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HYVINVOINNIN LAITOS

Marianne Gunell, Antti Hakanen, Janne Aittoniemi,
Jaana Kauppila, Kaisu Rantakokko-Jalava,
Anne-Mari Rissanen, Kerttu Saha, Martti Vaara,
Risto Vuento, Pentti Huovinen, Antti Nissinen
(Editors)

DISCUSSION PAPER

Antimicrobial Resistance in Finland

Finres 1997–2010

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Antimicrobial Resistance in Finland

Finres 1997–2010



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Editors: Marianne Gunell, Antti Hakanen, Janne Aittoniemi, Jaana Kauppila, Kaisu Rantakokko-Jalava, Anne-Mari Rissanen, Kerttu Saha, Martti Vaara, Risto Vuento, Pentti Huovinen ja Antti Nissinen.

Authors: Marianne Gunell, Antti Hakanen, Marjo Haanperä, Pentti Huovinen, Jari Jalava, Juha Kirveskari, Merja Marjamäki, Antti Nissinen, Kaisu Rantakokko-Jalava, Merja Rantala, Anne-Mari Rissanen, Kerttu Saha, Anja Siitonen, Hanna Soini, Martti Vaara, Risto Vuento, Jaana Vuopio ja Monica Österblad.

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Foreword

In your hand is a report collated by the Finnish Study Group for Antimicrobial Resistance (FiRe). It comprises antimicrobial resistance data on clinically important bacteria, collected during 14 years. The FiRe network was founded in the beginning of the 1990's, to collect specieswise susceptibility data from the Finnish clinical microbiology laboratories. The collection of data started in 1997, a year after all the laboratories had adopted the FiRe susceptibility testing standard, which corresponded to the CLSI standard, with some minor modifications. From this year onwards, susceptibility data has been collected into yearly reports of varying format, which have been distributed to the FiRe members and the partners from the pharmaceutical industry. The only previous print version was published in 1999. The FiRe laboratories adopted the EUCAST standard in the beginning of 2011, and this was therefore a good time to publish a summary of the FiRe-standard years, 1997-2010. This time period was used in the title; however, large-scale surveillance has been done for many bacteria during a much longer time. To give a better view of resistance development, we encouraged authors to also refer to other material. Thus an extensive review, comprising very varying timespans, on the susceptibility of the most significant pathogens in Finland, was put together.

In this report, the resistance development of 11 of the clinically most important pathogens is presented, as well as tuberculosis and salmonella data based on other surveillance systems. A presentation of the history and future of FiRe, and a description of the Finres database are also included.

Globally, the resistance situation has worsened, and the increase of multidrug resistant strains has made susceptibility testing an important part of successful and safe patient care. In the future, empirical antimicrobial treatment will be guided more and more by a knowledge of the local resistance situation. This report strives to respond to this need of knowledge. The situation in Finland is comparable to that in other Nordic countries, and good compared to the South and Eastern Europe; but ongoing surveillance and strategic planning are the prerequisites for stopping new resistance mechanisms from spreading among clinically important pathogens also here in Finland. Here, the voluntary surveillance done by the FiRe-laboratories is in a key position.

November 2012, Finland

The Authors

Preface to the English translation

This translation was done as a co-work by a number of the authors. In addition to translating the text, some minor corrections and clarifications were added.

October 2013,

The translators

Abstract

FiRe. Antimicrobial Resistance in Finland – Finres 1997–2010. National Institute for Health and Welfare (THL). Discussionpaper 43/2013. 61 pages. Helsinki, Finland 2013.

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In this report, we have presented resistance trends for 11 of the most clinically important pathogens collected in the Finres database between the years 1997 and 2010. The resistance situation among *Salmonella* spp. and *Mycobacterium tuberculosis* is also included. In addition, this report includes chapters on the history and future of FiRe, and a description of the Finres-database.

Antimicrobial resistance in *Streptococcus pneumoniae* has increased steadily during the previous decade. Macrolide resistance is so common that macrolides cannot be recommended as a first line treatment of pneumococcal infections. Although the number of penicillin-non-susceptible (I+R) strains is high, the proportion of highly penicillin-resistant strains of *S. pneumoniae* has remained low.

β -Lactamase mediated ampicillin resistance in *Haemophilus influenzae* has remained on a low level, and amoxicillin-clavulanate is still effective for the treatment of *H. influenzae* infections.

Moraxella catarrhalis –strains have remained susceptible to amoxicillin-clavulanate, and it is almost always susceptible to those *per os* antimicrobials (macrolides, tetracyclines and trimethoprim-sulfa) that can be used as alternatives for beta-lactams.

Group A streptococci are always penicillin-susceptible, but resistance to other antimicrobials do occur. Erythromycin resistance in *S. pyogenes* has markedly gone down since the 1990's, whereas clindamycin resistance has slightly increased, but is still at a low level.

A major reason for the increase in antimicrobial resistance in *Escherichia coli* is the increasing number of extended-spectrum beta-lactamase (ESBL) producing strains. The increase of β -lactam and fluoroquinolone resistance is worrying. Nitrofurantoin and mecillinam have remained effective against *E. coli* strains and only a few percent of the strains are resistant.

Despite the increasing trend in ESBL producing *Klebsiella pneumoniae* strains, cephalosporin, fluoroquinolone, aminoglycoside, and carbapenem resistance among *Klebsiella* spp. has remained low during the whole study period and there are no signs of increase.

Carbapenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter* spp. varies, whereas multi-drug resistance is a significant clinical problem. Carbapenem resistance in *P. aeruginosa* is increasing and needs to be carefully followed. Bacterial strains resistant to almost all available antimicrobials (PDR) are very rare in Finland, and so far MDR/PDR strains have only been detected as single findings or small outbreaks.

In Finland, the first **MRSA** hospital epidemics occurred in 1991 and until the mid-90's around 100 MRSA cases were detected annually. The number of MRSA cases increased sharply from 1997 to 2004, but this increase did not continue from 2005 onwards. The susceptibility to antimicrobials used for *Staphylococcus aureus* infections is good.

Of the β -lactams, only ampicillin, piperacillin and carbapenems are effective against *Enterococcus* spp. *Enterococcus faecalis* is almost always susceptible to these antimicrobials, whereas over 80 % of clinical *Enterococcus faecium* isolates are ampicillin resistant. Due to β -lactam resistance, vancomycin is the most important antimicrobial for the treatment of severe *E. faecium* infections. The first Finnish vancomycin-resistant *E. faecium* strains were discovered in 1992. The highest percentage (15 %) of vancomycin-resistant *E. faecium* was detected in 1997 (mainly due to a local outbreak) and thereafter vancomycin resistance has risen over 1 % only in 2000.

The first ciprofloxacin-resistant *Neisseria gonorrhoeae* strains in Finland were discovered in 1995 and ten years later the percentage of fluoroquinolone resistance was already over 50 %. Ceftriaxone however, is still clinically effective against *N. gonorrhoeae* infections. So far all clinical samples have been ceftriaxone-susceptible in Finland, but strains more resistant than the wild-type have started to appear.

The proportion of highly ciprofloxacin-resistant (MIC \geq 4 mg/l) *Salmonella enterica* strains have remained low (0–3 %) but the proportion of strains with reduced susceptibility (MIC \geq 0.125 mg/l) has markedly increased among both domestic and foreign *Salmonella* isolates. The reduced fluoroquinolone

susceptibility has increased mostly among strains acquired from Southeast Asia. This is worrisome since these reduced fluoroquinolone-susceptible isolates often also are ESBL-producers, making the treatment of severe *Salmonella* infections even more challenging.

The antimicrobial susceptibility of *Mycobacterium tuberculosis* strains isolated in Finland has remained good. The majority of MDR strains are isolated from patients with a foreign background, which reflects the increasing immigration from countries with a high incidence of tuberculosis. Resistance to second line tuberculosis antimicrobials is rare, and XDR strains which are resistant to nearly all tuberculosis antimicrobials have not yet been detected in Finland.

Keywords: FiRe, Finres, antimicrobial resistance, reduced susceptibility, bacteria

Authors of the Finres 1997–2010 report

The FiRe story

Antti Hakanen (THL), Antti Nissinen (KSKS) and Pentti Huovinen (TY, THL)

Description of the Finres –material

The FiRe-network

Marianne Gunell, Antti Hakanen (THL) and Antti Nissinen (KSKS)

Antimicrobial susceptibility testing

Monica Österblad, Antti Hakanen (THL) and Antti Nissinen (KSKS)

The collection of Finres data

Marianne Gunell (THL), Antti Nissinen (KSKS) and Kerttu Saha (SeKS)

Bacterial isolates and sample types in the Finres-report

Marianne Gunell (THL) and Antti Nissinen (KSKS)

Antimicrobial resistance in clinically important bacteria

Streptococcus pneumoniae

Merja Rantala (HY) and Jari Jalava (THL)

Haemophilus influenzae

Antti Nissinen (KSKS) and Risto Vuento (Fimlab)

Moraxella catarrhalis

Antti Nissinen (KSKS) and Risto Vuento (Fimlab)

Streptococcus pyogenes

Pentti Huovinen (TY) ja Jari Jalava (THL)

Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter spp.

Jari Jalava, Monica Österblad (THL), Martti Vaara, Juha Kirveskari (HUSLAB) and Anne-Mari Rissanen (ISLAB/KYS)

Staphylococcus aureus

Kaisu Rantakokko-Jalava (TYKSLAB) and Jaana Vuopio (THL, TY)

Enterococcus spp.

Kaisu Rantakokko-Jalava (TYKSLAB) and Jaana Vuopio (THL, TY)

Neisseria gonorrhoeae

Antti Nissinen (KSKS) and Antti Hakanen (THL)

Resistance surveillance based on research projects and other material

Salmonella

Marianne Gunell, Anja Siitonen and Antti Hakanen (THL)

Mycobacterium tuberculosis

Hanna Soini, Merja Marjamäki and Marjo Haanperä (THL)

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1 The FiRe story

Scientists have warned of antimicrobial resistant bacteria almost as long as antimicrobials have been in use. In Finland, antimicrobial susceptibility of bacterial isolates has been tested with the disc diffusion method since the 1960's, when the first laboratory method for routine susceptibility testing was published (1). In the 1970's and 1980's the first long-term susceptibility test results were collected and published at least in Turku and Helsinki. At the same time the first signs of antimicrobial resistance with clinical importance were discovered.

The first comparable study performed simultaneously in several geographical areas was the antimicrobial susceptibility study of otitis and tonsillitis pathogens, which was started in 1987 in KTL (The National Public Health Institute). In addition to being the basis of the thesis of hospital microbiologist Antti Nissinen, this study was the start of the studies on macrolide resistance in Group A streptococcus (*Streptococcus pyogenes*). During the collection of these Group A streptococcal isolates, Helinä Järvinen, a specialist in KTL in Turku, noticed the much higher resistance levels in Kaarina and Pöytyä (Turku district) compared to other areas (2). At a research group meeting in 1989, it was decided to expand the surveillance of erythromycin resistance in Group A streptococci to cover the whole of Finland, and therefore all clinical microbiology laboratories in Finland were invited to participate (3,4).

The first official FiRe (Finnish Study Group for Antimicrobial Susceptibility Testing) meeting was held in 1992 in Helsinki. Practically all Finnish clinical microbiology laboratories which perform antimicrobial susceptibility testing have participated in the FiRe network since the beginning (5). After the first meeting, FiRe has gathered together regularly twice a year, for an annual meeting and educational workshops on current topics on susceptibility testing and resistance surveillance. Participation in these meetings has always been abundant.



FiRe's 10th Anniversary meeting



Pentti Huovinen, the chair



The FiRe group in 2011

Administration

The first FiRe meeting set up a board to take care of FiRe's functions and chief physician Pentti Huovinen (KTL) was elected as chair. Hospital microbiologist Antti Nissinen (KSKS) was elected as secretary and coordinator. The other members of the first FiRe board were associate chief physician Marja-Leena Katila (KYS), specialist Heikki Hiekkaniemi (PKKS) and chief physician Martti Vaara (HYKS –laboratory diagnostics). This board was elected twice again and was in duty for ten years. In 2002, Martti Vaara was elected as chair and new members for the board were chief physician Henrik Jägerroos (LKS), specialist Ulla Kärkkäinen (KYS) and associate chief physician Risto Vuento (TAYKS). Heikki Kaukoranta and Antti Nissinen continued on the board, Antti Nissinen also as a coordinator. From the beginning the goal has been that the board should have representatives from all over the country, both from small and large FiRe-laboratories, as well as from the National Public Health Institute (KTL). Between 2005 and 2008 the chair of the board was chief physician Risto Vuento and board members were Antti Nissinen, Henrik

Jägerroos, Ulla Kärkkäinen and chief microbiologist Pauliina Kärpänoja (PHKS), hospital microbiologist Eveliina Tarkka (HUSLAB) and Jaana Vuopio-Varkila (KTL) as the new members.

In 2007, chief physician Antti Hakanen (KTL) became the coordinator, and since 2008 Antti Nissinen has been the chair of the FiRe board. Between 2008 and 2011 the board members were Henrik Jägerroos, Antti Hakanen, Ulla Kärkkäinen, Eveliina Tarkka, Risto Vuento and as new members, specialist Jaana Kauppila (OYS) and hospital microbiologist Päivi Suomala (MKS). Since 2011, in addition to the chair and coordinator, the members of the board have been specialist Janne Aittoniemi (Fimlab), Jaana Kauppila, specialist Kaisu Rantakokko-Jalava (TYKSLAB), specialist Anne-Mari Rissanen (ISLAB/KYS), hospital microbiologist Kerttu Saha (SeKS) and Martti Vaara. Since 2011 there has also been a secretary on the board, first senior researcher Miika Bergman (THL) and in 2012 senior researcher Marianne Gunell (THL). Since the beginning, the FiRe laboratory network has been “served” by the Antimicrobial Resistance Laboratory (KTL), from 2009 named the Antimicrobial Resistance Unit (THL). The FiRe network gather together for an annual meeting and educational workshops twice a year, and new board is elected every third year.

Table 1.1: The FiRe administration

Chairmen:	City	Years
Pentti Huovinen	Turku	1992–2002
Martti Vaara	Helsinki	2002–2005
Risto Vuento	Tampere	2005–2008
Antti Nissinen	Jyväskylä	2008–
FiRe-coordinators:		
Antti Nissinen	Jyväskylä	1992–2007
Antti Hakanen	Turku	2007–
FiRe -secretary:		
Miika Bergman	Turku	2011
Marianne Gunell	Turku	2012

Partners in cooperation – the financial basis of FiRe

During the years, FiRe has had 17 different pharmaceutical companies as partners in cooperation. With these companies FiRe has negotiated a cooperation contract, which has covered the costs of organizing meetings for a couple of years at a time. At the moment FiRe has cooperation contracts (via THL) with three pharmaceutical companies (Astellas Pharma, Orion Pharma and Pfizer). The cooperation contract has given the companies access to an annually published resistance map of the clinically most important bacterial isolates in Finland. This resistance map was called ‘Finres’, which is a descriptive and easily understood name both in Finland and abroad. It later got its own web site, www.finres.fi, which now also is the official FiRe web page. Between 2009 and 2012 FiRe has also got development project money from the Ministry of Social and Affairs and Health (STM) Appropriation for Surveillance of Infectious Diseases. The FiRe laboratories, however, have not received any extra funding for their surveillance activities.

The FiRe web page

FiRe has its own web page, www.finres.fi, for internal and external use. It contains information on current topics such as meetings and updates to standards. The most essential content are the guidelines on methods and interpretations for the use of laboratories, and the latest Finres-results. There is also a link to the extranet pages, available only to the FiRe network: they contain presentations from the meetings, and the Finres database tool. The www.finres.fi pages are on the THL web domain, and are updated by the Antimicrobial Resistance Unit (THL).

The harmonizing of breakpoints and the Finres-report

Collating a national antimicrobial resistance map based on routine material from the clinical laboratories was – in retrospect – a brilliant idea. The brilliance of this lay in the idea that the data was collected without any special clauses, so that every FiRe laboratory could participate straight from the beginning. To our knowledge this is unique: routine data from clinical laboratories are still not collected in any other country. The reason behind this is probably the mistrust of the quality of antimicrobial susceptibility testing. FiRe was aware of this weakness, but ensuring the quality and comparability of the results from each laboratory would have been such a massive and ungratifying work that we would still be in the starting blocks. While collecting the data as it was, we produced a map where every laboratory could compare their own data to others. Being part of this network in itself encourages laboratories to improve the quality of their own susceptibility results. In addition, when comparing one's results to others, deviations that might be caused by flawed methodology could be spotted; this would not have been possible without the pooled national data. In 1997, the first Finres-report was put together, after the nationwide adoption of a common susceptibility standard, which was completed the year before. This standard was based on the US CLSI (former NCCLS) standard.

At first, Finres was compiled by hand by Katrina Lager (KTL, Turku). During the 1990's, the FiRe laboratories gradually started to use WHONET, which is a free computer software provided by WHO, to collect and analyze their antimicrobial resistance results. This enabled FiRe to produce Excel-based reports: this method was used in 2005 and 2006. The mainly handmade Finres-report became history when the Finres data management project started in 2008, and the Finres database was created. This database and analysing tool was made by senior designer Piiia Peltola (KTL, Helsinki), who worked under the supervision of data administration manager Jaason Haapakoski, and this project was financed by FiRe. The Finres database was used during 2007-2011. In 2012, the Finres2 database project started. The aim of this project is to get automated and better analyzing and reporting tools. This project is financed by an Infectious Diseases Surveillance grant from the Ministry of Social Affairs and Health.

Although the most important task of the FiRe-network is to produce resistance surveillance data for clinicians, the Finres material has also been used in nine doctoral theses (Table 1.2) and about 30 scientific publications (Appendix 1). In addition to antimicrobial susceptibility testing, the link between antimicrobial consumption and resistance has been studied (6, 7, 8, 9).

Table 1.2: Doctoral theses which are based on or have used Finres material

Year	Author	University	Title of thesis
1994	Tuula Arstila	Turku	Beta-lactam resistance among <i>Escherichia coli</i> and <i>Klebsiella species</i> .
1994	Helena Seppälä	Turku	<i>Streptococcus pyogenes</i> ; erythromycin resistance and molecular typing.
1995	Antti Nissinen	Helsinki	Antimicrobial resistance of four major respiratory bacterial pathogens, <i>Streptococcus pyogenes</i> , <i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , and <i>Moraxella catarrhalis</i> .
1996	Tiina Leistevuo	Turku	Antimicrobial agents in the elderly: resistance of fecal aerobic gram-negative bacilli in the geriatric hospital and the community.
1998	Raija Manninen	Turku	Determination of antimicrobial resistance in five major clinical bacterial pathogens.
2000	Janne Kataja	Turku	Molecular epidemiology and resistance genes of macrolide-resistant beta-hemolytic streptococci.
2002	Marja Pihlajamäki	Turku	Antimicrobial resistance in <i>Streptococcus pneumoniae</i> , with special reference to macrolide resistance.
2009	Merja Rantala	Helsinki	Antimicrobial resistance in <i>Streptococcus pneumoniae</i> in Finland with special reference to macrolides and telithromycin.
2009	Sofia Forssten	Turku	Genetic basis and diagnostics of extended-spectrum beta-lactamases among <i>Enterobacteriaceae</i> in Finland.

The EUCAST-standard and the future of FiRe

Since the beginning of 2011, all FiRe laboratories use the common European EUCAST susceptibility testing standard. At the moment of writing, Finland was the only country in Europe where all laboratories used the EUCAST-standard, according to an international quality control questionnaire. The reason for the change of standard was the perception that EUCAST is a more up to date and more frequently updated standard than CLSI. The transition was facilitated by the unanimous decision by the FiRe group, and the long common history of harmonizing susceptibility testing methods. EUCAST is a project which is commonly financed by the European Centre for Disease Prevention and Control (ECDC) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the aim of EUCAST is to maintain the antimicrobial susceptibility testing standard as well as being a networking body for National Antimicrobial Susceptibility Testing Committees (NACs). FiRe is the official NAC of Finland.

Discussions and aiming for common goals have been peculiar to FiRe. Therefore it is not a surprise that the FiRe-meetings have been so popular during these 20 years. At these meetings, not only the findings of the Finres-report or current resistance issues are discussed, but also quality and comparability issues of susceptibility testing. After every meeting, the participants return home, having learned new things and being ever more convinced about the importance of this collaboration. It is a good basis to build on during the following years and decades.

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2 Defining the Finres data

2.1 The FiRe network

Right from the start, all Finnish clinical microbiology laboratories that perform susceptibility testing have been active in the network. At present FiRe is comprised of 24 laboratories (Table 2.1) and the bacteriological units of the National Institute for Health and Welfare. At most there have been 27 FiRe-laboratories, but as a result of fusions (Jorv hospital was merged with HUS in 2006, Medix PLC and Yhtyneet laboratories PLC were merged into Yhtyneet Medix Laboratories PLC in 2010) and other changes (Oulun Diakonissalaitos stopped doing susceptibility testing) the number was 24 in 2010. The majority are laboratories serving central hospitals (15) and university hospitals (5). In addition to these, also one university laboratory (Turku University) and three private laboratories are members. The 20 central and university hospital laboratories, who do blood cultures, have also participated in the ECDC-coordinated resistance surveillance programme (European Antimicrobial Resistance Surveillance Network – EARS-Net) from the start in 2010. Before this, 17 FiRe-laboratories took part in the EU-financed programme preceding this (European Antimicrobial Resistance Surveillance System – EARSS), which was coordinated by the Dutch National Institute for Public Health and the Environment (RIVM) during the years 1999-2009.

Table 2.1: The FiRe-laboratories in 2010

FiRe-laboratory	City	Hospital district
HUSLAB	Helsinki	Helsinki and Uusimaa
Fimlab	Tampere	Pirkanmaa
TYKSLAB	Turku	Varsinais-Suomi
OYSLAB	Oulu	Pohjois-Pohjanmaa
KESLAB/ Central Finland Central Hospital	Jyväskylä	Keski-Suomi
ISLAB/KYS	Kuopio	Pohjois-Savo
SataDiag/ Satakunta Central Hospital	Pori	Satakunta
Päijät-Häme Social and Health Group	Lahti	Päijät-Häme
Seinäjäki Central Hospital	Seinäjäki	Etelä-Pohjanmaa
Carea/ Kymenlaakso Central Hospital	Kotka	Kymenlaakso
Kanta-Häme Central Hospital	Hämeenlinna	Kanta-Häme
Vaasa Central Hospital	Vaasa	Vaasa
ISLAB/ Pohjois-Karjala Central Hospital	Joensuu	Pohjois-Karjala
Eksote/ Etelä-Karjala Central Hospital	Lappeenranta	Etelä-Karjala
Lappi Central Hospital	Rovaniemi	Lappi
ISLAB/ Mikkeli Central Hospital	Mikkeli	Etelä-Savo
Keski-Pohjanmaa Central Hospital	Kokkola	Keski-Pohjanmaa
Länsi-Pohja Central Hospital	Kemi	Länsi-Pohja
ISLAB/Savonlinna Central Hospital	Savonlinna	Itä-Savo
Kainuu Central Hospital	Kajaani	Kainuu
Yhtyneet Medix Laboratoriot PLC*	Espoo	
UTULab, Turku University *	Turku	
Vita-terveyspalvelut PLC*	Helsinki	
General practice Pulssi/ Terveystalo *	Turku	

*Do not participate in the EARS-Net surveillance.

2.1.1 Denominator data for the FiRe-laboratories

So far, the FiRe-network has not regularly collected data which could be used to eg. calculate resistance prevalence in relation to population size. However, already EARSS, and its successor EARS-Net have collected such data yearly from the participating laboratories. This data tells the coverage of each laboratory, if the laboratory serves one or several hospitals, and the number of blood cultures per year. Table 2.2 presents the data collected for 2010 (or 2011 if the numbers for 2010 were not available).

Table 2.2: Denominator data for the FiRe-laboratories

Clinical microbiology laboratory	Geographical area to serve	Population coverage	Total number of laboratory samples/year	Total number of blood cultures /year
HUSLAB	Helsinki and Uusimaa hospital district	1 500 000	1 160 000	91 000
Fimlab	Pirkanmaa hospital district	489 000	477 600	33 381
TYKSLAB	Varsinais-Suomi hospital district	470 000	187 000	25 419
OYSLAB	Pohjois-Pohjanmaa hospital district	400 000	114 000	28 500
KESLAB/ Central Finland Central Hospital	Keski-Suomi hospital district	273 000	130 251	15 085
ISLAB/ KYS	Pohjois-Savo hospital district	248 000	217 855	20 277
SataDiag/ Satakunta Central Hospital	Satakunta hospital district	225 000	128 495	14 844
Päijät-Häme Social and Health Group	Päijät-Häme hospital district	210 000	127 334	10 000
Seinäjäki Central Hospital	Etelä-Pohjanmaa hospital district	198 671	106 171	8 868
Careal/ Kymenlaakso Central Hospital	Kymenlaakso s hospital district	175 000	111 107	11 242
Kanta-Häme Central Hospital	Kanta-Häme hospital district	170 000	71 000	11 500
Vaasa Central Hospital	Vaasan hospital district	166 500	100 000	5 600
ISLAB/ Pohjois-Karjala Central Hospital	Pohjois-Karjala hospital district	160 000	110 000	9 500
Eksote/ Etelä-Karjala Central Hospital	Etelä-Karjala social and health district and Imatra city	130 000	100 353	9585
Lappi Central Hospital	Lappi s hospital district	118 000	77 443	9 955
ISLAB/ Mikkeli Central Hospital	Etelä-Savo hospital district	105 952	46 141	6 090
Keski-Pohjanmaa Central Hospital	Keski-Pohjanmaa hospital district	100 000	50 600	5 389
Kainuu Central Hospital	Kainuu region	78 000	48 900	5 000
Länsi-Pohja Central Hospital	Länsi-Pohja hospital district	65 000	26 361	4 636
ISLAB/ Savonlinna Central Hospital	Itä-Savon hospital district	45 000	15 902	3 437
Yhtyneet Medix Laboratoriot PLC	Whole Finland		128 500	-
UTULab, Turku University	Whole Finland, inc. Åland		120 000	-
Vita-terveyspalvelut PLC	Whole Finland		72 000	-
General practice Pulssi/ Terveystalo				-

2.2 Antimicrobial susceptibility testing

Antimicrobials either kill the microbe, or hinder its growth. All antimicrobials are not effective against all bacteria at the concentrations that can be achieved clinically. If all, or almost all, strains of a certain species are resistant to an antimicrobial, the species is intrinsically resistant. If the species usually is susceptible, but some strains develop resistance, they have acquired resistance. This can be measured in several ways. Phenotypic methods are used to see if the bacteria can grow in the presence of the antimicrobial.

In 1996, FiRe decided to introduce harmonised national breakpoints, based on the CLSI (then NCCLS) breakpoints, published in the USA. The CLSI standard was translated into Finnish, with some minor changes to suit local needs. This translation was named the FiRe-standard for domestic use – in international contexts the name CLSI was used. It was in use 1997-2010. It was updated in concert with the CLSI standard, with 1-2-year intervals. At the time, the CLSI standard was the best defined and updated standard available; it is still widely used also in Europe. In 2010, the European EUCAST standard was introduced, and by joint decision this was adopted by the FiRe-laboratories in the beginning of 2011.

The susceptibility testing standard defines the testing conditions to be used (growth medium, antibiotic concentrations of disks, the density of the bacterial suspension to be applied, incubation time and atmosphere), and the breakpoints used to interpret a strain as susceptible or resistant. The breakpoints are species-, antibiotic- and method-specific, and are published as tables. The tables of the FiRe-standard can be found on the FiRe webpages (www.finres.fi). The EUCAST tables are freely available at the EUCAST homepage (www.eucast.org).

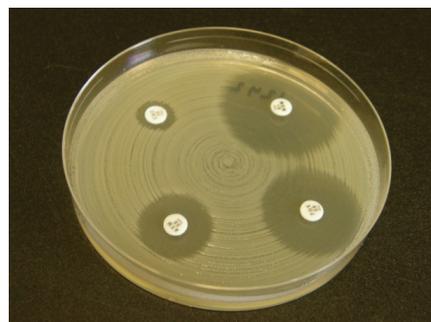
To set the breakpoints, EUCAST considers all of the following:

1. In what form the antimicrobial is administered.
2. The most common dosages.
3. Clinical indications and target organisms.
4. Species-specific distributions of susceptibility testing results, to determine the epidemiological cut-off (“ECOFF”) between resistant isolates and those without acquired resistance.
5. Pharmacokinetics and –dynamics
6. Information from modelling processes (eg. Monte Carlo simulations to assess the likelihood of achieving pharmacodynamic targets).
7. Outcome of treatment.
8. Ensuring that known resistance mechanisms are found.

Based on these facts, bacterial isolates are divided into resistant (R), intermediate (I), and susceptible (S).

2.2.1 The disk diffusion method

In disk diffusion, a bacterial suspension is spread evenly over the surface of an agar plate. The density most often used is McFarland 0.5, which is a turbidity standard roughly corresponding to 10^8 bacteria/mL in a 0.9 % NaCl solution. Filter paper disks containing the antimicrobials to be tested are placed on the plate. The antibiotic diffusing from the disk forms a gradient, which stabilizes in a couple of hours. For some antibiotics, this method does not work; the antibiotic does not dissolve sufficiently into the agar, or diffuses too slowly because of a large molecular mass. In these cases, other methods have to be used. The plates are placed in an incubator, the bacteria multiply,



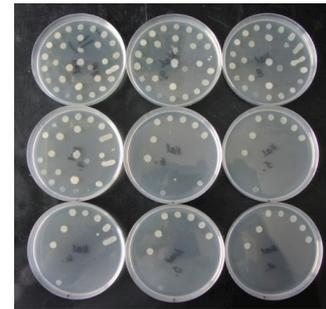
and gradually the growth becomes visible. The bacteria are usually unable to grow in the antibiotic-containing area close to the disk. This inhibition zone is inversely correlated to the smallest concentration of antibiotic that inhibits growth (or the MIC value, see below).

The disks used by FiRe and EUCAST are 6 mm in diameter, and a maximum of six disks can be placed on a standard 90 mm agar plate. After the incubation (usually 18 ± 2 h, $35\pm 2^\circ\text{C}$), the diameter of the inhibition zone is measured (in millimetres), and compared to the breakpoint table.

The perhaps most critical step in disk diffusion is the determination of the edge of the zone: exactly where does the growth stop? Usually the result is clear, but occasionally the edge is fuzzy, or there are separate colonies within the inhibition zone. In these cases, harmonised rules of interpretation are especially important. EUCAST has published a reading guide covering the most common cases (www.eucast.org).

2.2.2 MIC methods

The smallest concentration of antibiotic that inhibits growth, or minimum inhibitory concentration (MIC) can be determined by exposing the bacteria to stepwise increases in antibiotic concentration. After an overnight incubation (18 ± 2 h), the MIC is read as the lowest concentration of antibiotic where the bacteria are unable to grow.



The agar dilution method

A series of agar plates are made, where the antibiotic is added to the agar, and the concentration halves for each batch (eg. 64-32-16-8-4-2-1 mg/L). Bacterial suspensions are made similarly to the disk diffusion method. For each isolate, one drop (1-3 μL) is transferred to each plate in the series, and the plates are incubated overnight. This method is useful for studying many isolates at a time; 30-96 isolates can be fitted into one series (depending on inoculator and plate size).

The broth dilution method

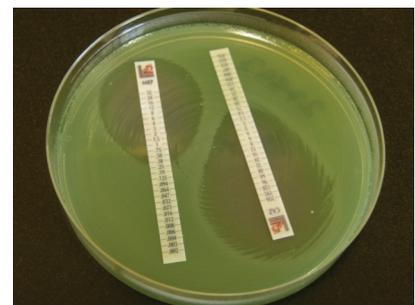


A series of dilutions of the antibiotic is made, using the same principle as in agar dilution, but broth is used instead of agar. Previously, test tubes were used (“macrodilution”), but nowadays microtiterplates are used (microdilution). One isolate is inoculated per well series. This method is the ISO-standardised reference method (“gold standard”) of antimicrobial susceptibility testing (ISO 20776).

The gradient strip method (eg. Etest)

There are commercial tests using paper or plastic strips, to which an antibiotic has been applied in such a way, that a gradient forms in the agar, when it is placed on a plate. The strips have printed MIC scales. The MIC is read at the intersection of the edge of the inhibition zone and the strip.

The gradient strip method works well, if the manufacturer has been able to calibrate the strip against the reference method for all concentrations. If no strains with the highest values were available when the calibration was done, results at the upper end of the strip can diverge significantly from the reference method. Even though the strip tests are based on agar diffusion, similarly to the disk diffusion method, the gradient on the strip largely bypasses the diffusion problems encountered in disk testing for some antibiotics.



Automated methods

Automated systems for identification and susceptibility testing have become common in clinical microbiology laboratories during the 2000's. Some systems use the microdilution principle, and these correspond most closely to the reference method. To save costs, the dilution series can sometimes be very short, which eg. can cause low-level resistance to go undetected, if the lowest concentration is too high. Other systems measure the growth dynamics of the isolates in the presence and absence of antibiotics, and calculate the MIC. Longer MIC series are possible, since the system can extrapolate between measurement points. This method, and its results, deviates more from the reference system.

2.3 The collection of Finres-material

2.3.1 Clinical samples and bacteria

The FiRe laboratories analyse the greater part of all clinical microbiology samples in Finland. These isolates comprise the Finres database. Bacteria that are tested only at local secondary hospitals, health care centres and general practice clinics are not included. Most of these are from urinary tract infections.

This does not have any remarkable effect on geographical or infection type variation in Finres-database. All laboratories perform sample type -specific cultures, using the Association of Finnish Local and Regional Authorities nomenclature. Clinically important bacterial findings are identified to species level, and when indicated, standard antimicrobial susceptibility testing (see chapter 2.2) is performed.

2.3.2 Antimicrobial susceptibility results

Antimicrobial susceptibility test results, the diameter of the inhibition zone (mm) or MIC-result (mg/l) is entered into the laboratory information system (LIS; e.g. Effic Mikrobiologia, SAMBA) as a feature of the bacterial finding. In some laboratories, the diameter of the inhibition zone is not measured if the diameter is so large/small that it can safely be directly interpreted as S/R. In that case, the result is only S or R, not the numerical value. This method is also used for parallel findings (blood culture). If the susceptibility testing is performed with an automated method (for example Vitek 2), MIC-values are automatically transferred to the LIS. Results from disc diffusion and E-tests are read and entered manually. The interpretation of susceptibility test results (S=susceptible, I=intermediately resistant, R=resistant), which are reported to the nursing unit, is based on the standard used (see chapter 2.2).

2.3.3 Resistance data files – the Finres-database

The FiRe laboratories convert their data from the laboratory database into a WHONET-compatible dBASE file, containing all clinically interesting bacterial strains and their antimicrobial susceptibility results (only results, not interpretations are included) and patient and sample information. The free WHONET software (www.whonet.org), designed for analysis of bacterial resistance, is in use in every FiRe laboratory. From this data file, the laboratories extract the subset of results that are included in the Finres database, and patient identifications are encrypted. These either monthly or annually collected files are exported to the Finres data system (Figure 2.1.) The data system combines the data files into a single national database file. Antimicrobial susceptibility results are transferred into the Finres database in the same form as they are in the WHONET file.

2.3.4 The Finres-report

According to international tradition (1) only the first bacterial isolate per patient of a certain sample type within a certain time scale (usually one year) are included in the Finres-report. FiRe laboratories use various methods for encrypting patient's identifier data, i.e. the encrypted identifier from a certain patient can be different if entered from different laboratories. Therefore it has been agreed that the first finding per patient and laboratory is included. The total number of tested isolates, the number of resistant isolates, and the resistance percentage with a 95% confidence interval are presented in the Finres-report. The resistance result from a certain laboratory is included in the Finres-report only if they have tested more than 50 % of their isolates against a certain antimicrobial, to avoid biasing caused by broader testing of certain types of subpopulations.

2.3.5 Quality assurance

Quality assurance has an important role in ensuring validity and comparability of susceptibility results. According to GLP (Good Laboratory Practice), in every clinical microbiology laboratory each new batch of reagents (growing media, antimicrobial disc/ -strip and MIC-card/ -well plate for automated readers) is tested with control strains recommended by CLSI (or EUCAST since 2011).

Comparability of results between different laboratories is best ensured by external quality assessment. External quality assessment services are provided by the domestic Labquality Ltd., and the British UKNEQAS. The FiRe laboratories also annually participate in the EARS-Net External Quality Assessment exercise, where laboratories are asked to determine the antimicrobial susceptibility of six EARS-Net pathogens (*S. pneumoniae*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *E. faecium*).

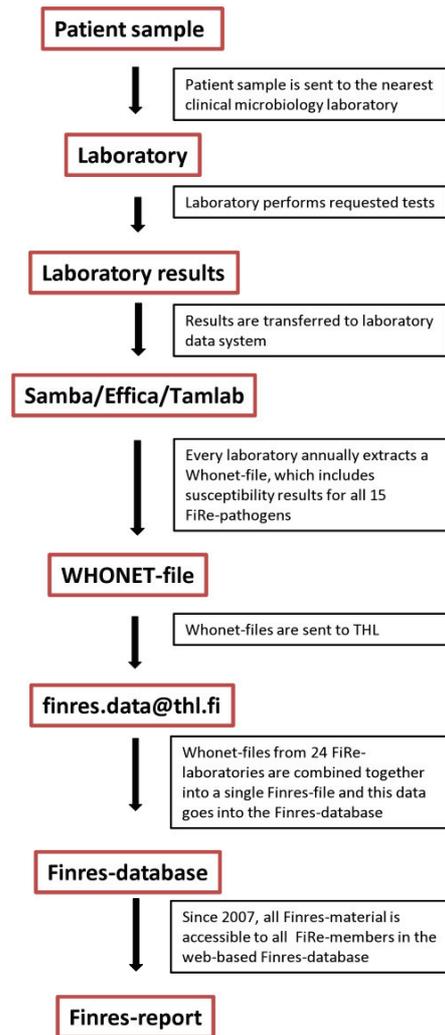


Figure 2.1: The collection of Finres-material

References:

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2.4 Bacterial isolates and sample types in the Finres-report

Annually collected antimicrobial susceptibility data on 15 of the clinically most important bacteria has been aggregated into Finres reports since 1997. Data has been collected from bacterial strains isolated from clinical samples, and susceptibility results have been produced by routine laboratory methods. An extensive collection of resistance data from all Finnish clinical laboratories started in 1997 when the FiRe laboratories had adopted a common standard for susceptibility testing. At the beginning, bacterial findings were divided into hospital and health care center isolates. This segmentation however, appeared to be too vague and therefore in 2005, the FiRe board decided to abandon it. At that time practically all laboratories had started sending their data in the WHONET format, which enabled a more precise and reliable classification (e.g. based on sample type and patient age). The number of bacterial isolates in the Finres-database has increased year by year; in 2010 the Finres-database included over 400 000 bacterial isolates and over 3 million antimicrobial susceptibility test results. The numbers of isolates of each bacterial species are found in Table 2.4.

For this Finres 1997–2010 –report, resistance data has been collected from the following bacteria-antimicrobial agent –combinations (the Finres pathogens *Klebsiella oxytoca*, *Enterobacter cloacae* and *Neisseria meningitidis* are not included.).

Streptococcus pneumoniae. Until 2004, Finres contains susceptibility data from all clinical *S. pneumoniae* findings. Since 2005, susceptibility data has been collected separately from blood isolates, and pus isolates collected from children under and over 5 years. Susceptibility data is collected for the following antimicrobials: oxacillin (screening test for penicillin resistance), erythromycin, clindamycin, tetracycline and trimethoprim-sulfa. Oxacillin-susceptible strains are interpreted as penicillin-susceptible, but a penicillin MIC result is always required if the strain is oxacillin-resistant.

Haemophilus influenzae. Since 1997, susceptibility data from all clinical *H. influenzae* isolates have been gathered for the following antimicrobials: ampicillin, amoxicillin-clavulanate, tetracycline and trimethoprim-sulfa. In addition, the β -lactamase production of the strain is noted. β -Lactamase-positive strains are always interpreted as ampicillin-resistant. If the strain is ampicillin-resistant but β -lactamase negative, the ampicillin susceptibility is based on the MIC result. In these cases, ampicillin resistance is interpreted as resistance to amoxicillin-clavulanate as well.

Moraxella catarrhalis. Since 1997, susceptibility data from all clinical *M. catarrhalis* isolates have been gathered for the following antimicrobials: ampicillin, amoxicillin-clavulanate, erythromycin, tetracycline and trimethoprim-sulfa. In addition, the β -lactamase production of the tested strain is taken into account. β -Lactamase positive strains are always interpreted as ampicillin-resistant.

Streptococcus pyogenes. Until 2004, Finres contains susceptibility data for all clinical *S. pyogenes* findings. Since 2005, susceptibility data has been collected separately from throat and pus isolates. Susceptibility data is collected for erythromycin and clindamycin.

Escherichia coli. Until 2004, Finres contains susceptibility data for all clinical *E. coli* findings, divided into hospital and health care center isolates. Since 2005, susceptibility data has been collected separately from blood and urine isolates, and divided into the patient groups under and over 75 years.

***Klebsiella* spp.** Until 2004, Finres contains susceptibility data from all *Klebsiella* findings, isolated from hospitals. Since 2005, susceptibility data has been collected from *Klebsiella pneumoniae*, isolated from urine and blood samples.

Susceptibility data on *E. coli* and *Klebsiella* spp. is collected for the following antimicrobials: For blood isolates ampicillin (*E. coli*), piperacillin-tazobactam, cefuroxime, cefotaxime/ceftriaxone, ceftazidime, imipenem/meropenem, tobramycin, netilmycin, norfloxacin/ofloxacin/ciprofloxacin/ levofloxacin and

trimethoprim-sulfa. For urine isolates mecillinam, cephalixin, cefuroxime, norfloxacin/ofloxacin/ciprofloxacin, nitrofurantoin, trimethoprim and trimethoprim-sulfa. If the laboratory identified the strain to be an ESBL producer, all 3rd generation cephalosporin S or I results are interpreted as R. This rule was changed after the adoption of the EUCAST standard.

Pseudomonas aeruginosa. Until 2004, Finres contains susceptibility data from all *P. aeruginosa* findings isolated from hospitals. Since 2005, susceptibility data has been collected from blood sample isolates. Susceptibility test data is presented for the following antimicrobials: piperacillin-tazobactam, ceftazidime, imipenem/meropenem, tobramycin and ciprofloxacin.

***Acinetobacter* spp.** Until 2004, Finres contains susceptibility data from all *Acinetobacter* spp. findings isolated from hospitals. Since 2005, susceptibility data has been collected from *Acinetobacter* sp. and *Acinetobacter baumannii* other than urine sample isolates. Susceptibility test data is presented for the following antimicrobials: imipenem/meropenem, tobramycin, netilmycin, ciprofloxacin/levofloxacin and trimethoprim-sulfa.

Staphylococcus aureus. Until 2004, Finres contains susceptibility data from all clinical *S. aureus* findings, divided into hospital and health care center isolates. Since 2005, susceptibility data has been collected separately from blood and pus isolates. Susceptibility data is presented for the following antimicrobials: oxacillin, erythromycin, clincamycin, tetracycline, trimethoprim-sulfa, tobramycin, netilmycin (blood isolates), vancomycin, rifampicin and fusidic acid.

***Enterococcus* spp.** Until 2004, Finres contains susceptibility data from all *E. faecalis* and *E. faecium* findings, isolated from hospitals. Since 2005, susceptibility data has been collected from all clinical *Enterococcus* spp. isolates without grouping to species level. Susceptibility data is presented for the following antimicrobials: ampicillin, vancomycin, teicoplanin and nitrofurantoin.

Neisseria gonorrhoeae. Finres contains susceptibility data from all clinical *N. gonorrhoeae* findings since 1997. Susceptibility data is presented for ciprofloxacin and cefotaxime/ceftriaxone.

Table 2.3: The annual variation in the number of bacterial isolates in the Finres-database (1997–2010)

Pathogen	Median	Mean value	Range
<i>S. pneumoniae</i>	4 703	4 742	4 217–5 845
<i>H. influenzae</i>	4 039	4 001	2 491–5 388
<i>M. catarrhalis</i>	1 936	2 058	1 559–2 731
<i>S. pyogenes</i>	11 962	13 088	8 007–21 058
<i>E. coli</i>	106 165	105 587	68 733–128 883
<i>Klebsiella</i> spp.	10 297	9 863	4 249–15 126
<i>P. aeruginosa</i>	8 166	9 120	7 091–13 455
<i>Acinetobacter</i> spp.	1 568	1 758	1 525–2 535
<i>S. aureus</i>	32 747	32 963	30 478–37 084
Enterococci	27 479	27 239	14 510–39 670
<i>N. gonorrhoeae</i>	163	155	100–219

3 Antimicrobial resistance in clinically important pathogens

3.1 Antimicrobial resistance in *Streptococcus pneumoniae* (1988–2010)

The pneumococcus

Typical infections:

- Pneumonia, otitis media, sinusitis, invasive infections (sepsis and meningitis)

The effect of the antimicrobial resistance situation on treatment options:

- The penicillin dose should be large and frequent enough
- Macrolides cannot be recommended for the treatment of pneumococcal infections without susceptibility testing

Streptococcus pneumoniae is one of the most common and important pathogens in our country. In Finland there are approximately 500 000 cases of otitis media annually (1) of which 26–60 % are caused by pneumococci (2). The incidence of community acquired pneumonia in different studies varies from 700 to 2000 cases / 100 000 inhabitants / year; pneumococci being the main cause (3). The incidence of invasive pneumococcal disease in Finland during the first decade of the 21st century has been 14–17 cases/100 000 inhabitants / year (4). The highest disease burden is focused on small children and elderly people. Invasive pneumococcal infections can be prevented by vaccination.

The FiRe network has followed the susceptibility of *S. pneumoniae* towards penicillin, erythromycin, clindamycin, tetracycline and trimethoprim-sulfa since 1988. When the surveillance started, resistance was practically non-existent. Since then, resistance to all of these antimicrobials has increased dramatically (Fig. 3.1), and there are no signs of a brighter future.

Penicillins

Penicillins are the most important antimicrobial group in the treatment of pneumococcal infections. FiRe has collected data separately for penicillin-resistant pneumococci (PEN R) and penicillin-non-susceptible (PEN I+R) pneumococci. In 1996, the proportion of penicillin-non-susceptible isolates was 6 %, and remained quite stable until 2000. Thereafter the proportion of PEN I+R pneumococci increased by approximately 1.3 % per year. In 2010, penicillin-non-susceptibility was detected in almost 20 % of all pneumococcal isolates (Fig. 3.1).

The proportion of penicillin-resistant *S. pneumoniae* has slightly increased since 2003, but has remained below 3 % until 2010, when this landmark was passed. The proportion of PEN R –strains isolated from blood and cerebrospinal fluid has remained under 2 % for the whole study period (Fig. 3.2).

Macrolides and lincosamides

Erythromycin resistance among *S. pneumoniae* isolates has increased substantially: between 1988 and 1990 less than 1 % of isolates were resistant whereas in 2009 already over 25 % were resistant (Fig. 3.1). Erythromycin resistance is even higher among pus isolates collected from small children (Fig. 3.3). Macrolide-resistant pneumococci are commonly penicillin non-susceptible and usually telithromycin susceptible (5).

Resistance to clindamycin is more uncommon than erythromycin resistance. Between 2000 and 2007, the proportion of clindamycin-resistant *S. pneumoniae* was 6–11 %, and in 2010 circa 13 %. Roughly one third of the erythromycin-resistant isolates are clindamycin-resistant. This correlates well with the most common macrolide resistance mechanism in Finland (5), which causes resistance to 14- and 15-membered

macrolides (i.e. erythromycin, roxithromycin, clarithromycin and azithromycin) but not to other macrolides, clindamycin and streptogramins.

Resistance to other antimicrobials

Resistance to tetracycline is as common as clindamycin resistance in *S. pneumoniae* isolates. Resistance to trimethoprim-sulfa is lower than to erythromycin (Fig. 3.1). Resistance to fluoroquinolones (levofloxacin, moxifloxacin) is still quite uncommon. In a *S. pneumoniae* material collected from the FiRe-laboratories in 2002, there were less than 2 % fluoroquinolone-resistant isolates (5) but in invasive isolates fluoroquinolone resistance has been detected in only sporadic cases (6).

Multidrug-resistance (non-susceptibility to penicillin, erythromycin and tetracycline) among blood and cerebrospinal fluid isolates was unknown at the end of the 1990's, and thereafter the proportion of multidrug-resistance has varied between 1.4 and 5.4 % (4). In pus, the proportion of multidrug-resistant isolates is somewhat higher (5). So far multidrug-resistant isolates have remained susceptible to fluoroquinolones and ceftriaxone (5, 6).

Summary

Antimicrobial resistance in *S. pneumoniae* has increased during the last decade. Macrolide resistance is so common that macrolides cannot be recommended for the treatment of *S. pneumoniae* infections without prior susceptibility testing. The good news is that penicillin resistance is still quite rare, and penicillins can be used for the treatment of non-invasive pneumococcal infections, as long as the proper dosage and frequency are used.

Antimicrobial resistance in *S. pneumoniae* varies among sample types, geographical location, bacterial clones and serotypes (5, 6). The hepta-valent pneumococcal conjugate vaccine was added to the national vaccination programme in autumn 2010. This vaccine covers up to 80 % of erythromycin resistant and penicillin-non-susceptible *S. pneumoniae* isolates (6). The conjugate vaccine is expected to decrease the number of invasive pneumococcal infections markedly. According to preliminary information, the number of severe pneumococcal infections among small children has already started to decrease. The future will show what effect the vaccine has on antimicrobial resistance in *S. pneumoniae*.

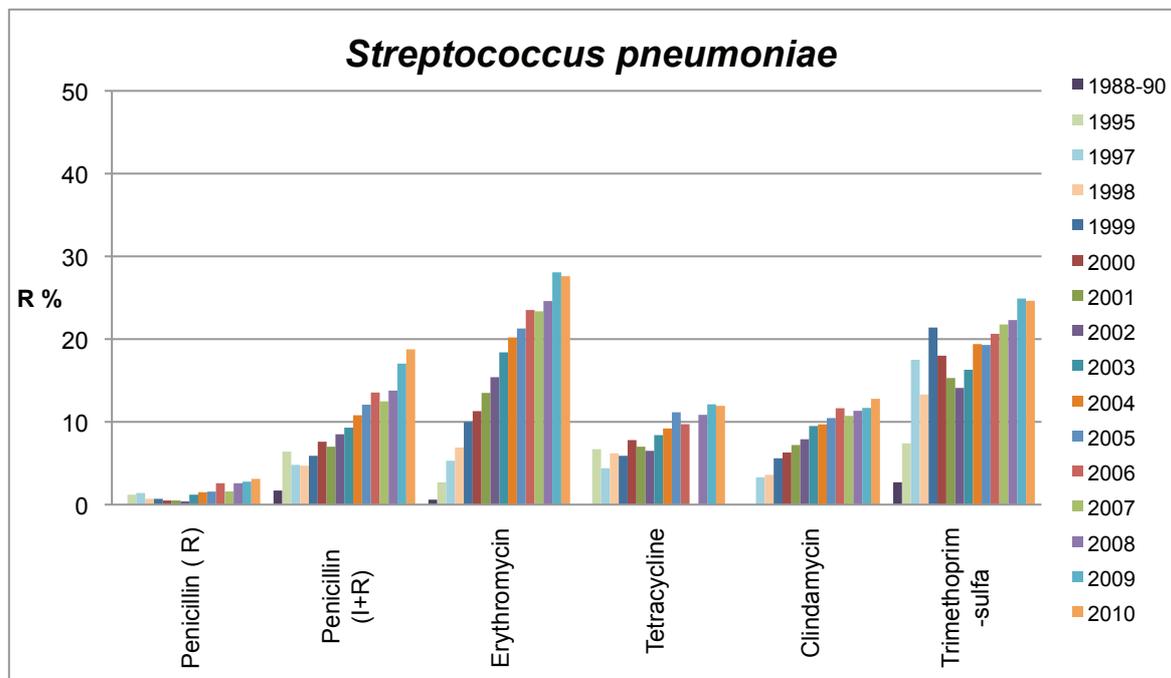


Figure 3.1: Antimicrobial resistance in *S. pneumoniae* between 1988 and 2010. PEN R: penicillin-resistant pneumococci, PEN I+R: penicillin-non-susceptible pneumococci (7,8).

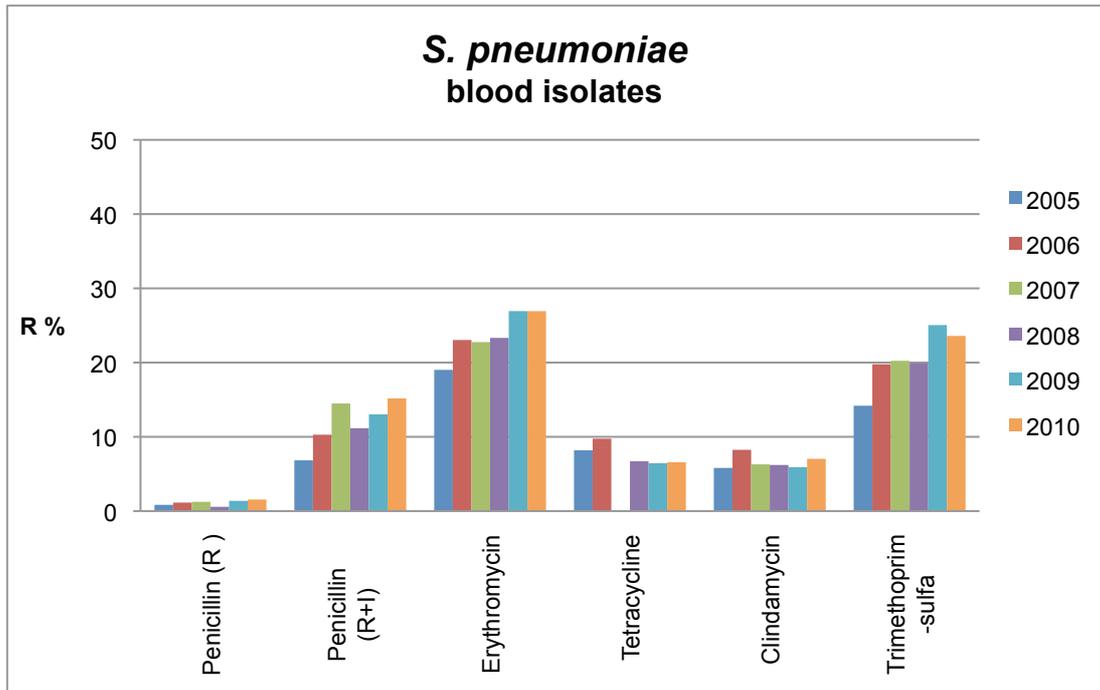


Figure 3.2: Antimicrobial resistance in *S. pneumoniae* blood isolates from 2005 to 2010.

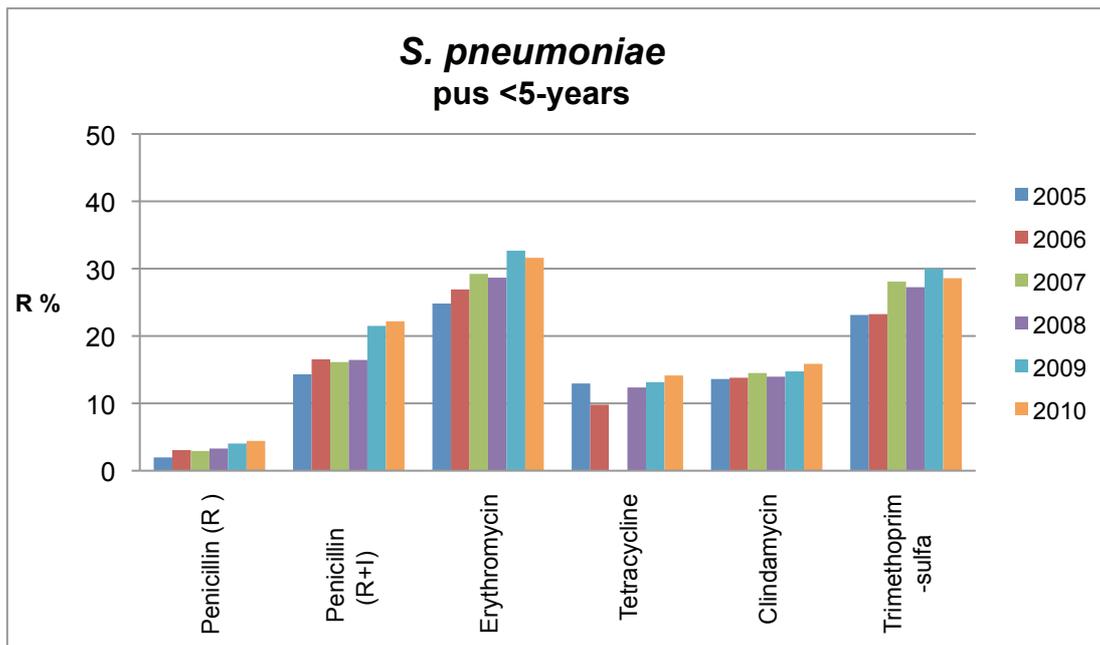


Figure 3.3: Antimicrobial resistance in *S. pneumoniae* pus isolates, collected from children under 5 years from 2005 to 2010.

References:

- (1) Niemelä M, Uhari M, Möttönen M and Pokka T. Costs arising from otitis media. *Acta Paediatr* 1999, 88, 553-556.
- (2) Kilpi T, Herva E, Kaijalainen T, Syrjänen R and Takala AK. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *Pediatr Infect Dis*, 2001, 20, 654-662.
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- (4) Tartuntataudit Suomessa 2010. Terveyden ja hyvinvoinnin laitos, Raportti 17/2011. <http://www.thl.fi/thl-client/pdfs/1d73f597-8188-4ff5-b33c-101d7e1c3e90>
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- (6) Siira L, Rantala M, Jalava J, Hakanen A, Huovinen P, Kaijanen T, Lyytikäinen O and Virolainen A. Temporal Trends of Antimicrobial Resistance and Clonality of Invasive *Streptococcus pneumoniae* Isolates in Finland, 2002 to 2006. *AAC*, 2009, 53, 2066-2073.
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- (8) Manninen R, Huovinen P, Nissinen A and the Finnish Study Group for Antimicrobial Resistance (FiRe). Increasing antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* in Finland. *J Antimicrob Chemother* 1997;40:387-392.

3.2 Antimicrobial resistance in *Haemophilus influenzae* (1978–2010)

H. influenzae

Typical infections:

- Otitis media, sinusitis, exacerbation of chronic obstructive pulmonary disease (COPD)

The effect of the antimicrobial resistance situation on treatment options:

- No effect at the moment

Nowadays *Haemophilus influenzae* is one of the most common causes of acute otitis media in small children, and acute sinusitis, along with *S. pneumoniae* and *Moraxella catarrhalis*. Septic infections and meningitis in small children, caused by serotype b (Hib) have become more uncommon due to the Hib-vaccination that was introduced into the national vaccination programme in the mid 1980's.

The most important and common antimicrobial resistance mechanism among *H. influenzae* is the beta-lactamase mediated ampicillin resistance. This resistance mechanism was described for the first time in the beginning of the 1970's, and in Finland in 1976 (1). Beta-lactamase mediated ampicillin resistance in *H. influenzae* is caused by the same TEM-1, or less frequently TEM-2, which cause ampicillin resistance also in *Escherichia coli*. Ampicillin resistance among *H. influenzae* spread rapidly and reached its present level (20 %, fig. 3.4) in the beginning of the 1980's (2, 3). It is on the same level elsewhere in the western industrialized world (6). Otitis media care guidelines recommending avoidance of eardrum perforation has been in use in Finland since the 1990's. This might have caused biasing of the collected material, since only complicated cases were cultured hereafter, and the bacteria might have been under selection pressure from previous antimicrobial treatments. Between 1994 and 1995, as preliminary pneumococcal vaccination research, 174 *H. influenzae* strains from unselected otitis samples were investigated in the Pirkanmaa hospital district, and this study showed that the prevalence of ampicillin resistance was 10 % among the first four infections and 30 % if the infant had had more than four infections (4).

Mutations in the penicillin-binding proteins (PBP) may also cause ampicillin resistance in *H. influenzae* isolates. These beta-lactamase-negative ampicillin-resistant (BLNAR) strains are also resistant to amoxicillin-clavulanate, and 2nd generation cephalosporins, which are not very efficient for the eradication of *H. influenzae* (5). The true prevalence of BLNAR strains is hard to get, since they have low-level resistance which is difficult to detect (a BLNAR strain resists only ten times more ampicillin than a susceptible strain). In Finland as well as in other industrialized countries the percentage of BLNAR strains has remained low at 1–2 % (6).

In addition to beta-lactam antimicrobials, *H. influenzae* infections can also be treated with doxycycline and trimethoprim-sulfa, whereas macrolides might not be effective (5) and according to EUCAST there is only a weak correlation between the MIC value and clinical effect. Tetracycline resistance is uncommon, but resistance to trimethoprim-sulfa has reached 15–20 % (Figure 3.5), in line with to other industrialized countries (6). Finnish Current Care Guidelines recommends the use of amoxicillin as the first line antimicrobial for the treatment of acute otitis media and sinusitis; this recommendation applies well to *H. influenzae* in the current resistance situation.

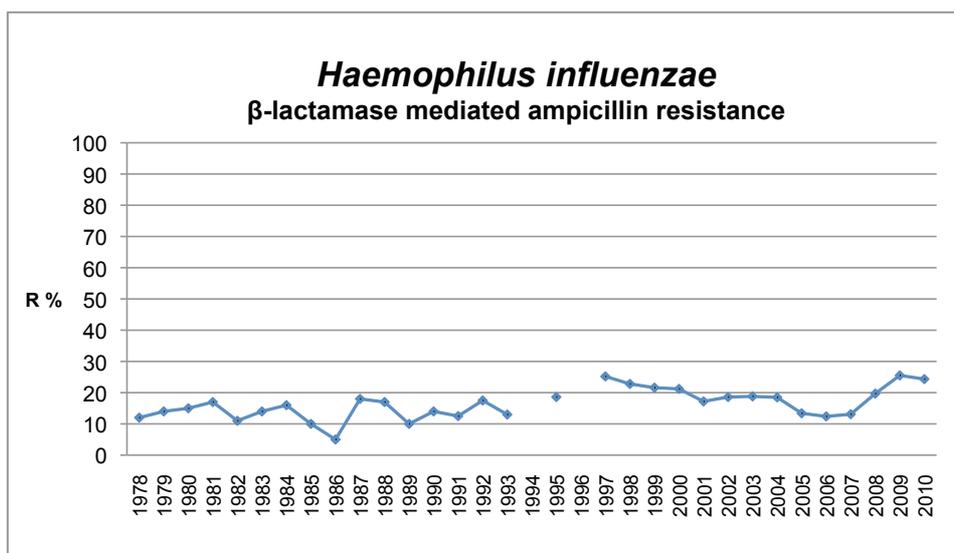


Figure 3.4: Beta-lactamase mediated ampicillin resistance in *H. influenzae* between 1978–2010 (2, 3).

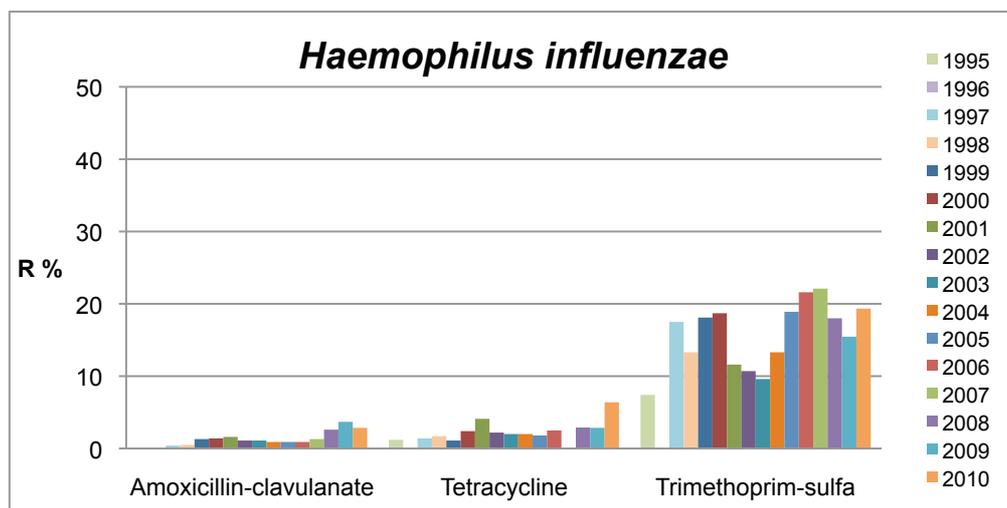


Figure 3.5: Antimicrobial resistance (other than beta-lactamase mediated) in *H. influenzae* between 1995 and 2010 (3).

References:

- (1) Siitonen A, Klossner M-L, Niemi E, Rantanen P. Meningitis caused by ampicillin-resistant *Haemophilus influenzae* type b. [in Finnish] Finnish Medical Journal 1977;32:2282-2285.
- (2) Nissinen A, Grönroos P, Huovinen P, Herva E, Katila M-L, Klaukka T, Kontiainen S, Liimatainen O, Oinonen S, Mäkelä PH. Developmet of beta-lactamase-mediated penicillin resistance in pediatric isolates of *Moraxella catarrhalis* in Finland, 1978-1993. Clin Infect Dis 1995;21:1193-6.
- (3) Manninen R, Huovinen P, Nissinen A, and the Finnish Study Group for Antimicrobial Resistance. Increasing antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* in Finland. J Antimicrob Chemother. 1997;40:387-92.
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- (5) Dagan R, Leibovitz E. Bacterial eradication in the treatment of otitis media. Lancet Infect Dis 2002; 2: 593-604
- (6) Tristram S, Jacobs MR, Appelbaum PC. Antimicrobial resistance in *Haemophilus influenzae*. Clin Microbiol Rev 2007;20:368-89.

3.3 Antimicrobial resistance in *Moraxella catarrhalis* (1978–2010)

M. catarrhalis

Typical infections:

- Otitis media, sinusitis, exacerbation of chronic obstructive pulmonary disease (COPD)

The effect of the antimicrobial resistance situation on treatment options:

- No effect at the moment

Moraxella catarrhalis is one of the most common causes of infant acute otitis media, acute sinusitis and exacerbation of chronic obstructive pulmonary disease (COPD) (1).

Almost all *M. catarrhalis* strains are ampicillin-resistant. Ampicillin resistance is caused by the production of a beta-lactamase, which to some extent also hydrolyses 2nd generation cephalosporins (cefuroxime, cefaclor). *M. catarrhalis* (previously *Branhamella catarrhalis*) was identified as a pathogen only a few decades ago, and at that time all Finnish strains were susceptible to penicillins. At the end of the 1970's, the first beta-lactamase producing *M. catarrhalis* strains appeared and thereafter beta-lactamase-mediated ampicillin resistance has increased rapidly (2, 3), reaching the present 90 % resistance level at the beginning of the 1990's (figure 3.6). All *M. catarrhalis* strains are still susceptible to amoxicillin-clavulanate. In addition, *M. catarrhalis* is almost totally susceptible to second line antimicrobials, macrolides, tetracyclines and trimethoprim-sulfa (Figure 3.7).

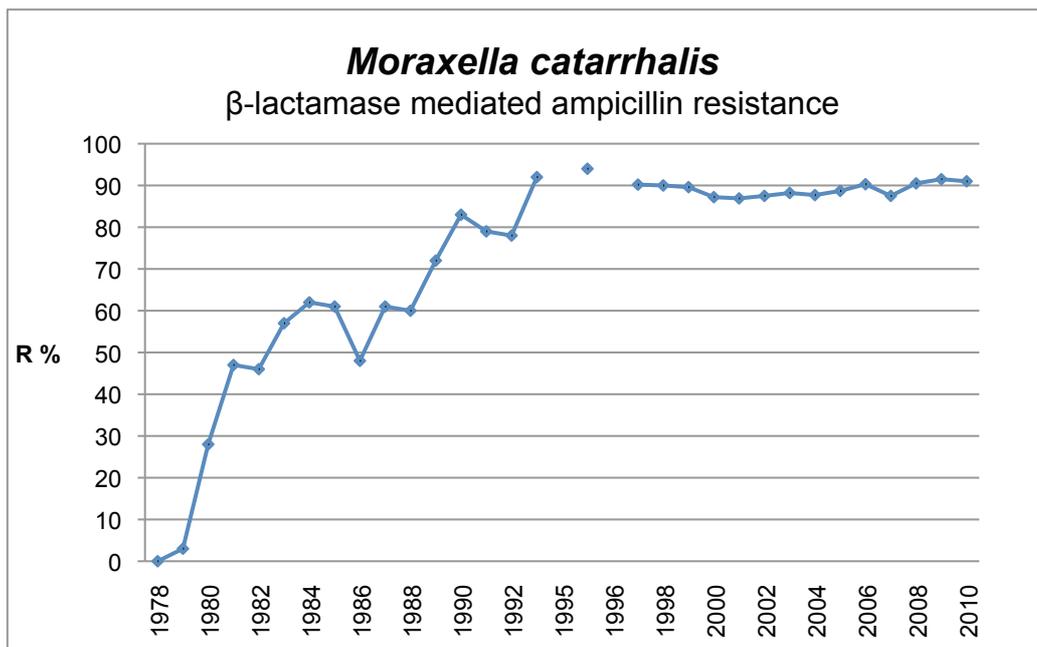


Figure 3.6: Beta-lactamase mediated ampicillin resistance in *M. catarrhalis* between 1978–2010 (2,3).

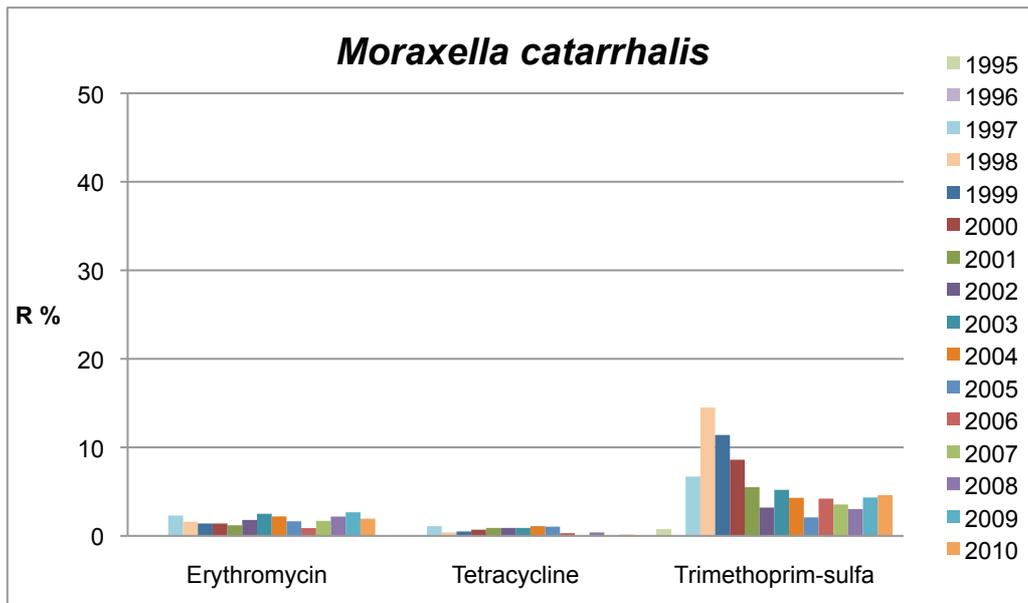


Figure 3.7: Erythromycin, tetracycline and sulfa/trimethoprim resistance in *M. catarrhalis* between 1995 and 2010 (3).

References:

- (1) Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. Clin Infect Dis 2009; 49: 124-31.
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3.4 Antimicrobial resistance in Group A streptococci (1991–2010)

S. pyogenes

Typical infections:

- Tonsillitis, skin- and soft-tissue infections, severe infections (sepsis, toxic shock)

The effect of the antimicrobial resistance situation on treatment options:

- No effect at the moment

Streptococcus pyogenes, also known as the group A streptococcus, causes throat and skin infections and sometimes also septic infections. Penicillin has been the first-line drug in the treatment of group A streptococcal infections for the last 70 years. During this time, penicillin or cephalosporin resistant strains have never been found, and *S. pyogenes* is thus consistently susceptible to these agents.

However, resistance to other antimicrobials occurs. Besides penicillin and 1st generation cephalosporins, macrolide antimicrobials and clindamycin can also be used for the treatment of group A streptococcal infections. Erythromycin is used for macrolide (azithromycin, clarithromycin and roxithromycin) susceptibility testing.

In the beginning of the 1990's, macrolide resistance in group A streptococci was at its highest level, in some regions over 40 %. Because of treatment failures – some even leading to serious complications demanding hospitalization – during macrolide treatment of infections caused by resistant strains, a recommendation was given to avoid the use of macrolides for the treatment of throat and skin infections (1). For outpatients with penicillin allergy, 1st generation cephalosporins were recommended as a first-line drug, if the patient had no previous anaphylactic reactions to penicillin derivatives. Following this recommendation, macrolide resistance fell to 5–10 % within a few years. In the 2000's, macrolide resistance decreased further, and has stabilized at 2–4 % between 2002 and 2010 (Figure 3.8).

Clindamycin resistance has remained at a low level, 0–2 %, from 2000–2010, but a slight increase can be detected. Clindamycin can be important in the treatment of severe group A streptococcal infections and for patients with penicillin allergy. Although clindamycin and macrolides have related mechanisms of action, clindamycin resistance is far more uncommon than macrolide resistance. This is due to the different resistance mechanisms towards these employed by the group A streptococcus.

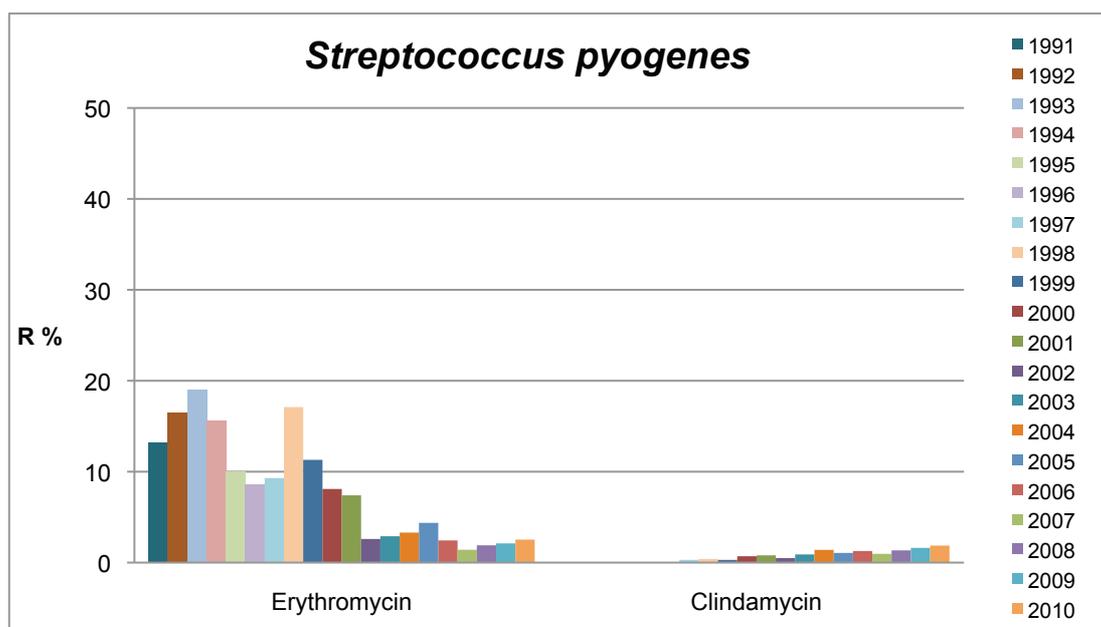


Figure 3.8: Antimicrobial resistance in Group A streptococci between 1991 and 2010.

References:

- (1) Seppälä H, Klaukka T, Vuopio-Varkila J, Muotiala A, Helenius H, Lager K, Huovinen P and the Finnish Study Group for Antimicrobial Resistance. The effects of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. *N Engl J Med* 1997;337:441-446.

3.5 Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp.

3.5.1 Background

The cell envelope of gram-negative bacteria consists of a cell wall built of an outer glycosylated lipid bilayer and a thin peptidoglycan layer, the periplasmic space, and an inner phospholipid membrane.

The outer lipid layer forms an efficient barrier to antimicrobial penetration. The development of new antimicrobials against infections caused by gram negative bacteria is therefore challenging. Since there are no new antimicrobials in sight, surveillance of the resistance towards antimicrobials now in use is particularly important.

Finres data has been collected since 1991. In the earliest material, ampicillin resistance was a notable exception amongst the otherwise very susceptible bacteria. The cause of this, the β -lactamase TEM-1, was common already then. Also trimethoprim resistance levels were fairly high. The situation has been worsening in the new millennium. The most important change has been the increase of strains producing extended spectrum β -lactamases (ESBL). They were found already in the 1990's, but mainly among *K. pneumoniae* strains, and in low numbers. The Finres data clearly shows the change that has occurred among *E. coli* strains. ESBL strains have become more prevalent, and the increase is steady among clinical strains.

Carbapenem-resistant *Enterobacteriaceae* strains have increased globally at an alarming rate. In Finland we have so far seen only sporadic cases involving carbapenemase-producing *E. coli*, *Klebsiella* spp. and *Enterobacter cloacae* strains. Among the non-fermenting rods *P. aeruginosa* and *Acinetobacter* spp., the situation is different; finding a carbapenem-resistant strain is no surprise, and small recurrent epidemics have already been seen. But strains producing carbapenemase enzymes are still rare. Effective antimicrobial treatment can still be found for the majority of infections caused by these strains, but strains resistant to all antimicrobials have been reported, mainly from the capital hospital district, HUS, and a few cases also from other parts of Finland. The Finres data clearly shows that the majority of Finnish strains are still susceptible to most antimicrobials.

3.5.2 Antimicrobial resistance among *Escherichia coli* 1997-2010

E. coli

Typical infections:

- Urinary tract infection, pyelonephritis, urosepsis, food borne intestinal infections

The effect of the antimicrobial resistance situation on treatment options:

- First line antimicrobials for urinary tract infections, especially mecillinam and nitrofurantoin, have remained effective. Resistance levels to fluoroquinolones have gone up.
- ESBL-producing multiresistant strains are increasing at a worrying pace; this decreases the available number of effective antimicrobials.

Escherichia coli is the clinically most significant species among the *Enterobacteriaceae*. It is the most common cause of lower urinary tract infection. It also causes serious infections, of which the most important is pyelonephritis and its complication urosepsis. In addition to anaerobes, *E. coli* is also important in intra-abdominal infections, such as peritonitis following an appendix perforation. Some strains cause food-borne intestinal infections.

The increase of ESBL-producing strains is a significant factor in the development of resistance in *E. coli*, since these strains are usually resistant, in addition to β -lactams, also to several unrelated antimicrobial

groups (fluoroquinolones, trimethoprim, aminoglycosides, tetracyclines). This multiresistance has important clinical consequences. Multiresistant strains increase mortality especially among seriously ill patients, prolong hospitalization, and increase costs (1). ESBLs are discussed in a separate section (3.5.4). Resistance of *E. coli* to carbapenems is so far very rare (section 3.5.5 and Figure 3.13).

Susceptibility data on *E. coli* in the Finres database was grouped first as either healthcare center or hospital isolates (the years 1997-2004, Figures 3.9 and 3.10), then as urinary tract or blood isolates (the years 2005-2010, Figures 3.11, 3.12, 3.13). The urinary tract isolates were further divided into isolates from patients younger than 75 years, and 75 years or over (Figures 3.11 and 3.12). As can be seen in these figures, ampicillin resistance is common (20-30%). In 2010 it was 34 % in blood isolates. This is explained by a high prevalence of ampicillin-hydrolyzing enzymes, especially TEM-1. Cephalothin is representing the first generation cephalosporins, and resistance is 8-10 % in this material. The spread of ESBL genes also affects ampicillin and cephalothin resistance levels. Mecillinam, usually used as the oral pivmecillinam, also belongs to the β -lactams, but according to current knowledge it is not affected by the TEM-1 enzyme. The majority (>96 %) of the *E. coli* strains in the Finres material is susceptible to mecillinam. Because of the increase of ESBL strains there has been a rising interest towards mecillinam in the treatment of lower urinary tract infections, and new research into the effectiveness of mecillinam will probably be reported in the near future. Nitrofurantoin has also remained effective against *E. coli*, and only a few percentages are resistant. It is also very effective for urinary tract infections, if the patient tolerates it. Nitrofurantoin cannot be used e.g. if kidney function is lowered. Fluoroquinolone resistance (to norfloxacin, ciprofloxacin or levofloxacin) is below 10 % in the Finres material. There is however a very worrying increasing trend, which might be explained by the increase of ESBL strains, which commonly are co-resistant also to fluoroquinolones. For trimethoprim, there was a decreasing trend before 2004. After that, the prevalence levelled out, and even increased among <75-year old patient isolates. The overall resistance level is now fairly high at 17–19 %.

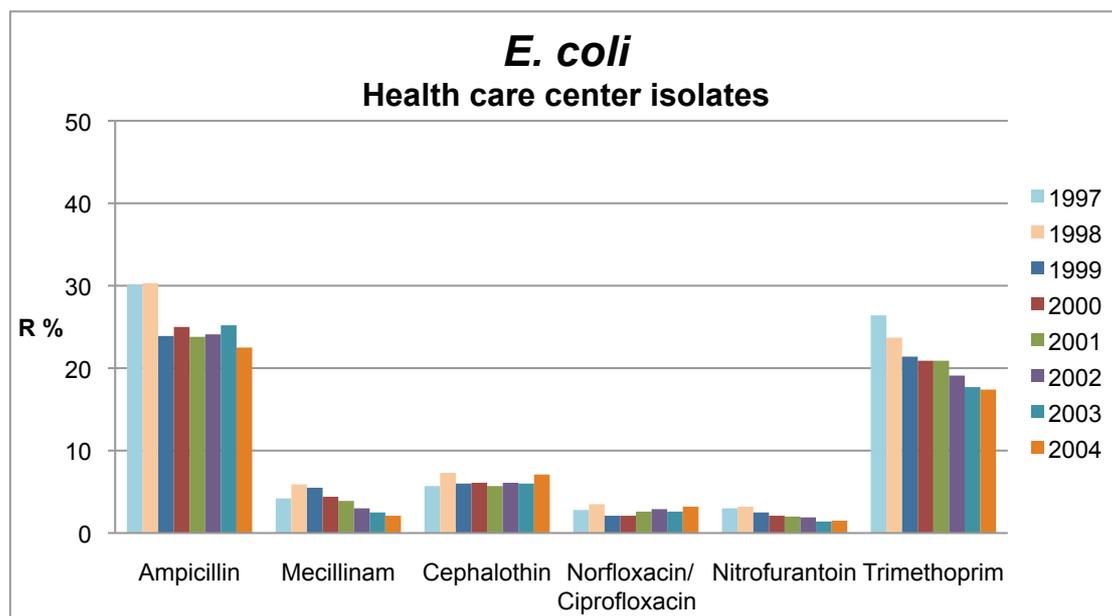


Figure 3.9: Antimicrobial resistance in *E. coli* strains, isolated from health care centers between 1997 and 2004.

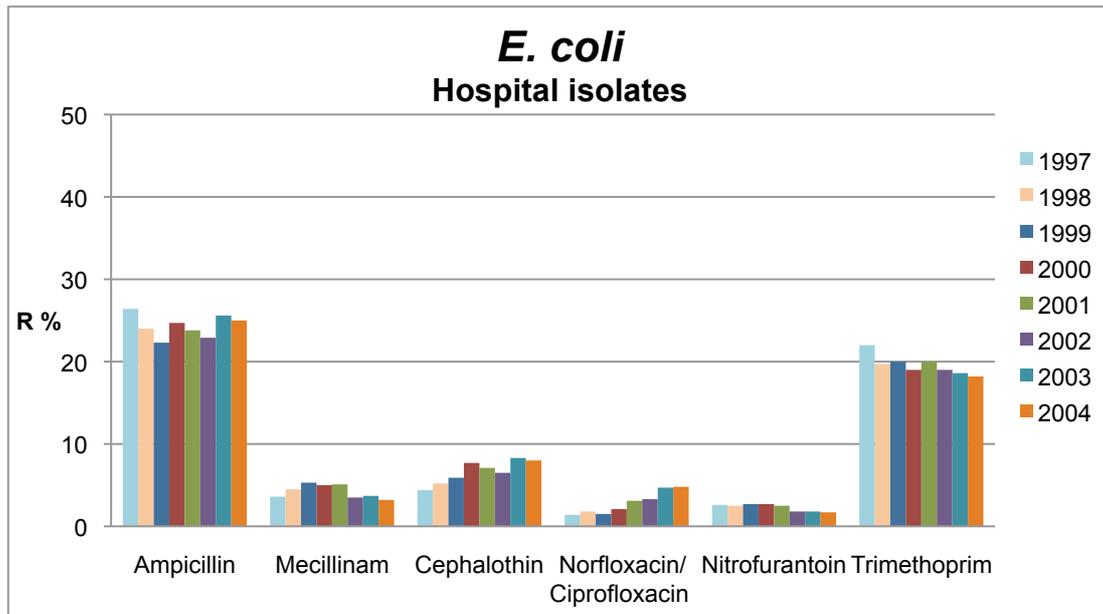


Figure 3.10: Antimicrobial resistance in *E. coli* strains, isolated from hospitals between 1997 and 2004.

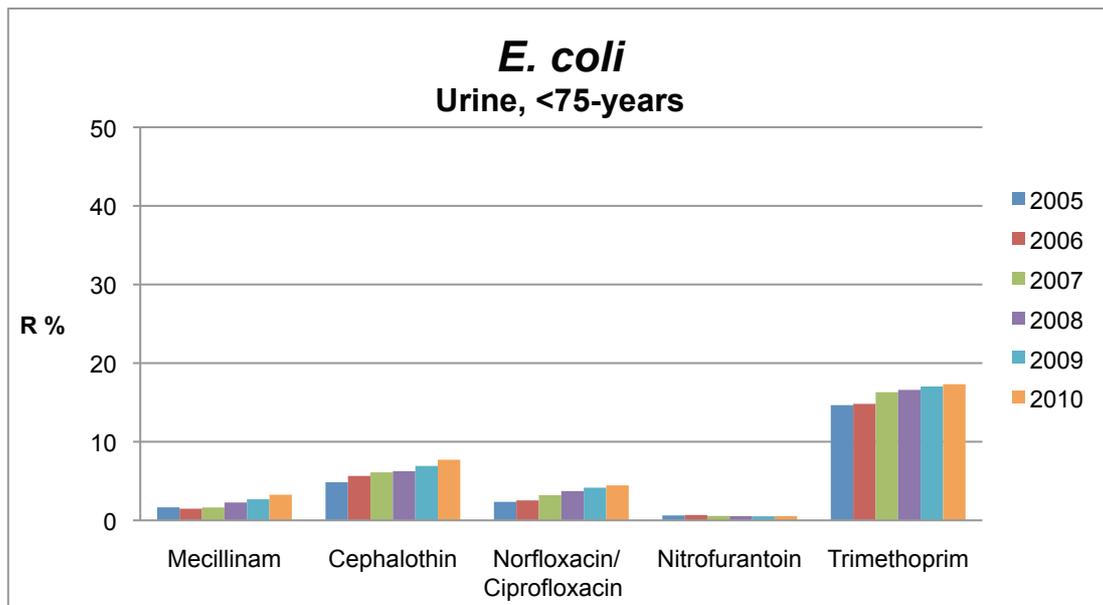


Figure 3.11: Antimicrobial resistance in *E. coli* strains, isolated from urine samples taken from patients under 75 years old (2005–2010).

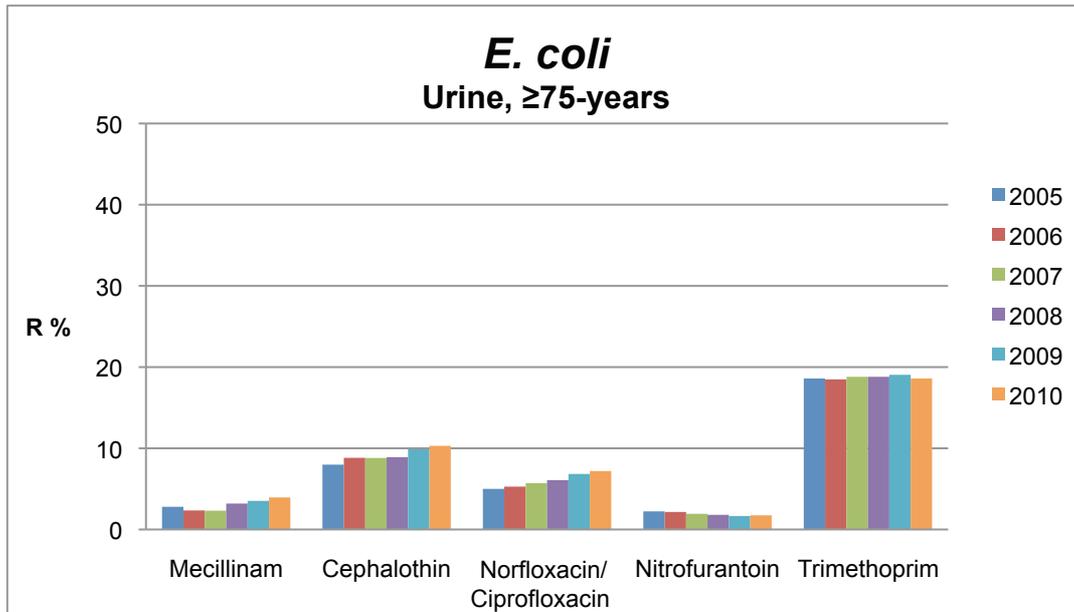


Figure 3.12: Antimicrobial resistance in *E. coli* strains isolated from urine samples taken from patients over 75 years old (2005–2010).

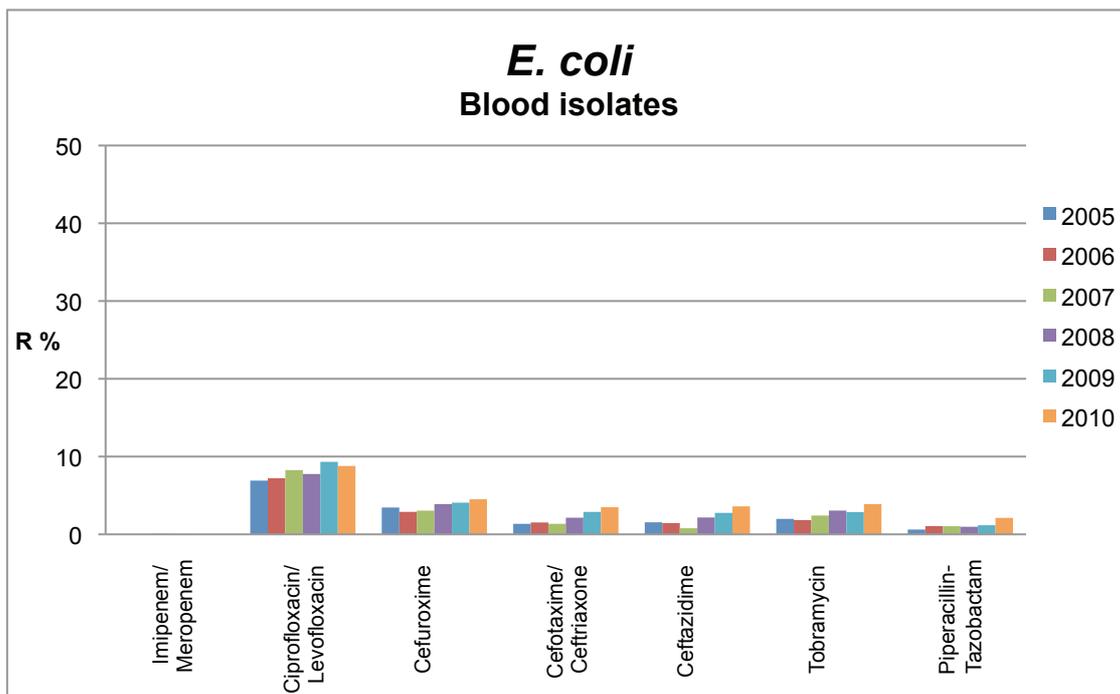


Figure 3.13: Resistance against the most important antimicrobials used in hospitals, in *E. coli* strains isolated from blood cultures.

References:

- (1) Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in *Enterobacteriaceae* bacteraemia: a systematic review and meta-analysis. *J Antimicrob Chemother* 2007 Nov;60(5):913-920.

3.5.3 Antimicrobial resistance among *Klebsiella* spp. 1997-2010

Klebsiella

Typical infections:

- Urinary tract infections, hospital acquired pneumonia, septic infections

The effect of the antimicrobial resistance situation on treatment options:

- No effect at present
- Sporadic almost panresistant strains may occur

Klebsiellas belong to the normal human intestinal and oral microbiota. A third of humans carry *Klebsiella* species in their gut, and hospitalization and antimicrobial use increase prevalence (1). *Klebsiellas* cause urinary tract infections, hospital acquired pneumonias and septic infections. The infecting strain is usually derived from the gut flora of the patient, but *klebsiellas* can persist in different environments, such as on the hands of personnel, wash basins and ventilators. Possible routes of infection are therefore many (2). *Klebsiellas* can be found also in food, soil and water (1), but pathogenicity, and probably also antimicrobial resistance rates, might be lower in such strains (3).

K. pneumoniae is the most common species in clinical samples. The former *Klebsiella* species *Raoultella terrigena* and *Raoultella planticola* are phenotypically so similar to *K. pneumoniae* that biochemical differentiation of these species may be difficult. The *raoultellas* were delineated as a new genus in 2001 (4), therefore the older Finres data comprise also these species. *Raoultella* spp. appears to be more common in Europe, compared to the US and Brazil (3). *K. oxytoca* is also commonly found in clinical samples. Phenotypical differentiation is straight forward and important because it differs slightly in its innate resistance.

Antimicrobial resistance based on Finres-data in 1997-2004 and 2005-2010

In 1997-2004, susceptibility data was collected on all *Klebsiella* spp. The data was divided into hospital and open care (healthcare center) isolates (Figure 3.14). From 2005 onwards, data on *K. pneumoniae* only has been collected, and this is divided into urinary tract (including hospital and open care) and blood isolates (Figures 3.15 and 3.16). Thus the two time periods are not directly comparable.

Resistance has remained low, below 5 %, to cephalosporins, fluoroquinolones, aminoglycosides and carbapenems during both time periods, and any clear changes cannot be seen. The data for nitrofurantoin and mecillinam is interesting: during the first time period, 1997-2004, there was a reduction in resistance. The number of tested strains is large; thus the finding is probably not due to statistical variation. E.g. the number of strains tested against mecillinam was 5188 in 1997, and varies between 3897 and 6802 during 2000-2004. The decline did not continue after 2005.

There was a transient increase in trimethoprim and trimethoprim-sulfa resistance during 2004-2006. The phenomenon was seen both among blood and urine isolates, and resistance levels were evenly distributed throughout the country. We have no explanation for this; one possibility might be the spread of a resistant clone. The Finres data does not suffice to prove this.

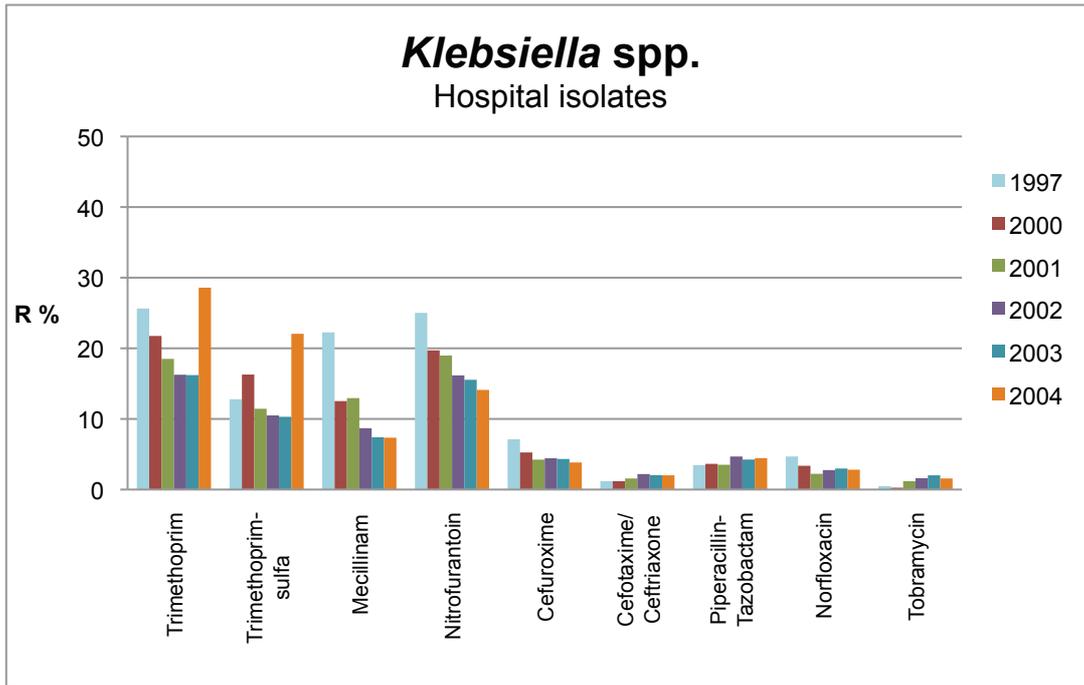


Figure 3.14: Antimicrobial resistance in *Klebsiella* spp., isolated from hospital patients 1997 – 2004.

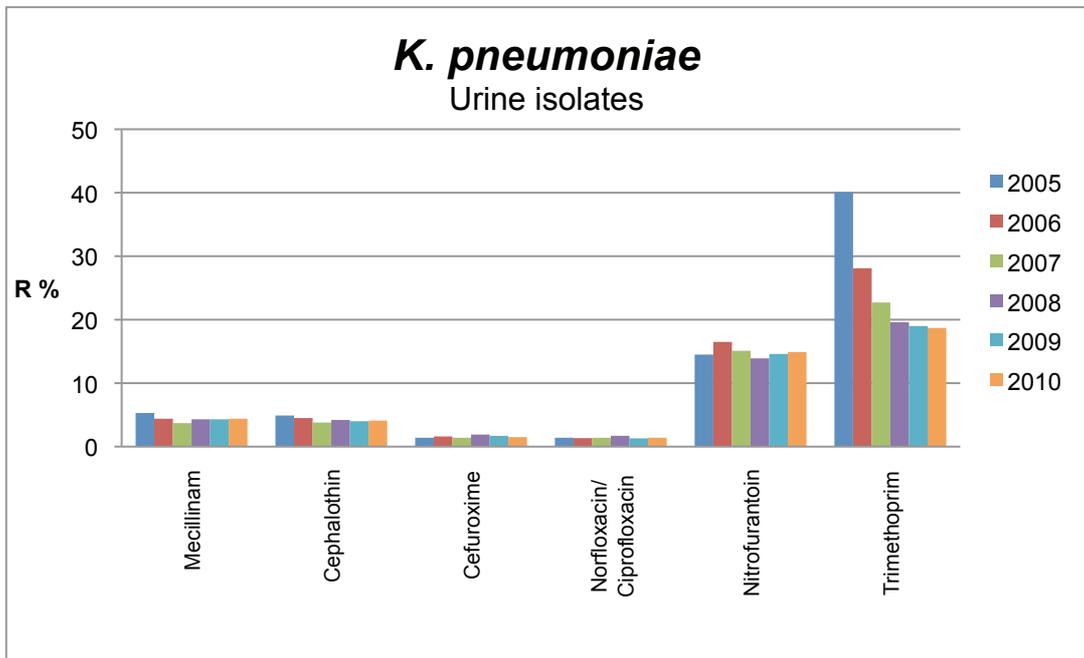


Figure 3.15: Antimicrobial resistance in *K. pneumoniae*, isolated from urine samples 2005 – 2010.

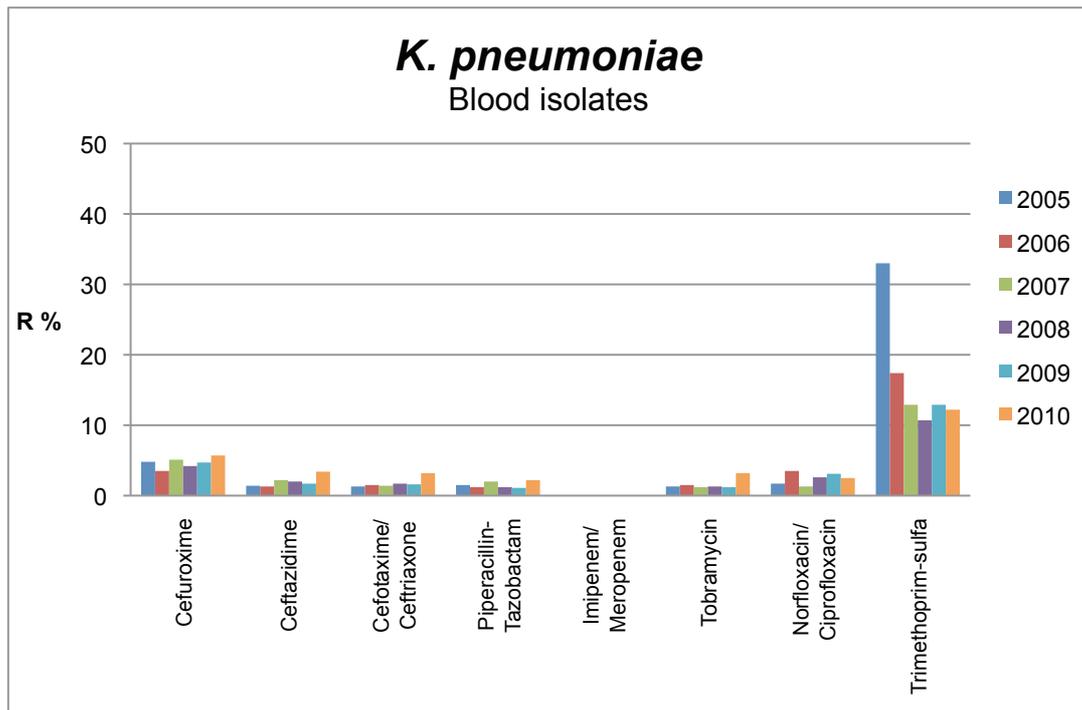


Figure 3.16: Antimicrobial resistance in *K. pneumoniae*, isolated from blood samples 2005 - 2010

References:

- (1) Janda JM. The Enterobacteria. 2nd ed. Washington D.C.: ASM Press; 2006.
- (2) Snitkin ES, Zelazny AM, Thomas PJ, Stock F, NISC Comparative Sequencing Program, Henderson DK, et al. Tracking a Hospital Outbreak of Carbapenem-Resistant *Klebsiella pneumoniae* with Whole-Genome Sequencing. *Sci Transl Med* 2012 Aug 22;4(148):148ra116.
- (3) Versalovic J editor. Manual of Clinical Microbiology. 10th ed. Washington D.C.: ASM Press; 2011.
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3.5.4 Bacteria producing extended-spectrum β -lactamases (ESBL)

β -Lactamases are enzymes produced by bacteria, which can break down antimicrobials having a β -lactam ring, e.g. penicillins, and thus render the bacterium resistant to this antimicrobial. Extended-spectrum β -lactamases (ESBL) can break down penicillins, extended-spectrum cephalosporins (e.g. ceftriaxone) and monobactams (aztreonam), but not carbapenems (imipenem, meropenem, ertapenem). ESBL genes are found in the family *Enterobacteriaceae*. Clinically most significant are ESBL producing *E. coli* and *K. pneumoniae* strains. ESBL producing strains are often also resistant to fluoroquinolones, aminoglycosides, trimethoprim and tetracycline (1), which complicates antimicrobial therapy.

The change in the epidemiological situation in Finland

There has been a global change in the prevalence of ESBL genes, and the species in which they occur. The first ESBL genes were found already in the beginning of the 1980's (2). They were usually found in *K. pneumoniae*, and the genes were ESBL variants of TEM and SHV genes (3). A fast increase in ESBL producing strains was noticed in the UK at the beginning of the 2000's. This was caused by *E. coli* strains carrying CTX-M genes.

In Finland, the development mirrors the situation globally. In the 1990's, ESBL strains were rare, and the genes similar to those then reported. In the FiRe report published in 1997, 0.6% of *E. coli* strains, and 2.3% of *K. pneumoniae* strains were resistant to 3rd generation cephalosporins (4). These strains had TEM and SHV genes. During the 2000's, the situation has changed, and the ESBL problem has worsened. In a representative material covering all of Finland from the years 2002-2004, CTX-M-group genes were already found in 90 % of all ESBL producing *E. coli* strains. Also in *K. pneumoniae*, CTX-M genes were the most common (5). ESBL production has been notified separately in the Finres data since 2007. The prevalence of ESBL strains is increasing steadily (Figures 3.17 and 3.18). In 2011, 4.6 % of *E. coli* blood isolates, and over 2 % of urine isolates were ESBL producers. The corresponding numbers for *K. pneumoniae* were 1.7 % and 1.0 %. The clear rising trend among *E. coli* isolates is not mirrored among *K. pneumoniae*. It is currently not known how widespread ESBL carriage in fecal flora in the community is in Finland, but e.g. travel can increase the risk of colonisation. *E. coli* is an important cause of uncomplicated open care urinary tract infections, and it is conceivable that the increase in ESBL infections is caused by more widespread colonisation of the Finnish population. *K. pneumoniae* strains are still more seen in hospital infections, and changes in prevalence are affected by transient hospital epidemics. The number of blood isolates is small, and thus even small changes can cause large variation in resistance percentages: e.g. the sudden rise in 2010 consists of 15 isolates (Figure 3.18).

The resistance among *E. coli* and *K. pneumoniae* against the most important hospital antibiotics is still relatively rare (Section 3.5.2 Figure 3.13 and Section 3.5.3 Figure 3.14). ESBL producing strains are multiresistant, and their increase affects the usefulness also of other antibiotics than β -lactams.

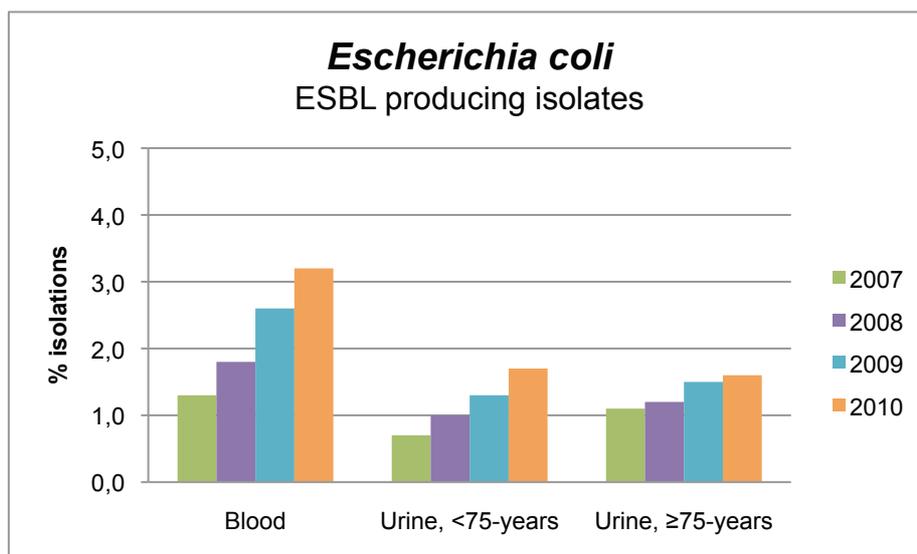


Figure 3.17: ESBL producing *Escherichia coli* isolates from 2007 to 2010.

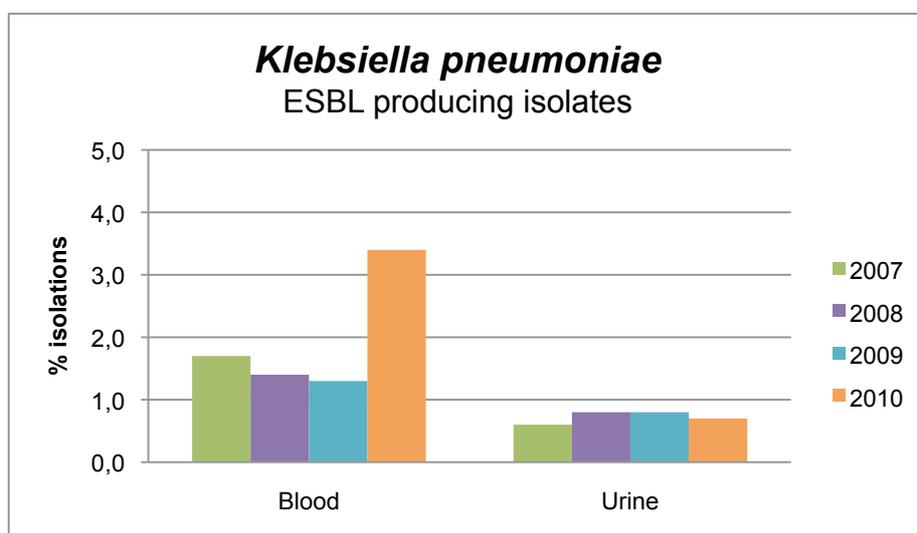


Figure 3.18: ESBL producing *Klebsiella pneumoniae* isolates from 2007 and 2010.

References:

- (1) Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, et al. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in the UK. *J Antimicrob Chemother* 2004 Oct;54(4):735-743.
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3.5.5 Carbapenem resistance

P. aeruginosa

Typical infections:

- Opportunistic hospital infections, skin and soft tissue infections

The effect of the antimicrobial resistance situation on treatment options:

- No major effects at present
- Nearly or completely panresistant strains may be found in the hospital environment

Acinetobacter

Typical infections:

- Pneumonia, skin and wound infections, urinary tract infections and opportunistic infections

The effect of the antimicrobial resistance situation on treatment options:

- No major effects at present
- Nearly or completely panresistant strains may be found in the hospital environment

Carbapenems (imipenem, meropenem, ertapenem) are considered last-resort antibiotics, since they are the only antimicrobial group that has remained broadly effective against gram-negative bacteria. They belong to the β -lactams, and are thus well tolerated and effective. However, resistance among gram-negative bacilli is increasing at an alarming rate globally. This concerns especially *Enterobacteriaceae* species such as *E. coli* and *K. pneumoniae*, but also the non-fermenting rods *P. aeruginosa* and *Acinetobacter baumannii* (1, 2). Gram-negative bacteria that are resistant to carbapenems are also very often resistant to several other antimicrobial classes, or even to all available antibiotics, which causes significant treatment problems (3). Resistance towards carbapenems can develop through several mechanisms, and enzymes which break down carbapenemases are the most important resistance mechanisms, that have to be monitored. This should be remembered especially for non-fermenting species, since other mechanisms are common among them. Carbapenemase-producing strains are found especially in the hospital environment, but according to recent findings they can also appear in community-acquired infections, when the patient has had contact to a country (e.g. India) where these bacteria are endemic.

Carbapenems (imipenem, meropenem, ertapenem) are considered last-resort antibiotics, since they are the only antimicrobial group that has remained broadly effective against gram-negative bacteria. They belong to the β -lactams, and are thus well tolerated and effective. However, resistance among gram-negative bacilli is increasing at an alarming rate globally. This concerns especially *Enterobacteriaceae* species such as *E. coli* and *K. pneumoniae*, but also the non-fermenting rods *P. aeruginosa* and *Acinetobacter baumannii* (1, 2). Gram-negative bacteria that are resistant to carbapenems are also very often resistant to several other antimicrobial classes, or even to all available antibiotics, which causes significant treatment problems (3). Resistance towards carbapenems can develop through several mechanisms, and enzymes which break down carbapenemases are the most important resistance mechanisms, that have to be monitored. This should be remembered especially for non-fermenting species, since other mechanisms are common among them. Carbapenemase-producing strains are found especially in the hospital environment, but according to recent findings they can also appear in community-acquired infections, when the patient has had contact to a country (e.g. India) where these bacteria are endemic.

In the surveillance of carbapenem resistance, *Enterobacteriaceae* and non-fermenters should be separated, both because of their differing pathogenicity and the often different mechanisms. In Sections 3.5.2 and 3.5.3, the resistance development of *E. coli* and *K. pneumoniae* from 2005 to 2010 are presented. As can be seen in Figures 3.13 and 3.16, there are practically no carbapenem resistant strains. At present, on average one carbapenemase-producing strain belonging to the family *Enterobacteriaceae* is isolated per month in Finland (4). The majority of patients have been abroad. The most common carbapenemase genes found are OXA-48/181, KPC, VIM and NDM.

In the case of non-fermenting gram-negative species, the carbapenems are an important antibiotic group, since these species have innate resistance to several other classes of antibiotics. The resistance

development towards the most important antimicrobials in *P. aeruginosa* and *Acinetobacter* spp. (*A. baumannii* being the most important) is presented in Figures 3.19 and 3.20. There are more carbapenem resistant strains than among *Enterobacteriaceae*. The levels vary, and there is no clear trend at least for *Acinetobacter*. For *P. aeruginosa*, there might be a slight increase; this must be closely monitored. Small local epidemics probably have an effect. Carbapenemase producing strains are occasionally found, but the majority of the resistance is caused by other mechanisms (unpublished data at THL and HUSLAB). There has so far been no outbreaks caused by carbapenemase-producing strains, but among immunocompromised patients, endemic clonal spread of both *Acinetobacter* and *P. aeruginosa* has been observed for a long time (HUSLAB unpublished data). When carbapenemases are found in *P. aeruginosa*, they are usually VIM or IMP metalloenzymes. In *A. baumannii*, OXA-carbapenemases have been found (unpublished data at THL and HUSLAB).

As appear from the Figures 3.19 and 3.20, and the Figures in Sections 3.5.2 and 3.5.3, most antimicrobials in use are still relatively effective against non-fermenting rods and *Enterobacteriaceae*. So far we have seen strains resistant to nearly all antimicrobials only as single isolations or small clusters. In these cases, polymyxins (e.g. colistin) might still be useful. Resistance data on colistin is not yet collected into the Finres database.

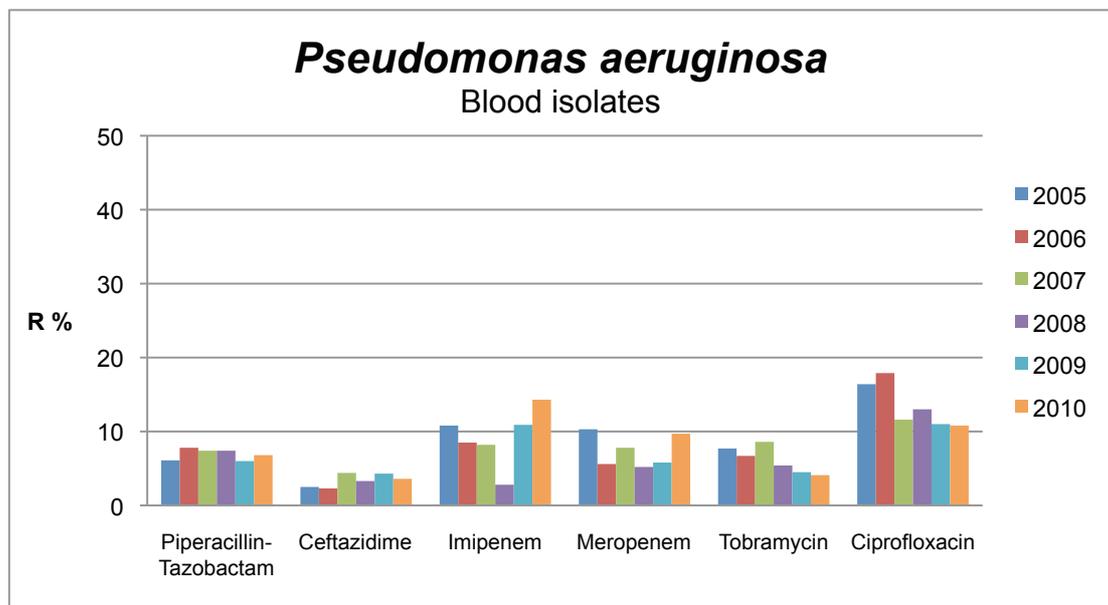


Figure 3.19: Antimicrobial resistance in *Pseudomonas aeruginosa*, 2005 – 2010.

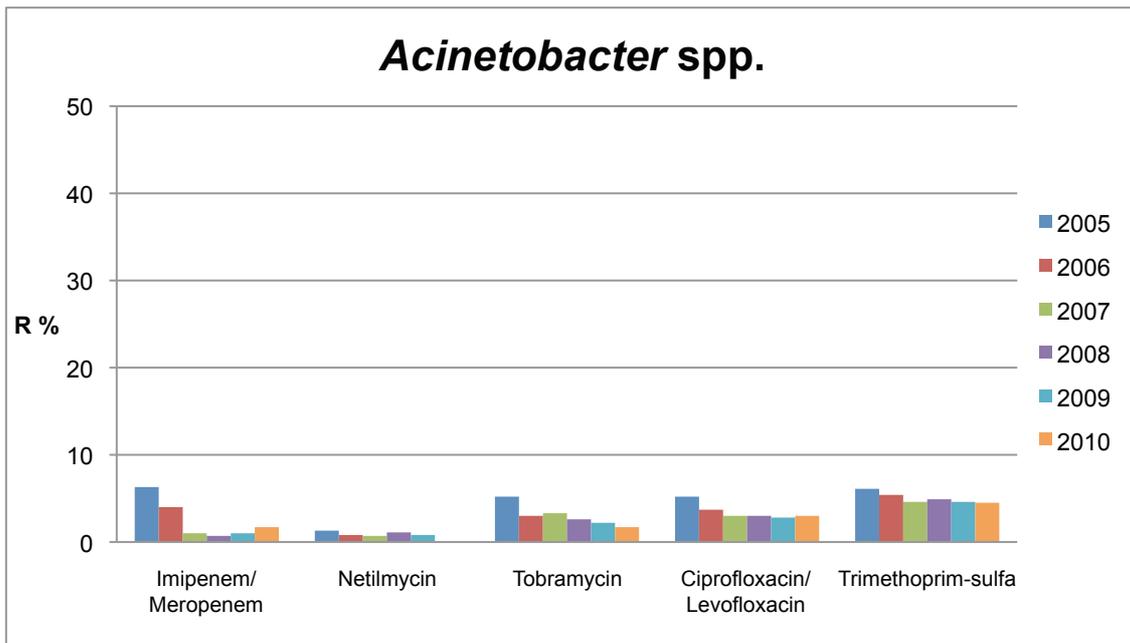


Figure 3.20: Antimicrobial resistance in *Acinetobacter* spp. from 2005 to 2010

References:

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- (3) Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality. *Antimicrob Agents Chemother* 2008 Mar;52(3):1028-1033.
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3.6 Antimicrobial resistance in *Staphylococcus aureus* (1991–2010)

S. aureus

Typical infections:

- Skin infections, cellulitis, sepsis, endocarditis

The effect of the antimicrobial resistance situation on treatment options:

- No effect at the moment at country level. The MRSA situation should be taken into consideration in certain geographical areas

At least half of all human beings carry *Staphylococcus aureus* in their nose flora either permanently or temporarily. The throat and moist skin areas can also be colonized. The source of *S. aureus* infections is commonly derived from the patient's own microbiota, but staphylococci can also be transferred between individuals via touching or indirectly via the environment.

S. aureus is the most important causer of purulent skin infections. Some *S. aureus* nasal carriers have recurrent skin abscesses. Local infection can spread into subcutaneous tissue as cellulitis, and proceed to bacteremia. Bacteremia can be followed by severe septic shock or/and metastatic abscesses in internal organs. Nowadays *S. aureus* is one of the most common causes of endocarditis, especially among drug users. *S. aureus* can cause also septic arthritis and osteomyelitis.

In the beginning of the antimicrobial era, *S. aureus* was susceptible even to penicillin, but the ability to degrade it spread rapidly among *S. aureus* strains. Penicillinase-resistant staphylococcal penicillins (among others methicillin, cloxacillin and oxacillin) were developed to solve this problem. The first methicillin-resistant *S. aureus* strains (MRSA) were detected already in the beginning of the 1960's, only a few years after methicillin had appeared on the market. Resistance to methicillin is caused by a protein involved in the cell wall synthesis, to which methicillin (and other betalactams) cannot bind and therefore cannot inhibit its function. Methicillin resistance is mediated by the *mecA* gene (and its recently found variants), and it is assumed that it can be transferred between strains. MRSA strains started to spread in hospitals all over the world in the 1980's. In Finland, the first MRSA hospital epidemic was detected in 1991, and by the mid 1990's around 100 MRSA cases were detected annually (1). From 1997 to 2004 the number of MRSA findings increased fairly rapidly: in hospitals the number of oxacillin-resistant *S. aureus* increased 2.6 times, from less than 2 % to nearly 5 % (Figure 3.21). Similar increase could be seen in healthcare center samples: the number of oxacillin-resistant strains increased from less than 1 % to 4 % (Figure 3.22). This increase was partly caused by the spread of two international clones in Finland, but also by the emergence of outpatient MRSA strains (2). Whereas MRSA strains that have circulated in hospitals globally for many years are commonly resistant to several antimicrobial groups, strains isolated from outpatients are usually only β -lactam resistant. The development of more sensitive laboratory detection methods probably also explains the increased proportion of these strains.

Luckily, the steep rise of MRSA did not continue between 2005 – 2010. The percentage of MRSA in both blood and pus isolates stabilized at 2–3 % (Figures 3.23 and 3.24). There is a significant geographical variation: in the Pirkanmaa region (Tampere), MRSA prevalence was 8.1 % in 2010, while in the rest of the country it was 0–2.7 % (3). In most hospitals invasive MRSA infections are still uncommon; the Pirkanmaa region accounted for nearly 40 % of these. We thus have slightly more invasive MRSA infections in Finland as compared to Sweden and Norway, where the proportion of MRSA is less than 1 % of all invasive *S. aureus* infections, but the number is still clearly lower than in Central and Southern Europe (4).

Strains resistant to vancomycin, the traditional first-line antibiotic against serious MRSA infections, have not been found in Finland. Also rifampicin, used in combination with other antimicrobials, can be used for the treatment of deep infections caused both by MRSA and "regular" *S. aureus* strains. In addition, *S. aureus* strains are almost always susceptible to aminoglycosides.

The susceptibility to antimicrobials that can be used to treat mild *S. aureus* infections is also good: only about 5 % of all *S. aureus* strains are macrolide-resistant and 3–4 % clindamycin-resistant. Resistance to tetracyclines (1–2 %) and trimethoprim-sulfa (<1 %) is even more uncommon. The topical antibiotic fusidic acid is effective against over 95 % of *S. aureus* strains.

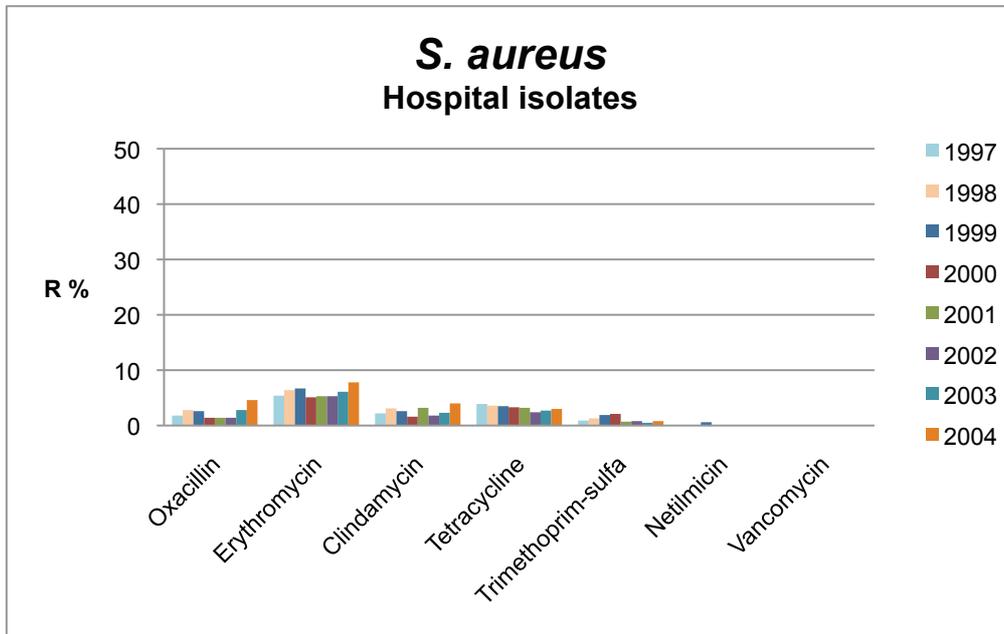


Figure 3.21: Antimicrobial resistance in *S. aureus* strains, isolated from hospitals 1997 – 2004.

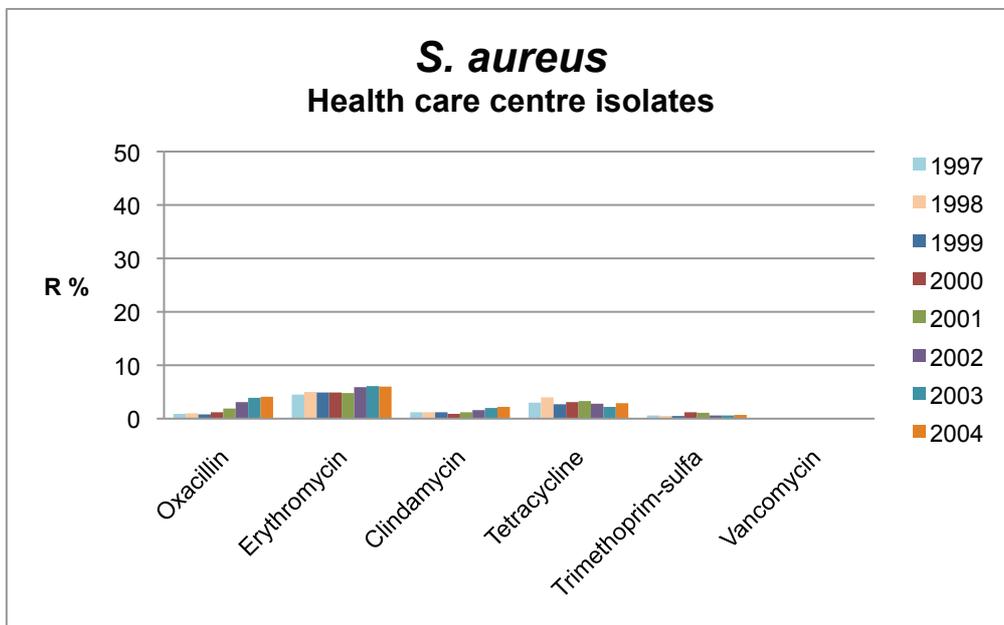


Figure 3.22: Antimicrobial resistance in *S. aureus* strains, isolated from health care centers 1997 – 2004.

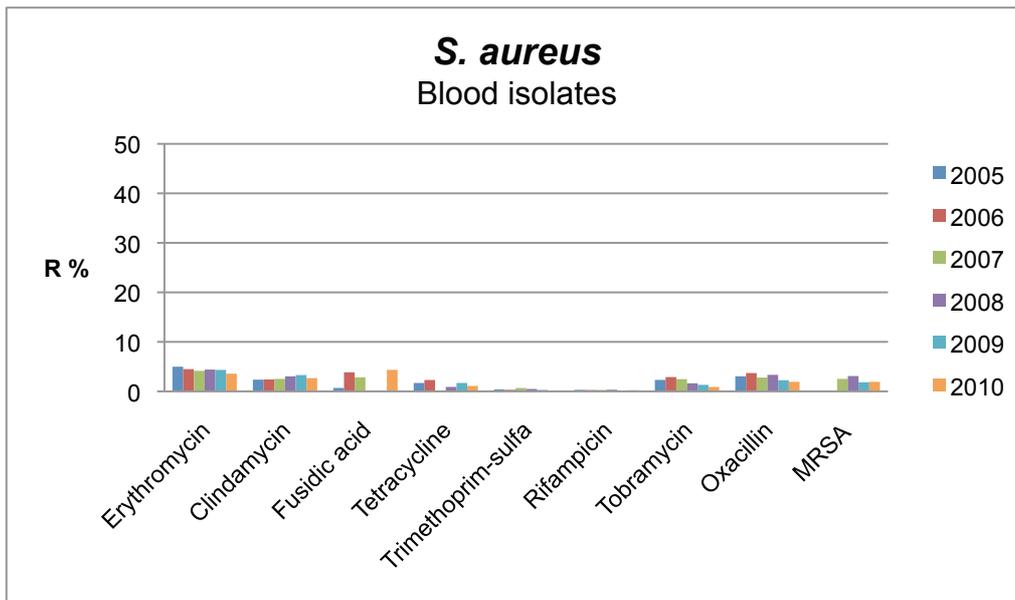


Figure 3.23: Antimicrobial resistance in *S. aureus* blood isolates, 2005 – 2010.

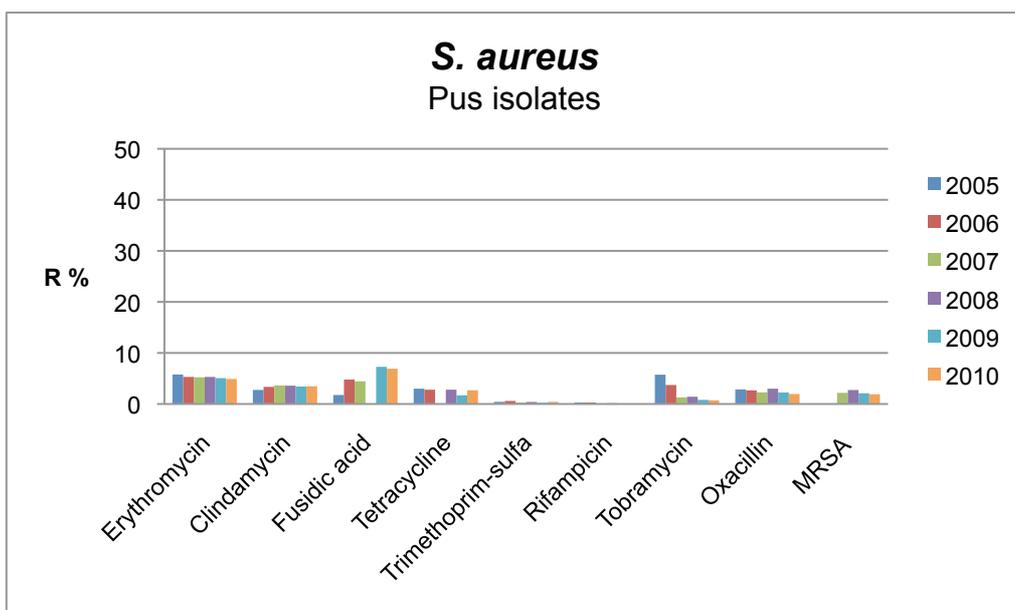


Figure 3.24: Antimicrobial resistance in *S. aureus* pus isolates, 2005 – 2010.

References:

- (1) Vuopio-Varkila J, Kotilainen P. Valtaako moniresistentti *Staphylococcus aureus* Suomenkin sairaalat? *Duodecim* 1996;112(10):851
- (2) Kerttula A-M, Lyytikäinen O, Karden-Lilja M, Ibrahim S, Salmenlinna S, Virolainen A, Vuopio-Varkila J. Mationwide trends in molecular epidemiology of methicillin-resistant *S. aureus*, Finland, 1997-2004. *BMC Infectious Diseases* 2007, 7:94.
- (3) Laine J, Huttunen R, Vuento R, Arvola P, Levola R, Vuorihuhta M, Syrjänen J, Vuopio J, Lumio J. Methicillin-resistant *Staphylococcus aureus* epidemic restricted to one health district in Finland: a population-based descriptive study in Pirkanmaa, Finland, years 2001-2011. *Scand J Infect Dis* 2013; 45:45-53.
- (4) European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2010. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECEC;2011:30-32.

3.7 Antimicrobial resistance in *Enterococcus* spp. (1991–2010)

Enterococci

Typical infections:

- Urinary tract infections (especially catheter-related), cellulitis, intra-abdominal and pelvic infections, sepsis, endocarditis

The effect of the antimicrobial resistance situation on treatment options:

- No effect at the moment

Enterococcus spp. are an important part of intestinal microbiota of humans and many animals. Enterococci are able to adapt to many different growth conditions, and they are intrinsically resistant to many antimicrobials. Enterococci have traditionally been regarded as low-grade pathogens and thus infections are more common in immune-compromised patients, following procedures where the natural barriers of the body have been broken, or after antimicrobial treatments which have decreased the numbers of protective bacteria. Urinary tract infections caused by *Enterococcus* spp. are commonly linked to catheterization or other invasive procedures. *Enterococcus* spp. can also cause hospital-acquired bacteremia and a substantial part of bacterial endocardites. Enterococci also appear in the mixed flora of abscesses in the abdominal and pelvic area, and in chronic wounds. Up to 80 % of enterococcal infections are caused by *E. faecalis*, which is the clinically most important enterococcus, and of the remaining part, most are caused by *E. faecium*. Since the 1990's, the role of *E. faecium* especially in hospital infections has increased. Molecular techniques have shown that this is due to the hospital-adapted *E. faecium* clone CC17, which has exceptionally well-developed virulence and resistance determinants (1).

The penicillin-binding proteins (PBP) in the enterococcal cell wall naturally bind β -lactams very weakly. Of the β -lactam antimicrobials, only ampicillin, piperacillin and imipenem are effective against *Enterococcus* spp. *E. faecalis* is nearly always susceptible to all these, whereas over 80 % of clinical *E. faecium* strains are ampicillin-resistant. Since enterococci from urine samples are not identified to species level in every laboratory, it cannot be deduced whether the increase in ampicillin resistance in the 2000's is due to an increased proportion of *E. faecium* strains, or an increasing level of ampicillin resistance within this species (Figure 3.25).

Due to the widespread β -lactam resistance, vancomycin is the most important antimicrobial agent for the treatment of severe *E. faecium* infections. During the 1990's the number of vancomycin-resistant *E. faecium* strains increased steeply especially in hospitals in the United States, where VRE is one of the most important causes of hospital infections. In Finland, the first VRE cases were detected in 1992 (2). According to the National Infectious Diseases Register, the highest number of VRE cases (over 300 cases) was detected in 1997 (figure 3.26). The large VRE epidemics in the 1990's were located in the capital healthcare district, and around 2000 in the Vaasa region; in the 2000's VRE outbreaks have also been detected in Northern Ostrobothnia and in the South-West. The National Infectious Diseases Register also includes screening results from carriers, and thus the real number of VRE infections is difficult to obtain from that register. In the Finres material, screening samples are not included, and only one bacterial finding/sample type/patient/year is included. Here the highest percentage of VRE in the top year 1997 was 15 %, among *E. faecium* strains (n= 1974, species identified). Subsequently the percentage of VRE-strains has been over 1 % only in 2000, when 1.6 % of *E. faecium* strains (n=2700) were vancomycin-resistant (Figure 3.26). When all *Enterococcus* spp. isolates are pooled together, this percentage appears as a very tiny bar (Figure 3.25). As elsewhere, the majority of VRE cases are *E. faecium* but vancomycin-resistant *E. faecalis* also exists.

Nitrofurantoin resistance among *Enterococcus* spp. has increased markedly since 2005 (Figure 3.25). This phenomenon has been seen mainly in *E. faecium*, but the reason is unknown. On the other hand, nitrofurantoin susceptibility testing in *E. faecium* has been found to be very unreliable, and therefore no definitive conclusions can be made about the resistance levels. Since *E. faecium* is also commonly resistant to fluoroquinolones, there are sometimes no oral antimicrobials available to treat urinary tract infections caused by this bacterium.

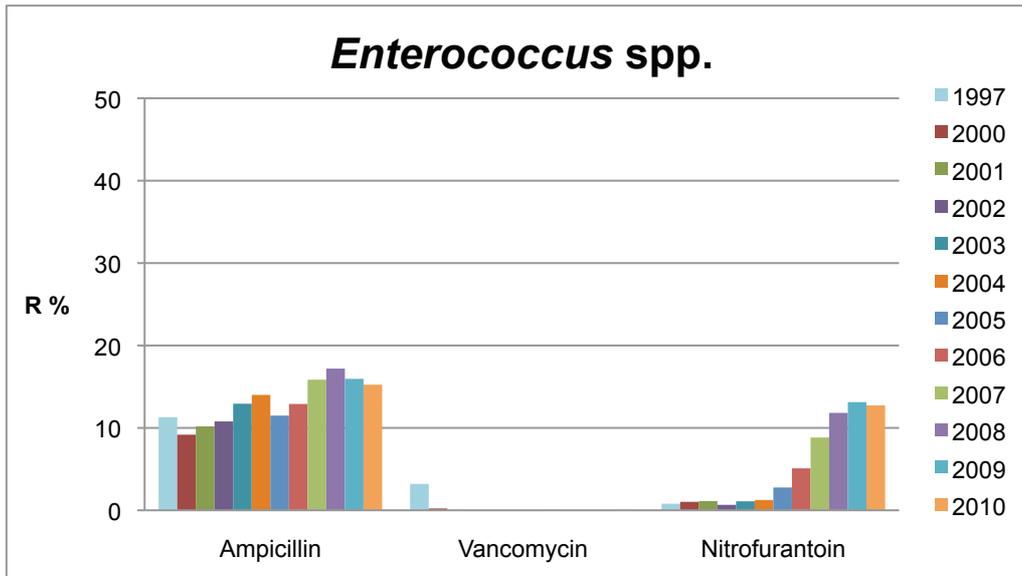


Figure 3.25: Antimicrobial resistance in *Enterococcus* spp. between 1997 and 2010.

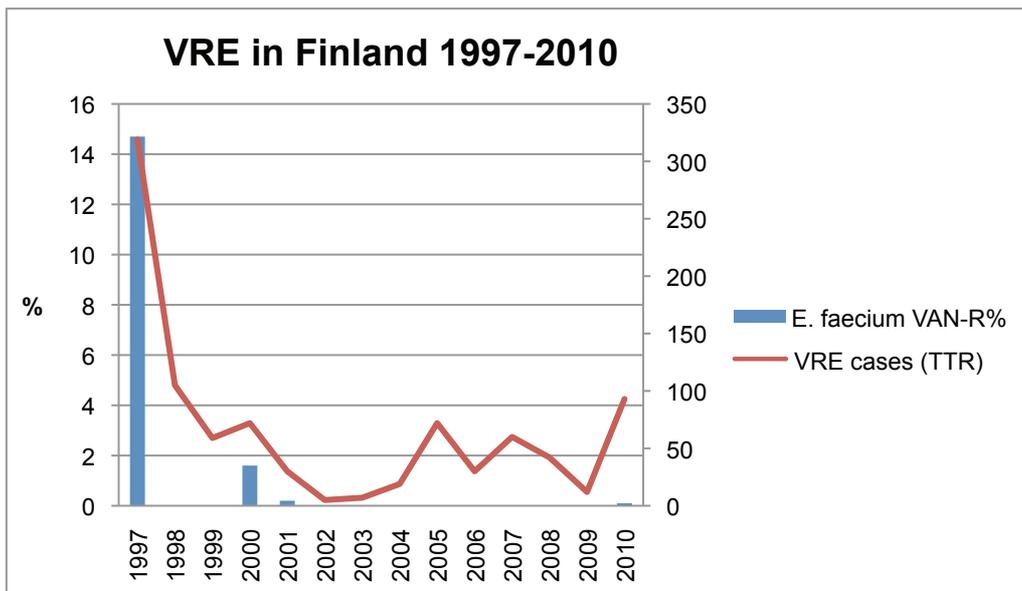


Figure 3.26: VRE cases in Finland (including both clinical and carrier isolates): The percentage of vancomycin-resistant *E. faecium* represented by bars (left y-axis) and the number of VRE cases reported to the National Infectious Diseases Register is represented by a line (right y-axis).

References:

- (1) Top J, Willems R, Bonten M. Emergence of CC17 *Enterococcus faecium*: from commensal to hospital-adapted pathogen. FEMS Immunol Med Microbiol 2008;52:297–308
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3.8 Antimicrobial resistance in *Neisseria gonorrhoeae* (2000–2010)

N. gonorrhoeae

Typical infections:

- Gonorrhoea: purulent (sometimes symptom-free) urethritis, epididymitis, oviduct infection, throat gonorrhoea, eye gonorrhoea, rectal infection

The effect of the antimicrobial resistance situation on treatment options:

- Gonorrhoea can no longer be treated with fluoroquinolones
- 3rd generation cephalosporins need to be used with higher doses

Neisseria gonorrhoeae is still among the most common sexually transmitted diseases in the world. Although endemic gonorrhoea was practically eradicated in Finland in the 1990's, isolated cases have been reported evenly throughout the 2000's, around 240 cases per year (1). The majority of cases are "souvenirs" from abroad, with some minor secondary spread. Therefore local antimicrobial stewardship programmes would have no effect on the development of resistance.

After the increase in penicillin resistance among *N. gonorrhoeae*, fluoroquinolones appeared on the market at the end of the 1980s, and became the drug of choice for gonorrhoea all over the world. Thus a Finnish treatment recommendation published in 1986 (2), which still recommended the use of penicillins, had to be replaced already in 1989 by a recommendation where ciprofloxacin was the first-line treatment (3). The first ciprofloxacin-resistant *N. gonorrhoeae* strains in Finland were described in 1995 (4) and ten years later the proportion already exceeded 50% (Figure 3.27). After 20 years of use, ciprofloxacin has come to the end of its road in the treatment of gonorrhoea in Finland.

Penicillin resistance in *N. gonorrhoea* is most commonly caused by mutations in the genes encoding penicillin-binding proteins which gradually increase the level of resistance. For a long time this was countered by increasing dosages. At the beginning of the penicillin era at the end of the 1940's, gonorrhoea could be treated with a dose of 60 000 units, whereas at the end of the 1980's the dosage needed was 40 times higher (2). Ceftriaxone has so far maintained its clinical efficacy against *N. gonorrhoeae*. But resistance is gradually increasing (5), and in 2011 the first fully ceftriaxone-resistant strain of *N. gonorrhoeae* was described in Japan (6). Such strains have also been reported in Europe lately.

So far all tested patient isolates have been ceftriaxone susceptible in Finland, but strains with reduced susceptibility have started to appear (Figure 3.28). By increasing the dosage (as with the penicillins!), we can manage for a while.

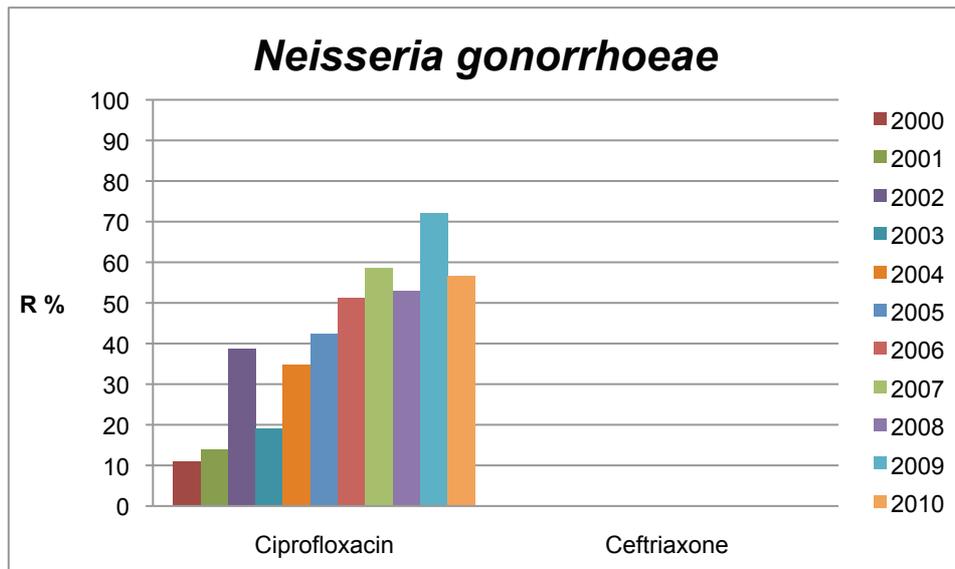


Figure 3.27: Ciprofloxacin and ceftriaxone resistance in *Neisseria gonorrhoeae* from 2000 to 2010.

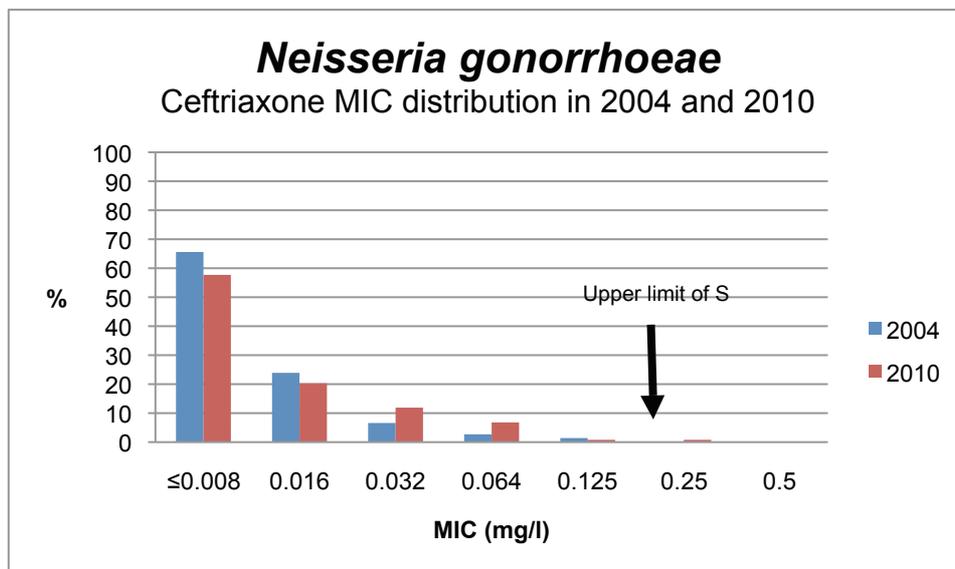


Figure 3.28: Ceftriaxone MIC distribution of *Neisseria gonorrhoeae* in 2004 and 2010.

References:

- (1) Tartuntautirekisteri (www.thl.fi)
- (2) Grönroos P, Jähkölä M, Järvinen H *et al.* Suomen gonokokkien mikrobilääkeresistenssi ja komplisoitumattoman tippurin hoito vuonna 1985. Suomen lääkärilehti 1986;8:731-
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4 Resistance surveillance based on research projects and other materials

4.1 Antimicrobial resistance in *Salmonella enterica* isolates from 1995 to 2009

Salmonella

Typical infections:

- Enterites, serious septic general infections

The effect of the antimicrobial resistance situation on treatment options:

- Mildly symptomatic patients with no underlying diseases should not be treated with antibiotics.
- Travellers, especially from the Southeast Asia, with severe symptoms should be treated according to susceptibility results, and if starting empiric treatment, keep in mind the large proportion of fluoroquinolone nonsusceptible strains, and the possibility of strains resistant to 3rd generation cephalosporins (ESBL/AmpC).

The development of antimicrobial resistance in *Salmonella enterica* isolates has been monitored at the Antimicrobial Resistance Unit at the National Institute for Health and Welfare (formerly the National Public Health Institute) since 1996. During these years, three important phenomena have been detected: an increase in strains with reduced fluoroquinolone susceptibility, and also more frequent isolation of both *qnr*- and ESBL-producing *S. enterica* isolates. All of these originate from Southeast Asia.

Reduced fluoroquinolone susceptibility

In 1995 to 2003, reduced fluoroquinolone susceptibility (CIP ≥ 0.125 mg/L) among foreign *S. enterica* isolates increased from 4 % to 47 %, whereas the increase among domestic isolates was from 0 % to 15 %. The increase was most marked among isolates collected from travellers returning from Southeast Asia and especially from Thailand, from 6 % to 66 %. From the 1990's to the beginning of the 2000's, all *S. enterica* isolates with reduced fluoroquinolone susceptibility were highly resistant to nalidixic acid (NAL ≥ 64 mg/L) and the resistance was caused by mutations in the *gyrA* gene (1,2).

The Qnr-phenotype

Since 2003, reduced ciprofloxacin susceptibility has become rarer in all study populations, and in 2009 only 10 % of domestic, 30 % of foreign and 35 % of isolates with Southeast Asian origin had reduced fluoroquinolone susceptibility (Figure 4.1&4.2). At the same time a new resistance phenotype arrived: after 2003, we have found *S. enterica* isolates with reduced ciprofloxacin susceptibility that are sensitive or only low-level resistant to nalidixic acid (NAL ≤ 32 mg/L) (3). This new resistance phenotype is plasmid mediated, and the *qnr* genes are easily transferred from one isolate to another. Isolates with gyrase mutations were easily detected thanks to their nalidixic acid resistance. Isolates with the *qnr* phenotype are missed if only nalidixic acid is tested; also the ciprofloxacin susceptibility must be determined. Between 2003 and 2008, the percentage of *qnr* isolates was around 10%, but decreased to 5% in 2009 (Figure 4.2). Practically all *qnr* positive *S. enterica* isolates originate from Southeast Asia, mainly Thailand and Malaysia (4).

ESBL-producing S. enterica

In the 1990's, *S. enterica* isolates with reduced cefotaxime susceptibility were found rarely. In the 2000's, and especially 2005 onwards, the proportion of cefotaxime nonsusceptible isolates has increased substantially, already being over 1% in 2009. Over 50% of these have the ESBL phenotype; the other half

are AmpC producers. Again, the reduced susceptibility is mainly concentrated to *S. enterica* isolates originating from Southeast Asia. In addition, the CTX-M ESBL genes are commonly linked to *qnr*.

Azithromycin resistance

Due to increased resistance to fluoroquinolones and cephalosporins, other antimicrobials, like azithromycin, have been tested for the treatment of *Salmonella* infections. Although *Salmonella* is intrinsically resistant to erythromycin, the macrolide derivative azithromycin has shown good *in vitro* activity against *S. enterica* isolates. Between 2003 and 2008, less than 2 % of the tested *S. enterica* isolates were resistant to azithromycin (AZM ≥ 32 mg/L), whereas among reduced fluoroquinolone susceptible isolates azithromycin resistance was >5 % (5).

MDR

From 2000 to 2009, the ASSuT resistance profile (resistance to AMP, STR, SUL and TCY) was detected in 8.1 % of domestic and 6.0 % of foreign *S. enterica* isolates, and the ACSSuT (CHL in addition to ASSuT) resistance profile was detected in 5.0 % of domestic and 2.6 % of foreign *S. enterica* isolates. Among foreign *S. enterica* isolates, 71 and 56 % of the isolates with ASSuT and ACSSuT resistance profile, respectively, originated from Southeast Asia.

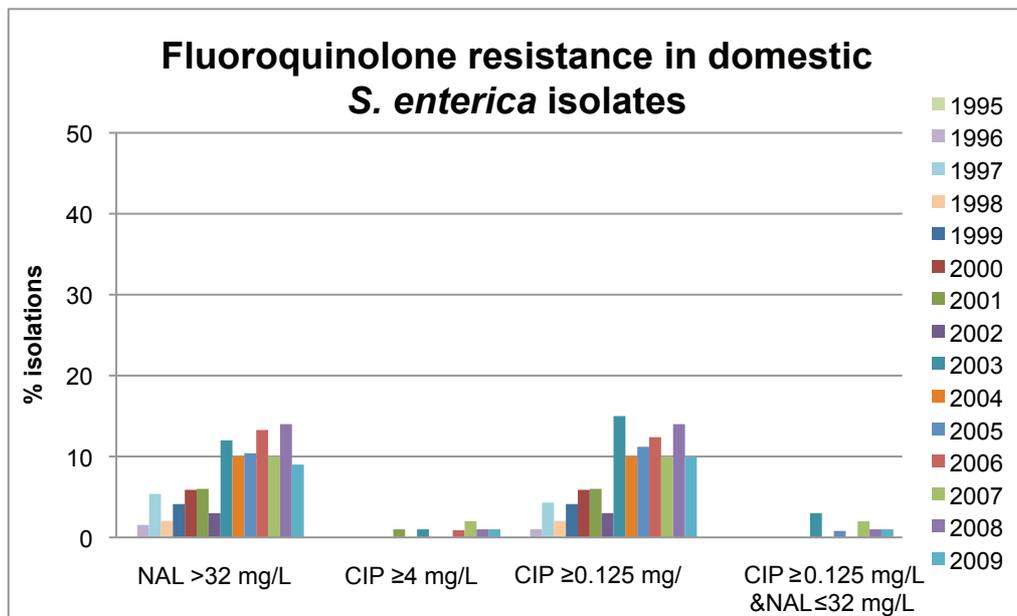


Figure 4.1: Fluoroquinolone resistance in domestic *S. enterica* isolates 1995–2009.

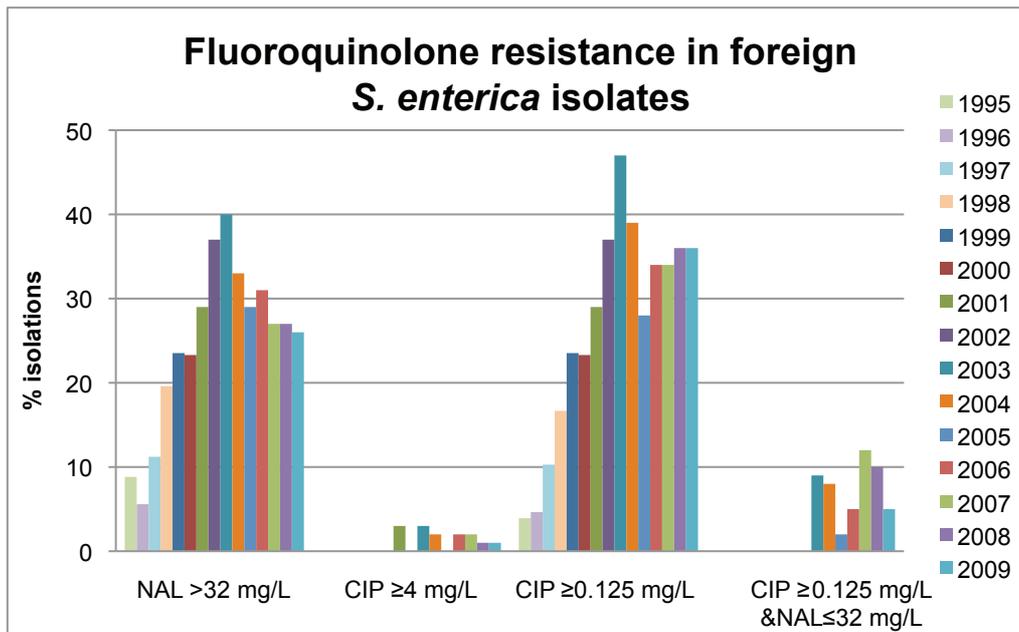


Figure 4.2: Fluoroquinolone resistance in foreign *S. enterica* isolates 1995–2009.

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4.2 Antimicrobial resistance *Mycobacterium tuberculosis*, 1991–2010

Mycobacterium tuberculosis

Typical infections:

- Tuberculosis

The effect of the antimicrobial resistance situation on treatment options:

- MDR strains are started on a combination of five antibiotics

The level of drug resistance for *Mycobacterium tuberculosis* in Finland has remained low, despite the situation in the neighboring countries Russia and Estonia, where isolates resistant to the two most important TB drugs, isoniazid and rifampin (MDR isolates), are common. However, the number of resistant isolates has slowly increased. In 1991 the proportion of *M. tuberculosis* isolates resistant to any first-line TB drug (any resistance) was 2.0%, whereas in 2010 it was 7.5%. MDR isolates have been reported in 0-6 cases (0-2.4% from all isolates) yearly. The majority of MDR isolates have been reported from foreign-born patients, reflecting the situation of increased immigration from high-incidence TB countries to Finland. Resistance to second-line TB drugs is still rare, and no XDR isolates were reported in Finland during the years 1991-2010 (Marttila, Vasankari).

All *M. tuberculosis* strains isolated in Finland are submitted to the Mycobacterial Reference Laboratory at the National Institute for Health and Welfare (THL). The drug susceptibility testing has been performed with the agar proportion method or with the MD Bactec MGIT 960 method, according to the recommendations of the WHO.

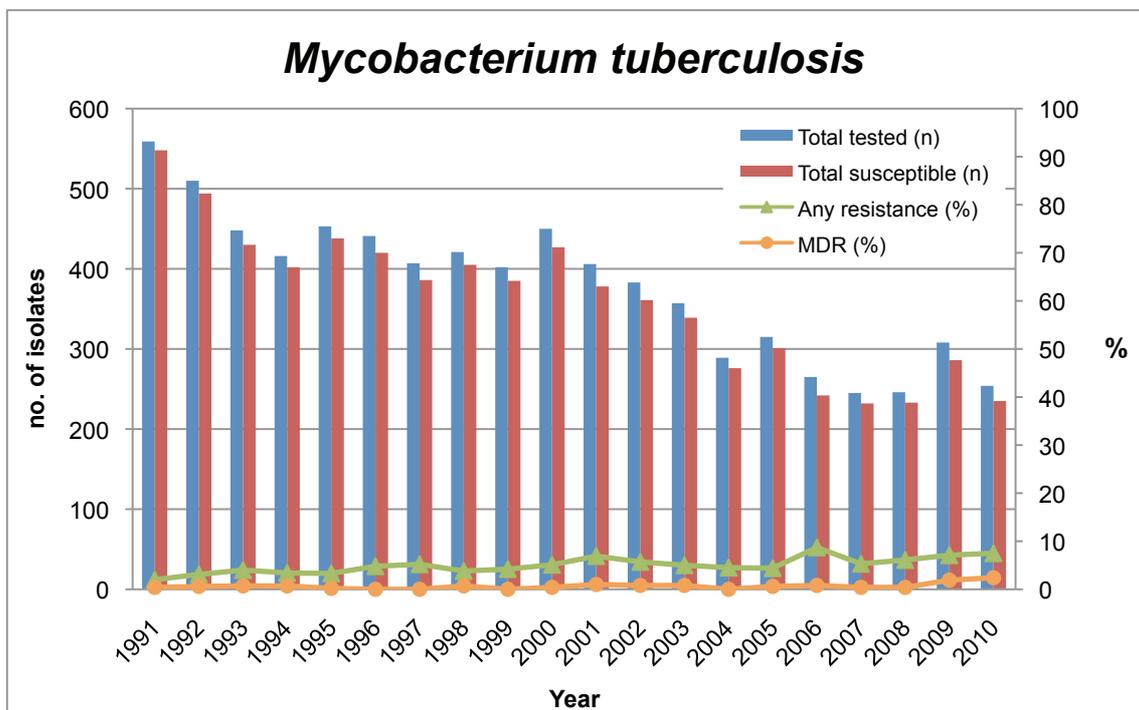


Figure 4.3: Antimicrobial resistance of *Mycobacterium tuberculosis* isolates in Finland in 1991-2010.

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Appendix 2. SIR antimicrobial breakpoints used in the report

Bacterium	Abbreviation	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
		S ≤	R >		S ≥	R <
<i>S. pneumoniae</i>	Penicillin (PEN)	0,064	2	10	6	0
	Erytromycin (ERY)	0,25	1	15	21	15
	Klindamycin (CLI)	0,25	1	2	19	15
	Tetracycline (TCY)	2	8	30	23	18
	Trimethoprim-Sulfa (SXT)	0,5	4	1-2	19	15
<i>H. influenzae</i>	Ampicillin (AMP)	1	4	10	17	13
	Amoxicillin-clavulanic acid (AMC)	4	8	2	17	13
	Tetracycline (TCY)	2	8	30	29	25
	Trimethoprim-Sulfa (SXT)	0,5	4	1-2	16	10
<i>M. catarrhalis</i>	Ampicillin (AMP)	8	32	10	17	13
	Amoxicillin-clavulanic acid (AMC)	8	32	20	18	13
	Erytromycin (ERY)	0,5	8	15	23	13
	Tetracycline (TCY)	4	16	30	19	14
	Trimethoprim-Sulfa (SXT)	2	4	1-2	16	10
	<i>S. pyogenes</i>	Erytromycin (ERY)	0,25	1	15	21
	Klindamycin (CLI)	0,25	1	2	19	15
<i>E. coli</i>	Ampicillin (AMP)	8	32	10	17	13
<i>K. pneumoniae</i>	Piperacillin-tazobactam (TZP)	16	128	100	21	17
<i>Acinetobacter</i> spp.	Cefalothine (CEP)	8	32	30	18	14
	Cefuroxime (CXM)	8	32	30	18	14
	Cefotaxime (CTX)	8	64	30	23	14
	Ceftriaxone (CRO)	8	64	30	21	13
	Ceftazidime (CAZ)	8	32	30	18	14
	Imipenem (IPM)	4	16	10	16	13
	Meropenem (MEM)	4	16	10	16	13
	Tobramycin (TOB)	4	16	10	15	12
	Netilmicin (NET)	8	32	30	15	12
	Norfloxacin (NOR)	4	16	10	17	12
	Ciprofloxacin (CIP)	1	4	5	21	15
	Levofloxacin (LVX)	2	8	5	17	13
	Trimethoprim-Sulfa (SXT)	2	4	1-2	16	10
	Mecillinam (MEC)	8	32	10	15	11
	Nitrofurantoin (NIT)	32	128	300	17	14
Trimethoprim (TMP)	8	16	5	16	10	
<i>P. aeruginosa</i>	Piperacillin-tazobactam (TZP)	64	128	100	18	17
	Ceftazidime (CAZ)	8	32	30	18	14
	Imipenem (IPM)	4	16	10	16	13
	Meropenem (MEM)	4	16	10	16	13

	Tobramycin (TOB)	4	16	10	15	12
	Ciprofloxacin (CIP)	1	4	5	21	15
<i>S. aureus</i>	Oxacillin (OXA)	2	4	1	13	10
	Erythromycin (ERY)	0,5	8	15	23	13
	Klindamycin (CLI)	0,5	4	2	21	14
	Tetracycline (TCY)	4	16	30	19	14
	Trimethoprim-Sulfa (SXT)	2	4	1-2	16	10
	Tobramycin (TOB)	4	16	10	15	12
	Netilmicin (NET)	8	32	30	15	12
	Vankomycin (VAN)	2	16	30	15	0
	Rifampicin (RIF)	1	4	5	20	16
	Fusidic acid (FUS)	0,5	2	-	-	-
<i>Enterococcus spp.</i>	Ampicillin (AMP)	8	16	10	17	16
	Vankomycin (VAN)	4	32	30	17	14
	Teicoplanin (TEC)	8	32	30	14	10
	Nitrofurantoin (NIT)	32	128	300	17	14
<i>N. gonorrhoeae</i>	Ciprofloxacin (CIP)	0,064	1	5	41	27
	Cefotaxime (CTX)	0	0,5	30	31	0
	Ceftriaxone (CRO)	0	0,25	30	35	0
<i>Salmonella spp.</i>	Ciprofloxacin (CIP)	0,125	4	5	21	15
	Nalidixic acid (NAL)	16	32	30	19	13