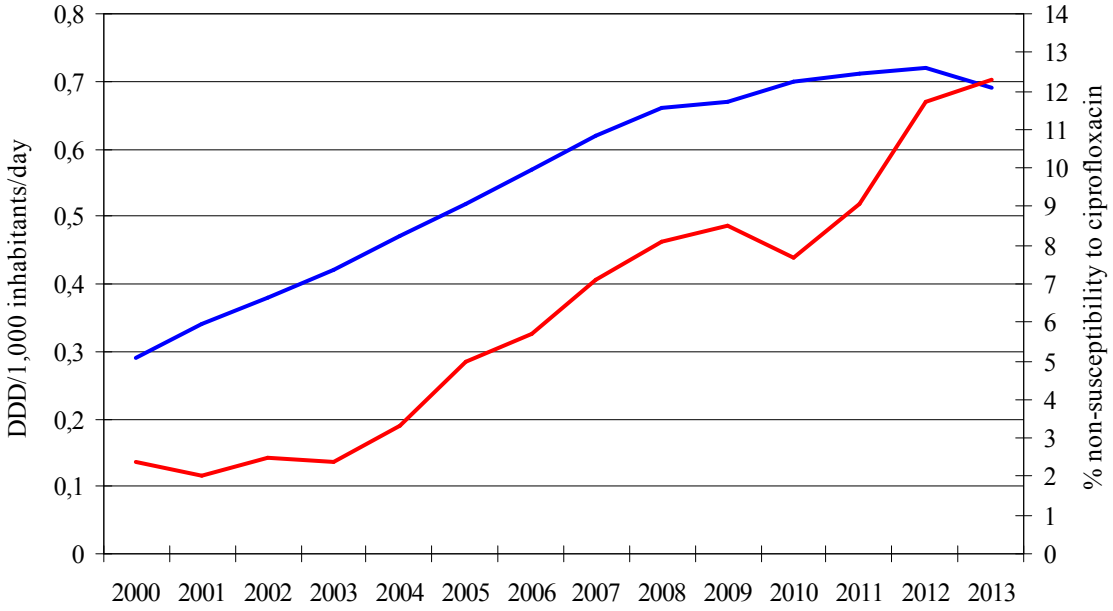


FIGURE 48. Usage of ciprofloxacin (blue) and prevalence of ciprofloxacin non-susceptibility in *Escherichia coli* blood culture isolates as defined by the 2014 breakpoints (red) 2000-2013.

Escherichia coli in urine

TABLE 29. *Escherichia coli* urinary tract isolates in 2013 (n=839). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	66.6	-	33.4
Mecillinam	≤ 8	> 8	95.0	-	5.0
Amoxicillin-clavulanic acid*	≤ 32	> 32	91.9	-	8.1
Cefuroxime	≤ 8	> 8	95.4	-	4.6
Cefotaxime	≤ 1	> 2	97.6	0.0	2.4
Ceftazidime	≤ 1	> 4	97.7	0.6	1.7
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	97.2	0.1	2.7
Ciprofloxacin	≤ 0.5	> 1	92.7	0.1	7.2
Nitrofurantoin	≤ 64	> 64	99.0	-	1.0
Trimethoprim	≤ 2	> 4	79.2	0.4	20.4
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	80.5	0.8	18.7
ESBL	Negative	Positive	97.9	-	2.1

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Urinary tract isolates of *E. coli* have been included in the surveillance programme every year since NORM was established in 2000. The prevalence of resistance for 2013 is shown in Table 29 and the results for 2000-2013 are shown in Figure 49. As for *E. coli* blood culture isolates, wild type isolates were reclassified as susceptible to ampicillin from 2012 and to cefuroxime from 2014. All results since 2000 have been recategorised accordingly.

The resistance rates among urinary tract isolates have remained relatively stable over the last ten years, but are slowly drifting upward for most antibiotics. The prevalence of resistance to ampicillin has gradually increased from approximately 25% to 35%. Around 20% of *E. coli* isolates are resistant to trimethoprim and trimethoprim-sulfamethoxazole with a possible decrease from 2012 to 2013. The prevalence of resistance to mecillinam increased from 4.2% in 2012 to 5.0% in 2013, but susceptibility test results are notoriously difficult to reproduce for this agent and the observed changes may thus not reflect real differences in prevalence.

Ciprofloxacin is used as a second-line agent for urinary tract infections in Norway. The prevalence of non-susceptibility has been relatively stable around 3.5-5.5% over the last years. This prevalence was 7.3% in 2013 with 0.1% intermediate susceptibility and 7.2% resistance, thus confirming the increase seen from 2011 to 2012. The corresponding rates for blood culture isolates were 0.5% intermediate susceptibility and 11.8% resistance in 2013. The persistent discrepancy between urinary tract isolates and isolates from bloodstream infections suggests that systemic infections are caused by selected pathogenic lineages with increased virulence and accumulation of mutations in gyrase and/or topoisomerase genes, whereas urinary tract isolates are more representative of the wild type normal flora. The steadily increasing resistance rates are obviously a cause for great concern.

Of note, 8.1% of the isolates were resistant to amoxicillin-clavulanic acid when using the newly issued breakpoints for uncomplicated urinary tract infections. When recategorising previous results, this is an increase from 4.3% in 2012. Further surveys are needed to determine whether this is a true increase or is due to technical difficulties in the interpretation of zone sizes.

In total, 18 isolates (2.1%) were reported as ESBL producers. This prevalence is unchanged from 2012 (2.2%). As seen in Figure 50, the prevalence of *E. coli* ESBL is still significantly lower in urine than in blood culture isolates (5.0%), but there is an increasing trend in both specimen types. The ESBL-positive isolates were retrieved from ten different laboratories in all parts of the country. Fourteen isolates were retrieved from samples submitted by general practitioners, while the others were found in hospitalised patients (n=2) or patients visiting outpatient clinics (n=2). The ESBL strains were generally resistant to ampicillin (18/18), cefuroxime (18/18) and cefotaxime (18/18), and non-susceptible to ceftazidime (15/18). Most isolates were registered as *in vitro* susceptible to mecillinam (17/18). The clinical relevance of this finding is doubtful, since mecillinam is not stable for most beta-lactamases. Many of the ESBL isolates were non-susceptible to quinolones (10/18) and trimethoprim-sulfamethoxazole (11/18), but remained susceptible to nitrofurantoin (18/18) and gentamicin (14/18). All isolates were susceptible to carbapenems. All urinary tract *E. coli* ESBL isolates were molecularly characterised which revealed a predominance of CTX-M groups 1 (n=9) and 9 (n=6). Single isolates harboured SHV-ESBL, derepressed chromosomally encoded AmpC, or wild type AmpC enzymes.

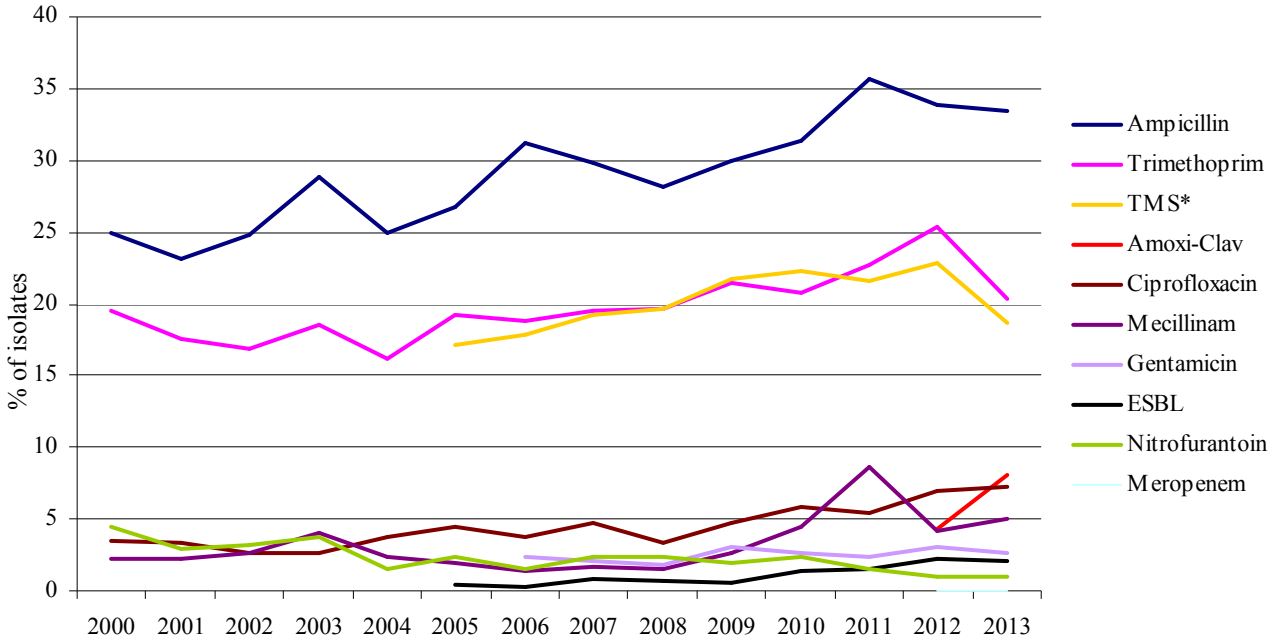


FIGURE 49. Prevalence of resistance to various antimicrobial agents in urinary tract *E. coli* isolates 2000-2013. Isolates previously classified as intermediately susceptible to ampicillin and cefuroxime have been recategorised as susceptible according to 2014 EUCAST guidelines. *TMS=Trimethoprim-sulfamethoxazole.

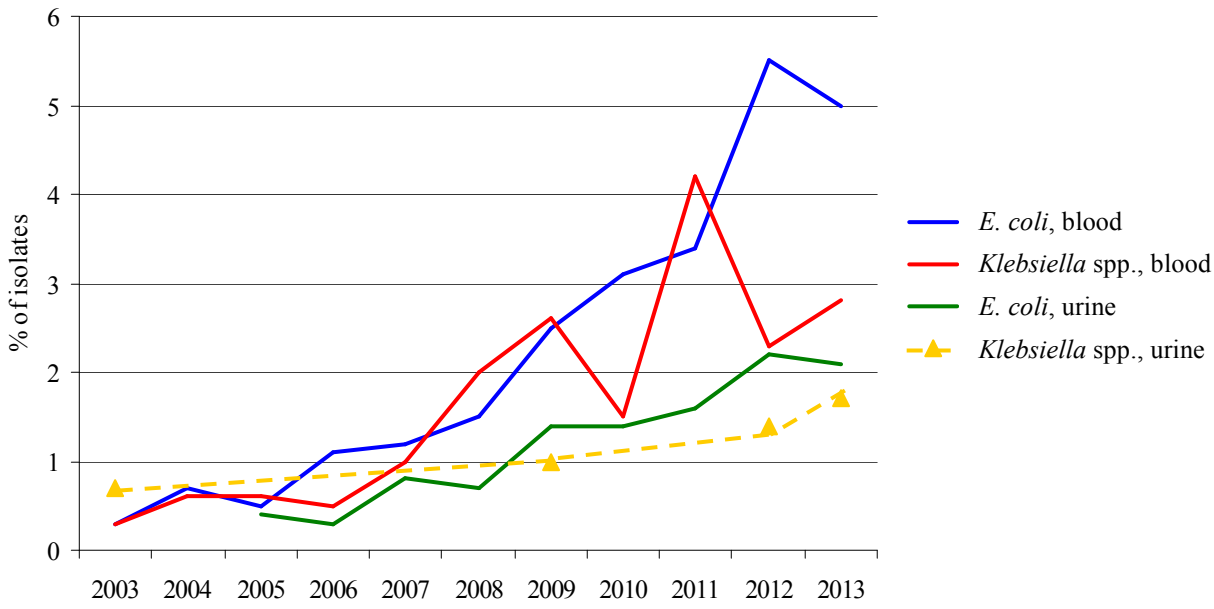


FIGURE 50. Prevalence of ESBL production among *E. coli* and *Klebsiella* spp. isolates from blood and urine 2003-2013.

Klebsiella spp. in blood cultures

TABLE 30. *Klebsiella* spp. blood culture isolates in 2013 (n=652). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Piperacillin-tazobactam	≤ 8	> 16	89.4	7.1	3.5
Cefuroxime	≤ 8	> 8	91.7	-	8.3
Cefotaxime	≤ 1	> 2	96.7	0.8	2.5
Ceftazidime	≤ 1	> 4	96.5	0.9	2.6
Cefepime	≤ 1	> 4	97.1	1.1	1.8
Meropenem	≤ 2	> 8	99.7	0.0	0.3
Gentamicin	≤ 2	> 4	98.3	0.6	1.1
Ciprofloxacin	≤ 0.5	> 1	95.4	1.7	2.9
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	87.9	1.5	10.6
ESBL	Negative	Positive	97.2	-	2.8

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 31. *Klebsiella pneumoniae* blood culture isolates in 2013 (n=454). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Piperacillin-tazobactam	≤ 8	> 16	89.0	8.4	2.6
Cefuroxime	≤ 8	> 8	92.3	-	7.7
Cefotaxime	≤ 1	> 2	97.4	0.2	2.4
Ceftazidime	≤ 1	> 4	96.7	0.7	2.6
Cefepime	≤ 1	> 4	97.8	0.7	1.5
Meropenem	≤ 2	> 8	99.6	0.0	0.4
Gentamicin	≤ 2	> 4	98.0	0.9	1.1
Ciprofloxacin	≤ 0.5	> 1	94.3	2.4	3.3
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	85.5	1.5	13.0
ESBL	Negative	Positive	97.4	-	2.6

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 32. *Klebsiella oxytoca* blood culture isolates in 2013 (n=125). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Piperacillin-tazobactam	≤ 8	> 16	91.2	3.2	5.6
Cefuroxime	≤ 8	> 8	90.4	-	9.6
Cefotaxime	≤ 1	> 2	96.0	3.2	0.8
Ceftazidime	≤ 1	> 4	97.6	1.6	0.8
Cefepime	≤ 1	> 4	96.8	1.6	1.6
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	99.2	0.0	0.8
Ciprofloxacin	≤ 0.5	> 1	99.2	0.0	0.8
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	95.2	0.0	4.8
ESBL	Negative	Positive	99.2	-	0.8

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The surveillance of *Klebsiella* spp. in blood cultures included 454 *K. pneumoniae* (69.6%), 125 *K. oxytoca* (19.2%), and 73 (11.2%) isolates not identified to the species level, giving a total of 652 *Klebsiella* spp. isolates (Tables 30-32). There were a higher proportion of unspeciated isolates and fewer *K. pneumoniae* than in 2012. As for *E. coli*, the Norwegian Working Group for Antibiotics (NWGA) has from 2014 redefined the *Klebsiella* spp. wild type as susceptible to cefuroxime in accordance with EUCAST protocol. Breakpoints for the other antimicrobial agents included in the *Klebsiella* surveillance protocol were not changed in 2013.

The majority of *Klebsiella* spp. isolates remained susceptible to aminoglycosides. The peak of 4.6% non-susceptibility observed in 2011 reverted to 1.0% in 2012 and 1.7% in 2013. The prevalence of non-susceptibility to aminoglycosides was similar in *K. oxytoca* isolates (0.8%) and *K. pneumoniae* (2.0%) in 2013. Aminoglycoside resistance in common *Enterobacteriaceae* species is a cause for great concern as aminoglycosides have traditionally been used in the empirical regimen for treatment of septiceamia in Norway.

The overall prevalence of resistance to ciprofloxacin has been stable at 3-4% when taking into account the changes in breakpoints and interpretive criteria. The 4.6% non-susceptibility (1.7% intermediate susceptibility and 2.9% resistance) observed in 2013 was at the same level as in previous years. Non-susceptibility to ciprofloxacin is still more common in *K. pneumoniae* (5.7%) than in *K. oxytoca* (0.8%). Non-susceptibility to trimethoprim-sulfamethoxazole remained unchanged at 12.1% in 2013 compared to 12.9% in 2012. As for ciprofloxacin, the prevalence of resistance to trimethoprim-sulfamethoxazole was significantly lower in *K. oxytoca* (4.8%) than in *K. pneumoniae* (13.0%).

A comparison of ESBL rates and non-susceptibility to beta-lactam antibiotics between species is complicated by the diagnostic challenges of the chromosomal K1 beta-lactamase in *K. oxytoca*. Most *Klebsiella* spp. isolates were susceptible to cefotaxime (96.7%), ceftazidime (96.5%) and the beta-lactam/beta-lactamase inhibitor combination piperacillin-tazobactam (89.4%), see Figure 51. The rates of non-susceptibility to third generation cephalosporins were at the same level as in previous years.

As for *E. coli*, the detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefotaxime and ceftazidime disks. Isolates with reduced zone diameters were further characterised by combination MIC gradient tests. The prevalence of phenotypically confirmed ESBL isolates remained stable at 2.8% compared to 2.3% in 2012 (Figure 50). The 18 ESBL isolates originated from 10 different laboratories and were identified as *K. pneumoniae* (n=12, 2.6%), *K. oxytoca* (n=1, 0.8%) or *Klebsiella* sp. (n=5, 6.8%). The ESBL isolates were generally non-susceptible to cefuroxime (16/18), ceftazidime (17/18) and cefotaxime (16/18), and co-resistance was frequently seen to ciprofloxacin (10/18), trimethoprim-sulfamethoxazole (14/18) and gentamicin (8/18). Many isolates were intermediately (7/18) or even fully (5/18) susceptible to piperacillin-tazobactam. Molecular characterisation of the ESBL isolates at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-Res) confirmed the presence of CTX-M groups 1 (n=11) and 9 (n=1). The remaining isolates contained SHV-ESBL variants (n=4) or hyper-producing wild type SHV (n=1). Two *K. pneumoniae* isolates (0.3%) displayed reduced susceptibility to meropenem and contained KPC and OXA-48 determinants compatible with carbapenemase production.

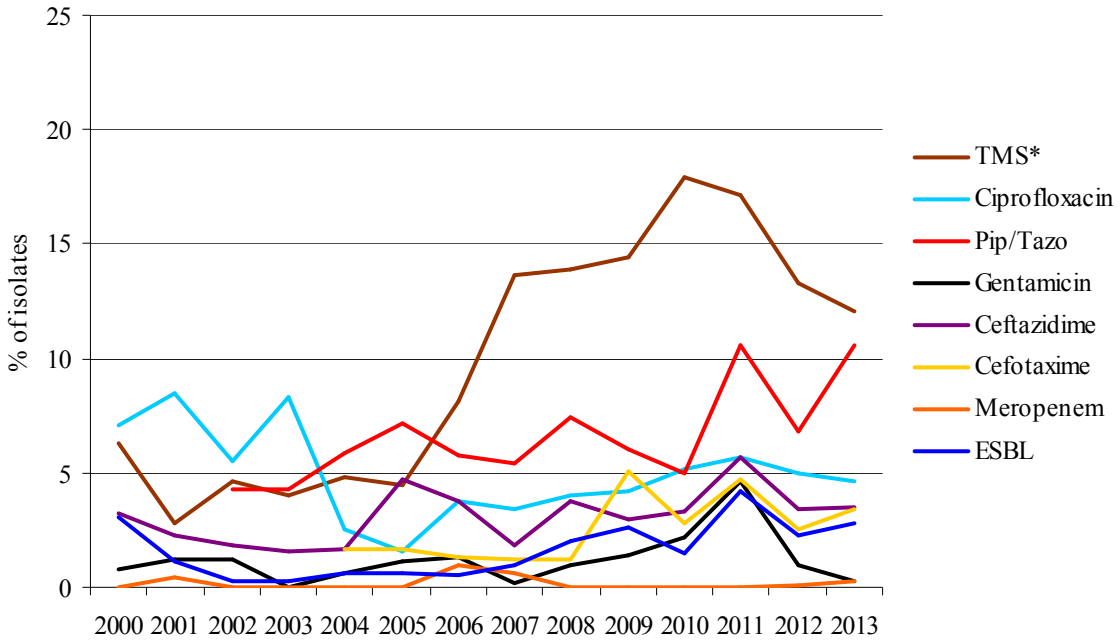


FIGURE 51. Prevalence of non-susceptibility to various antimicrobial agents in *Klebsiella* spp. blood culture isolates 2000-2013. *TMS=Trimethoprim-sulfamethoxazole.

Klebsiella spp. in urine

TABLE 33. *Klebsiella* spp. urinary tract isolates in 2013 (n=886). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Mecillinam	≤ 8	> 8	92.3	-	7.7
Amoxicillin-clavulanic acid*	≤ 32	> 32	91.2	-	8.8
Piperacillin-tazobactam	≤ 8	> 16	92.1	6.0	1.9
Cefuroxime	≤ 8	> 8	94.5	-	5.5
Cefotaxime	≤ 1	> 2	97.5	0.7	1.8
Ceftazidime	≤ 1	> 4	97.6	0.7	1.7
Meropenem	≤ 2	> 8	99.9	0.0	0.1
Gentamicin	≤ 2	> 4	97.9	0.6	1.5
Ciprofloxacin	≤ 0.5	> 1	96.1	1.6	2.3
Trimethoprim	≤ 2	> 4	82.9	1.1	16.0
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	87.2	1.1	11.7
ESBL	Negative	Positive	98.3	-	1.7

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Klebsiella spp. isolates have previously been included in the NORM surveillance programme in 2001, 2003, 2009 and 2012. Due to methodological changes and adjustment of breakpoints it is not possible to directly compare the results from 2009 and 2012 with the ones from 2001 and 2003. There are no *Klebsiella* spp. breakpoints for nitrofurantoin.

In general, the rates of resistance to urinary tract antibiotics were slightly lower in *Klebsiella* spp. than in *E. coli* isolates (Tables 33-35). The vast majority of isolates are still susceptible to gentamicin (97.9% compared to 98.1% in 2012), ciprofloxacin (96.1% compared to 96.5% in 2012), and meropenem (99.9%). The comparable rates for *E. coli* were 97.2% for gentamicin, 92.7% for ciprofloxacin and 100% for meropenem. Susceptibility to

trimethoprim (82.9% compared to 81.5% in 2012) and trimethoprim-sulfamethoxazole (87.2% compared to 76.4% in 2009) were similar to the findings in *E. coli* (79.2% and 80.5%, respectively).

All *Klebsiella* isolates are inherently resistant to ampicillin due to the chromosomal SHV beta-lactamase. Wild type strains are since 2014 categorised as susceptible to cefuroxime in accordance with EUCAST protocol. As for *Klebsiella* spp. blood culture isolates, ESBL detection in urinary tract isolates was based on non-susceptibility to cefotaxime and/or ceftazidime and subsequent confirmatory ESBL MIC gradient tests. Only 15 isolates were reported as ESBL positive of which 14 were *K. pneumoniae* and one was unspeciated. The 15 ESBL isolates were retrieved from nine different laboratories and originated from hospitals (n=10), general practices (n=2), outpatient clinics (n=2) and an unknown location (n=1). The 1.7% ESBL rate (2.5% in *K. pneumoniae*) represented a modest increase from 1.4% in 2012 and was lower than the 2.8% rate (2.6% in *K. pneumoniae*) found in blood culture isolates. The 15 ESBL isolates were generally non-susceptible to trimethoprim (n=12), trimethoprim-sulfamethoxazole (n=13) and ciprofloxacin (n=13), but many remained susceptible to gentamicin (n=6), mecillinam (n=12) and piperacillin-tazobactam (n=7). Molecular characterisation confirmed the presence of CTX-M groups 1 (n=12) and 9 (n=2), as well as an isolate with an SHV-ESBL enzyme (n=1). A single ESBL positive *K. pneumoniae* isolate was resistant to meropenem, but carbapenemase production could not be detected. Phenotypical carbapenem resistance in this isolate was probably caused by the CTX-M genotype in combination with reduced permeability and/or efflux mechanisms.

TABLE 34. *Klebsiella pneumoniae* urinary tract isolates in 2013 (n=557). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Mecillinam	≤ 8	> 8	91.6	-	8.4
Amoxicillin-clavulanic acid*	≤ 32	> 32	91.7	-	8.3
Piperacillin-tazobactam	≤ 8	> 16	92.3	6.8	0.9
Cefuroxime	≤ 8	> 8	95.3	-	4.7
Cefotaxime	≤ 1	> 2	97.3	0.2	2.5
Ceftazidime	≤ 1	> 4	97.2	0.5	2.3
Meropenem	≤ 2	> 8	99.8	0.0	0.2
Gentamicin	≤ 2	> 4	97.7	0.5	1.8
Ciprofloxacin	≤ 0.5	> 1	95.9	1.4	2.7
Trimethoprim	≤ 2	> 4	80.8	1.1	18.1
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	84.9	1.3	13.8
ESBL	Negative	Positive	97.5	-	2.5

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 35. *Klebsiella oxytoca* urinary tract isolates in 2013 (n=104). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Mecillinam	≤ 8	> 8	95.2	-	4.8
Amoxicillin-clavulanic acid*	≤ 32	> 32	95.2	-	4.8
Piperacillin-tazobactam	≤ 8	> 16	94.2	1.9	3.8
Cefuroxime	≤ 8	> 8	95.2	-	4.8
Cefotaxime	≤ 1	> 2	98.1	1.9	0.0
Ceftazidime	≤ 1	> 4	100.0	0.0	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Ciprofloxacin	≤ 0.5	> 1	99.0	1.0	0.0
Trimethoprim	≤ 2	> 4	95.2	1.9	2.9
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	97.1	1.0	1.9
ESBL	Negative	Positive	100.0	-	0.0

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

The $ESBL_{CARBA}$ (carbapenemase) situation in Norway

The ongoing global dissemination of multidrug-resistance among common Gram-negative pathogens such as the Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* has been highlighted as a major concern in several recent reports (1-3). Of particular concern is the emergence and dissemination of $ESBL_{CARBA}$ beta-lactamases which are enzymes with the ability to inactivate carbapenems and in most cases all beta-lactam antibiotics (4). The genes encoding $ESBL_{CARBA}$ beta-lactamases disseminate through mobile genetic elements (e.g. plasmids) frequently harbouring genes causing resistance to other classes of antimicrobial agents. Consequently, isolates with $ESBL_{CARBA}$ beta-lactamases are multidrug-resistant and we are now observing isolates that are resistant to all clinically relevant antimicrobial agents. $ESBL_{CARBA}$ beta-lactamases can be subdivided into three groups $ESBL_{CARBA-A}$, $ESBL_{CARBA-B}$ (metallo-beta-lactamases) and $ESBL_{CARBA-D}$ (5). The dominating $ESBL_{CARBA}$ beta-lactamases includes KPC ($ESBL_{CARBA-A}$), NDM, VIM and IMP ($ESBL_{CARBA-B}$) and OXA-carbapenemases ($ESBL_{CARBA-D}$) which include the OXA-48-like, OXA-23-like, OXA-24/-40-like and OXA-58-like beta-lactamases (4).

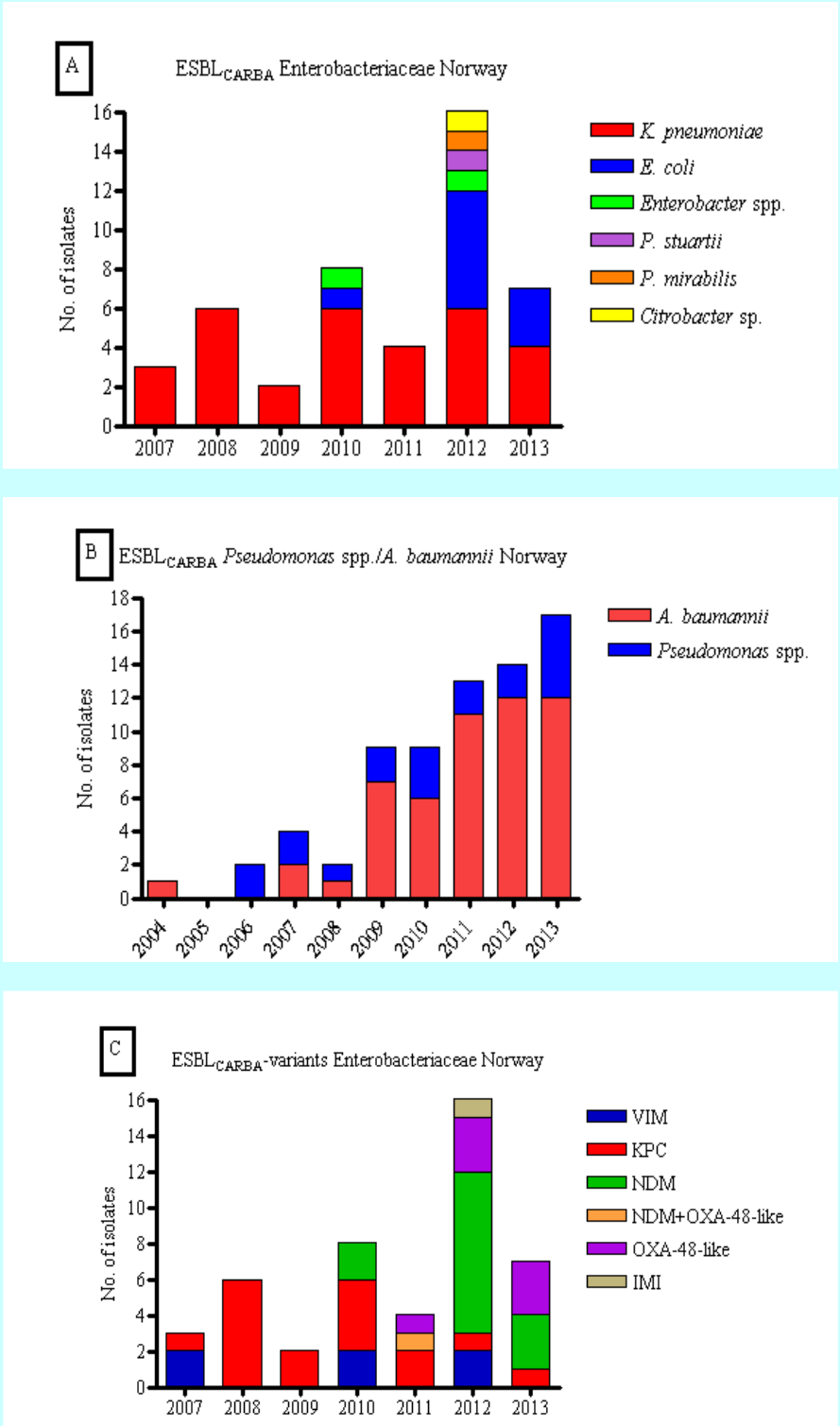


FIGURE 52. A and B: number of $ESBL_{CARBA}$-producing Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter. baumannii* isolates submitted to K-Res. C: Specific $ESBL_{CARBA}$-variants among $ESBL_{CARBA}$-producing Enterobacteriaceae.

Detection and identification of $ESBL_{CARBA}$ in the diagnostic clinical laboratory are challenging for several reasons. The contribution of $ESBL_{CARBA}$ beta-lactamases to carbapenem susceptibility varies between different enzymes and host species. $ESBL_{CARBA}$-producing Enterobacteriaceae isolates are frequently categorised as susceptible according to the clinical breakpoints and separate screening breakpoints need to be applied (6). Further, variability in the activity of $ESBL_{CARBA}$ beta-lactamases towards other beta-lactams and to beta-lactamase inhibitors complicates the detection. For example OXA-48-like enzymes have limited or no activity against 3rd generation cephalosporins and there are no available inhibitor for phenotypic detection (7). Recently, simple and rapid biochemical tests have been reported but they require further evaluation due to reports of false-negative results (8). Molecular detection is also challenging due to the great diversity of $ESBL_{CARBA}$ enzymes.

The prevalence of $ESBL_{CARBA}$ and subtypes varies significantly between geographical regions and countries (4,9). For example KPC enzymes are dominating in countries such as Israel, Italy, China and certain areas of the United States, while OXA-48-like enzymes are dominant in North African countries, Turkey as well as in many European countries. Among the $ESBL_{CARBA-B}$ enzymes NDM is dominating in the Indian subcontinent while VIM is dominating in Greece. In Norway, The Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-Res) has followed the emergence of $ESBL_{CARBA}$ since the first identification of an $ESBL_{CARBA}$-producing *A. baumannii* isolate in 2004. In 2012 $ESBL_{CARBA}$-producing isolates were included in the Norwegian Surveillance System for Communicable Diseases (MSIS). Currently the number of $ESBL_{CARBA}$-producing isolates in Norway is low (Figure 52) and the majority of cases is associated with travel or hospitalisation abroad. However, cases with no clear link to import are also observed. In 2013, a total of 24 $ESBL_{CARBA}$-producing isolates were identified (Figure 52A and 52B). The number of isolates does not reflect the number of cases as single patients have been found to be infected or colonised with $ESBL_{CARBA}$-producing isolates of different species. Among the $ESBL_{CARBA}$-producing Enterobacteriaceae the diversity of different $ESBL_{CARBA}$ enzymes and species has increased over the years reflecting the global situation (Figure 52A and 52C).

Although the number of $ESBL_{CARBA}$-producing isolates identified in Norway is currently low, the increasing dissemination on a global scale warrants a strong focus on detection of these isolates to implement targeted infection control measures to limit the spread. One small long-term outbreak has already occurred in Norway (10) and cases of intra-hospital and in-between hospitals spread have been observed. The association with hospitalisation and/or travel abroad may warrant active screening of patients to identify colonisation that could lead to further spread, especially in vulnerable settings (ie ICUs, burn units etc.). Active screening of patients to reduce the transmission of multidrug-resistant Gram-negatives in the endemic setting is debated, but is strongly recommended in the epidemic setting by the European Society of Clinical Microbiology and Infectious Diseases (11).

References:

1. World Health Organization (WHO). Antimicrobial resistance: global report on surveillance. 2014.
2. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013. 2013.
3. European Centre for Disease Prevention and Control. Carbapenemase-producing bacteria in Europe: interim results from the European Survey on carbapenemase-producing Enterobacteriaceae (EuSCAPE) project. 2013.
4. Patel, G. and R. A. Bonomo. "Stormy waters ahead": global emergence of carbapenemases. Front Microbiol. 2013;4:48.
5. Giske, C. G., A. S. Sundsfjord, G. Kahlmeter, *et al.* 2009. Redefining extended-spectrum β-lactamases: balancing science and clinical need. J.Antimicrob.Chemother. 2009;63:1-4.
6. Miriagou, V., G. Cornaglia, M. Edelstein, *et al.* Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin.Microbiol.Infect. 2010;16:112-122.
7. Poirel, L., A. Potron, and P. Nordmann. OXA-48-like carbapenemases: the phantom menace. J.Antimicrob.Chemother. 2012;67:1597-1606.
8. Dortet, L., L. Poirel, and P. Nordmann. Rapid identification of carbapenemase types in Enterobacteriaceae and *Pseudomonas* spp. by using a biochemical test. Antimicrob.Agents Chemother. 2012;56:6437-6440.
9. Glasner, C., B. Albiger, G. Buist, A. A. *et al.* Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. Euro.Surveill 2013;18:pii=20525.
10. Tofteland, S., U. Naseer, J. H. Lislevand, *et al.* A long-term low-frequency hospital outbreak of KPC-producing *Klebsiella pneumoniae* involving intergenus plasmid diffusion and a persisting environmental reservoir. PLoS.One. 2013;**8**:e59015.
11. Tacconelli, E., M. A. Cataldo, S. J. Dancer, *et al.* ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. Clin.Microbiol.Infect. 2014;20 Suppl 1:1-55.

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Prevalence of $ESBL_{M-C}$ (plasmid-mediated AmpC) among *Escherichia coli* and *Klebsiella pneumoniae* isolates from NORM 2010-2012

AmpC beta-lactamases can be divided into two clinically relevant groups, the intrinsic chromosomal AmpC beta-lactamases (cAmpCs) and plasmid-mediated AmpCs here designated $ESBL_{M-C}$ (1,2). cAmpCs are present in many Gram-negative bacteria including a majority of Enterobacteriaceae such as *Citrobacter freundii, Enterobacter cloacae, Escherichia coli, Hafnia alvei, Morganella morganii, Providencia stuartii, Serratia marcescens, Shigella boydii, Shigella flexneri, Yersinia enterocolitica* as well as *Acinetobacter baumannii,* and *Pseudomonas aeruginosa* (1). In contrast, some species within Enterobacteriaceae including *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, and *Salmonella* spp. do not possess an intrinsic AmpC beta-lactamase. In many Enterobacteriaceae the expression of cAmpC genes is low, but can be induced by beta-lactams (1). Further, mutations in regulatory genes or in the promoter region as well as insertions of genetic elements in the promoter region can cause cAmpC overexpression (3).

The $ESBL_{M-C}$ beta-lactamases are cAmpCs that have been captured from their chromosomal origin through the association with mobile genetic elements (e.g. transposons and plasmids) and can consequently disseminate between bacteria (1). On the basis of amino acid sequence the $ESBL_{M-Cs}$ can be divided into different groups (CMY, FOX, ACC, LAT, MIR, ACT, MOX, BIL and DHA). Within some of these groups several variants have been described. CMY-2 is the most prevalent $ESBL_{M-C}$ variant both on a global scale and in Norway (1,4). In general, $ESBL_{M-C}$ genes are constitutively expressed, but the expression of some $ESBL_{M-Cs}$, DHA in particular, has been shown to be inducible (1).

AmpC beta-lactamases are characterised by their activity towards penicillins, 1^{st}-3^{rd} generation cephalosporins including cephamycins (e.g. cefoxitin) while the activity towards 4^{th} generation cephalosporins, monobactams and carbapenems is limited (1). In addition they are inhibited by cloxacillin and boronic acid which is used for phenotypic detection. Currently only molecular methods are able to distinguish between cAmpC and $ESBL_{M-C}$.

To analyse the prevalence of $ESBL_{M-C}$ among *E. coli* and *K. pneumoniae* in Norway, isolates from urine and blood culture in NORM 2010-2012 with reduced susceptibility (intermediate susceptible or resistant) to ceftazidime and/or cefotaxime and classified as $ESBL_A$-negative in NORM were collected and analysed at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-Res). In total, 45 *E. coli* and 38 *K. pneumoniae* isolates were collected. In addition isolates identified with $ESBL_{M-C}$ from the yearly analysis of putative $ESBL_A$ isolates in NORM were included in the calculations of prevalence. *E. coli* isolates with a phenotypic AmpC profile but negative for $ESBL_{M-C}$ on molecular analysis were interpreted as cAmpC.

The results show that the prevalence of $ESBL_{M-C}$ among *E. coli* isolates is currently low ranging from 0-0.4% depending on clinical specimen type and year (Table 36). No trend towards an increase could be observed during the time period. It should be noted that the results must be interpreted with caution as the number of isolates is low and that the yearly analysis of $ESBL_{M-C}$ among putative $ESBL_A$ isolates was not carried out on urine isolates in 2012. Further, cefoxitin which is used as a marker for AmpC-production is not included in NORM. Consequently, the total prevalence could be underestimated. Among $ESBL_{M-C}$ positive isolates, CMY-2 was dominating followed by DHA. The prevalence of cAmpC was also low and varied from 0-0.6% depending on the year and specimen type. A previous analysis of isolates from NORM in 2004 which then included reduced susceptibility to cefoxitin showed a higher prevalence of cAmpC (1.0%)(5). In 2004 no $ESBL_{M-C}$-positive isolates were identified. It has previously been shown that isolates with $ESBL_{M-C}$ often show more co-resistance than isolates with cAmpC (6). This was also observed in this collection where 75% of the $ESBL_{M-C}$-positive isolates were resistant to ≥2 other antimicrobial classes as compared to only 12% among *E. coli* overexpressing cAmpC.

TABLE 36. Prevalence of $ESBL_{M-C}$ and cAmpC overexpression among *E. coli* isolates in NORM 2010-2012.

Species/clinical specimen	$ESBL_{M-C}$	cAmpC
E. coli urine 2010 (n=1,093)	0.4%	0.3%
E. coli urine 2011 (n=940)	0.1%	0.0%
E. coli urine 2012 (n=955)	0.0%	0.2%
E. coli blood 2010 (n=1,359)	0.1%	0.4%
E. coli blood 2011 (n=1,438)	0.1%	0.6%
E. coli blood 2012 (n=1,646)	0.2%	0.4%

Among the *K. pneumoniae* isolates no $ESBL_{M-C}$ genes were identified indicating a very low prevalence.

In conclusion the prevalence of $ESBL_{M-C}$ among *E. coli* and *K. pneumoniae* isolates in Norway is currently low, but the prevalence of $ESBL_{M-C}$ in *E. coli* may have increased since 2004. As observed globally CMY-2 is the dominating $ESBL_{M-C}$ in Norway and *E. coli* isolates with $ESBL_{M-C}$ are frequently more often resistant to other non-beta-lactam antibiotics. Finally it should be noted that some of the isolates analysed in this collection were $ESBL_A$-positive although they were reported as $ESBL_A$-negative in NORM, and that for a significant number of isolates the reduced susceptibility to ceftazidime and/or cefotaxime could not be reproduced.

The results have also been presented in a separate report from K-Res (http://www.unn.no/rapporter-fra-k-res/category35882.html).

References:

1. Jacoby, G. A. AmpC β-lactamases. Clin.Microbiol.Rev. 2009;22:161-82.
2. Giske, C. G., A. S. Sundsfjord, G. Kahlmeter, *et al.* Redefining extended-spectrum β-lactamases: balancing science and clinical need. J.Antimicrob.Chemother. 2009;63:1-4.
3. Haldorsen, B., B. Aasnaes, K. H. Dahl, *et al.* The AmpC phenotype in Norwegian clinical isolates of *Escherichia coli* is associated with an acquired IS*Ecp1*-like *ampC* element or hyperproduction of the endogenous AmpC. J.Antimicrob.Chemother. 2008;62:694-702.
4. Naseer, U., B. Haldorsen, G. S. Simonsen, *et al.* Sporadic occurrence of CMY-2-producing multidrug-resistant *Escherichia coli* of ST-complexes 38 and 448, and ST131 in Norway. Clin.Microbiol.Infect. 2010;16:171-178.
5. Tofteland, S., K. H. Dahl, B. Aasnaes, *et al.* A nationwide study of mechanisms conferring reduced susceptibility to extended-spectrum cephalosporins in clinical *Escherichia coli* and *Klebsiella* spp. isolates. Scand.J.Infect.Dis. 2012;44:927-933.
6. Edquist, P., M. Ringman, B. O. Liljequist, *et al.*Phenotypic detection of plasmid-acquired AmpC in *Escherichia coli* - evaluation of screening criteria and performance of two commercial methods for the phenotypic confirmation of AmpC production. Eur.J.Clin.Microbiol.Infect.Dis. 2013;32:1205-10.

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Haemophilus influenzae in blood cultures and cerebrospinal fluids

TABLE 37. *Haemophilus influenzae* in blood cultures and cerebrospinal fluids in 2013 (n=79). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 1	> 1	82.3	-	17.7
Amoxicillin-clavulanic acid	≤ 2	> 2	94.9	-	5.1
Cefuroxime	≤ 1	> 2	48.1	32.9	19.0
Cefotaxime	≤ 0.12	> 0.12	98.7	-	1.3
Ceftriaxone	≤ 0.12	> 0.12	100.0	-	0.0
Ciprofloxacin	≤ 0.5	> 0.5	98.7	-	1.3
Chloramphenicol	≤ 2	> 2	98.7	-	1.3
Tetracycline	≤ 1	> 2	100.0	0.0	0.0
Trimethoprim-sulfamethoxazole*	≤ 0.5	> 1	83.6	2.5	13.9
Penicillin G (mm)	≥ 12	< 12	70.9	-	29.1
Cefaclor (mm)	≥ 23	< 23	73.4	-	26.6
Nalidixic acid (mm)	≥ 23	< 23	100.0	-	0.0
Beta-lactamase	Negative	Positive	84.8	-	15.2

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 38. *Haemophilus influenzae* in blood cultures and cerebrospinal fluids in 2013 (n=79). Distribution (%) of MICs (mg/L) and zone diameters for penicillin G, cefaclor and nalidixic acid (mm).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Ampicillin						5.1	36.7	32.9	7.6	2.5	1.3			2.5		11.4
Amoxi-clav**						1.3	3.8	45.6	29.1	15.2	2.5	1.3		1.3		
Cefuroxime							1.3	2.5	44.3	32.9	3.8	7.6	2.5	1.3	1.3	2.5
Cefotaxime		1.3	15.2	39.2	31.6	11.4	1.3									
Ceftriaxone	6.3	35.4	45.6	7.6	5.1											
Ciprofloxacin	2.5	12.7	59.5	24.1					1.3							
Chloramph.			1.3					3.8	84.8	8.9			1.3			
Tetracycline					1.3		11.4	86.1	1.3							
TMS***			5.1	36.7	27.8	8.9	3.8	1.3	2.5		1.3			12.7		

	< 11	11	12	13	14	15	16	17	18	19	20	21	22	23	24	≥ 25
Penicillin G	29.1		1.3	1.3	2.5	20.3	8.9	12.7	5.1	5.1	5.1	2.5	3.8	1.3		1.3
Cefaclor	7.6		1.3	2.5	1.3		2.5	2.5					8.9	12.7	7.6	53.2
Nalidixic acid																100.0

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **Amoxi-clav=Amoxicillin-clavulanic acid. ***TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Systemic isolates of *H. influenzae* have not previously been included in the NORM programme, but the Reference Laboratory at the Norwegian Institute of Public Health will provide resistance data for this species on a yearly basis from 2013. Isolates from respiratory tract specimens were surveyed by NORM in 2001, 2004, 2007 and 2011, and the test panels for isolates originating from systemic and localised infections are the same to facilitate comparison. The present results are interpreted according to EUCAST guidelines which have remained unchanged since NORM 2011.

A total of 79 isolates were recovered from blood cultures (n=74) or cerebrospinal fluids (n=5), all representing unique patients (Tables 37-38). Beta-lactamase production was detected in 15.2% of the isolates which is slightly higher than the 12.3% seen in respiratory tract isolates in 2011. The gradual increase of beta-lactamase producers thus appears to continue. A total of 14/79 isolates were resistant to ampicillin, and beta-lactamase production was present in 12 of them. Three of these isolates were concomitantly resistant to amoxicillin-clavulanic acid and cefuroxime, thus indicating a combination of beta-lactamase production and chromosomal resistance mechanisms. The two beta-lactamase negative, ampicillin resistant strains were both cefuroxime resistant suggesting a chromosomal basis for beta-lactam resistance. Only one of them was resistant to amoxicillin-clavulanic acid.

Cefuroxime MIC > 2 mg/L has been suggested as the most precise indicator for alterations in the wild type PBP3 sequence. A total of 15 isolates (19%) displayed this phenotype compared to 17% in respiratory tract isolates in 2011, but a majority of these isolates were susceptible to ampicillin (10/15) and amoxicillin-clavulanic acid (12/15). A single blood culture isolate was resistant to cefotaxime with an MIC of 0.25 mg/L. This isolate was beta-lactamase negative and ampicillin susceptible, but resistant to cefuroxime. The ceftriaxone MIC was 0.064 mg/L and thus well within the susceptible range.

Different substrates have been suggested for screening of beta-lactam resistance in *H. influenzae*. The penicillin G 1 IU disk (PCG1) successfully identified all ampicillin and amoxicillin-clavulanic acid resistant isolates, but missed two cefuroxime resistant strains. Eleven out of 67 beta-lactamase negative isolates were resistant to PCG1; two of these were resistant to ampicillin and all were non-susceptible to cefuroxime. The breakpoints for the cefaclor disk test are calibrated for beta-lactamase positive isolates; cefaclor correctly identified all three cefuroxime resistant isolates in addition to three isolates where cefuroxime resistance was not verified. The results illustrate the continuing challenges in defining the optimal algorithm for beta-lactam susceptibility testing in *H. influenzae*.

As previously seen in respiratory tract isolates, resistance to ciprofloxacin (1.3%), chloramphenicol (1.3%) and tetracycline (0.0%) was at a very low level. The unexpected occurrence of ciprofloxacin resistance (MIC 1 mg/L) in a nalidixic acid susceptible isolate was not further investigated. The 13.9% resistance to trimethoprim-sulfamethoxazole is lower than 22.0% seen in respiratory tract isolates in 2011, but as the difference is mainly due to low-level resistance just above the breakpoint in 2011 one may speculate technical difficulties with the test methodology.

Neisseria meningitidis in blood cultures and cerebrospinal fluids

TABLE 39. *Neisseria meningitidis* in blood cultures and cerebrospinal fluids in 2013 (n=23). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.064	> 0.25	43.5	56.5	0.0
Ceftriaxone	≤ 0.125	> 0.125	100.0	-	0.0
Ciprofloxacin	≤ 0.032	> 0.064	100.0	0.0	0.0
Chloramphenicol	≤ 2	> 4	100.0	0.0	0.0
Rifampicin	≤ 0.25	> 0.25	100.0	0.0	0.0

TABLE 40. *Neisseria meningitidis* in blood cultures and cerebrospinal fluids in 2013 (n=23). Distribution (n) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G**			1	4	5	6	7									
Ceftriaxone	22	1														
Ciprofloxacin	17	6														
Chloramph.								6	17							
Rifampicin	12	7	4													
Azithromycin					1	1	1	10	9	1						
Tetracycline					1	6	9	1	6							
Sulfonamide							1		5	1	1				1	14

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **Pen G=Benzylpenicillin.

RESULTS AND COMMENTS

N. meningitidis from blood cultures and cerebrospinal fluids have not previously been included in NORM, but as for systemic *H. influenzae* isolates the Reference Laboratory at the Norwegian Institute of Public Health will provide data on *N. meningitidis* on a yearly basis from 2013. The results are presented in Tables 39-40.

A total of 23 isolates were recovered from cerebrospinal fluids (n=9) and blood cultures (n=14). All isolates were from separate patients. The isolates belonged to serogroups B (n=9), C (n=6), Y (n=7) and E (n=1). Thirteen isolates displayed penicillin G MIC of 0.125 or 0.25 mg/L and were thus intermediately susceptible. The genetic basis for non-susceptibility was not determined, but was most likely caused by alterations in the penicillin-binding protein 2 (PBP2) encoded by *penA*. Of note, the majority of serogroup B isolates (7/9) were susceptible to penicillin G whereas all serogroup Y isolates (7/7) were intermediately susceptible.

Sulfonamide resistance has been widespread in *N. meningitidis* since the 1960s. EUCAST has not defined clinical breakpoints for this agent, but the MIC distributions clearly demonstrate a high prevalence of acquired resistance among Norwegian isolates. All isolates were fully susceptible to ceftriaxone, ciprofloxacin, chloramphenicol and rifampicin.

Neisseria gonorrhoeae

TABLE 41. *Neisseria gonorrhoeae* from all specimen types in 2013 (n=225). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.064	> 1	1.8	50.2	48.0
Ceftriaxone	≤ 0.125	> 0.125	98.2	-	1.8
Cefixime	≤ 0.125	> 0.125	89.3	-	10.7
Azithromycin	≤ 0.25	> 0.5	41.4	37.3	21.3
Ciprofloxacin	≤ 0.032	> 0.064	26.2	0.0	73.8
Tetracycline	≤ 0.5	> 1	9.8	20.4	69.8
Spectinomycin	≤ 64	> 64	100.0	-	0.0
Beta-lactamase	Negative	Positive	67.6	-	32.4

TABLE 42. *Neisseria gonorrhoeae* from all specimen types in 2013 (n=225). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G**		0.4		1.3	0.4	3.1	18.2	13.3	15.1	17.8	5.8	4.0	3.6	16.9		
Ceftriaxone	3.1	17.8	23.6	16.0	24.0	13.8	1.8									
Cefixime			33.8	18.2	21.3	16.0	8.9	1.8								
Azithromycin				1.3	4.9	13.3	21.8	37.3	18.7	0.9	0.4	0.9	0.4			
Ciprofloxacin	6.2	13.3	4.4	2.2		0.4			1.8	4.0	6.7	8.0	6.2	46.7		
Tetracycline						0.4	3.6	5.8	20.4	24.4	6.2	1.3	2.7	18.2	7.1	9.8
Spectinomycin												14.7	80.0	5.3		

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. **Pen G=Benzylpenicillin.

RESULTS AND COMMENTS

Neisseria gonorrhoeae was previously surveyed in 2003 and 2010. Oslo University Hospital will provide resistance data for Norwegian *N. gonorrhoeae* isolates on a yearly basis beginning in 2013. All isolates from all specimen types were included in the survey, but only a single isolate was accepted from each patient. The microbiological data could not be linked to information from the Norwegian Surveillance System for Communicable Diseases (MSIS).

A total of 225 isolates were available for analysis. The isolates were reported to originate from urethra (n=145), cervix uteri (n=22), anus (n=21), throat (n=6), eye (n=2) or "uknown/others" (n=29). A total of 187 isolates (83.1%) originated from men, whereas only 38 (16.9%) were recovered from women. The geographical location where the infection was acquired was in most cases unknown to the laboratory. From MSIS it is known that gonococcal infections frequently are acquired abroad with secondary transmission in sexual networks within Norway. There is an ongoing outbreak among men who have sex with men, but the strains linked to this outbreak could not be identified within the NORM protocol.

The results from susceptibility testing are presented in Tables 41-42. As in 2010, the majority of isolates were intermediately susceptible (55.0% in 2010, 50.2% in 2012) or resistant (42.7% in 2010, 48.0% in 2012) to penicillin G according to EUCAST breakpoints. Seventy-three isolates (32.4%) produced beta-lactamase and were phenotypically resistant (n=70) or intermediately susceptible (n=3) to penicillin G. This is a slight increase from 2010 (28.4%). Most beta-lactamase positive isolates (68/73, 93.2%) were also non-susceptible to ciprofloxacin. In addition, 38 isolates were resistant and 110 were intermediately susceptible to penicillin G in spite of being beta-lactamase negative. This may be caused by alterations in penicillin binding proteins (PBPs) and/or reduced permeability through the outer cell membrane.

The survey included the two cephalosporins ceftriaxone and cefixime. Four isolates (1.8%) were categorised as resistant to ceftriaxone and displayed an MIC of 0.25 mg/L. Ceftriaxone resistant isolates from Norway have previously been linked to treatment failure. Twenty-four (10.7%) isolates were resistant to the oral cephalosporin cefixim which is no longer recommended for empirical treatment in Norway. The results confirm the emergence of cephalosporin non-susceptible gonococci in Norway, which is of course extremely alarming from both a clinical and a public health perspective. The current treatment guidelines consist of a combination of ceftriaxone and azithromycin. It should be noted that 58.6% of the isolates were categorised as non-susceptibilible to azithromycin including all four ceftriaxone non-susceptible isolate which had MICs for azithromycin of 0.5 (n=1) or 1 mg/L (n=3).

Ciprofloxacin was previously used for empirical treatment of gonorrhoeae acquired outside South-East Asia. The 61.1% prevalence of ciprofloxacin resistance seen in 2010 had in 2013 increased to 73.8%. Ciprofloxacin is consequently not a viable alternative except in cases where susceptibility has been documented. All strains were susceptible to the aminoglycoside spectinomycin.

Staphylococcus aureus in blood cultures

TABLE 43. *Staphylococcus aureus* blood culture isolates in 2013 (n=1,155). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	95.6	0.2	3.9
Clindamycin	≤ 0.25	> 0.5	97.9	0.6	1.5
Fusidic acid	≤ 1	> 1	95.2	-	4.8
Ciprofloxacin	≤ 1	> 1	97.1	-	2.9
Gentamicin	≤ 1	> 1	99.7	-	0.3
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	98.8	1.0	0.3
Tetracycline	≤ 1	> 2	95.9	0.6	3.5
Tigecycline	≤ 0.5	> 0.5	99.3	0.0	0.7
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.2	0.3	0.5
Beta-lactamase	Negative	Positive	27.9	-	72.1
Cefoxitin screen	Negative	Positive	99.7	-	0.3
MRSA (*mecA*)	Negative	Positive	99.7	-	0.3

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Four methicillin resistant *S. aureus* (MRSA) isolates were detected in the NORM surveillance system in 2013 (Table 43) corresponding to a prevalence of 0.3%. This is at the same level as in 2008 (0.7%), 2009 (0.4%), 2010 (1.0%), 2011 (0.5%) and 2012 (1.0%). The resistance phenotype was confirmed by *mecA* PCR in all cases. The isolates originated from three different hospitals.

Laboratory screening for MRSA in NORM is performed using cefoxitin disks. All MRSA isolates had cefoxitin zone diameters below the screening breakpoint. Only a single MRSA isolate was concomitantly resistant to erythromycin and tetracycline. All MRSA isolates were fully susceptible to clindamycin, ciprofloxacin, gentamicin, linezolid, trimethoprim-sulfamethoxazole, rifampicin and fusidic acid. The results from susceptibility testing of all Norwegian MRSA isolates are presented on page 76. No methicillin susceptible *S. aureus* (MSSA) isolates were reported to have cefoxitin zone diameters below the screening breakpoint.

The findings are in accordance with reports from the databases of the participating laboratories where 16 out of 1,633 (1.0%) *S. aureus* blood culture isolates were MRSA. None of the 18 *S. aureus* isolates recovered from cerebrospinal fluids were methicillin resistant, thus bringing the total number of systemic MRSA isolates to 16/1,651 (1.0%).

A total of 47 *S. aureus* isolates (4.1%) were non-susceptible to erythromycin. This is at the same level as 4.3% in 2011 and 3.7% in 2012. The macrolide resistance phenotypes of 41 of these isolates were determined by the double disk diffusion (DDD) test. Seven isolates (17%) was constitutively MLS_B resistant, 25 (61%) were inducibly MLS_B resistant and nine (22%) displayed efflux mediated M type resistance. These figures represent 0.7%, 2.6% and 0.9% of all *S. aureus* isolates from blood cultures, respectively. The proportion of constitutively MLS_B resistant isolates has apparently increased.

The prevalence of resistance to fusidic acid of 4.8% was at the same level as 5.9% in 2012 and 4.2% in 2011. The 2.9% prevalence of ciprofloxacin resistance was also at approximately the same level as 2.0% in 2011 and 2.5% in 2012. There were no significant changes for gentamicin, rifampicin or trimethoprim-sulfamethoxazole. All isolates were fully susceptible to linezolid. Eight isolates (0.7%) were phenotypically resistant to tigecycline, but seven of these had zone diameters just below the breakpoint. Vancomycin was not included in the susceptibility test panel in 2013.

Figure 53 shows the prevalence of non-susceptibility to various antimicrobials. A total of 72.1% of the isolates were beta-lactamase positive which is unchanged from previous years. A subgroup analysis revealed only minor differences in resistance rates to other antimicrobials between beta-lactamase positive and beta-lactamase negative isolates.

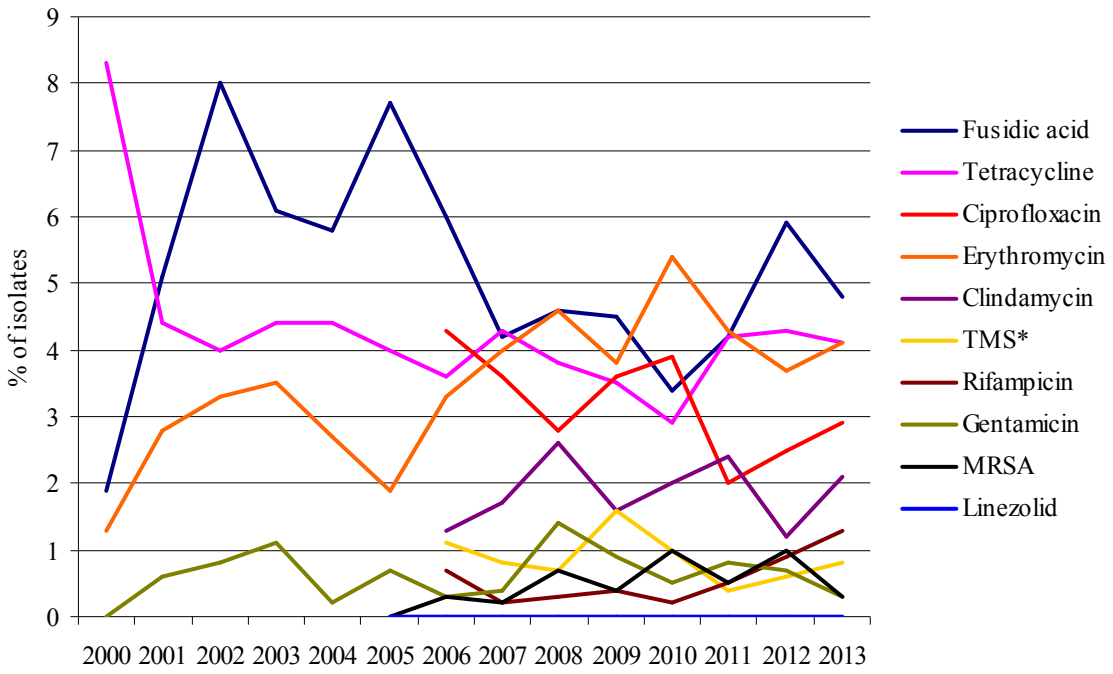

FIGURE 53. Prevalences of non-susceptibility to various antimicrobial agents among *Staphylococcus aureus* blood culture isolates 2000-2013. Doxycycline was replaced by tetracycline in 2006. *TMS=Trimethoprim-sulfamethoxazole.

Staphylococcus aureus in wound specimens

TABLE 44. *Staphylococcus aureus* isolates from wound specimens in 2013 (n=867). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	93.9	0.0	6.1
Clindamycin	≤ 0.25	> 0.5	98.0	0.6	1.4
Fusidic acid	≤ 1	> 1	91.0	-	9.0
Ciprofloxacin	≤ 1	> 1	97.3	-	2.7
Gentamicin	≤ 1	> 1	99.7	-	0.3
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	99.4	0.3	0.3
Tetracycline	≤ 1	> 2	95.1	1.0	3.9
Tigecycline	≤ 0.5	> 0.5	99.8	0.0	0.2
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.5	0.2	0.3
Beta-lactamase	Negative	Positive	23.8	-	76.2
Cefoxitin screen	Negative	Positive	98.8	-	1.2
MRSA (*mecA*)	Negative	Positive	98.8	-	1.2

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

S. aureus from wound specimens were screened for methicillin resistance by the cefoxitin disk method in the same way as blood culture isolates. Ten out of 867 (1.2%) isolates were confirmed as MRSA by *mecA* PCR. The prevalence was at the same level as in 2011 (1.3%) and 2012 (0.7%), and also as in blood cultures (0.3%, see above). The MRSA isolates originated from patients admitted to a hospital ward (n=1), a nursing home (n=1), general practitioners (n=6) and unknown locations (n=2) in different parts of the country. All MRSA isolates were fully susceptible to clindamycin, fusidic acid, gentamicin, rifampicin, trimethoprim-sulfamethoxazole, tigecycline and linezolid. Three isolates displayed resistance ciprofloxacin, and two of these were also resistant to erythromycin. Two isolates were monoresistant to erythromycin or tetracycline. No isolates were reported with zone diameters below the cefoxitin screening breakpoint without being confirmed as MRSA by *mecA* PCR. This indicates a high specificity of the cefoxitin screen as well as a low prevalence of *mecC* MRSA (see page 76).

The high prevalence of resistance to fusidic acid in *S. aureus* wound isolates remained essentially unchanged at 9.0% in 2013 compared to 9.9% in 2011 and 9.5% in 2012 (Table 44 and Figure 54). This confirms that the gradually declining prevalence of fusidic acid resistance has now levelled off after the epidemic which peaked at 25.0% in 2004. The prevalence of resistance to fusidic acid is still significantly lower in blood culture isolates (4.8%).

For other antimicrobial agents such as trimethoprim-sulfamethoxazole, gentamicin, rifampicin, and tetracycline there were only minor changes from 2012 to 2013, and the prevalence of non-susceptibility was in general similar for blood culture isolates and isolates from wound specimens. All isolates were susceptible to linezolid.

A total of 53 (6.1%) isolates were non-susceptible to erythromycin which is at the same level as 5.9% in 2012. Fifty-two of these isolates were further examined for determination of resistance phenotype. The majority (33/52, 63% of macrolide resistant isolates) were inducibly resistant to clindamycin, thus representing the $iMLS_B$ phenotype. Minor proportions were either constitutively resistant to clindamycin (n=8) or low-level resistant to erythromycin (n=11), expressing efflux mediated M type resistance. The findings are in accordance with the results from previous years.

A total of 76.2% of the isolates were beta-lactamase positive compared to 76.7% in 2012. Resistance to fusidic acid was more common among the 661 beta-lactamase positive isolates (9.5%) than among the 206 beta-lactamase negative ones (7.3%). A similar trend was seen for erythromycin (6.4% vs 5.3%), tetracycline (5.6% vs 2.9%), ciprofloxacin (3.2% vs 1.0%) and clindamycin (2.1% vs 1.5%). There were no significant differences for other antimicrobial agents.

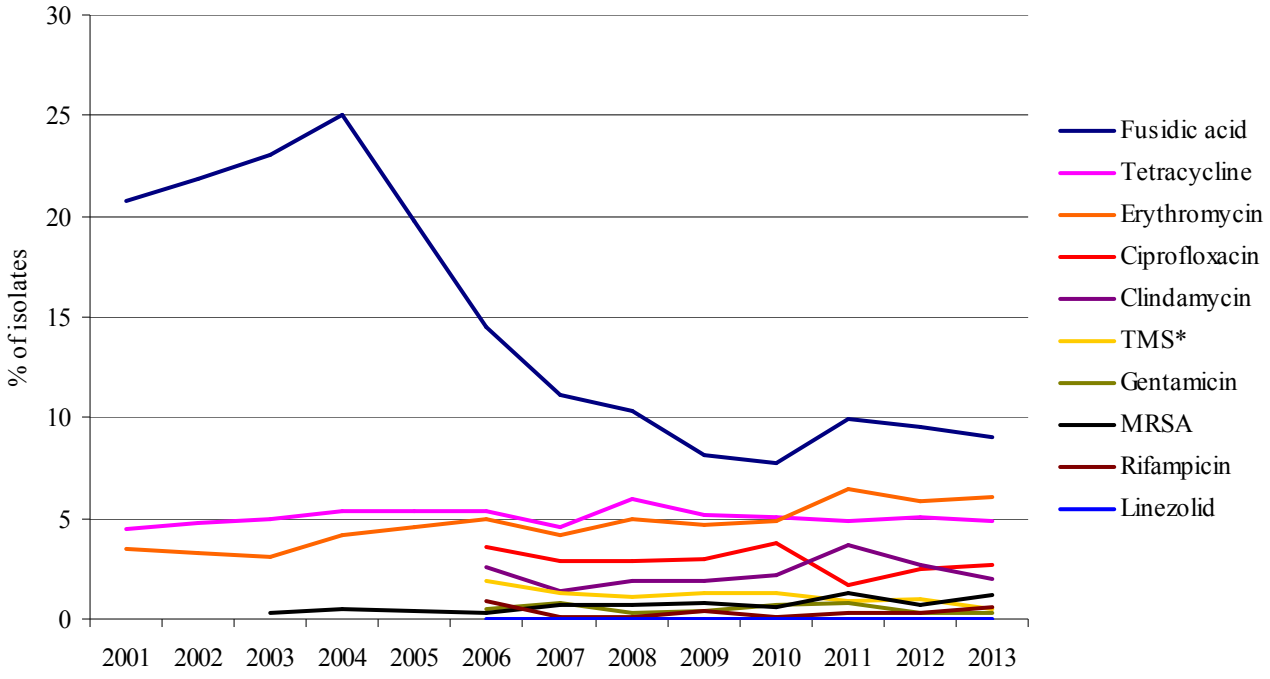

FIGURE 54. Prevalence of non-susceptibility to various antimicrobial agents among *Staphylococcus aureus* wound isolates 2001-2013. Doxycycline was replaced by tetracycline in 2006. *TMS=Trimethoprim-sulfamethoxazole.

Methicillin resistant *Staphylococcus aureus* (MRSA) infections in Norway 2013

Infections with methicillin resistant *Staphylococcus aureus* (MRSA) have been notifiable in Norway since 1995 and colonisation from 2005. A total of 1,482 cases of MRSA were reported in 2013 (29 per 100,000 person-years). Among these, 659 (44%) had an infection and 823 were colonised (Figure 55).

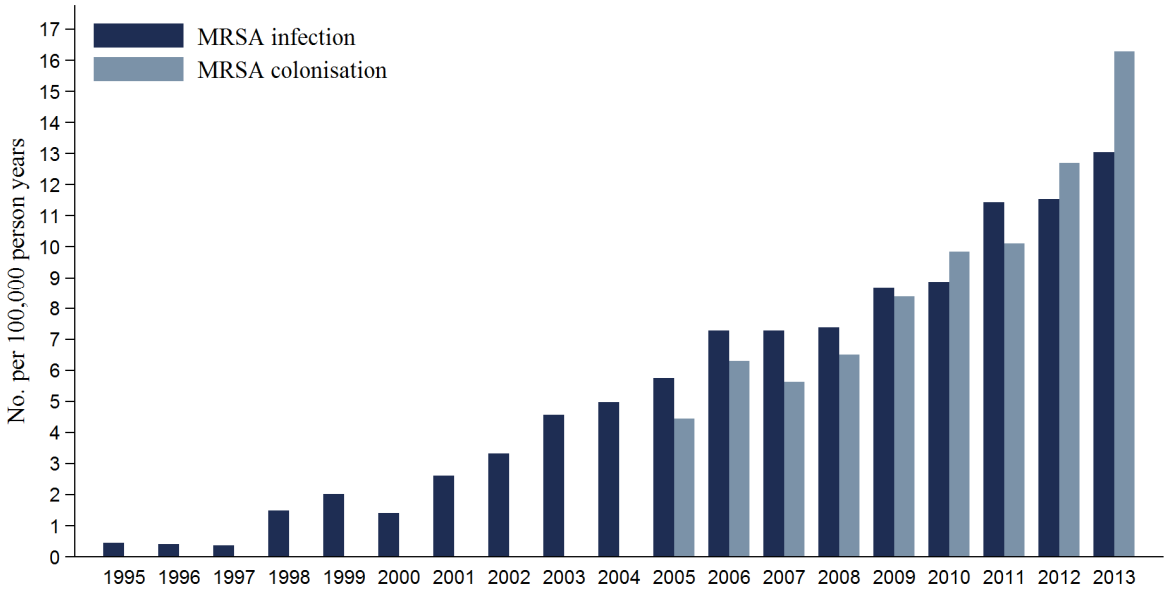

FIGURE 55. Number of MRSA cases per 100,000 person-years in Norway 1995-2013, by infection and colonisation.

The notification rate of MRSA increased by 21% from 2012 to 2013, compared with a mean increase per year of 18% in the former five years. The increase last year is mainly seen among notified cases of MRSA colonisation.

The main objective of the Norwegian MRSA infection control measures is to prevent MRSA from becoming endemic in health care institutions. Among persons notified with MRSA in 2013, 292 (20%) were inpatients at the time of diagnosis, while 79 (5%) were residents in nursing homes and 1099 (75%) were diagnosed in the community. Sixty of the reported MRSA cases in 2013 were health-care workers. The increase in notified MRSA cases is mainly attributed to persons infected abroad or in the community, while there has been no increase in cases associated with Norwegian health-care institutions in the last eight years (Figure 56).

An outbreak with livestock associated MRSA (LA-MRSA) in Norwegian swine herds was identified in 2013. The investigation discovered colonised pigs in 19 farms and one abattoir. The contact tracing resulted in 30 persons diagnosed with the same MRSA strain as the animals (*spa*-type t034 or t12359). In total 46 cases of LA-MRSA were notified to MSIS in 2013.

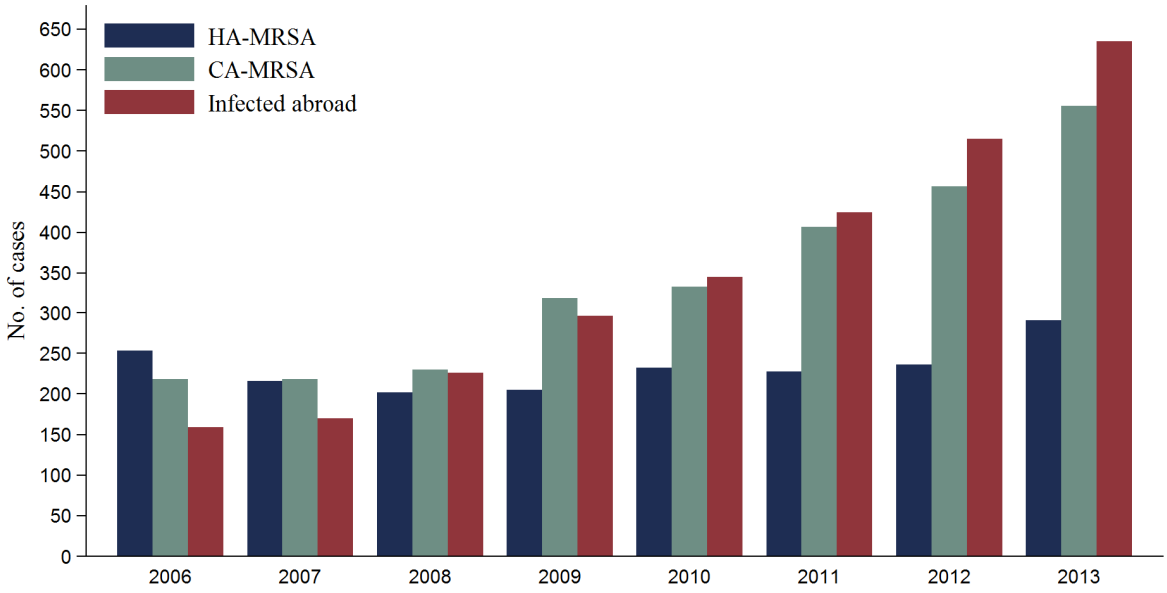

FIGURE 56. Reported cases of MRSA infections and colonisations in Norway 2006-2013, by health-care associated, community associated and imported cases.

The Reference Laboratory for MRSA, St. Olav University Hospital, Trondheim, received 1,539 MRSA isolates from different patients in 2013. A total of 238 different spa-types were identified and the six most frequent were (spa-type, n (%)): t002, n=156 (10.1%), t019, n=156 (10.1%), t008, n=96 (6.2%), t223, n=76 (4.9%), t127, n=63 (4.1%) and t437, n=45 (2.9%). A total of 116 spa-types were reported as single events. Based on spa-type, all isolates were allocated to MLST clonal complex. A total of 1,136 isolates (73.8%) occurred in the six most prevalent clusters (CC, n (%)): CC5, n=284 (18.5%), CC30, n=246 (16.0%), CC8, n=210 (13.7%), CC22, n=189 (12.3%), CC1, n=116 (7.5%), and CC88, n=91 (5.9%). The Reference Laboratory found 46 LA-MRSA (CC398) in humans (33 spa-type t034, 11 t011 and 2 t12359) and 1 isolate positive for *mecC* (spa-type t1535).

Susceptibility testing was performed on 1,528 MRSA isolates collected in 2013 with the EUCAST 2013 disk diffusion method and analysed with breakpoints from NWGA 2013 (Table 45). 370 strains (24.2%) were sensitive to all antibiotics tested except beta-lactams. The highest proportions of resistance were found for erythromycin (27.3%) followed by tetracycline (24.6%) and norfloxacin (23.3%). In total, 18.9% of the strains were resistant to clindamycin, of which 52.7% were inducibly resistant. The lowest rates of resistance were found towards mupirocin (0.5%) and rifampicin (1.2%). No strains were resistant to linezolid.

TABLE 45. Susceptibility characterisation of methicillin resistant *Staphylococcus aureus* (MRSA) from 2013 (n=1,528). Standard agar diffusion method ad modum EUCAST 2013. Breakpoints from NordicAST 2013.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	72.6	0.3	27.1
Clindamycin	≤ 0.25	> 0.5	78.7	2.4	18.9
Fusidic acid	≤ 1	> 1	87.7	-	12.3
Norfloxacin	≤ 4	> 4	76.7	-	23.3
Gentamicin	≤ 1	> 1	89.0	-	11.0
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	98.1	0.7	1.2
Tetracycline	≤ 1	> 2	74.9	0.5	24.6
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	95.1	2.7	2.2
Mupirocin	≤ 1	> 256	78.1	21.4	0.5
Ceftaroline	≤ 1	> 1	82.2	-	17.8

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

Enterococcus spp. in blood cultures

TABLE 46. *Enterococcus* spp. blood culture isolates in 2013 (n=622). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 4	> 8	74.4	0.5	25.1
Imipenem	≤ 4	> 8	72.8	2.4	24.8
Gentamicin*	≤ 128	> 128	-	70.9	29.1
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	99.7	0.0	0.3
Vancomycin (any genotype)	≤ 4	> 4	96.3	-	3.7
Vancomycin (Van A or VanB)	Negative	Positive	99.4	-	0.6

*The wild type is defined as intermediately susceptible.

TABLE 47. *Enterococcus faecalis* blood culture isolates in 2013 (n=413). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 4	> 8	100.0	0.0	0.0
Imipenem	≤ 4	> 8	98.3	1.5	0.2
Gentamicin*	≤ 128	> 128	-	76.4	23.6
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	99.8	0.0	0.2
Vancomycin (VanA or VanB)	Negative	Positive	98.8	-	0.2

*The wild type is defined as intermediately susceptible.

TABLE 48. *Enterococcus faecium* blood culture isolates in 2013 (n=171). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 4	> 8	11.6	1.7	86.7
Imipenem	≤ 4	> 8	9.2	4.6	86.1
Gentamicin*	≤ 128	> 128	-	53.2	46.8
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	99.4	0.0	0.6
Vancomycin (VanA or VanB)	Negative	Positive	98.2	-	1.8

*The wild type is defined as intermediately susceptible.

RESULTS AND COMMENTS

As in previous years, enterococci were analysed both as a group and separately for *E. faecalis* and *E. faecium*. The results for each species are microbiologically more valid as resistance rates differ significantly between *E. faecalis* and *E. faecium*. However, overall rates of resistance are of interest when formulating empirical treatment strategies because they include the probability of systemic infections with each enterococcal species. The overall results for enterococci are presented in Table 46. The surveillance in NORM 2013 included 413 (66.4%) *E. faecalis* isolates (69.3% in 2012), 171 (27.5%) *E. faecium* isolates (25.9% in 2012) and 38 (6.1%) unspeciated enterococcal isolates (4.9% in 2012). The ratio of *E. faecalis* to *E. faecium* isolates was 3.5 in 2009, 2.5 in 2010, 2.1 in 2011, 2.7 in 2012 and 2.4 in 2013. Further surveillance will show whether the declining proportion of *E. faecalis* among enterococcal isolates has now stabilised. The number of isolates not speciated to the genus level or identified as *E. faecalis* or *E. faecium* has decreased over the last five years. The panel of antimicrobial agents examined and the breakpoints for interpretation remained unchanged from 2012 to 2013.

E. faecalis was universally susceptible to ampicillin (Table 47). The prevalence of resistance to ampicillin in *E. faecium* remained relatively stable at 86.7% compared to 79.8% in 2012 and 81.1% in 2011 (Table 48). As expected, the results for imipenem closely mirrored those for ampicillin.

The prevalence of high-level gentamicin resistance (HLGR) in *E. faecalis* was 23.6% which is essentially unchanged from 25.6% in 2012 (Figure 57), and the prevalence of HLGR in *E. faecium* has apparently stabilised around 45-50%. Almost all (79/81) HLGR *E. faecium* isolates were concomitantly non-susceptible to ampicillin. Conversely, 79 of 153 (51.6%) ampicillin non-susceptible *E. faecium* also displayed HLGR. These findings are similar to the results from previous years. The strong linkage between ampicillin resistance and HLGR may indicate the continuing presence of the internationally disseminated *E. faecium* clonal complex (CC) 17 which is non-susceptible to ampicillin and often harbors high-level resistance to aminoglycosides and vancomycin. The wide dissemination of high-level gentamicin resistance in enterococci is of great concern as it abolishes the bactericidal synergy between aminoglycosides and beta-lactams often used for treatment of severe enterococcal infections.

Transferable vancomycin resistance has not yet been endemically established in clinical enterococcal isolates in Norway, but recent outbreaks have occurred in the western and south-eastern parts of the country. Twenty-three blood culture isolates were reported as vancomycin resistant in NORM 2013 (3.7%), but only one *E. faecalis* (VanB) and three *E. faecium* (2 VanB, 1 VanA) isolates contained transferable glycopeptide resistance confirmed by positive PCRs. The remaining vancomycin resistant isolates were registered as either *E. gallinarum* (n=12) or *E. casseliflavus* (n=7) which are inherently low-level resistant to vancomycin due to expression of the VanC ligase. All enterococcal isolates in NORM 2013 were fully susceptible to linezolid as opposed to the previous year when several resistant isolates were detected.

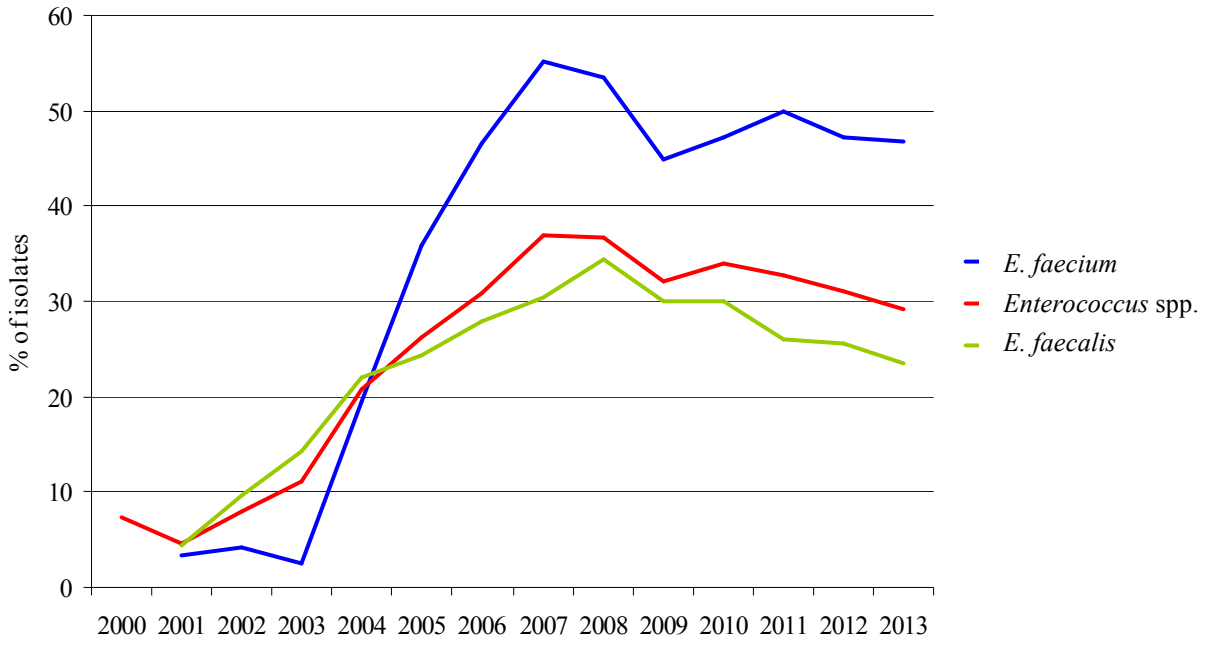

FIGURE 57. Prevalence of high-level resistance to gentamicin in blood culture isolates of *E. faecalis*, *E. faecium* and all enterococci combined during 2000-2013. The breakpoint for high-level resistance was decreased from R ≥ 1,024 mg/L to R > 128 mg/L in 2004.

Is the epidemiology of vancomycin resistant enterococci in Norway changing?

Over the last decades enterococci have attracted increased attention due to issues concerning resistance [1]. Resistance to glycopeptides (especially vancomycin) has caused major concern because of the limited further therapeutic options and fear of horizontal spread of the resistance mechanism to other species e.g. *S. aureus* [2,3]. In Europe the epidemiology of vancomycin resistant enterococci (VRE) has been characterised by less resistant isolates than in North America and early on by a resistance pattern similar to isolates from animal husbandry. The early isolates were highly polyclonal and believed to be associated with the use of avoparcin as a growth promoter in the farming industry [4], leading to a ban on avoparcin in 1997. Nevertheless, in south and central Europe the occurrence of VRE in clinical isolates has continued to increase [1]. Northern Europe has been a low prevalence area with respect to most antimicrobial resistant microbes including VRE [1]. However, in 2007 Sweden experienced a large increase in the number of VRE cases, largely due to nosocomial cases from a limited number of hospitals in the Stockholm area [5].

VRE in the MSIS surveillance system

Since 1995, VRE infections have been notifiable to the Norwegian Surveillance System for Communicable Diseases (MSIS) at the Norwegian Institute of public Health (NIPH). From 2005 it has been mandatory to notify of all positive findings including carrier status. Cases (infections and carriers) are notified by both the laboratories and the clinicians to the NIPH. This information is compiled into one unique record in MSIS. The system covers the entire Norwegian population. Infections and carriage with linezolid resistant enterococci are also notifiable. Cases related to outbreaks are given specific outbreak-based codes in MSIS. The MSIS register is person-based and relies on each person having an individual and unique ID-number. Data are collected as to age, sex, region, hospital-admission status, material for isolation, infection status (carrier or infection) and where they were infected. There is also a section for free text.

VRE epidemiology in Norway

Until 2010, Norway had only registered 0-10 sporadic cases annually. This changed with the first large-scale hospital outbreak of VRE in Norway in 2010 (*E. faecium*, *van*B), which also spread to neighbouring hospitals and one hospital outside the region. The outbreak is still on-going and by the end of 2013, 418 cases had been notified to MSIS. In 2011, a small VRE outbreak (*E. faecium, van*A), localised to a dialysis unit, was rapidly contained (15 cases notified to MSIS). The second large-scale hospital outbreak in Norway occurred in 2012. This outbreak (*E. faecium, van*A) has not reported any new cases between May 2013 and the end of 2013. Active screening procedures are still ongoing and the outbreak is likely contained through the extensive interventions applied. Seventy-six cases from this outbreak have been notified to MSIS until the end of 2013. The VRE epidemiology in Norway is summarised in Figure 58.

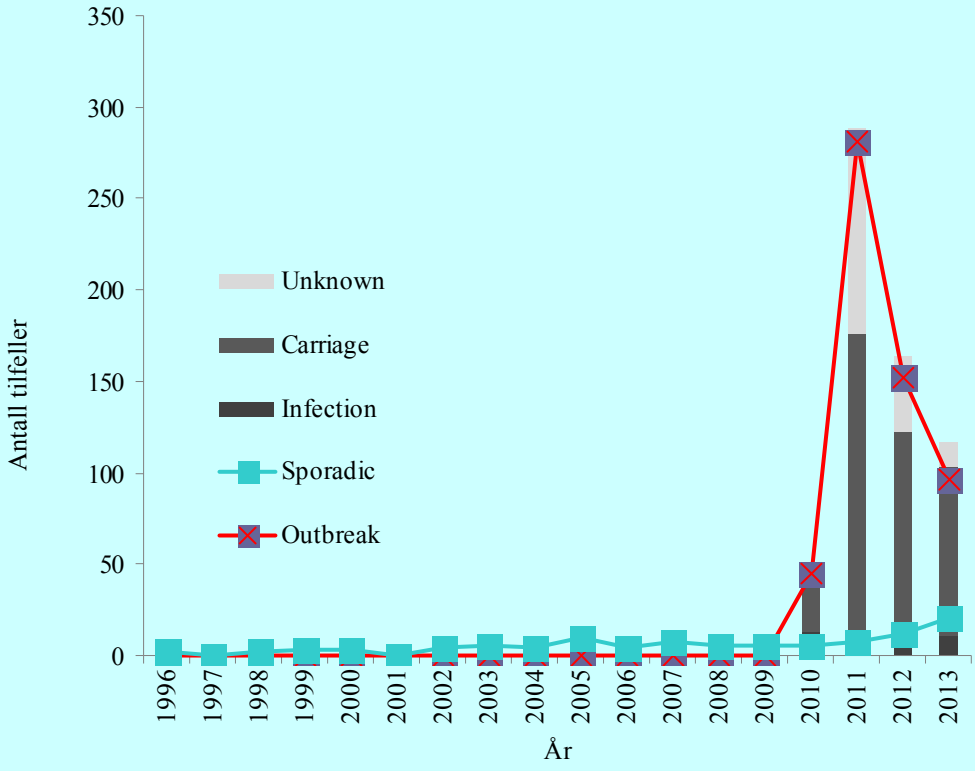

FIGURE 58. Annual number of cases of vancomycin resistant enterococci reported to the Norwegian Surveillance System for Communicable Diseases (MSIS) during 1996-2013. The bars indicate whether cases represent infection, carriage only or unknown clinical status. Coloured lines classify number of cases as sporadic (blue) and outbreak related (red).

Norway has no national reference laboratory for VRE. Microbiological diagnostic- and susceptibility testing is done by the individual hospital clinical microbiology laboratories in accordance with the NordicAST recommendations [http://www.nordicast.org/]. However, screening associated with outbreaks presents special challenges and no common approach exists. The methods vary according to species and mechanism involved. Methods involving overnight incubation in enrichment broth are commonly used, but in outbreak settings these have been supplemented by commercial and in-house direct molecular detection to accomplish speedy triage. The bias introduced by this variation in screening, predominantly affects detection and the absolute number of cases detected between outbreaks.
The VRE epidemiology in Norway is strongly biased by the active screening and contact tracing associated with outbreak control measures and the resulting inevitable increase in screening activity. A central feature of the VRE outbreak epidemiology is the carrier to infection ratio of 10 to 1 or higher, warranting not only liberal screening criteria in the epidemic setting, but also highlights the ability of VRE to disseminate in vulnerable populations. Despite the bias introduced by contact tracing, a small increase in non-outbreak related notifications is apparent. It is likely that the VRE epidemiology in Norway will change if outbreaks are not contained. Although not unlikely, there is little evidence that the outbreak index cases are imported from abroad. It is, however, less likely that they were introduced to the hospitals from prevalent cases in the general population and the strict infection control measures seem to be justified. Continued vigilance and prompt outbreak containment are crucial measures if Norway is to maintain a low prevalence of VRE in the coming decades.

References:

1. European Centre for Disease Prevention and Control (2013) Antimicrobial resistance surveillance in Europe 2012. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC.
2. Rossi F, Diaz L, Wollam A, Panesso D, Zhou Y, et al. (2014) Transferable vancomycin resistance in a community-associated MRSA lineage. N Engl J Med 370: 1524-1531.
3. Limbago BM, Kallen AJ, Zhu W, Eggers P, McDougal LK, et al. (2014) Report of the 13th Vancomycin-Resistant Staphylococcus aureus Isolate from the United States. Journal of Clinical Microbiology 52: 998-1002.
4. Willems RJL, Homan W, Top J, van Santen-Verheuvel M, Tribe D, et al. (2001) Variant esp gene as a marker of a distinct genetic lineage of vancomycinresistant Enterococcus faecium spreading in hospitals. The Lancet 357: 853-855.
5. Soderblom T, Aspevall O, Erntell M, Hedin G, Heimer D, et al. (2010) Alarming spread of vancomycin resistant enterococci in Sweden since 2007. Euro Surveill 15: pii = 19620.

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Evaluation of antimicrobial susceptibility methods in detection of low-level vancomycin resistance in enterococci: A Scandinavian multicentre study

Acquired vancomycin resistance in enterococci (VRE) is currently due to either of eight different gene clusters (*vanA,B,D,E,G,L,M,N*). VanA VRE is most prevalent worldwide, but infections with VanB-type VRE (mainly *E. faecium*) are predominant in Australia and have shown a dramatic increase in several European countries [1-6].
The *vanB*-type VRE is inducible and expresses various levels of resistance to vancomycin (MIC 4-1024 mg/L) and susceptibility to teicoplanin (MIC ≤ 2 mg/L) *in vitro* [7]. The wide range of vancomycin MICs in VanB-type enterococci is well known and has also been observed within the same clone during outbreaks [2,8]. The EUCAST MIC clinical breakpoints for *Enterococcus* spp. are susceptible (S) ≤ 4 mg/L and resistant (R) > 4 mg/L for vancomycin and S ≤ 2 mg/L and R > 2 mg/L for teicoplanin. The inducible moderate to low vancomycin MIC phenotypes of *vanB*-type VRE challenge current phenotypic detection methods. It is important to detect these VRE isolates as glycopeptide treatment of infections caused by such isolates may lead to treatment failure.

A well-characterised diverse collection of *Enterococcus faecalis* (n=12) and *Enterococcus faecium* (n=18) with and without non-susceptibility to vancomycin was examined blindly in Danish (n=5), Norwegian (n=13) and Swedish (n=10) laboratories using the EUCAST disk diffusion method (n=28), and the CLSI agar screen (n=18) or the VITEK 2 system (n=5) [9]. The EUCAST disk diffusion method and CLSI agar screen performed significantly (p=0.02) better than VITEK 2 (Table 49).

The performance of the EUCAST disk diffusion method was challenged by differences in vancomycin inhibition zone sizes as well as the experience of the personnel to interpret fuzzy zone edges as an indication of vancomycin resistance (Figure 59). Laboratories using Oxoid ($p<0.0001$) and Merck Mueller-Hinton agar (p=0.027) for disk diffusion performed significantly better compared to laboratories using the BBL MH II medium. Laboratories using Difco BHI for CLSI agar screen performed significantly better (p=0.017) than those using Oxoid BHI.

In conclusion, our results demonstrate acceptable performance by both the EUCAST disk diffusion and CLSI agar screen methods (sensitivity 0.93 and specificity 0.94-0.98) in detection of low-level *vanB* VRE. Importantly, the agar disk diffusion method requires personnel trained in interpretation of zone edges. Moreover, the CLSI agar screen method requires careful monitoring of the vancomycin concentration in the plates.

TABLE 49. Number of very major (VME) and major errors (ME), sensitivity and specificity calculated for each detection method as well as for each type of agar medium used

Method (no. of laboratories)	No. VMEs/ total no. of isolates with *van* genotype (%)	Sensitivity (95% CI)	No. MEs/ total no. of susceptible isolates (%)	Specificity (95% CI)
EUCAST disk diffusion (28) Oxoid MH (16) BBL MH II (10)	53/756 (7.0) 14/432 (3.2) 37/270 (14)	0.93 (0.91-0.95) 0.97 (0.94-0.98) 0.86 (0.81-0.90)	2/84 (2.4) 0/48 (0.0) 2/30 (6.7)	0.98 (0.91-1) 1.0 (0.91-1) 0.93 (0.76-0.99)
CLSI agar screen (18) BHI Difco (8) BHI Oxoid (5)	32/486 (6.6) 9/216 (4.2) 15/135 (11)	0.93 (0.91-0.95) 0.96 (0.92-0.98) 0.89 (0.82-0.93)	3/54 (5.6) 0/24 (0.0) 0/15 (0.0)	0.94 (0.84-0.99) 1.0 (0.83-1) 1.0 (0.75-1)
VITEK 2 (5)	18/135 (13)	0.87 (0.79-0.92)	0/15 (0.0))	1.0 (0.75-1)

VMEs; strains classified as S when containing a *van* genotype, MEs; strains classified as R when containing no *van* genotype.

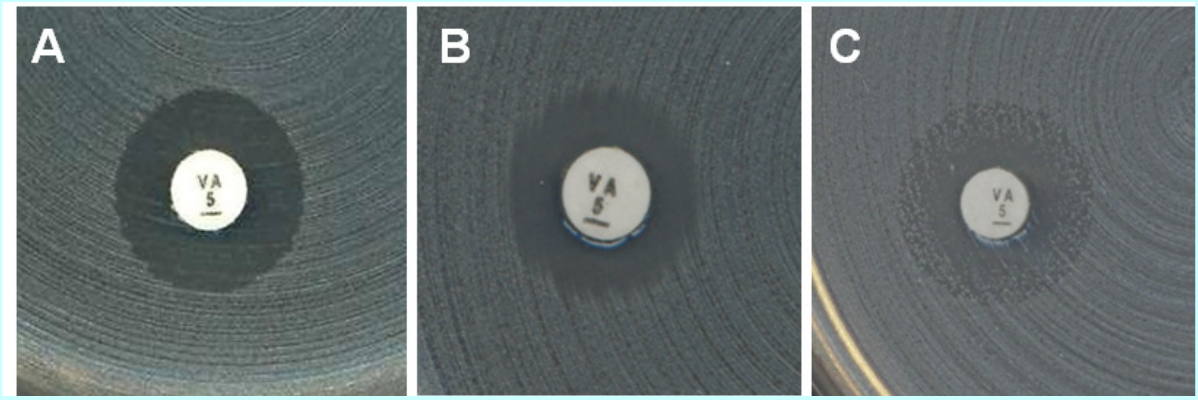

FIGURE 59. Examples of disk diffusion inhibition zones for *Enterococcus spp.* with 5 µg vancomycin disk. A) Sharp zone edge and zone diameter ≥ 12 mm should be reported as susceptible. B) Fuzzy zone edge or C) colonies within zone should be reported as resistant even if the zone diameter is ≥ 12 mm.

References:

1. M. Granlund, C. Carlsson, H. Edebro, K. Emanuelsson, and R. Lundholm, Nosocomial outbreak of vanB2 vancomycin-resistant Enterococcus faecium in Sweden, J. Hosp. Infect., 62 (2006) 254-256.
2. G. Werner, I. Klare, C. Fleige, U. Geringer, W. Witte, H. M. Just, and R. Ziegler, Vancomycin-resistant vanB-type Enterococcus faecium isolates expressing varying levels of vancomycin resistance and being highly prevalent among neonatal patients in a single ICU, Antimicrob. Resist. Infect. Control, 1 (2012) 21.
3. N. Bourdon, M. Fines-Guyon, J. M. Thiolet, S. Maugat, B. Coignard, R. Leclercq, and V. Cattoir, Changing trends in vancomycin-resistant enterococci in French hospitals, 2001-08, J. Antimicrob. Chemother., 66 (2011) 713-721.
4. T. Söderblom, O. Aspevall, M. Erntell, G. Hedin, D. Heimer, I. Hökeberg, K. Kidd-Ljunggren, Å. Melhus, B. Olsson-Liljequist, I. Sjögren, J. Smedjegård, J. Struwe, S. Sylvan, K. Tegmark-Wisell, and M. Thore, Alarming spread of vancomycin resistant enterococci in Sweden since 2007, Euro. Surveill, 15 (2010) pii=19620.
5. E. K. Bjørkeng, G. Rasmussen, A. Sundsfjord, L. Sjöberg, K. Hegstad, and B. Söderquist, Clustering of polyclonal VanB-type vancomycin-resistant Enterococcus faecium in a low-endemic area was associated with CC17-genogroup strains harbouring transferable vanB2-Tn5382 and pRUM-like repA containing plasmids with axe-txe plasmid addiction systems, APMIS, 119 (2011) 247-258.
6. P. D. Johnson, S. A. Ballard, E. A. Grabsch, T. P. Stinear, T. Seemann, H. L. Young, M. L. Grayson, and B. P. Howden, A sustained hospital outbreak of vancomycin-resistant Enterococcus faecium bacteremia due to emergence of vanB E. faecium sequence type 203, J. Infect. Dis., 202 (2010) 1278-1286.
7. K. Hegstad, T. Mikalsen, T. M. Coque, L. B. Jensen, G. Werner, and A. Sundsfjord, Mobile genetic elements and their contribution to the emergence of antimicrobial resistant Enterococcus faecalis and E. faecium, Clin. Microbiol. Infect., 16 (2010) 541-554.
8. A. Sivertsen, H. Billström, Ö. Melefors, B. Ollson Liljequist, K. Tegmark-Wisell, M. Ullberg, V. Özenci, A. Sundsfjord, and K. Hegstad, A multicentre hospital outbreak in Sweden caused by introduction of a vanB transposon into a stably maintained pRUM-plasmid in an Enterococcus faecium ST192 clone, PLoS ONE, In press (2014).
9. K. Hegstad, C. G. Giske, B. Haldorsen, E. Matuschek, K. Schonning, T. M. Leegaard, G. Kahlmeter, and A. Sundsfjord, Performance of the EUCAST disk diffusion method, the CLSI agar screen method, and the Vitek 2 automated antimicrobial susceptibility testing system for detection of clinical isolates of Enterococci with low- and medium-level VanB-type vancomycin resistance: a multicenter study, J. Clin. Microbiol., 52 (2014) 1582-1589.

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Streptococcus pneumoniae in blood cultures and cerebrospinal fluids

TABLE 50. *Streptococcus pneumoniae* in blood cultures and cerebrospinal fluids in 2013 (n=608). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.064	> 2	96.9	3.0	0.2
Cefotaxime	≤ 0.5	> 2	99.8	0.2	0.0
Ceftriaxone	≤ 0.5	> 2	99.8	0.2	0.0
Erythromycin	≤ 0.25	> 0.5	96.4	0.0	3.6
Clindamycin	≤ 0.5	> 0.5	97.5	-	2.5
Tetracycline	≤ 1	> 2	96.2	0.8	3.0
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	92.4	3.8	3.8
Chloramphenicol	≤ 8	> 8	99.0	-	1.0
Oxacillin screen (mm)	≥ 20	< 20	95.1	-	4.9

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 51. *Streptococcus pneumoniae* in blood cultures and cerebrospinal fluids in 2013 (n=608). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G		0.5	53.0	38.5	4.9	1.0	1.8	0.2			0.2					
Cefotaxime		0.8	63.5	29.1	2.3	3.0	1.2		0.2							
Ceftriaxone		1.2	75.2	17.4	2.3	2.8	1.0		0.2							
Erythromycin					10.2	80.8	5.4			0.2	0.7	0.2	0.2			2.5
Clindamycin					9.2	70.1	18.3									2.5
Tetracycline					1.6	86.7	7.4	0.2	0.3	0.8		0.3	1.3	1.5		
TMS**						2.5	67.1	20.4	2.5	3.8	0.7	0.5	0.2	2.5		
Chloramph.							0.2			37.7	61.2		0.8	0.2		
Norfloxacin										6.4	64.5	28.9	0.2			

	< 20	20	21	22	23	24	25	26	27	28	29	30	31	32	33	≥ 34
Oxacillin disk	4.9	0.8	0.7	0.5	1.0	2.3	7.6	7.2	19.7	22.4	13.0	15.8	3.8	0.3		

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. Non-shaded rows indicate that breakpoints have not been defined. **TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The results are summarized in Tables 50-51 and Figures 60-61. All systemic *S. pneumoniae* isolates submitted to the National Reference Laboratory for Pneumococci at the Norwegian Institute of Public Health during 2013 were included in the surveillance protocol. Twenty-two isolates were recovered from cerebrospinal fluids, and six of these were found in patients who concomitantly had positive blood cultures. Both blood culture isolates and isolates from cerebrospinal fluids were included from patients with positive cultures from both specimen types.

Norwegian breakpoints for pneumococci are in accordance with EUCAST, and these remained unchanged in 2013 as defined by MIC values. The results for penicillin G, cefotaxime and ceftriaxone were interpreted according to the general breakpoints for pneumococci (R > 2 mg/L for all three substances; S ≤ 0.06, S ≤ 0.5 and S ≤ 0.5 mg/l, respectively). The isolates from cerebrospinal fluids were in addition categorised according to breakpoints for meningitis (R > 0.064, R > 0.5 and R > 0.5 mg/L, respectively).

A total of 3.0% (18/608) of *S. pneumoniae* isolates were intermediately susceptible to penicillin G (MIC 0.125-0.5 mg/L), and a single blood culture isolate (0.2%) was classified as resistant with an MIC of 4 mg/L. This isolate also displayed intermediate susceptibility to cefotaxime and ceftriaxone (both MIC 1 mg/L). All the intermediately susceptible isolates were recovered from blood cultures and were suscpetible to 3rd generation cephalosporins. Non-susceptibility to penicillin G has gradually increased over the last years, but the 3.2% prevalence in 2013 is a significant decrease from 6.3% in 2012.

The oxacillin screening disk is often used to discriminate between penicillin susceptible and non-susceptible isolates. All penicillin G non-susceptible isolates were resistant to oxacillin. Conversely, 11/589 penicillin G susceptible isolates were oxacillin resistant. The sensitivity and specificity of the screening test were thus 100.0% and 98.1%, respectively. Many of the penicillin non-susceptible *S. pneumoniae* isolates were also non-

susceptible to erythromycin (7/19), trimethoprim-sulfamethoxazole (10/19), and tetracycline (8/19).

The prevalence of macrolide non-susceptibility decreased from 6.0% in 2012 to 3.6% in 2013, the same level as the two previous years (Figure 60). The macrolide resistance phenotype was further characterised in erythromycin non-susceptible isolates. Three (16% of erythromycin non-susceptible isolates, 0.5% of all isolates) displayed a phenotype compatible with efflux-based M-type resistance to erythromycin only. The remaining isolates were either constitutively (n=15, 79% of erythromycin non-susceptible isolates, 2.5% of all isolates) or inducibly (n=1, 5% of erythromycin non-susceptible isolates, 0.2% of all isolates) resistant to clindamycin, thus indicating the presence of *erm*-encoded MLS resistance to both macrolides and lincosamides. The distribution of MLS phenotypes was not significantly altered from 2012 to 2013. The low number of isolates analysed precludes any firm conclusions, but the results may suggest a continuing predominance of *erm*-mediated macrolide resistance as opposed to the *mef*-dominated peak 2002-2009 (Figure 61).

The 7.6% prevalence of non-susceptibility to trimethoprim-sulfamethoxazole is a further increase from 4.3% in 2010, 5.7% in 2011 and 6.5% in 2012. The prevalence of non-susceptibility to tetracycline has decreased from 6.5% in 2012 to 3.8% in 2012 (Figure 60). The vast majority of isolates (99.0%) remained susceptible to chloramphenicol which was earlier often used for empirical treatment of meningitis in Norway. The low prevalence of high-level norfloxacin resistance (Table 51) reflects the very limited use of levofloxacin and moxifloxacin for respiratory tract infections in Norway.

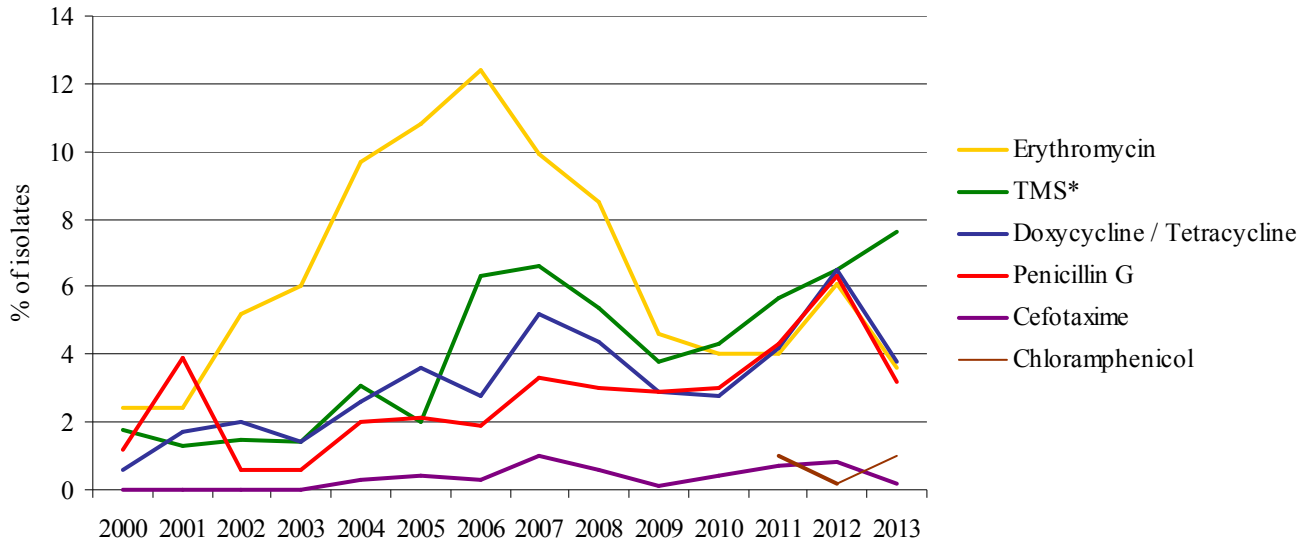


FIGURE 60. Prevalence (%) of non-susceptibility to various antimicrobial agents in *Streptococcus pneumoniae* blood culture isolates during 2000-2013. Doxycycline was substituted by tetracycline in 2005. All results are categorised according to the 2014 breakpoint protocol. *TMS=Trimethoprim-sulfamethoxazole.

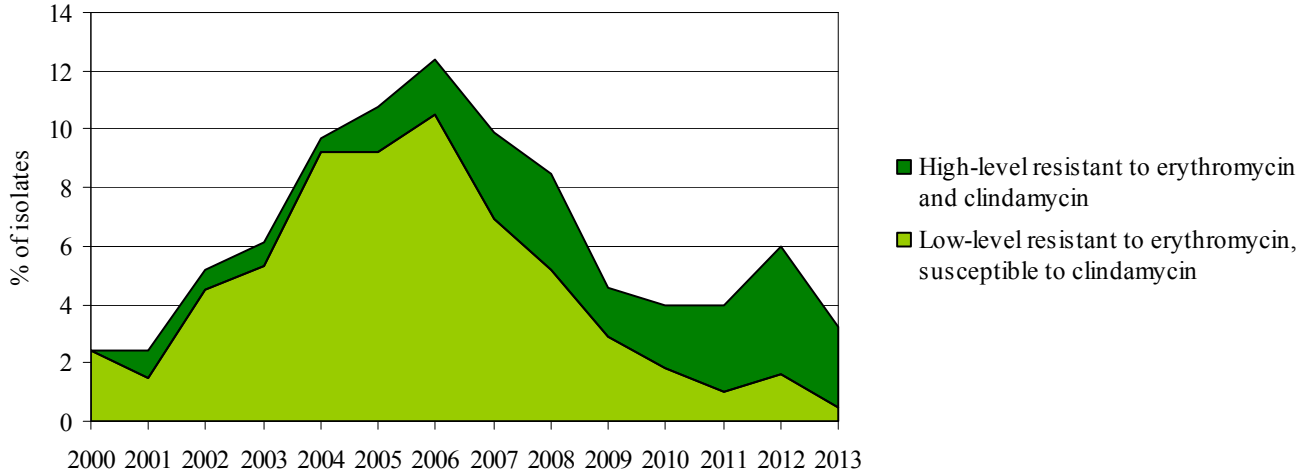


FIGURE 61. Prevalence (%) of non-susceptibility to erythromycin and clindamycin in *Streptococcus pneumoniae* blood culture isolates during 2000-2013.

Streptococcus pyogenes in blood cultures

TABLE 52. *Streptococcus pyogenes* in blood cultures in 2013 (n=162). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	98.1	0.0	1.9
Clindamycin	≤ 0.5	> 0.5	99.4	-	0.6
Tetracycline	≤ 1	> 2	85.2	0.0	14.8
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	96.3	0.6	3.1

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 53. *Streptococcus pyogenes* in blood cultures in 2013 (n=162). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G		0.6	81.5	17.9												
Erythromycin					35.8	56.8	5.6				0.6		0.6			0.6
Clindamycin				1.2	36.4	61.7										0.6
Tetracycline					9.9	63.0	12.3					0.6	4.3	8.0	1.9	
TMS**					2.5	10.5	34.6	45.1	3.7	0.6	0.6			2.5		

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. Non-shaded rows indicate that breakpoints have not been defined. **TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

S. pyogenes blood culture isolates have not been surveyed in NORM since 2006. However, from 2013 the Reference Laboratory at the Norwegian Institute of Public Health will provide resistance data for systemic *S. pyogenes* isolates on a yearly basis. The Norwegian breakpoints for haemolytic streptococci are in accordance with EUCAST. For several antimicrobial agents, breakpoints have changed since 2006 but the following comments are based on comparable interpretations using the present NWGA recommendations.

As expected, all isolates were fully susceptible to penicillin G. The prevalence of resistance to erythromycin (1.9%) and clindamycin (0.6%) was at the same level as in 2006 (0.7% and 0.7%, respectively). Two of the three macrolide resistant isolates had phenotypes compatible with inducible (n=1) and constitutive (n=1) MLS_B resistance, whereas the characteristics of the third isolate were not recorded. The prevalence of tetracycline resistance has increased from 8.6% in 2006 to 14.8% in 2013, whereas the prevalence of non-susceptibility to trimethoprim-sulfamethoxazole has remained relatively stable at 3.9% in 2013 compared to 4.9% in 2006. For further comments, see below under non-systemic isolates.

Streptococcus pyogenes in specimens from the respiratory tract and wounds

TABLE 54. *Streptococcus pyogenes* in respiratory tract specimens in 2013 (n=261). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	94.6	0.0	5.4
Clindamycin	≤ 0.5	> 0.5	96.9	-	3.1
Tetracycline	≤ 1	> 2	93.1	0.4	6.5
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	99.6	0.4	0.0

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 55. *Streptococcus pneumoniae* in respiratory tract specimens in 2013 (n=261). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G	1.9	50.2	45.6	0.8	0.4	0.8	0.4									
Erythromycin				0.8	18.8	63.2	11.9						0.4	0.4		4.6
Clindamycin			0.8	10.0	42.5	40.6	2.3	0.8	0.8			0.8				1.5
Tetracycline			0.4		37.9	49.4	5.0	0.4		0.4			2.3	3.8		0.4
TMS**		0.4	2.3	8.0	22.2	28.4	30.3	6.5	1.5	0.4						

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. Non-shaded rows indicate that breakpoints have not been defined. **TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 56. *Streptococcus pyogenes* in wound specimens in 2013 (n=243). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	95.9	0.0	4.1
Clindamycin	≤ 0.5	> 0.5	98.4	-	1.6
Tetracycline	≤ 1	> 2	86.0	0.0	14.0
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	98.8	0.0	1.2

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 57. *Streptococcus pneumoniae* in wound specimens in 2013 (n=243). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G	0.8	49.0	46.9	0.4	0.8	2.1										
Erythromycin				1.2	15.2	68.3	11.1					0.8	0.4		0.4	2.5
Clindamycin			0.8	10.7	51.0	33.7	1.6	0.4								1.6
Tetracycline				0.4	31.3	51.4	2.9					0.8	6.6	5.3	1.2	
TMS**			2.5	14.4	18.1	27.6	25.9	7.4	2.9					1.2		

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. Non-shaded rows indicate that breakpoints have not been defined. **TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Streptococcus pyogenes (beta-haemolytic streptococci group A - GAS) from wounds and respiratory tract specimens have previously been surveyed in NORM in 2002, 2004, 2006 and 2008. The results from 2013 are presented in Tables 54-57 and the trends during 2002-2013 in Figure 62. The relevant breakpoints have remained unchanged since the last survey in 2008, and the results for all years are interpreted according to the 2014 NWGA protocol.

Penicillin G non-susceptibility has never been detected in group A streptococci, and the highest MIC value in this study was 0.25 mg/L which is equivalent to the breakpoint for susceptibility. Most isolates displayed MICs of 0.008-0.016 mg/L.

Macrolide resistant group A streptococci have been a problem in many European countries. In NORM, the prevalence of erythromycin resistance has remained relatively stable below 5%, but increased 2008-2013 from 3.1% to 5.4% and from 1.2% to 4.1% in respiratory tract and wound isolates, respectively. The prevalence of resistance to clindamycin has similarly increased from 0.8% to 3.1% and from 0.4% to 1.6% in respiratory tract and wound isolates, respectively. In total, 24 non-systemic isolates were erythromycin resistant and were classified as either inducibly (9/24, 1.8% of all isolates) or constitutively (12/24, 2.4% of all isolates) MLS_B resistant. In addition, three isolates displayed a phenotype compatible with *mef*-encoded efflux. A single isolate had clindamycin MIC of 1 mg/L but was susceptible to erythromycin (MIC 0.125 mg/L). This could be caused by alterations in ribosomal proteins but was not further explored.

As seen in Figur 62, the prevalence of non-susceptibility to tertacycline in isolates from wound specimens is 14.0%. This corresponds to 14.8% in blood culture isolates, but is consistently higher than in respiratory tract isolates (7.0% in 2013). Conversely, systemic isolates have a higher prevalence of non-susceptibility to trimethoprim-sulfamethoxazole (3.7%) than isolates from wounds (1.2%) and the respiratory tract (0.4%). One may speculate that differences in resistance rates between isolates from different clinical conditions is caused by clonal variation, but further studies are needed to support this hypothesis.

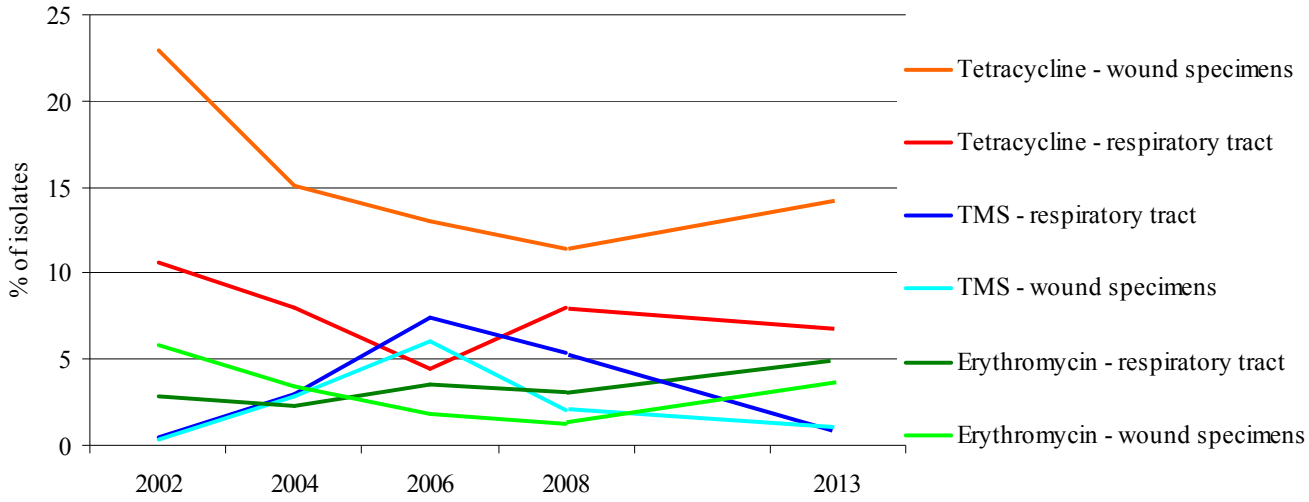


FIGURE 62. Prevalences of non-susceptibility to various antimicrobial agents in *Streptococcus pyogenes* from wound specimens and the respiratory tract in 2002-2013. Doxycycline was used in 2002 and 2006, but was replaced by tetracycline in 2008 and 2013. All data are categorised according to the 2014 EUCAST breakpoint protocol.

Mycobacterium tuberculosis

A total of 401 cases of infection with *M. tuberculosis* complex (not BCG) were reported to the Norwegian Surveillance System for Communicable Diseases (MSIS) in 2013. Sixteen of the cases had been treated with anti-TB drugs previously. Nine additional cases had previous TB but had not been treated. There were six MDR-TB* cases, one of which was also resistant to moxifloxacin and amicacin, hence categorized as XDR-TB**. Five of the six MDR-TB cases were treated for the first time; the last one had previously been treated.

Three hundred and eighteen cases were confirmed by culture followed by susceptibility testing of the strain isolated. The results are presented in Table 58. The cases are registered in MSIS the year in which the first culture positive test was taken.

TABLE 58. Antimicrobial susceptibility of 318 isolates of *M. tuberculosis* complex (not *M. bovis* (BCG)) isolated from human infections in 2013. Figures from 2012 are shown in parenthesis.

Origin of birth	No. of cases	No. of isolates	Resistance to antimicrobial agents (No. of isolates)					
			Isoniazid	Rifampicin	Ethambutol	Streptomycin	Pyrazinamid	MDR TB*
Norway	53 (55)	35 (42)	0 (1)	0 (0)	0 (0)	8 (4)	0 (0)	0 (0)
Europe excl. Norway	29 (28)	28 (22)	3 (5)	2 (3)	1 (1)	3 (6)	2 (2)	2 (3)
Asia	135 (116)	106 (82)	15 (7)	2 (2)	2 (0)	13 (9)	8 (5)	2 (1)
Africa	177 (176)	142 (131)	13 (15)	3 (3)	0 (2)	17 (19)	4 (4)	2 (3)
America	4 (3)	4 (3)	0 (0)	0 (0)	0 (0)	2 (0)	0 (0)	0 (0)
Unknown	3 (0)	3 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	401 (378)	318 (280)	31 (28)	7 (8)	3 (3)	43 (38)	14 (11)	6 (7)
Proportion of resistant isolates (%)			9.7 (10.0)	2.2 (2.9)	0.9 (1.2)	13.5 (13.6)	4.4 (3.9)	1.9 (2.5)

* MDR-TB: Multi-drug resistant tuberculosis, resistant to at least rifampicin and isoniazid.
** XDR-TB: Extensively drug-resistant tuberculosis, resistant to at least rifampicin and isoniazid plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).

Candida spp. in blood cultures

TABLE 59. Antimicrobial susceptibility of *Candida albicans* blood culture isolates (n=113). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	100.0	0.0	0.0
Voriconazole*	≤ 0.125	> 0.125	100.0	-	0.0
Anidulafungin*/**	≤ 0.03	> 0.03	100.0	-	0.0
Micafungin*/***	≤ 0.016	> 0.016	97.3	-	2.7

* Recommended breakpoints by the European Commitee on Antimicrobial Susceptibility Testing – EUCAST.
** There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin as well as micafungin are considered susceptible.
***The MIC of the resistant isolates were given before EUCAST breakpoints for micafungin were defined, and the isolates considerred susceptibile (anidulafungin susceptibile).

TABLE 60. *Candida albicans* blood culture isolates (n=113). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B				0.9		36.3	0.9	59.3	2.7								
Fluconazole					2.7	20.4	48.7	28.3									
Voriconazole	36.3	54.0	9.7														
Anidulafungin	62.8	31.9	5.3														
Micafungin**	2.7	25.7	69.0	2.7													
Caspofungin***				3.5	15.0	47.8	33.6										

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
**The MIC of the resistant isolates were given before EUCAST breakpoints for micafungin were defined, and the isolates considerred susceptibile (anidulafungin susceptibile).
***There are no European breakpoints for caspofungin. Strains susceptible to anidulafungine as well as micafungin are considered susceptible.

TABLE 61. Antimicrobial susceptibility of *Candida glabrata* blood culture isolates (n=26). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 0.002	> 32	0.0	88.4	11.5
Anidulafungin*/**	≤ 0.06	> 0.06	100.0	-	0.0
Micafungin*	≤ 0.03	> 0.03	100.0	-	0.0

* Recommended breakpoints by the European Commitee on Antimicrobial Susceptibility Testing - EUCAST.
** There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin as well as micafungin are considered susceptible.
There is insufficient evidence that *C.glabrata* is a good target for therapy with voriconazol and no clinical breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.

TABLE 62. *Candida glabrata* blood culture isolates (n=26). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B				3.8		7.7	11.5	61.5	15.4								
Fluconazole							3.8	11.5	7.7	7.7	30.8	19.2	7.7			3.8	7.7
Voriconazole**			15.4	7.7	30.8	11.5	7.7	11.5	7.7		3.8		3.8				
Anidulafungin	15.3	15.4	61.5	3.8	3.8												
Micafungin		30.8	61.5	7.7													
Caspofungin***						23.1	65.4	11.5									

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
**There is insufficient evidence that *C .glabrata* is a good target for therapy with voriconazol and no breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.
***There are no European breakpoints for caspofungin. Strains susceptible to anidulafungine are considered susceptible.

TABLE 63. Antimicrobial susceptibility of *Candida tropicalis* blood culture isolates (n=18). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	100.0	0.0	0.0
Voriconazole*	≤ 0.125	> 0.125	100.0	-	0.0
Anidulafungin*/**	≤ 0.06	> 0.06	100.0	-	0.0

* Recommended breakpoints by the European Commitee on Antimicrobial Susceptibility Testing – EUCAST.
** There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin are considered susceptible to caspofungin.
There is insufficient evidence that *C. tropicalis* is a good target for therapy with micafungin and no breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.

TABLE 64. *Candida tropicalis* blood culture isolates (n=18). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B							11.1	50.0	38.9								
Fluconazole						5.5	22.2	38.9	16.7	16.7							
Voriconazole		5.5	22.2	22.2	33.3	16.7											
Anidulafungin	11.0	5.5	83.3														
Micafungin**		5.5	27.8	66.7													
Caspofungin***						27.8	72.2										

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
**There is insufficient evidence that *C. tropicalis* is a good target for therapy with micafungin and no breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.
***There are no European breakpoints for caspofungin. Strains susceptible to anidulafungine are considered susceptible to caspofungin.

TABLE 65. Antimicrobial susceptibility of *Candida parapsilosis* blood culture isolates (n=10). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	90.0	-	10.0
Voriconazole*	≤ 0.125	> 0.125	100.0	-	0.0
Anidulafungin*	≤ 0.002	> 4	0.0	70.0	30.0
Micafungin*	≤ 0.002	> 2	0.0	80.0	20.0

* Recommended breakpoints by the European Commitee on antimicrobial susceptibility testing - EUCAST.
Susceptibility testing for caspofungin is not recommended as this species is a poor target for therapy with this drug. Isolates may be reportet as resistant without prior testing.

TABLE 66. *Candida parapsilosis* blood culture isolates (n=10). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B							50.0	40.0	10.0								
Fluconazole							40.0	20.0	20.0	10.0				10.0			
Voriconazole		60.0	10.0	10.0		20.0											
Anidulafungin							20.0	20.0	10.0	20.0		20.0		10.0			
Micafungin							30.0	10.0	40.0	20.0							
Caspofungin**							10.0	30.0	20.0	20.0	20.0						

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined.
**Susceptibility testing for caspofungin is not recommended as this species is a poor target for therapy with this drug. Isolates may be reportet as resistant without prior testing.

RESULTS AND COMMENTS

The National Mycology Reference Laboratory received 176 *Candida* isolates of eight different *Candida* species isolated from blood stream infections in 171 patients during 2013 compared to 186 isolates of ten *Candida* species in 2012. *Candida albicans* is still the most common *Candida* species observed (n=113, 64.2%) followed by *Candida glabrata* (n=26, 14.8%), *Candida tropicalis* (n=18, 10.2%) and *Candida parapsilosis* (n=10, 5.7%). In total, non-albicans strains were isolated in 63 cases (35.8%) compared to 59 isolates (31.7%) in 2012.

All isolates were susceptibility tested for amphotericin B, fluconazole, voriconazole, caspofungin, anidulafungin and micafungin by E-test according to the manufacturer's instructions (AB bioMérieux). The National Mycology Reference Laboratory uses E-test for MIC determinations and does not perform the EUCAST standardized broth micro-dilution method routinely. The results are summarised in Tables 59-66.

In general, species identification predicts the susceptibility pattern of the yeast. All but two *C. kruzei* isolates tested were susceptible to amphotericin B. The obseved MIC 2 mg/L of the two isolates suggests that they are likely to be resistant.

In Norway, acquired fluconazole resistance is still rare, observed in only one *C. parapsilosis* isolate in a patient with persistent infection. In 2013, EUCAST set new *C. glabrata* breakpoints for fluconazole ($S \leq 0.002$ mg/L, R > 32 mg/L) categorising the wild type as intermediately susceptible. An elevated MIC > 32 mg/L was observed in 17.4% of our isolates, and the occurrence of hetero-resistant *C. glabrata* to fluconazole was low (n=1). The number of *C. glabrata* isolates from blood is still low, 26 isolates compared to 23 isolates in 2013. Only four isolates of *C. kruzei* were referred to the National Mycology Reference Laboratory. This species is always considered resistant as it is a poor target for fluconazole therapy.

There is still insufficient evidence that *C. glabrata* and *C. kruzei* are good targets for therapy with voriconazole, and no breakpoints are set. EUCAST recommends reporting the MIC value without S, I and R categorisation. Except from these two species, all isolates were found susceptible to voriconazole.

In 2013, species-specific European breakpoints were defined for micafungin for *C. albicans, C. glabrata* and *C. parapsilosis.* The clinical significance of observed higher MIC in *C. tropicalis, C. kruzei* and *C. guilliermondii* is unknown, but there is insufficient evidence to indicate whether the wild type populations of these species can be considered susceptible to micafungin. EUCAST also modified species-specific breakpoints for *C. parapsilosis* and echinocandins, categorising the wild type as intermediately susceptible to micafungin and anidula-fungin in order to accommodate use of these drugs in some clinical situations. *C. parapsilosis* and *C. guilliermondii* are still not considered as good targets for therapy with caspofungin, and should be reported as resistant. For caspofungin EUCAST, breakpoints have not been established.

All *C. albicans* isolates were susceptible to all drugs tested, with the exception of observed elevated MIC to micafungin (MIC 0.032 mg/L) in three isolates. The significance is uncertain as the MIC values of the resistant isolates were given before EUCAST breakpoints for micafungin were defined, and the isolates were considered susceptibile due to anidulafungin susceptibility.

Appendix 1:
Collection of data on usage of antimicrobial agents in animals

Data sources

Feed additives

Until 2003, the Norwegian Agricultural Inspection Service was responsible for the collection of data on sales of antimicrobial growth promoters and coccidiostats as feed additives, while from that time the Norwegian Food Safety Authority has been in charge of collecting such data. Reliable data on the use of different substances and categories of feed additives was obtained from these sources.

Antimicrobial agents for therapeutic use

In Norway, veterinary antimicrobial agents for therapeutic use in domestic animals or farmed fish are prescription drugs only. Moreover, veterinary antimicrobial agents have to be dispensed through pharmacies, which are supplied by drug wholesalers only. An exemption from the pharmacy/wholesalers monopoly has been granted for medicated feeds (i.e. feeds into which drugs for therapeutic use are mixed prior to sales). Medicated feeds have to be prescribed by veterinarians and produced by feed mills authorised by the Norwegian Medicines Agency. In Norway, medicated feeds produced and supplied by feed mills are only used for farmed fish. Medicated feeds for livestock are not produced in feed mills due to the small size of livestock herds compared to most other European countries. However, herd/flock treatment of livestock with antimicrobial agents is possible, again subject to veterinary prescription, through the drinking water or as a top dressing on the feed. The sales figures for veterinary antimicrobial agents from wholesalers and feed mills are thought to roughly equal the use of these drugs. Sales figures for veterinary antimicrobial agents are therefore used as a synonym for usage of veterinary antimicrobial agents. Drug wholesalers and feed mills report their sales figures to the Norwegian Institute of Public Health. This reporting was made mandatory from January 1st 2002. Number of items sold for the various veterinary drug formulations, strengths and package sizes were obtained from the Norwegian Institute of Public Health and calculated to express kg active substance.

Drug classification system

The Anatomical Therapeutic Chemical vet (ATCvet) classification system was used to categorise veterinary medicinal products (http://www.whocc.no/atcvet).

Unit of measurement

The amount of active substance denoted in kilograms was chosen as the unit of measurement. Veterinary antimicrobial drug usage was calculated from sales figures for delivery of antimicrobials from wholesalers to pharmacies, and from feed mills. The data for benzyl penicillin salts and esters (procaine penicillin and penethamate hydriodide) were converted to the corresponding values for benzyl penicillin.

Inclusion criteria – veterinary drugs

The veterinary drugs included for terrestrial animals were all the approved veterinary antimicrobial products belonging to the following ATCvet groups: QA07AA (gastrointestinal infections) (no product in ATCvet group QA07AB on the market in Norway), QG01AA+AE (uterine infections) (no products in ATCvet groups QG51AC, -AE, -AX, -BA, -BC or -BE on the market in Norway), and QJ (antimicrobial agents for systemic use that includes intramammary dose applicators (QJ51)). Additionally, antimicrobial products sold on special exemption from market authorisation have been included following a case by case assessment. Sales of antimicrobial agents as medicated feeds and as premixes, both intended for use in farmed fish, belonging to QJ are presented separately. An exemption has been made for an antimicrobial premix approved for farmed fish only (trimethoprim-sulfadiazine 1:5) but sold solely for use in terrestrial animals since 1995 (unpublished data). Consequently, the sales of the antimicrobial agents in terrestrial animals reported for the years 1993-2005 were underestimated, although only slightly. However, the updated usage figures for 1995-2005 correlated highly positive (r=0.998) with the data reported previously for these years confirming the formerly reported reduction in the usage of antimicrobial agents in terrestrial animals. Dermatological preparations (QD) and preparations intended for sensory organs (QS) are not included in the material. Human antimicrobial preparations are used in small animal practice. However, data on the use of such antimicrobial preparations in animals are not included in this report as such sales cannot be separated from sales intended for use in humans.

Analysis of the data

In order to assess the overall use of antimicrobial VMPs for terrestrial food producing animals and for pets, sales of products approved for companion animals only (tablets and pasta) have been separated form the total sales.

Appendix 2:
Collection of data on human usage of antimicrobial agents

Data sources
In Norway, antimicrobials are prescription only medicines, and only allowed sold through pharmacies. These data are collected from three databases: the Norwegian drug wholesales statistics database, the hospital pharmacies drug statistics database and the Norwegian prescription database (NorPD).

The wholesales database covers total sales of antimicrobials in Norway and is based on sales of medicaments from drug wholesalers to pharmacies and health institutions in Norway. The figures presented should be regarded as maximum figures based on the assumption that all medicaments sold are actually consumed. The actual drug consumption will probably be somewhat lower. The Norwegian Institute of Public Health collects the data. Data on drug use from wholesalers is available since the beginning of the seventies.

Data on antibacterial use in hospitals are retrieved from *Sykehusapotekenes Legemiddelstatistikk* (the hospital pharmacies drug statistics database) which is a cooperation of LIS, Legemiddel Innkjøp Samarbeid (Drug Purchasing Cooperation) and the four regional pharmaceutical health trusts operating the hospital pharmacies in Norway. *Sykehusapotekenes Legemiddelstatistikk* collects sales data from each pharmacy delivering drugs to hospitals. Data are collected as sales from the pharmacy to hospital wards. Data are available since 2006.

Data on the use in ambulatory care are retrieved from NorPD, a nation-wide prescription database. This database includes all prescriptions being prescribed to out-patients in Norway. These data give us the exact population prevalence of antibacterial use in ambulatory care. The Norwegian Institute of Public Health collects the data. More information is available at www.fhi.no. Data are available from 2004.

Drug Classification
The data are categorised according to the ATC classification system (1). Defined Daily Doses (DDDs) are employed as units of measurement. The ATC/DDD index of 2014 is used.

Unit of measurement
The ATC/DDD system is recommended by the WHO to serve as a tool for drug utilisation research in order to improve quality of drug use. One component of this is the presentation and comparison of drug consumption statistics at international and other levels.
The use of defined daily dose, DDD, as a unit of measurement, simplifies and improves the evaluation of drug consumption over time, nationally and internationally. The DDD is a theoretical unit of measurement, and does not necessarily reflect the recommended or Prescribed Daily Dose.

The basic **definition** of the unit is:
The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults.

The DDDs for the antibacterials are as a main rule based on the use in infections of moderate severity. Some antibacterials are only used in severe infections and their DDDs are assigned accordingly. The DDDs assigned are based on daily treatment.

Inclusion criteria
The antibacterials for human use included in this report belong to ATC group J01 antibacterials for systemic use. Oral vancomycin (A07AA09), rifaximin (A07AA11) and oral and rectal metronidazole (P01AB01) are also included. For antifungals, only ATC-group J02; antimycotics for systemic use is included. Of the antimycobacterials, only rifampicin is included. The content in plain products and in combinations is calculated and data are presented as total amount of rifampicin used. Antibacterials used in dermatological preparations (ATC group D) and preparations intended for sensory organs (ATC group S) are not included in the material, except for mupirocin, which is included in one table.

References
1. WHO Collaborating Centre for Drug Statistics Methodology. ATC index with DDDs 2014. WHO Collaborating Centre, Oslo

Appendix 3:
Sampling, microbiological methods and data processing in NORM-VET

Sampling strategy
Altogether, 201 clinical isolates of *Staphylococcus pseudintermedius* from dogs were included for susceptibility testing. Each of the five laboratories of the Norwegian Veterinary Institute (NVI) collected isolates from clinical submissions.
The indicators *Escherichia coli* and *Enterococcus* spp. from layer and turkey flocks were collected from samples obtained by the Norwegian *Salmonella* control programme for live animals. From each flock a piece of a boot swab was analysed. A total of 204 and 131 samples were collected from layer and turkey flocks, respectively. The samples were also used for selective isolation of ESBL producing *E. coli* and for VRE. In addition, *E. coli* isolates were obtained from turkey meat samples bought at retail. Altogether 156 samples were collected, and the samples were also used for selective isolation of ESBL producing *E. coli*. *E. coli* was collected from 191 faecal dog samples collected at five different veterinary clinics around Norway, and the samples were also used for selective isolation of ESBL producing *E. coli*. Only one sample from each individual or production unit was included.

S. pseudintermedius
The clinical isolates from dogs included for the NORM-VET programme were confirmed as *S. pseudintermedius* using PCR (Sasaki et al. 2010). Isolates with oxacillin MIC above cut-off (suspected isolates) were subjected to further identification including PCR for detection of the *mec*A/*mec*C genes (Poulsen 2003, Stegger 2012).

Indicator isolates of *E. coli*
Sample material, i.e. boot swabs from layers and turkey holdings was mixed with sterile distilled water prior to plating onto the surface of MacConkey agar and incubated at 37°C for 24h. *E. coli* from turkey meat was isolated by plating on lactose-bromthymol blue agar plates after enrichment in MacConkey broth (see description under ESBL producing *E. coli*). Faecal samples from dog was plated directly onto lactose-bromthymol blue agar and incubated at 37°C for 24h. Typical colonies were subcultured on blood agar (Heart infusion agar, Difco) containing 5% bovine blood and incubated at 37°C for 24h. Colonies were identified as *E. coli* by typical colony appearance, lactose fermentation, a positive indole reaction, and negative oxidase reactions.

ESBL producing *E. coli*
From the suspension of sample material (layers and turkey) used for *E. coli* isolation, 0.1 ml was plated onto MacConkey agar (Difco) containing 1 mg/L cefotaxime and MacConkey agar containing 2 mg/L ceftazidime. Meat samples from poultry of approximately 5 g were cultured in MacConkey broth for 24 h at 41°C before plating out on MacConkey agar with 1 mg/L cefotaxime and MacConkey agar with 2 mg/L ceftazidime. Sample material from dog was plated directly onto the selective plates. The agar plates were incubated at 37°C for 24-48h. Presumptive ESBL positive *E. coli* were further investigated by disk diffusion (Beckton Dickinson) and subjected to PCR (Pérez-Pérez et al, 2002) and DNA sequencing. PCR positive colonies were confirmed as *E. coli* using PCR (Heininger 1999).

***Enterococcus* spp.**
Sample material was plated on Slanetz & Bartley agar (Oxoid) and incubated at 41°C for 24-48h. Colonies from each positive sample were selected, and the isolates confirmed as *Enterococcus* spp. by phenotypic characterisation and negative catalase test. The isolates were further identified to the species level as *E. faecalis* or *E. faecium* using PCR (Dutka-Malen et al. 1995).

Vancomycin resistant *Enterococcus* spp.
Sample material was plated on Slanetz & Bartley agar (Oxoid) with 32 mg/L vancomycin and incubated at 41°C for 48h. Presumptive positive colonies were selected and confirmed as *Enterococcus* spp. by phenotypic characterisation and negative catalase test. The isolates were identified to the species level as *E. faecalis* or *E. faecium* and tested for the *vanA* gene using PCR (Clark et al. 1993, Dutka-Malen et al. 1995).

Susceptibility testing
Isolates were tested for antimicrobial susceptibility at NVI, Oslo. MIC values were obtained using the VetMIC™ microdilution method (Dep. of Antibiotics, National Veterinary Institute, Sweden). Epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 20.02.2014) were used, except for ciprofloxacin for *E. coli*, and trimethoprim for *S. pseudintermedius*. For these, and for additional antimicrobial agents not defined in the EUCAST recommendations, cut-off values were defined on the basis of the actual MIC distributions obtained in the NORM-VET programme (see also Appendix 6). *S. pseudintermedius* isolates that were erythromycin resistant and clindamycin susceptible on the VetMIC™ plate, were further investigated for inducible clindamycin resistance using disk diffusion with clindamycin and erythromycin (EUCAST recommendations).

Quality assurance systems
The following susceptible bacteria were included as quality controls on a regular basis: *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213. The following resistant bacteria were tested on a regular basis: *E. coli* CCUG 37382, *E. coli* K8-1 (ESBL), *E. coli* K5-20 (AmpC), *E. faecium* CCUG 33829. The results were approved according to reference values given by EUCAST when available. Additional control strains were included when necessary. The laboratories at NVI are accredited according to the requirements of NS-EN ISO/IEC 17025 and participate in quality assurance programmes for veterinary pathogens (Veterinary Laboratories Agency Quality Assurance Unit. Loughborough, UK) and for resistance monitoring (EURL for Antimicrobial Resistance in Denmark).

Data processing
Susceptibility data were recorded and stored in the sample registration system at NVI as discrete values (MIC). Data management and analysis was performed in SAS Enterprise guide 4.3 (SAS Institute Inc., Cary, NC, USA) and the 95% confidence intervals were calculated by the exact binomial test using R v. 2.15.21 (R Development Core Team, 2012).

Appendix 4:
Sampling, microbiological methods and data processing of zoonotic and non-zoonotic enteropathogenic bacteria in NORM and NORM-VET

Sampling strategy - animals

Salmonella
Samples from animals were collected according to the Norwegian *Salmonella* control programme for live animals. Additional isolates were obtained from clinical submissions or necropsies at the Norwegian Veterinary Institute (NVI). One isolate of each serovar per incident was included for susceptibility testing.

Campylobacter jejuni
Caecal samples were collected from positive flocks of broiler in the Norwegian action plan against *Campylobacter* (www.vetinst.no). One isolate from each flock was included for susceptibility testing.

Sampling strategy - humans

All human isolates of *Salmonella, Yersinia enterocolitica* and *Shigella* were obtained from clinical specimens. One isolate per patient or one isolate per recognised outbreak was included for susceptibility testing.

Campylobacter isolates from a selection of a little less than 10% of campylobacteriosis cases registered were submitted in the following way: Five regional laboratories submitted the first five independent isolates each month to the NRL for Enteropathogenic Bacteria at the Norwegian Institute of Public Health.

Isolation and identification of bacteria
Isolation and identification of *Salmonella* spp. from animals was carried out at the NVI according to ISO 6579:2002/Amd.1:2007: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.

The reference analyses on human clinical isolates of enteropathogenic bacteria were performed according to conventional methods described in standard reference literature (e.g. Murray PR & al.: Manual of Clinical Microbiology, 8th edition ASM-Press, Washington 2003 and Ewing WH: Edwards and Ewing's Identification of *Enterobacteriaceae*, 4. edition, Elsevier, New York 1986).

Susceptibility testing
Isolates from animals were tested for antimicrobial susceptibility at NVI, Oslo. MIC values were obtained using the VetMIC™ microdilution method (Dep. of Antibiotics, National Veterinary Institute, Sweden).

Salmonella spp., *Yersinia* spp. and *Shigella* spp. isolates from humans were susceptibility tested at the NRL for Enteropathogenic Bacteria at the NIPH by agar disk diffusion tests according to the EUCAST standardised method for AMR testing of non-fastidious bacteria. *Campylobacter* isolates from humans were tested for antimicrobial susceptibility using MIC Test Strips (Liofilchem).

For animal isolates, epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 20.02.2014) were used. For additional antimicrobial agents not defined in the EUCAST recommendations, cut-off values were defined on the basis of the actual MIC distributions obtained in the NORM-VET programme (see also Appendix 6).

For human isolates EUCAST clinical or epidemiological breakpoints for Enterobacteriaceae, version 4.0 2014 were used if established, otherwise local epidemiological cut-off values were used.

Quality assurance systems
NVI and the Reference Laboratory for Enteropathogenic Bacteria/NIPH have a quality assurance system according to the requirements of NS-EN ISO/IEC 17025. The participating laboratories at NVI are accredited according to the requirements of NS-EN ISO/IEC 17025 and participate in external quality assurance programmes for veterinary pathogens (Veterinary Laboratories Agency Quality Assurance Unit Loughborough, UK) and for resistance monitoring (EURL for Antimicrobial Resistance in Denmark).

The NRL for Enteropathogenic Bacteria at the NIPH is accredited according to the requirements of NS-EN ISO/IEC 17025. *E. coli* ATCC 25922 was used as quality control strain for AMR testing of non-fastidious Enterobacteriaceae. The NRL participated in the external quality assessment programme for *Salmonella* spp. and antimicrobial susceptibility testing organized by ECDC.

Data processing
Susceptibility data were recorded and stored in the sample registration system at NVI. The susceptibility data were stored as discrete values (MIC). Data management and analysis was performed in SAS v 9.4 (SAS Institute Inc., Cary, NC, USA) and the 95% confidence intervals were calculated by the exact binomial test using R v. 2.15.21 (R Development Core Team, 2012).

The NRL at the NIPH stored susceptibility data of human isolates as either millimeter zone diameters or MIC values. Data analysis was performed in SPSS version 20 (SPSS Inc. Chicago, USA).

Appendix 5:
Sampling, microbiological methods and data processing in NORM

General considerations

NORM is based on a combination of periodic sampling and testing in primary diagnostic laboratories and annual results from national reference laboratories for specific microoganisms. Isolates are included from defined clinical conditions such as respiratory tract infections, wound infections, urinary tract infections, and septicaemiae. For enteric infections see Appendix 4. 2013 was the fourteenth year of surveillance, and all 21 diagnostic laboratories in Norway participated in the surveillance system in addition to seven reference laboratories. All diagnostic laboratories followed the same sampling strategy and used identical criteria for the inclusion of microbial strains. Only one isolate per patient and infectious episode was included unless otherwise stated. All microbes were identified using conventional methods as described in the ASM Manual of Clinical Microbiology. The surveillance period started in the beginning of January, and consecutive isolates were included for defined time periods for each surveillance category. The surveillance categories and sampling periods in 2013 were as follows: *E. coli* in blood cultures (6 months); *Klebsiella* spp., *Staphylococcus aureus* and *Enterococcus* spp. in blood cultures (9 months); *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Neisseria meningitidis* and *Candida* spp. from blood cultures and cerebrospinal fluids (12 months); *S. pyogenes* from respiratory tract specimens and wounds (3 weeks); *S. aureus* from wound specimens (1 week); *E. coli* from urinary tract infections (2 days); *Klebsiella* spp. from urinary tract infections (3 weeks); *Mycobacterium tuberculosis* and *Neisseria gonorrhoeae* from all samples (12 months).

S. pneumoniae, S. pyogenes, H. influenzae and *N. meningitidis* from blood cultures and cerebrospinal fluids were analysed at the the Norwegian Institute of Public Health in Oslo. *Candida* spp. isolates from blood cultures were analysed at Oslo University Hospital, Rikshospitalet. *N. gonorrhoeae* isolates were characterised at Oslo University Hospital, Ullevål. MRSA isolates were analysed at St. Olav University Hospital in Trondheim. ESBL-producing *Enterobacteriaceae* were genetically characterised at University Hospital of North Norway in Tromsø. *M. tuberculosis* was further analysed at the Norwegian Institute of Public Health and Oslo University Hospital (Ullevål and Rikshospitalet).

Susceptibility testing

E. coli, *Klebsiella* spp., *Enterococcus* spp. and *S. aureus* isolates were examined according to the EUCAST disk diffusion standard using antibiotic disks and Mueller Hinton II agar from either Oxoid or Beckton Dickinson. Suitable antibiotics were selected for each bacterial species, and the results were interpreted according to the breakpoints of the Norwegian Working Group on Antibiotics (NWGA). The NWGA breakpoints are harmonised with EUCAST. All *S. aureus* isolates were tested for beta-lactamase production by nitrocefin disks, acidometric agar plates (3.6 mg/L penicillin G and phenol red) or the clover leaf test. *Enterococcus* spp. isolates were screened for glycopeptide resistance using a vancomycin 6 mg/L BHI agar. *S. pneumoniae*, *S. pyogenes*, *H. influenzae*, *N. meningitidis* and *N. gonorrhoeae* isolates were susceptibility tested using MIC gradient tests (bioMerieux or Liofilchem) on MH II agar supplemented with 5% lysed horse blood, or GC agar with 1% haemoglobin and Isovitalex (*N. gonorrhoeae*). Susceptibility testing of *Candida* spp. isolates was performed by MIC gradient tests using RPMI agar containing 2% glucose and MOPS. Resistance values were recorded either as mm inhibition zone sizes or MIC values in order to monitor trends in the occurrence of resistance.

For *M. tuberculosis*, all isolates were tested using BACTEC MGIT 960 systems. All three test laboratories participate in the WHO external DST quality control programme. The same three laboratories, St. Olavs Hospital and Haukeland University Hospital also perform tests for mutations in the *rpoB* gene to detect rifampicin resistance.

Confirmation of resistance phenotypes

E. coli and *Klebsiella* spp. with reduced susceptibility to 3rd generation cephalosporins were examined for ESBL production using ESBL combination MIC gradient tests according to the instructions of the manufacturer (Liofilchem). ESBL positive strains from blood cultures were subjected to PCR and DNA sequencing for determination of ESBL genotype. *S. aureus* isolates with reduced susceptibility to cefoxitin were examined by *mecA* PCR for confirmation of methicillin resistance (MRSA). *Enterococcus faealis* and *E. faecium* isolates displaying growth on the vancomycin screening agar were examined by *van* PCRs. Erythromycin resistant *S. pneumoniae*, *S. pyogenes* and *S. aureus* isolates were analysed for determination of MLS phenotype using the double disk diffusion (DDD) synergy assay with erythromycin and clindamycin disks.

Quality control

The following strains were used for quality control: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 (ESBL positive), *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *S. pneumoniae* ATCC 49619, *S. pneumoniae* TIGR4, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 (heterogeneous MRSA), *S. aureus* CCUG 35600 (homogeneous MRSA), *N. gonorrhoeae* CCUG 41811, *C. albicans* ATCC 90028.

Data processing

The specially designed eNORM computer programme was used for the registration and storage of patient data, sample data and resistance data. The results were further analysed by WHONET 5.3 with the aid of the BacLink programme, both developed by Dr. John Stelling. The distribution of microbial species in blood culture was based on extraction of routine data from the laboratory information systems of the participants. All isolates of the same species recovered within one month after the initial finding were considered duplicates and omitted from the survey. No attempts were made to evaluate the clinical significance of each finding.

Appendix 6:
Definitions and classification of resistances used in this report

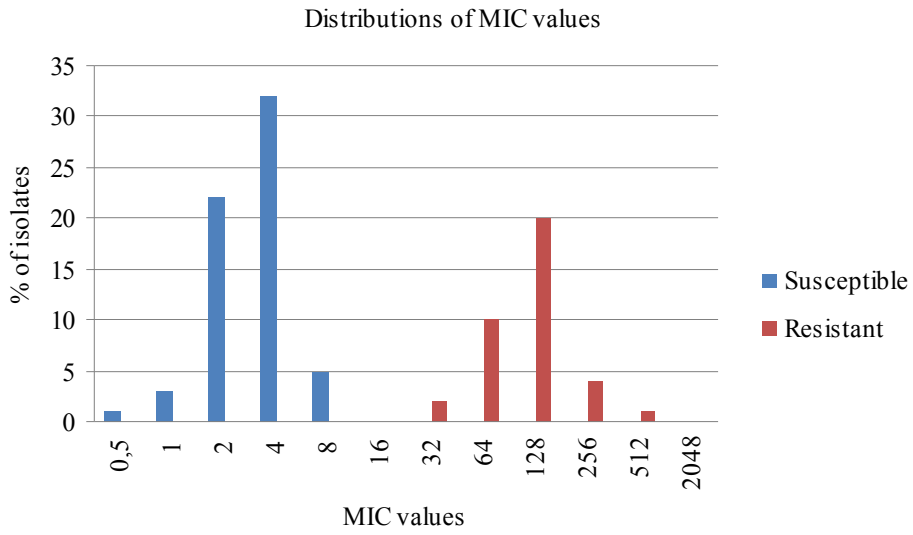
General guidelines for the reader of this report

The results presented in the NORM and NORM-VET programme are not directly comparable. This is because the sampling between the programmes and also the classification of resistance differ between the programmes. For the classification within NORM, clinical breakpoints are used and for the classification within NORM-VET epidemiological cut-off values are used. EUCAST definitions of clinical breakpoints and epidemiological cut-off values are presented at the web page: http://www.srga.org/Eucastwt/eucastdefinitions.htm.

The terms and usage of these two ways of classification of resistance are further explained below. The epidemiological breakpoint would normally be lower for MIC values and higher for disk diameters than the clinical breakpoins. However this is not always the case.

Epidemiological cut-off values

The epidemiological cut-off values are mainly used by epidemiologists and could indicate emerging resistance in the bacterial populations. Based on the distribution of the minimum inhibitory concentration (MIC) or the inhibition zone diameter distribution, each bacterial population could (in an ideal case) be divided into two populations by a biphasic curve as shown in the example below. The curve to the left (blue) shows the susceptible or wild type distribution whereas the curve to the right (red) shows the resistant or non-wild type distribution. The green line indicates a possible epidemiological cut-off value applicable to the distributions in the example.



However, for several bacterial populations and corresponding tested antimicrobial substances these distributions may be overlapping. A part of the population within the overlapping area may carry resistance mechanisms and others not. In the area with the non-wild type distribution, new resistance mechanisms are responsible for the resistance either alone or in addition to the resistance mechanisms present at lower MIC values. In order to establish MIC values for each specific bacterial population and antimicrobial agent, large amounts of data are collected and assessed. In the NORM-VET part of this report we have mainly used the epidemiological cut-off values recommended by EUCAST. However, for some combinations of bacteria and antimicrobial agents these were not applicable to our data, which probably are influenced by the VETMIC plates provided by the manufacturer. In these cases epidemiological cut-off values defined on the basis of the actual MIC distributions obtained in the NORM-VET programme were used.

Clinical breakpoints

Clinical breakpoints are defined in order to indicate if a treatment of a specific pathogen is likely to succeed or not. Other factors like dosage and formulations also affect the clinical result. The MIC values are ideally obtained for the pathogen *in vitro*, and this is compared with the predetermined clinical breakpoint to determine whether the organism is likely to respond *in vivo*.

Term used to describe antimicrobial resistance levels

In this report the level of resistance in the NORM-VET programme have been classified according to the levels presented in The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012 by EFSA Journal 2014; 12(3):3590 as follows:

Rare:	<0.1%
Very Low:	0.1% to 1%
Low:	>1% to 10%
Moderate:	>10% to20%
High:	>20% to 50%
Very high:	>50% to 70%
Extremely high:	>70%

Appendix 7: Cut-off values NORM-VET

Epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 20.02.2014) were used. For additional antimicrobial agents not defined in the EUCAST recommendations, cut-off values were defined on the basis of the actual MIC distributions obtained in the NORM-VET programme.

Antimicrobials	Resistant MIC (mg/L)	*Campylobacter jejuni*	*Salmonella* spp.	*Escherichia coli*	*Enterococcus faecium*	*Enterococcus faecalis*	*Staphylococcus pseudintermedius**
Ampicillin	> 4				■	■	
	> 8		■	■			
Bacitracin	> 32				■	■	
Cefotaxime	> 0.25			■			
	> 0.5		■				
Ceftazidime	> 0.5			■			
	> 2		■				
Cefalothin	> 1						■
Chloramphenicol	> 16		■	■			■
	> 32				■	■	
Ciprofloxacin	> 0.06		■	■			
	> 0.5	■					
	> 1						■
Clindamycin	> 0.25						■
	> 0.5						
Colistin	> 2		■	■			
Erythromycin	> 1						■
	> 4	■			■	■	
Florfenicol	> 16		■	■			
Fusidic acid	> 0.5						■
Gentamicin	> 2	■	■	■			■
	>32				■	■	

Antimicrobials	Resistant MIC (mg/L)	*Campylobacter jejuni*	*Salmonella* spp.	*Escherichia coli*	*Enterococcus faecium*	*Enterococcus faecalis*	*Staphylococcus pseudintermedius**
Kanamycin	> 8			■			■
	> 16		●				
	> 1024				●	●	
Linezolid	>4				■	■	
Nalidixic acid	> 16	■	■	■			
Narasin	> 2					■	
	> 4				■		
Oxacillin	> 2						■
Sulfamethoxazole	> 64			■			
	> 256		●				
Streptomycin	> 4	■					
	> 16		■	■			
	> 128				■		
	> 512					■	
Tetracycline	> 1	■					■
	> 4				■	■	
	> 8		■	■			
Trimethoprim	> 2		■	■			
	> 8						●
Vancomycin	>4				■	■	
Virginiamycin	>4				■		
	>32					■	

Squares: Cut-off values recommended by EUCAST
Filled circles: Cut-off values not defined by EUCAST - defined on the basis of the actual MIC distributions obtained in the NORM-VET programme.
*For *Staphylococcus pseudintermedius* the cut-off values for *Staphylococcus aureus* was used.

Appendix 8: Breakpoints NORM

NORM data are categorised according to the breakpoints of the Norwegian Reference Group on Antibiotic Susceptibility Testing (NWGA) which are harmonised with EUCAST breakpoints. NWGA breakpoints are available at www.antibiotikaresistens.no.

Antimicrobials	MIC (mg/L) S	MIC (mg/L) R	*Escherichia coli*	*Klebsiella* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp	*Haemophilus influenzae*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
Amphotericin B	≤ 1	> 1							■							■	■	■	■
Ampicillin	≤ 4	> 8											■						
	≤ 8	> 8	■		■		■												
						■[#]													
Amoxi-Clav*	≤ 2	> 2																	
	≤ 32	> 32	■	■					■										
Anidulafungin	≤ 0.002	> 4																	■
	≤ 0.03	> 0.03														■			
	≤ 0.06	> 0.06															■	■	
Azithromycin	≤ 0.25	> 0.5									■								
Cefaclor									■[#]										
Cefepime	≤ 1	> 4	■	■															
Cefixime	≤ 0.125	> 0.125									■								
Cefoxitin												■[#]							
Cefotaxime	≤ 0.125	> 0.125							■										
	≤ 0.5	> 2												■					
	≤ 1	> 2	■	■															
Ceftaroline	≤ 1	> 1										■							
Ceftazidime	≤ 1	> 4	■	■															
Ceftriaxone	≤ 0.125	> 0.125							■	■	■								
	≤ 0.5	> 2												■					
Cefuroxime	≤ 1	> 2							■										
	≤ 8	> 8	■	■															
Chloramphenicol	≤ 2	> 2							■										
	≤ 2	> 4								■									
	≤ 8	> 8			■		■							■					
						■[#]													
Ciprofloxacin	≤ 0.032	> 0.064									■								
	≤ 0.5	> 0.5						■	■										
	≤ 0.5	> 1	■	■	■		■												
	≤ 1	> 1										■							
						■[#]													
Clindamycin	≤ 0.25	> 0.5										■							
	≤ 0.5	> 0.5												■	■				
Erythromycin	≤ 0.25	> 0.5												■	■				
	≤ 1	> 2										■							
	≤ 4	> 4						■											
Fluconazole	≤ 0.002	> 32															■		
	≤ 2	> 4														■		■	■

Antimicrobials	MIC (mg/L)		*Escherichia coli*	*Klebsiella* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp.	*Haemophilus influenzae*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
	R	R																	
Fusidic acid	≤ 1	> 1										■							
Gentamicin	≤ 1	> 1										■							
	≤ 2	> 2						■#											
	≤ 2	> 4	■	■															
	≤ 128	> 128											■						
Imipenem	≤ 4	> 8											■						
Linezolid	≤ 4	> 4										■	■						
Mecillinam	≤ 8	> 8	■	■															
Meropenem	≤ 2	> 8	■	■															
Micafungin	≤ 0.002	> 2																	■
	≤ 0.016	> 0.016														■			
	≤ 0.032	> 0.032															■		
Mupirocin	≤ 1	> 256										■#							
Nalidixic acid					■#	■#	■#	■#	■#										
Nitrofurantoin	≤ 64	> 64	■																
Norfloxacin	≤ 4	> 4										■#							
Oxacillin														■#					
Penicillin G	≤ 0.064	> 0.25								■									
	≤ 0.064	> 1									■								
	≤ 0.064	> 2												■					
	≤ 0.25	> 0.25													■				
									■#										
Pip-Tazo**	≤ 8	> 16	■	■															
Rifampicin	≤ 0.06	> 0.5										■							
	≤ 0.25	> 0.25								■									
Spectinomycin	≤ 64	> 64									■								
Tetracycline	≤ 1	> 2							■			■		■	■				
	≤ 2	> 2						■											
					■#	■#	■#												
Tigecycline	≤ 0.25	> 0.5											■						
	≤ 0.5	> 0.5										■							
	≤ 0.5	> 1									■								
	≤ 1	> 2	■																
Trimethoprim	≤ 2	> 4	■	■															
TMS***	≤ 0.5	> 1							■										
	≤ 1	> 2												■	■				
	≤ 2	> 4	■	■	■		■					■							
						■#													
Vancomycin	≤ 2	> 2																	
	≤ 4	> 4											■						
Voriconazole	≤ 0.125	> 0.125														■		■	■

Epidemiological cut-off value based on the wild type distribution by EUCAST. * Amoxi-Clav= Amoxicillin-Clavulanic acid. ** Pip-Tazo=Piperacillin-Tazobactam. *** TMS Trimethoprim-sulfamethoxazole. Breakpoints for the e combination are given for the trimethoprim component only.

Appendix 9: References used in this report

- Angeletti, S., Lorino, G., Gherardi, G., Battistoni, F., Cesaris, M., Dicounzo, G., 2001. Routine Molecular Identification of Enterococci by Gene-Specific PCR and 16S Ribosomal DNA sequencing. J. Clin. Microbiol., 39 vol 2: 794-797.
- Briñas L, Zarazaga M, Sáenz Y, Ruiz-Larrea F, Torres C. Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob Agents Chemother. 2002 Oct; 46(10): 3156-63.
- Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob Agents Chemother. 1993 Nov; 37(11): 2311-7.
- Dutka-Malen, S., Evers, S., Courvalin, P., 1995. Detection of Glycopeptide Resistance Genotype and Identification to the Species Level of Clinically Relevant *Enterococci* by PCR. J. Clin. Microbiol., 33: 24-27.
- Dutka-Malen, S., Evers S., Courvalin, P., 1995. *ERRATUM:* Detection of Glycopeptide Resistance Genotype and Identification to the Species Level of Clinically Relevant Enterococci by PCR. J. Clin. Microbiol., 33(5): 1434.
- Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother. 2005 Jul; 56(1): 115-21.
- Heininger, A., Binder, M., Schmidt, S., Unertl, K., Botzenhart, K., Döring G. PCR and Blood Culture for Detection of *Escherichia coli* Bacteriemia in Rats. J Clin Microbiol. 1999 August; 37(8): 2479–2482.
- Multiplex PCR for the detection of the *mec*A gene and the identification of *Staphylococcus aureus* (http://www.crl-ar.eu/233-protocols.htm)
- NORM/NORM-VET 2004. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2014. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2007. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2014. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2008. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2014. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2011. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2014. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2012. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2014. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002 Jun; 40(6): 2153-62.
- Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotika A, Kawakami T, Fukata T, Hiramatsu K. 2010. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol 2010 Mar; 48(3): 765-9.
- Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new mecA homologue $mecA_{LGA251}$. CMI. 2011 18, 395-400.

ISSN: 1502-2307 (print) / 1890-9965 (electronic)

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