ANNUAL REPORT

EARS-Net

2009

DRAFT

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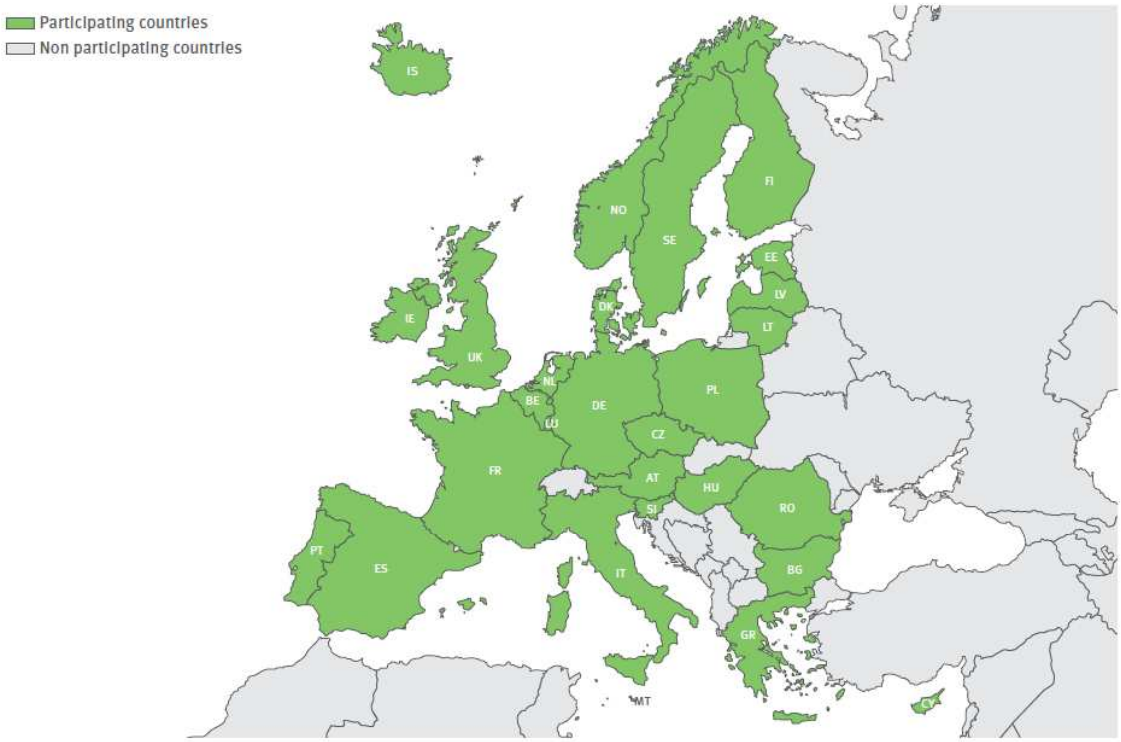
Table of contents EARS-Net Annual Report 2009

Countries participating in EARS-Net 2009



AT	Austria	FR	France	NL	Netherlands
BE	Belgium	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	PL	Poland
CY	Cyprus	IE	Ireland	PT	Portugal
CZ	Czech Republic	IS	Iceland	RO	Romania
DE	Germany	IT	Italy	SE	Sweden
DK	Denmark	LT	Lithuania	SI	Slovenia
EE	Estonia	LU	Luxembourg	UK	United Kingdom
ES	Spain	LV	Latvia		
FI	Finland	MT	Malta		

List of national institutions and organisations participating in EARS-Net

AUSTRIA	BELGIUM
Federal Ministry of Health Medical University Vienna Elisabethinen Hospital, Linz	Scientific Institute of Public Health University of Antwerp
BULGARIA	**CYPRUS**
Alexander University Hospital, Sofia National Center of Infectious and Parasitic Diseases	Nicosia General Hospital
CZECH REPUBLIC	**DENMARK**
National Institute of Public Health	Statens Serum Institute
ESTONIA	**FINLAND**
Health Board East-Tallinn Central Hospital Tartu University Hospital	National Institute for Health and Welfare
FRANCE	**GERMANY**
Pitie-Salpetriere Hospital National Institute for Public Health Surveillance	Robert Koch Institute
GREECE	**HUNGARY**
Hellenic Pasteur Institute National School of Public Health National and Kapodistrian University of Athens, Medical School	National Centre for Epidemiology
IRELAND	**ICELAND**
Health Protection Surveillance Centre (HPSC)	Centre for Health Security and Infectious Disease Control Landspitali University Hospital
ITALY	**LATVIA**
National Institute of Public Health	Paul Stradins Clinical University Hospital State Agency "Infectology Center of Latvia"
LITHUANIA	**LUXEMBOURG**
Center for Communicable Diseases and AIDS National Public Health Surveillance Laboratory	National Health Laboratory
MALTA	**NETHERLANDS**
Hospital of Malta	National Institute for Public Health and the Environment
NORWAY	**POLAND**
University Hospital of North Norway Norwegian Institute of Public Health St. Olav University Hospital Trondheim	National Medicines Institute
PORTUGAL	**ROMANIA**
National Institute of Health Dr. Ricardo Jorge Ministry of Health Directorate General of Health	National Institute of Research and Development for Microbiology and Immunology "Cantacuzino" Institute of Public Health
SLOVENIA	**SPAIN**
National Institute of Public Health University of Ljubljana	Health institute Carlos lll National Centre of Epidemiology
SWEDEN	**UNITED KINGDOM**
Swedish Institute for Infectious Disease Control	Health Protection Agency Northern Ireland Healthcare Associated Infection Surveillance Centre

Chapter 1.

General introduction

EARS-Net is a European wide network of national surveillance systems, providing European reference data on antimicrobial resistance for public health purposes. The network is coordinated and funded by the European Centre for Disease Prevention and Control (ECDC).

The surveillance of antimicrobial resistance within the EU is carried out in agreement with Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 and Regulation (EC) no 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for Disease Prevention and Control.

The coordination of EARS-Net, the European Antimicrobial Resistance Surveillance Network (former EARSS), was transferred from the Dutch National Institute for Public Health and the Environment (RIVM) to the European Centre for Disease Prevention and Control (ECDC) in January 2010. At the same time, EU Member States were requested to nominated disease specific contact points for antimicrobial resistance.

At ECDC, the management and coordination of EARS-Net is done by the Section for Antimicrobial Resistance and Healthcare Associated infections. Scientific guidance and supported to the coordination of the network is provided the EARS-Net Coordination Group (previous EARSS advisory board) composed of individual experts selected among the nominated disease specific contact points.

EARS-Net consists of national antimicrobial resistance surveillance networks in Europe and conducts surveillance of antimicrobial susceptibility in seven major invasive pathogens of public health importance: *Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

The national networks systematically collect data from clinical laboratories in their own countries. At present EARS-Net includes over 900 public-health laboratories serving over 1400 hospitals in Europe. The national networks upload the data to a central database at ECDC (The European Surveillance System - TESSy).

EARS-Net maintains an interactive database at the ECDC website (www.ecdc.europa.eu) and publishes annual reports on the occurrence of antimicrobial resistance in Europe. This constitutes an important source of information on antimicrobial resistance for policy makers, scientists, doctors and the public.

Summary

This is the first Annual Report of EARS-Net after the transition of EARSS to ECDC by 31st December 2009. This report represents the continuation of the series of highly valued EARSS Annual Reports published by the network since 2001.

During the last decade, antimicrobial resistance has moved steadily to a more and more prominent position on the public health agenda in Europe. The surveillance of antimicrobial resistance conducted previously by EARSS, and currently by EARS-Net, has played an important role to provide documentation of the occurrence and spread of antimicrobial resistance, and to increase awareness of the problem at the political level, among public health officials and in the scientific community.

Based on the antimicrobial resistance data reported to EARS-Net by twenty eight countries in 2009, and on the trend analyses including EARSS data from previous years, the resistance situation in Europe displays large variation depending on pathogen type, antimicrobial substance and geographic region.

In 2009, the most concerning resistance results come from the rapidly decreasing susceptibility of invasive *Escherichia coli* to basically all antimicrobial agents included in the EARS-Net surveillance except carbapenems, and from the high prevalence of resistance in *Klebsiella pneumonia* to third gen cephalosporins, fluoroquinolone and aminoglycosides. In half of the reporting countries, the proportion of multi resistant *K. pneumonia* isolates (combined resistance to third generation cephalosporins, fluoroquinolones and aminoglycosides) is above 10%, and a few countries are now also reporting high proportions of resistance to carbapenems. These antibiotics have been widely used in many countries due to the increasing rate of ESBLs producing Enterobacteriaceae with a consequent impact on the emergence of carbapenemase production (VIM, KPC and NDM-1), especially in *K. pneumoniae.*

The highest resistance proportions in *E. coli* were reported for aminopenicillins ranging up to 66%. Irrespective of the high level of resistance, proportions continue to increase even in countries already presenting resistance well above 50%. Resistance to 3rd generation cephalosporins in *E. coli* has also increased significantly during the last four years in more than half of the reporting countries. This resistance is directly linked to the high proportions (85% - 100%) of ESBL positive among the resistant isolates reported by eleven of twelve countries reporting on ESBL in 2009.

Other trends in the occurrence of resistance reported to EARS-Net brings hope that national efforts on infection control and efforts targeted at containment of resistance may in some cases bring the development of resistance to a halt, or even reverse undesirable resistance trends, as exemplified by

the development for MRSA. Even though the proportion of MRSA among *Staphylococcus aureus* is still above 25% in ten out of twenty eight countries, the occurrence of MRSA is stabilizing or decreasing in some countries and a sustained decrease of MRSA was observed in Austria, France, Ireland, Latvia and UK.

Furthermore, the United Kingdom has shown a consistent reduction of resistant proportions in *K. pneumoniae* for all the antibiotic classes under surveillance, and in a few countries (Greece, Germany, Italy and France) the efforts to control glycopeptide resistance in *Enterococcus faecium* seem to be successful and resulting in a continuous decrease of proportions of resistant isolates. Meanwhile, high-level aminoglycoside resistance in *Enterococcus faecalis* seems to stabilize at a relatively high level. The majority of countries reported proportions of resistant isolates between 30% and 50%.

For *Streptococcus pneumoniae*, non-susceptibility to penicillin is generally stable in Europe and non-susceptibility to macrolides has declined in six countries while no country reported increasing trends. For *Pseudomonas aeruginosa,* high proportions of resistance to fluoroquinolones, carbapenems and combined resistance have been reported by many countries especially in Southern and Eastern Europe.

For several antimicrobial and pathogen combinations e.g. fluoroquinolone resistance in *E. coli, K. pneumoniae, P. aeruginosa* and for MRSA, a north to south gradient is evident in Europe. In general, lower resistance proportions are reported in the north and higher proportions in the south of Europe likely reflecting differences in infection control practices, presence or absence of legislation regarding prescription of antimicrobials and other factors know to influence the occurrence of resistance. However, for *K. pneumoniae*, Increasing trends of resistance to specific antibiotic classes and of multi resistance have been observed also in northern European countries, like Denmark and Norway, with a traditionally prudent approach to the antibiotic use.

In addition to the regular trend analysis and situation overview, this 2009 EARS-Net report features a new focus chapter providing in-depth analysis for *E. coli* and MRSA. These analyses are based exclusively on data from laboratories reporting consistently over several years. The in-depth analysis confirms a consistent rise in multi drug resistance and reveals a steady and significant decline of antimicrobial susceptibility in *E. coli* over several years. For MRSA the observed decline likely reflects the efficacy of infection control measures at hospital level, and may even leave some hope for the success of containment strategies in other areas.

In conclusion the data reported to EARS-Net for 2009 by the participating countries provides a knowledge baseline on the occurrence of antimicrobial resistance in Europe and documents the unfortunate and steadily diminishing antimicrobial treatment options for major bacterial pathogens.

Chapter 2.

Focus chapter: *Escherichia coli* and *Staphylococcus aureus*: bad news and good news

Analysis of data from laboratories reporting continuously from 2002 to 2009

The results presented in this focus chapter are based on data from 198 laboratories that reported susceptibility results of *Escherichia coli* and *Staphylococcus aureus* continuously from 2002 to 2009. These laboratories are located in 22 countries participating in EARS-Net. Their long standing contribution to the surveillance of antimicrobial resistance allow for accurate comparison of proportions of resistant isolates and number of reported infections over time. The results presented in this focus chapter may be slightly different from those obtained from the full group of laboratories reporting to EARS-Net.

Key points

- A significant decline of antimicrobial susceptibility was observed for *E. coli* between 2002 and 2009 with a concomitant and continuous increase in the number of reported blood stream infections (increase of 71% from 2002 to 2009).
- *S. aureus* showed a different tendency with a significant decrease of the proportion of meticillin resistance (MRSA) and a slight increase in the number of reported blood stream infections.
- The trends observed for *E. coli* could suggest an incremental burden of disease caused by this microorganism while the reduction of the MRSA proportion and the containment of the number of *S. aureus* infections could result from efficacy of infection control measures at hospital level.
- Even though an overall decreasing trend for MRSA was evident, not all countries contribute to this result. Efforts to reduce MRSA occurrence should remain a priority irrespective of decreasing trends.

Escherichia coli and *Staphylococcus aureus* are the most frequent causes of blood stream infections (BSIs). The temporal trends of resistance and the trends in the incidence of BSIs caused by these microorganisms, observed through the EARSS/EARS-Net data, are described for the period 2002-2009.

The antimicrobial susceptibility of *E. coli* BSI isolates shows an alarming Europe-wide decline as previously reported by the European Antimicrobial Resistance Surveillance System (EARSS)(1). Increasing resistance in *E. coli* and combined resistance of invasive and non-invasive isolates is reported in several national European surveillance reports (2-5). At the same time, the proportion of meticillin resistant *S. aureus* (MRSA) has showed a significant decrease in many European countries.

The numbers of BSIs caused by MRSA, as reported by the mandatory surveillance system in United Kingdom, decreased by 56% between 2004 and 2008 (6). A similar reduction of the rate of health care–associated invasive MRSA infections has been observed at population level in the US (7).

A total of 198 laboratories in 22 countries reported continuously from 2002 to 2009. The number of laboratories per country ranged between 1 (Iceland and Malta) and 33 (Czech Republic) (Table 2.1).
Considering the whole group of selected laboratories, the reported number of *E. coli* BSIs, increased by 71% from 10688 in 2002 to 18240 in 2009. In the same period *S. aureus* BSIs showed a 34% increase from 7855 to 10503 (Figure 2.1); this increase was observed in most but not all countries (Table 2.1).
During this interval the proportion of third generation cephalosporin resistant *E. coli* increased significantly from 1.7% to 8% ($p<0.001$) and the proportion of MRSA decreased from 21.5% to 19.7% ($p<0.001$) (Figure 2.2). The trends of resistance proportions at country level were consistent with those of the whole group of 198 laboratories in 18 countries out of 22 for *E. coli* and in 7 countries out of 22 for *S. aureus*.
Combined resistance in *E. coli* (defined as resistance to 2, 3 and 4 antibiotic classes reported to EARS-Net) showed a significant increase (Figure 2.3) ($p<0.001$) and became the dominant phenotype in most countries, whereby single resistance diminished from 37.1% in 2002 and 35.8% in 2009 ($p<0.001$). The proportion of isolates susceptible to all four antibiotic classes decreased from 51.4% in 2002 to 41.7% in 2009 ($p<0.001$).

The decline of antimicrobial activity in *E. coli* was evident both through the observed increase of combined resistance and through the reduction of full susceptibility to the antimicrobials included in the analysis. In the same time period and considering the same data source, a significant decrease of meticillin resistance was observed for *S. aureus*. For this species, the number of BSIs increased less (+34%) than for *E. coli* BSI (+71%). Importantly, most of the rise (38% of 71%) in *E. coli* BSIs appeared to be due to isolates resistant to two or more antibiotics. Furthermore, the increase in the number of BSIs was similar for meticillin susceptible *S. aureus* (MSSA, 37%) and for fully susceptible *E. coli* (39%).
Despite the possible limitations of the presented results (see the paragraph “Limitations of the analysis”), the trends of third generation cephalosporins and combined resistance are relevant findings that deserve further consideration. According to the results it appears that, during the study period, the emergence and spread of combined resistance is the main factor that influences the decline of antimicrobial activity against *E. coli*. In the period 2002-2009, only an increase of

combined resistance with a concurrent relative reduction of the proportion of single resistance was observed. The resistant sub-population with the largest relative growth in the period 2002-2009, was the resistance to all the four antibiotic classes under surveillance: this pattern increased more than fivefold from 0.6% to 3.4%. This trend suggests that inside the subpopulation of resistant isolates there was a continuous relative growth of combined resistance possibly caused by the addition of resistance traits to strains that were already resistant to at least one among the considered antibiotic classes. This trend may be explained by the spread of multidrug-resistant plasmids which also contain genes for ESBL (extended-spectrum beta-lactamase) production (8). This is a serious concern since, if ESBL is not contained, the use of carbapenems will increase favoring the emergence of carbapenemase producing enterobacteria.

The growing number of *E. coli* BSIs indicates an increasing burden of disease caused by this microorganism. A similar trend in the number of reported cases of *E. coli* BSIs has been observed in England, Wales and Northern Ireland by the national voluntary surveillance scheme, in the period 2004-2008. The increase (38%) observed by the British surveillance system is greater than the 16% increase in all BSIs reported in the country during the same time period (9).

In conclusion, the reported data show a significant increase of antimicrobial resistance in *E. coli* invasive isolates and an overall increase in BSIs caused by this organism. *S. aureus* shows a different tendency with a significant decrease of the proportion of meticillin resistance (MRSA) and a slight increase in the number of reported blood stream infections. Importantly, the containment of MRSA has been a primary target in several European countries and the US which resulted in a significant reduction in the number of *S. aureus* infections.

Methods of analysis

- Data referring to *E. coli* and *S. aureus* BSIs were extracted for laboratories reporting susceptibility results continuously for both pathogens to EARSS/EARS-Net during the period 2002-2009. Countries with no laboratories participating for the entire period or with small amount of data (less than 20 isolates per pathogen and year) were not included in the analysis. Only the first isolate per patient, pathogen and year was included.
- The number of BSIs caused by *E. coli* and *S. aureus* and the proportions of third generation cephalosporin resistant *E. coli* and of MRSA was calculated for each year of the period 2002-2009. Three additional antibiotic classes (aminopenicillins, aminoglycosides and fluoroquinolones) were also considered for *E. coli* to assess the patterns of combined resistance of this pathogen.
- The significance of the temporal trends for resistance proportions was evaluated by the Cochran-Armitage test for trend.

Limitations of the analysis

- Even considering a stable group of laboratories, it is still possible that a change in the population coverage of these laboratories has occurred over time.
- The different trends observed for *E. coli* and *S. aureus* could also be explained by ascertainment bias leading to higher reporting of *E. coli* infections and caused by an increase of empirical treatment failures triggering delayed diagnostic procedures (blood culture).
- The indicator used to monitor the resistance trend was the SIR (sensitive, intermediate, resistant) interpretation since the actual MIC (minimum inhibitory concentration) values are not systematically made available by participating laboratories. Reporting MICs instead of the SIR interpretation - defined by clinical breakpoints - would improve the monitoring of dynamic and subtle changes of antimicrobial susceptibility.

Table 2.1 - Number of *Escherichia coli* and *Staphylococcus aureus* isolates per country reported in 2002 and 2009 by laboratories participating continuously to the network during this time interval.

		Escherichia coli (No. isolates)		*Staphylococcus aureus* (No. isolates)	
Country	No. labs	first year -2002	last year - 2009	first year -2002	last year - 2009
AT	10	468	886	454	664
BE	9	300	687	331	354
BG	7	76	110	67	83
CZ	33	1439	2243	1028	1414
DE	2	115	226	96	160
EE	5	49	224	74	157
ES	19	1666	2411	632	1079
FI	5	647	902	333	353
FR	12	1445	2081	959	1292
GR	22	452	1127	291	566
HU	14	214	531	273	591
IE	15	684	1438	856	916
IS	1	46	96	54	51
IT	3	179	335	187	175
LU	4	124	220	68	83
MT	1	74	159	87	86
NL	4	405	412	301	213
NO	7	670	1217	387	597
PT	8	326	972	359	762
SE	3	458	357	308	401
SI	9	350	782	232	424
UK	5	501	824	478	82

Figure 2.1 - Yearly number of blood stream infections caused by *Escherichia coli* and *Staphylococcus aureus*

EARSS/EARS-Net 2002-2009 (22 countries/198 labs)

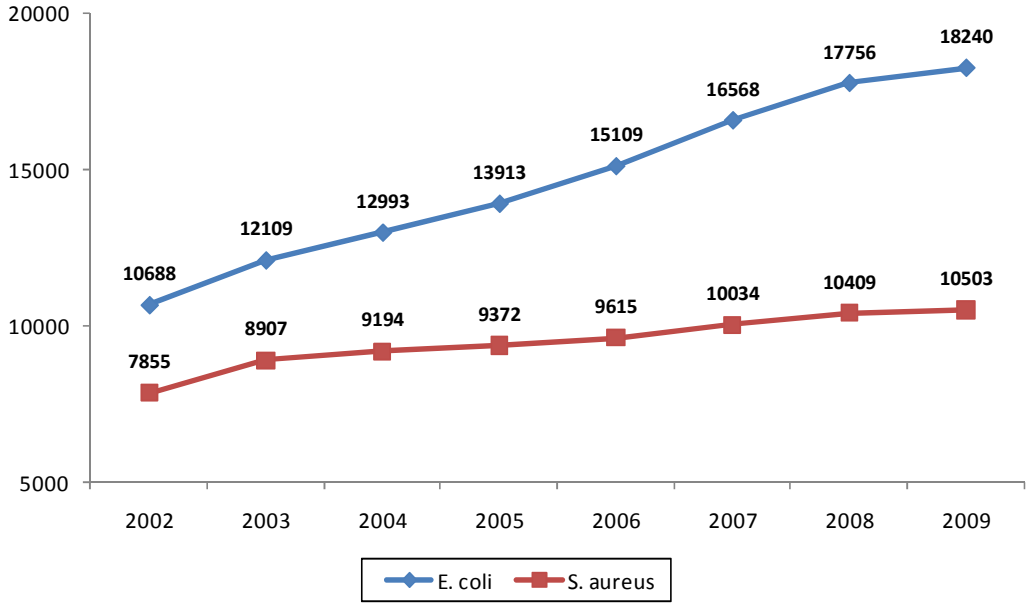


Figure 2.2 - Proportion of 3rd gen. cephalosporin-resistant *Escherichia coli* (CREC) and meticillin-resistant *Staphylococcus aureus* (MRSA)

EARSS/EARS-Net 2002-2009 (22 countries/198 labs)

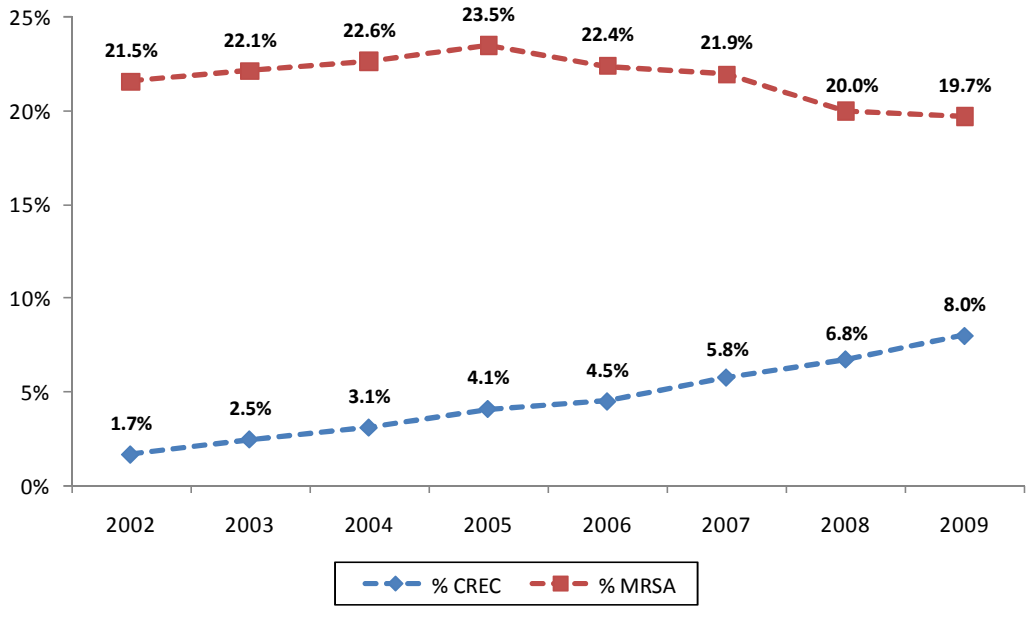
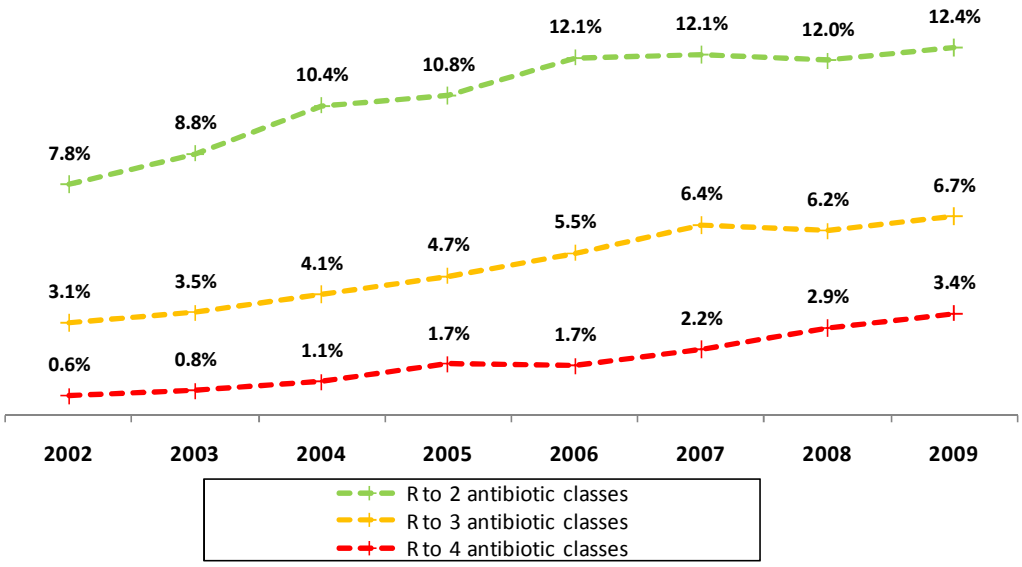


Figure 2.3 - Combined resistance of *Escherichia coli* to aminopenicillins, 3rd gen. cephalosporins, fluoroquinolones and amynoglicosides

EARSS/EARS-Net 2002-2009 (22 countries/198 labs)



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Chapter 3.

External Quality Assessment Exercise (EQA) 2009

1. Introduction

Since 2000, EARSS has been organizing external quality assessment (EQA) exercises of antibiotic susceptibility testing in collaboration with UK NEQAS (United Kingdom National External Quality Assessment Service). UK NEQAS is based at the Health Protection Agency, Colindale, London (UK) and is a non-profit organization with more than 35 years experience with external quality assessment in different countries (www.ukneqasmicro.org.uk).

The rationale of these EQA exercises is,

i) to assess the ability of participating laboratories to identify antimicrobial resistance of clinical and public health importance,

ii) to determine the accuracy of susceptibility test results reported by individual laboratories and

iii) to decide on the overall comparability of routinely collected test results between laboratories and countries and thus provide the means for justifying the pooling and comparison of antimicrobial susceptibility test (AST) data across Europe.

During the latest EQA, held in the second half of 2009, a panel of 6 strains was included. The strains were characterized and tested in two reference laboratories (Addenbrookes Hospital Cambridge, and the Antimicrobial Resistance Research Laboratory (AMRL) of the Health Protection Agencey, Colindale, London.) .Both reference laboratories confirmed MICs and interpreted the results according to frequently used breakpoint criteria such as CLSI and EUCAST, as indicated in each of the species' chapters.

2. Results

Strain panels were distributed to 886 participating laboratories , who were asked to report the clinical susceptibility categorization (S, I, R) according to the guideline used. The laboratories returned 775 (87%) reports, which is equivalent to the returne rate of previous years. Turkey was

not able to perform the tests, due to difficulties shipment problems. Figure 3.1 shows the proportion of participating laboratories returning reports per country.

All results were collected via the UK NEQAS internet website. Results were analyzed and considered 'concordant' if the reported categorization agreed with the interpretation of the reference laboratories.

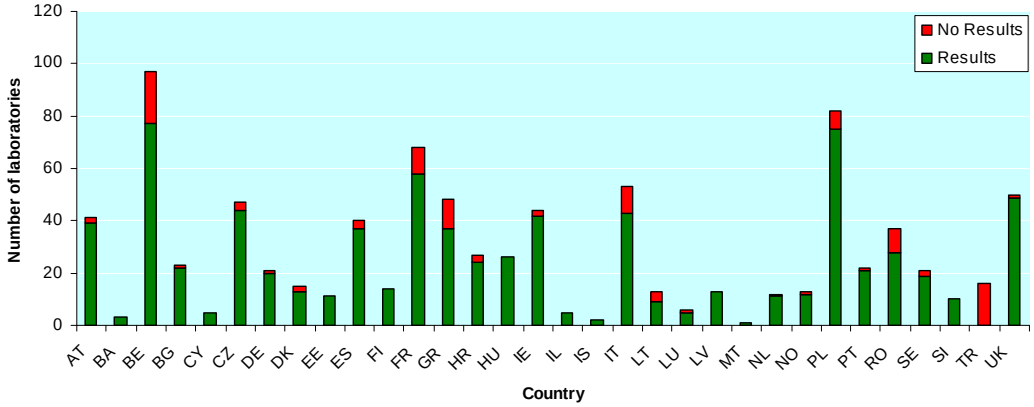

Figure 3.1. Proportion of participating laboratories returning reports per country. Red bars indicate those which did not return results.

For the determination AST results, laboratories used automated methods (35%), disc diffusion tests (33%), or combined methods (23%). For species identification, 60% used automated and 40% used conventional methods. The majority of laboratories are appyling CLSI guidelines (68%), and some countries use national guidelines like France (SFM), UK (BSAC), and Sweden (SRGA). The use of EUCAST guidelines was mainly reported by laboratories that utilise automated diagnostic test systems already calibrated for EUCAST breakpoints. At the same time, harmonisation efforts between the different national guideline committees have accomplished a satisfactory degree of agreement and therefore previous discrepancies between different guidelines have become less significant. Figure 3.2 shows the adherence to (inter)national guidelines by number of laboratories per country.

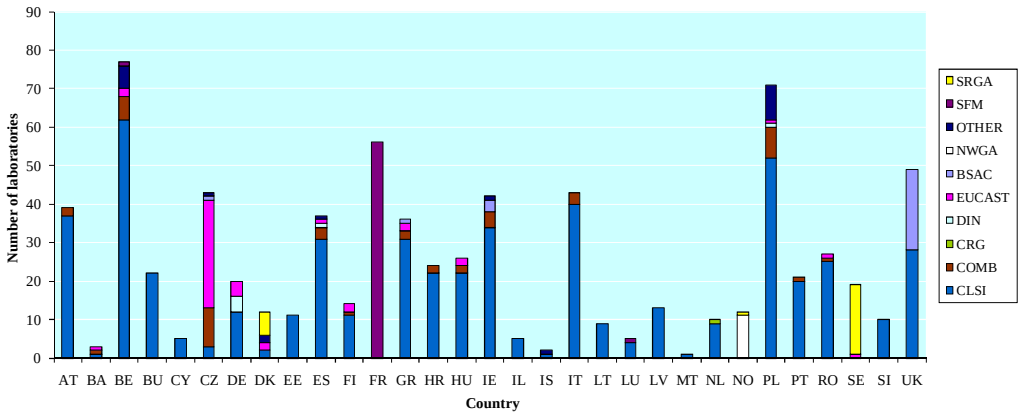

Figure 3.2. Adherence to guidelines*: number of laboratories per country

BSAC, British Society for Antimicrobial Chemotherapy; CRG, (Dutch) Commissie Richtlijnen Gevoeligheidsbepalingen; DIN, Deutsche Industrie Norm; EUCAST, European Committee on Antimicrobial Susceptibility Testing; NCCLS/CLSI, (American) Clinical and Laboratory Standards Institute; NWGA, Norwegian Working Group on Antimicrobials; SFM, Comité de l'Antibiogramme de la Société Française de Microbiologie; SRGA, Swedish Reference Group for Antibiotics.

2.1. Specimen 9448

This specimen consisted of an *Escherichia coli* with CTX-M-14 ESBL production. Unlike most TEM- and SHV-derived ESBLs, CTX-M enzymes are more active against cefotaxime than ceftazidime. This organism should clearly be interpreted as resistant to cefotaxime (MIC ≥128 mg/L) using EUCAST (S ≤1, R >2 mg/L), and CLSI (S ≤8, R ≥64 mg/L) guidelines. With ceftazidime (MIC 2 mg/L) the isolate appears intermediate by EUCAST guidelines (S ≤1, I 2-8, R >8 mg/L) and susceptible by standard CLSI guidelines (S ≤8, I 16, R ≥32 mg/L). However, current EUCAST expert rules recommend that ESBL-producing isolates are reported resistant if they appear intermediate in routine tests and intermediate if they appear susceptible. Resistance should be reported if CLSI ESBL screening methods are used as recommended.

Detection of reduced susceptibility to cefotaxime and ceftriaxone was not a problem and <1% participants reported the organism susceptible to these agents. With ceftazidime, reports of intermediate (15%) and susceptible (6%) were more common. A large majority (98%) of participants testing for ESBLs correctly reported the organism ESBL-positive.

This organism was susceptible to piperacillin/tazobactam in MIC tests (MIC 2-4 mg/L). Despite some reports of clinical efficacy, there has been considerable debate about whether ESBL-producing strains from serious infections should be reported susceptible to ß-lactamase inhibitor combinations

when they appear susceptible in routine tests. While the majority of participants (71%) reported the organism susceptible to piperacillin/tazobactam, significant proportions reported intermediate (9%) or resistant (20%).

Table 3.1. *Escherichia coli* **(9448*):* Minimal inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories.**

Antibiotic agent	MIC range ref. lab.		Intended interpretation	Overall
	from	to	EUCAST/CLSI	concordance (%)
Amikacin	1	1	S	98
Amoxicillin	NT*		R	93
Ampicillin	>128	>128	R	100
Cefotaxime	128	>128	R	97
Ceftazidime	2	2	R (expert rule)	80
Ceftriaxone	>128	>128	R	99
Ciprofloxacin	64	128	R	100
ESBL			positive	98
Gentamicin	32	64	R	99
Imipenem	0.12	0.12	S	100
Meropenem	≤0.03	≤0.03	S	99
Piperacillin	≥128	≥128	R	99
Piperacillin/tazobactam	2	4	S	71
Tobramycin	4	>128	I-R/S-I-R	

*Not tested, result inferred from ampicillin.

In reference tests this organism was resistant to gentamicin, highly variable in susceptibility to tobramycin (reference MICs 4 to >128 mg/L) and susceptible to amikacin. It is likely that this reflects production of an ANT (2'') enzyme, expression of which can be variable. EUCAST expert rules suggest that such isolates should be reported resistant to tobramycin if they appear intermediate. While 99% participants reported the organism gentamicin resistant and 98% amikacin susceptible, for tobramycin, 30%, 28% and 42% were reporting resistant, intermediate and susceptible, respectively (Table 3.1).

2.2. Specimen 9449 *Klebsiella pneumoniae*

This organism is a *Klebsiella pneumoniae* with a high level β-lactamase production; not only penicillin resistant, but also reduced susceptibility to ß-lactamase inhibitor combinations. Piperacillin-tazobactam MICs of 64 to ≥128 mg/L for this organism indicate resistance by EUCAST breakpoints and intermediate/resistant by CLSI breakpoints. Among participants, 70% reported the organism resistant, 25% intermediate and 5% susceptible. ESBL production was incorrectly reported by 9% participants and this was frequently associated with incorrect reporting of resistance to cefotaxime, ceftriaxone and ceftazidime by these participants (Table 3.2).

Table 3.2. *Klebsiella pneumoniae* (9449*):* Minimal inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories.

Antibiotic agent	MIC range ref. lab.		Intended interpretation	Overall
	from	to	EUCAST/CLSI	concordance (%)
Amikacin	64	64	R	97
Ampicillin/amoxicillin	128	≥128	R	100
Cefotaxime	0.12	0.5	S	92
Ceftazidime	0.12	0.25	S	93
Ceftriaxone	0.5	0.5	S	93
Ciprofloxacin	0.015	0.03	S	100
ESBL			negative	91
Gentamicin	64	128	R	99
Imipenem	0.12	0.12	S	99
Meropenem	≤0.03	≤0.03	S	99
Piperacillin	64	≥128	I/R	99
Piperacillin/tazobactam	64	128	I/R	95
Tobramycin	16	128	R	99

2.3. Specimen 9450 *Streptococcus pneumoniae*

This organism is a *Streptococcus pneumoniae* with reduced susceptibility to penicillin (MIC 0.25 mg/L). For *S. pneumoniae* with no mechanism of resistance to penicillin, MICs are ≤0.06 mg/L. EUCAST guidelines divide reduced susceptibility to penicillin into intermediate (penicillin MIC 0.12-2 mg/L) and resistant (MIC >2 mg/L) categories. However, the interpretation of susceptibility to penicillin depends on whether the isolate is from a patient with meningitis or other infections (most commonly pneumonia). Strains with intermediate susceptibility are treatable with the high doses of penicillin, ampicillin or amoxicillin routinely used to treat pneumonia. Hence such strains may be reported susceptible in this situation. Patients with meningitis caused by strains with intermediate susceptibility to penicillin are unlikely to respond to therapy, and hence such strains should be reported as resistant in this situation.

Overall, 92% participants reported resistance in the oxacillin screening test for penicillin resistance. A further 3% reported the isolate intermediate to oxacillin although the oxacillin screening test does not distinguish penicillin intermediate and resistant isolates and published guidelines do not include an intermediate category.

The participants' results for penicillin will include those that only screened for reduced susceptibility with oxacillin discs, those that screened with oxacillin and confirmed with a penicillin MIC, and those that tested with other methods. Overall, 76% reported the isolate as being of intermediate susceptibility to penicillin, with a further 8% reporting the isolate resistant. Susceptibility reported to clinicians for this organism -if isolated from cases of meningitis or pneumonia- indicates that many participants do interpret test results to suit the clinical situation. 85% reported the isolate as resistant when the isolate was from a case of meningitis and 70% as susceptible when the isolate was from a case of pneumonia. However, significant numbers of participants interpreted the organism as intermediate in susceptibility to penicillin irrespective of whether the isolate was from meningitis (11% reported intermediate) or from pneumonia (26% reported intermediate).

Table 3.3. *Streptococcus pneumoniae* (9450*):* Minimal inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories.

Antibiotic agent	MIC range ref. lab.		Intended interpretation	Overall
	from	to	EUCAST/CLSI	concordance (%)
Cefotaxime	0.06	0.12	S	98
meningitis			S	96
pneumonia			S	99
Ceftriaxone	0.06	0.12	S	98
meningitis			S	95
pneumonia			S	98
Ciprofloxacin	1	1	I/-	27
Clindamycin	0.12	0.12	S	99
Erythromycin	0.12	0.25	S	99
Oxacillin			R	92
Penicillin	0.25	0.25	I	76
meningitis			R	85
pneumonia			S	70

With EUCAST breakpoints for ciprofloxacin wild type organisms such as this (MIC 1 mg/L) are reported intermediate in susceptibility to ciprofloxacin, reflecting the need for treatment with high doses. CLSI do not give ciprofloxacin breakpoints for *S. pneumoniae*. This uncertainty was reflected in the high discrepancy rate in reporting for this agent (69% susceptible, 27% intermediate and 4% resistant) (Table 3.3).

2.4. Specimen 9451 *Pseudomonas aeruginosa*

This organism is a *Pseudomonas aeruginosa* with borderline resistance to piperacillin-tazobactam (MIC 32-64 mg/L) but susceptible to ceftazidime, a susceptibility profile seen with some β-lactamase -producing strains. This organism produces the PSE-4 (CARB-1) ß-lactamase. The borderline resistance to piperacillin-tazobactam was reflected in the variation in susceptibility reported by participants, with 49% reporting susceptible, 12% intermediate and 39% resistant. The different

categorisation largely relates to the breakpoint differences between EUCAST (susceptible ≤16 mg/L, resistant >16 mg/L) and CLSI (susceptible ≤64 mg/L, resistant ≥128 mg/L) (Table 3.4).

Table 3.4. *Pseudomonas aeruginosa* **(9451): Minimal inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories.**

Antibiotic agent	MIC range ref. lab. from	to	Intended interpretation EUCAST/CLSI	Overall concordance (%)
Amikacin	1	8	S	100
Ceftazidime	1	2	S	98
Ciprofloxacin	0.12	0.5	S	100
Gentamicin	1	4	S	97
Imipenem	1	2	S	99
Meropenem	0.5	2	S	99
Piperacillin/tazobactam	32	64	R/S	39
Tobramycin	0.5	1	S	99

2.5. Specimen 9452 *Pseudomonas aeruginosa*

This organism is a *Pseudomonas aeruginosa* resistant to gentamicin and susceptible to other reference agents tested. Some laboratories experienced problems with piperacillin-tazobactam testing in that overall 14% participants reported the isolate intermediate and 2% resistant. The reason for the problems is not obvious as the isolate was clearly susceptible in reference tests (Table 3.5).

Table 3.5. *Pseudomonas aeruginosa* (9452*): Minimal inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories.

Antibiotic agent	MIC range ref. lab. from	to	Intended interpretation EUCAST/CLSI	Overall concordance (%)
Amikacin	1	4	S	99
Ceftazidime	1	2	S	99
Ciprofloxacin	0.12	0.25	S	100
Gentamicin	16	64	R	99
Imipenem	1	2	S	99
Meropenem	0.5	2	S	99
Piperacillin/tazobactam	4	8	S	84
Tobramycin	0.5	1	S	99

2.6. Specimen 9453 *Staphylococcus aureus*

This organism is a methicillin resistant *Staphylococcus aureus* (EMRSA 6) and most participants correctly reported resistance. There was no significant difference in reliability of detection of methicillin resistance in tests with oxacillin (94% participants reported resistance) and cefoxitin (95% participants reported resistance).

About a third of participants reported a result for methicillin (97% resistant) but it may be that oxacillin or cefoxitin were actually tested. Erythromycin reference MICs were very variable (4->128 mg/L), probably because resistant colonies of this organism grew slowly. MICs in this range indicate resistance by EUCAST breakpoints and intermediate/resistant by CLSI breakpoints; 93% participants reported the organism resistant and only 6% intermediate (Table 3.6).

Table 3.6. *Staphylococcus aureus* (9453*):* Minimal inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories.

Antibiotic agent	MIC range ref. lab.		Intended interpretation	Overall
	from	to	EUCAST/CLSI	concordance(%)
Cefoxitin	8	16	R	95
Ciprofloxacin	0.5	0.5	S	95
Erythromycin	4	≥128	I/R	93
Fucidic acid	0.06	0.12	S	100
Gentamicin	0.25	0.50	S	99
Methicillin	Nt*		R	97
Oxacillin	8	32	R	94
Penicillin	1	4	R	99
Rifampicin	0.004	0.008	S	99
Teicoplanin	1	1	S	99
Tetracycline	64	64	R	97
Vancomycin	1	2	S	99

*Not tested, result inferred from oxacillin and cefoxitin.

3. Conclusions

In this eighth EARSS EQA exercise, participation of the laboratories was high. The results show that routinely reported results as collected by EARSS in most instances have sufficient accuracy to provide good estimates of overall resistance prevalence and trends. The overall concordance was high, except in case of borderline susceptibility, when various guidelines reveal remaining discrepancies in routine susceptibility testing, and when a breakpoint was recently changed. The latter was found for penicillin susceptibility of *Streptococcus pneumoniae* in relation to the source of the isolate, where the isolate was regularly reported non sensitive for pneumonia incorrectly. Furthermore, differences in interpretation of results were found for Piperacillin-Tazobactam (as frequently noted previously).

EARSS MT would like to thank UK NEQAS for Microbiology, the reference laboratories, the members of the Advisory Board, the country coordinators for the swift distribution of the strains, and all the participating laboratories for their excellent response rate.

Chapter 4. EARS-Net laboratory/hospital denominator data 2009

Introduction

For correct interpretation of the EARS-Net resistance data, accurate background information is important. Therefore, hospital activity data and denominator data are collected as previously done by EARSS.

Methods

Questionnaires, in digital Excel version, were sent to the EARS-Net contact points at the beginning of June 2010. The contact points distributed the questionnaires to the participating laboratories and hospitals in their country. Information was collected on the total number of blood culture sets processed in the laboratories, and the number of hospital beds for each participating hospital, the type of hospital, the bed occupancy and the number of admissions. The national data managers received the completed questionnaires, compiled them and produced the final format suitable for uploading in TESSy. Laboratories have been defined as reporting denominator data if they have provided the number of blood culture sets performed for one or for more than one hospital; hospitals have been defined as reporting denominator data if they have provided the number of beds.

Participation

Twelve out of 28 countries reporting antimicrobial resistance results returned hospital denominator data while eleven countries returned laboratory denominator data. Considering these responding countries, 189 of the 266 laboratories (71%) and 526 of the 656 hospitals (80%) reporting antimicrobial susceptibility results for the 12 countries in 2009, also provided denominator data (Figures 4.1-4.2, Tables 4.1-4.3). Some denominator data of laboratories and hospitals not participating to antimicrobial resistance surveillance have been reported (Figure 4.1) but were not included in the analysis.

Population coverage

The population coverage of antimicrobial resistance data at country level is not reported this year because of the low number of countries submitting denominator data and because of possible limitations in the use of the data on population coverage:

- Laboratories/hospitals reporting antimicrobial susceptibility data do not always provide denominator data and this could give a biased figure of the country population coverage since it can be calculated only for laboratories/hospitals with denominator data.
- Laboratories and hospitals cluster in big cities and for this reason, some of the catchment areas overlap. This could lead to double counts which could artificially increase the estimated coverage.

The coverage at European level possibly increased in 2009 compared to the previous year since the number of reporting laboratories increased from 825 in 2008 to 916 in 2009. A high coverage at country level is very important to properly describe the country profile and it also allows a more accurate calculation of the culture rate and the MRSA incidence rate. Calculation of the MRSA incidence rate (not included in the 2009 report) would be based on the assumption that each hospital is served by one laboratory. Therefore, in cases where the laboratory denominator data is referring to a specific hospital (with patient days available), calculation of the culture rate for that hospital is possible. Similarly, having AMR data referring to a specific hospital (with patient days available) would enable calculation the MRSA rate for that hospital.

However, looking into the available data this assumption seems not always correct. Some hospitals are served by more than one laboratory; it occurs mainly in UK (where the hospital code often refers to a trust instead of a single hospital) but there are examples also in Ireland and Hungary. The implication of the relation “one hospital to more than one laboratory” is that, if one hospital is reported in the AMR and denominator data while only one of the laboratories serving this hospital is reported, then the culture rate and the MRSA incidence rate will be underestimated.

Hospital denominator information

The total number of hospital beds for the hospitals reporting AMR results and providing denominator data in different countries ranged from 1,313 in Cyprus to 143,433 in France, reflecting the size of the country as well as the rate of participation to EARS-Net and the rate of response to the questionnaires. The proportion of ICU beds over total hospital beds shows wide variation according with the country, ranging from 1% in UK to 9% in Cyprus. The median length of stay was 6.3 days with a minimum in UK (3.4 days) and a maximum in Malta (12.7 days). The annual occupancy rate exceeded 75% in 8 out of 12 countries. Only Cyprus, Latvia, UK and Poland reported an annual occupancy rate below 75% (Table 4.1).

Hospital characteristics

Both the size of a hospital and the level of specialization can influence the proportion of resistance. As can be seen from Table 4.2 and Figure 4.2, the distribution of size and specialization level of hospitals varied considerably between the reporting countries. This does not necessarily reflect different distributions of the origin of EARS-Net blood cultures per country, because not all hospitals contribute evenly to the EARS-Net database. On the other hand, this diversity can indicate differences in case-mix which may confound comparison of AMR results between countries.

The type of hospital and the size of hospital are not always linked and it is not rare, especially in small countries, that university hospitals have less than 500 beds.

Laboratory denominator information

In 2009, 753,195 blood culture sets were processed in the EARS-Net laboratories responding to the questionnaire. The median culturing frequency was 21.6 blood culture sets per 1,000 patient days in 2009. The highest rate was observed in Portugal (50.7) and the lowest in Hungary (1.5). In comparison with previous year, the number of blood culture sets taken per 1,000 patient days increased in Cyprus and decreased in Hungary and UK, with no or slight changes in the other 8 reporting countries (Table 4.3). The differences observed in UK and Hungary when comparing 2008 and 2009 can also originate from the increasing number of reporting hospitals in the two countries: in Hungary 14 laboratories reported in 2008 and 23 in 2009, while in UK the number of reporting laboratories was 10 in 2008 and 20 in 2009. The BSIs ascertainment is strongly linked to the blood culture rate. Therefore, the very wide range of culture rate observed in the countries providing denominator data could have implications on inter-country comparison of both the incidence rate of infections, which could be underestimated in some countries, and of the proportion of resistance. In particular, the proportion of resistance could be overestimated if, in a country, there is a frequent use of empiric therapy also for invasive infections and if the cultures are more likely to be performed in patients not responding to the empiric treatment.

Conclusions

In summary, the situation as assessed from denominator data reported in 2009 is similar to 2008, with only few significant changes.

For future improvement of the denominator data collection and analysis, it would be important to address the following issues:

- Increase the number of countries reporting denominator data
- Increase the number of hospital and laboratories participating within countries

- Improve the data quality for the variable HospitalId (unique identifier for the hospital within each laboratory)
- Improve the estimation of the coverage of the EARS-Net surveillance e.g. by using estimations done at the national level based on knowledge of the country specific situation

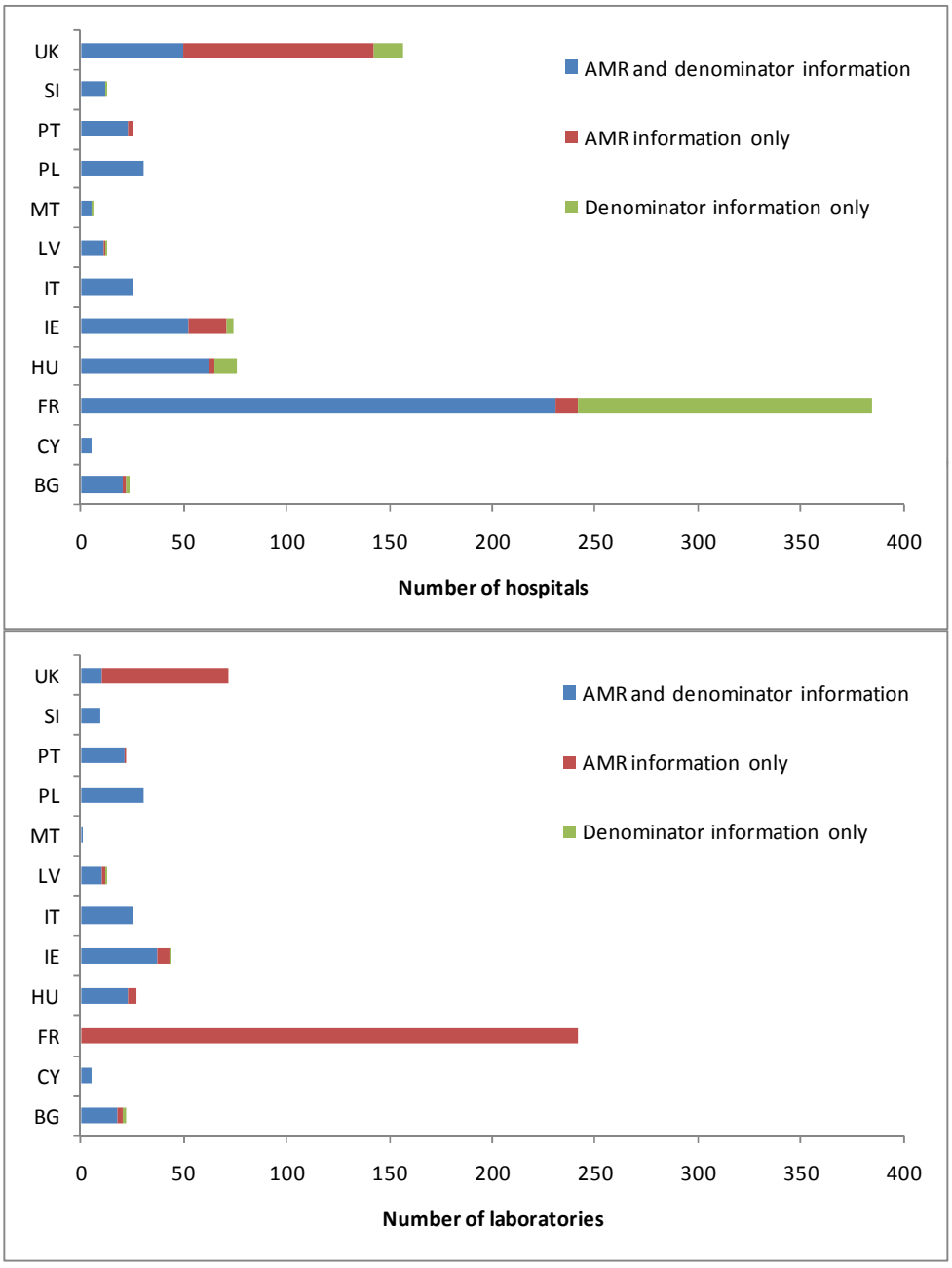

Figure 4.1. Number of laboratory and hospitals reporting AMR and/or denominator data in 2009

Table 4.1. Hospital denominator data for 2009

Country code	Hospitals reporting (denominator/AMR data)	Total number of bed	Proportion of ICU beds (%)	Annual occupancy rate (%)	Median length of stay (days)	IQR length of stay (days)
BG	(20/22)	9326	8	77	6.3	5.4-6.9
CY	(5/5)	1313	9	72	5.5	5.4-5.7
FR	(231*/242)	143433	5	83	7.7	6.4-9.2
HU	(62/65)	40749	2	76	7.8	6.5-9.6
IE	(52/71)	12175	3	87	5.8	4.6-7.1
IT	(25/25)	15358	5	84	-	-
LV	(11/12)	5251	2	68	6.8	5.6-7.4
MT	(5/5)	1861	5	79	12.7	5.9-38.6
PL	(30/30)	15045	2	70	5.5	4.4-6.2
PT	(23/25)	10885	5	79	7.3	5.5-8.6
SI	(12/12)	6559	5	79	5.4	4.8-6.2
UK	(50/142)	60775	1	74	3.4	2.7-6.1

* 178 hospitals in France reported data for a six months period.

Table 4.2. Hospital characteristics for 2009

Country code	Hospitals reporting (denominator/AMR data)	Proportion of hospitals by level of care (%)				
		Tertiary level	Secondary level	Primary level	Other	Unknown
BG	(20/22)	50	35	5	10	0
CY	(5/5)	20	20	40	20	0
FR	(231/242)	22	78	0	0	0
HU	(62/65)	50	29	15	6	0
IE	(52/71)	17	52	12	17	2
IT	(25/25)	68	28	4	0	0
LV	(11/12)	36	55	0	0	9
MT	(5/5)	20	20	0	60	0
PL	(30/30)	87	10	3	0	0
PT	(23/25)	57	26	4	13	0
SI	(12/12)	17	50	17	17	0
UK	(50/142)	6	20	6	18	50

Primary level or district hospital: Has few specialties, limited laboratory services; bed capacity ranges from 30 to 200 beds. Secondary level, or provincial hospital: Highly differentiated by function with five to ten clinical specialties; bed capacity ranging from 200-800 beds. Tertiary level or central / regional hospital. Highly specialized staff and technical equipment; clinical services are highly differentiated by function; may have teaching activities; bed capacity ranges from 300 to 1,500 beds. Other: hospitals for a specific patient population, like a military hospital, or hospitals with any single specialty, like a burns unit; Unknown=not available.

Chapter 5

Streptococcus pneumoniae

Clinical and epidemiological importance

Streptococcus pneumoniae is a common cause of disease, especially among young children, elderly people and patients with compromised immune functions. The clinical spectrum ranges from upper airway infections such as sinusitis, and otitis media to pneumonia and invasive blood stream infections and meningitis. Since *S. pneumoniae* is the most common cause of pneumonia worldwide, morbidity and mortality are high and annually approximately 3 million people are estimated to die of pneumococcal infections.

Pneumococci carry a variety of virulence factors that facilitate adherence and transcytosis of epithelial cells. The cell wall of pneumococci is coated with a viscous polysaccharide slime layer termed the capsule. This is the most important virulence factor, because it protects the bacteria from the adhesion of opsonising antibodies and the destruction by leucocytes. Capsular polysaccharides are highly diverse and play an important role in immune evasion. Around 80 different serotypes have been described. The serotype distribution varies with age, disease and geographical region. Interestingly, serotypes most frequently involved in pneumococcal disease or colonization in infants are also most frequently associated with antimicrobial resistance.

Resistance mechanisms

Beta-lactam antibiotics bind to cell wall synthesizing enzymes, so called penicillin-binding proteins (PBPs) and interfere with the biosynthesis and remodeling of the bacterial cell wall during cell growth and division. The mechanism of penicillin resistance in *S. pneumoniae* consists of alterations in PBPs, which results in reduced affinity to this class of antibiotics. Alterations in PBPs occur in a stepwise fashion which causes different degrees of resistance proceeding from reduced susceptibility through low-level clinical resistance – conventionally termed intermediate* (I) to full clinical resistance (R). Although intermediately resistant strains are clearly less susceptible than

* Microorganisms are defined as intermediate by a level of antimicrobial activity with uncertain clinical effect. Occasionally, this can be overcome if antibiotics can be administered at a higher dose and/or are concentrated at the infected body site.

sensitive strains, in absence of meningitis, infections with these strains are often successfully treated with high doses of penicillin or other beta-lactam compounds.

Macrolide, Lincosamine and Streptogramin (MLS) antibiotics are chemically distinct, but all bind to a ribosomal subunit inhibiting the initiation of mRNA binding and thus act as protein synthesis inhibitors. In *S. pneumoniae* two resistance mechanisms against MLS antibiotics have been reported: i) The acquisition of an erythromycin ribosomal methylation gene (*erm*) results in a posttranscriptional modification of the 23S subunit of ribosomal RNA, which blocks the binding of the macrolide to the ribosome. Once expression of the gene is induced, this often results in high-level resistance (MICs>128 mg/L) to macrolide, lincosamines and streptogramin B, termed MLS_B resistance (40;43). ii) The acquisition of a macrolide efflux system gene (*mef*E) results in the excretion of the antimicrobial, and effectively reduces intracellular erythromycin, azithromycin and clarithromycin to subinhibitory concentrations (24). In contrast to beta-lactam resistance, macrolide resistance via these mechanisms (particularly for MLS_B) provide very high MICs, and cannot be overcome by increasing the dosages of antibiotics.

Since *S. pneumoniae* is the most frequent cause of community-acquired pneumonia and can clinically not easily be distinguished from lower airway infections caused by other pathogens, empirical treatment of community-acquired lower respiratory infections needs to be active against pneumococci and should take the local prevalence of antimicrobial resistance into account. Habitual prescription of non-beta-lactam compounds is therefore typical in countries where penicillin resistance has been frequently reported. Such reactive prescribing increases the selection pressure for alternative antibiotics such as macrolides and novel fluoroquinolones. It is therefore no surprise to see a dynamic antimicrobial resistance picture emerge in different European countries. At the same time, the existence of frequent dual beta-lactam/macrolides resistance, particularly among children's serotypes, assures that in practice the use of drugs of any of these families will increase resistance for the members of the other one, and so the extended use of macrolides has been considered as a major driver for the increase in beta-lactam resistance.

Even though a certain small decrease in penicillin-resistance was detected in some countries *before* the introduction of the PCV7 vaccine, the widespread use of this vaccine is probably an important factor that may have influenced the decrease in antibiotic resistance levels, eliminating the infections (and more importantly, the children's carriage) of frequent "classic" resistant serotypes, 14, 6B, 19F and 23F, all of them covered by PCV7. The distribution of serotypes detected in this report 2009 (almost identical to that of 2008) includes serotypes 1 (15%), 19A (12%), 7F (11%), 3 (8%), 12F (7%), 6A (6%), 14 (5%). Even though a limited number of countries have provided

serotyping data, ad hoc studies in other EU countries as France, Spain, Greece, Norway and Portugal confirms the current generality of this pattern.This shift indicates the effect of the PCV7 vaccine, selecting the non-vaccine serotypes, and more importantly the serotype 19A. In fact the "classic diversity pattern" of well-adapted types of S. pneumoniae clones in children has been maintained, being these clones "disguised" under the PCV7-non-covered 19A capsule type. At least 10 non-19A serotypes had a 19A capsular switch. Eventually the introduction of PCV13 vaccine (covering 19A) will produce a new reduction in antibiotic resistance in S. pneumoniae.

Results

Penicillin

- Twenty-seven countries reported 11055 isolates of which 828 were non-susceptible to penicillin; 344 of the 828 non-susceptible isolates were identified as resistant. One country (Malta) reported less than 10 isolates and, therefore, it was not included in the map.
- Proportion of non susceptibility was: below 1% in three countries, between 1-5% in nine countries, between 5-10% in two countries, between 10-25% in seven countries and between 25-50% in five countries (Fig 5.1, Tab 5.1).
- Trends in the 2006-2009 period have been calculated for 22 countries. Three countries (Bulgaria, Ireland and Luxemburg) reported a significant increasing trend with proportions of non susceptibility to penicillin which, in 2009, were 37%, 19% and 19%, respectively. In one of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.4).
- Significant decreasing trends have been observed for Belgium(*), France and Spain with proportions of non susceptibility to penicillin which, in 2009, were <1%, 27% and 22%, respectively. In two of these countries the trend is significant also when considering only data from laboratories consistently reporting all four years (Fig 5.4).

(*) The proportion of *Streptococcus pneumoniae* non-susceptible to penicillin reported by Belgium dropped from 8% in 2008 to <1% in 2009. This is largely due to the fact that the clinical breakpoints (CLSI) used to determine SIR have changed. The laboratory that performs all the susceptibility tests started using the new CLSI clinical breakpoints in the beginning of 2009. During the entire EARS-Net surveillance, the same method of susceptibility testing has been used, only clinical breakpoints have changed.

Macrolides

- Twenty-seven countries reported 10934 isolates of which 1469 were non-susceptible to macrolides. Two countries (Malta and Poland) reported less than 10 isolates and, therefore, they were not included in the map.
- Proportion of non susceptibility was: between 1-5% in seven countries, between 5-10% in four countries, between 10-25% in nine countries and between 25-50% in five countries (Fig 5.2, Tab 5.1).
- Trends in the 2006-2009 period have been calculated for 22 countries. No country reported a significant increasing trend (Fig 5.5).
- Significant decreasing trends have been observed for six countries. In four of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.5). In 2009, these six countries reported proportion of resistance to macrolides: between 1-5% in one case (United Kingdom), between 5-10% in two cases (Netherlands and Norway), between 10-25% in two cases (Belgium and Italy) and between 25-50% in one case (France).

Dual non susceptibility (penicillin and macrolides)

- Twenty-seven countries reported 10577 isolates tested for penicillin and macrolides. In 2009, 5% of isolates had dual non susceptibility to the two considered antibiotic classes. Two countries (Malta and Poland) reported less than 10 isolates (therefore not included in figure 5.3) (fig 5.3: map on dual non-susceptibility is still missing in this draft - to be handmade and inserted).
- Proportion of dual non susceptibility was: below 1% in seven countries, between 1-5% in seven countries, between 5-10% in three countries, between 10-25% in six countries and between 25-50% in two countries (Fig 5.3, Tab 5.1).
- Trends in the 2006-2009 period have been calculated for 22 countries. A significant increase has been observed for Ireland that, in 2009, reported a proportion of dual non susceptibility of 12%. A significant decreasing trend has been observed for Belgium (proportion of dual non susceptibility in 2009 was <1%) (Fig 5.6).

Serogroups

- Six countries reported 2959 *S. pneumoniae* isolates with identification of the serotype/serogroup (Tab 5.2).
- In 2009 data, the serogroup 1 is the most prevalent (16% of total) followed by serogroups 19 and 7 (both 11% of total), serogroup 3 (8%), serogroup 12 (7%) and serogroups 6, 14, 9 and 22 (all at 5%) (Fig 5.7, Tab 5.2).
- Dual non-susceptibility has been mainly retrieved in serogroups 14, 19, 6, 9 and 23. Single non-susceptibility to penicillin in serogroups 14, 9, 19 and 6. Single non-susceptibility to macrolides in serogroups 1, 19, 14, 6, 33, 15, 9, 23, 11, 3 and 12 (Fig 5.7, Tab 5.2).

Conclusions

In 2009, the proportion of non-susceptibility to penicillin remained generally stable in Europe with three countries reporting significant increasing trends and other three reporting significant decreasing trends. Fourteen of 26 countries reported proportions of non-susceptibility below 10%. The proportion of non-susceptibility to macrolides has significantly declined in six countries while no country reported increasing trends. Nevertheless, fourteen countries out of twenty-five reported proportion of non susceptibility above 10%.

The dual non-susceptibility to penicillin and macrolides was above 10% in eight countries out twenty-five countries reporting more than 10 isolates.

The highest proportions of non-susceptibility of *S. pneumoniae* to penicillin and/or macrolides were reported by countries of Southern and Eastern Europe with Finland as the only Northern exception.

So far, the serogroup data reported to EARS-Net should not be regarded as representative for Europe in general; only six countries reported more than 30 isolates with serogroup information and more than 60% results were provided by Belgium. The serogroups distribution reported in 2009, is similar to the previous year. Most non-susceptible isolates belong to few serogroups, especially serogroups 1, 19, 14, 6 and 9. The serogroup 14, which is particularly important for in relation to penicillin resistance, was the most frequent serogroup only in Ireland (12%) (Fig 5.7, Tab 5.2).

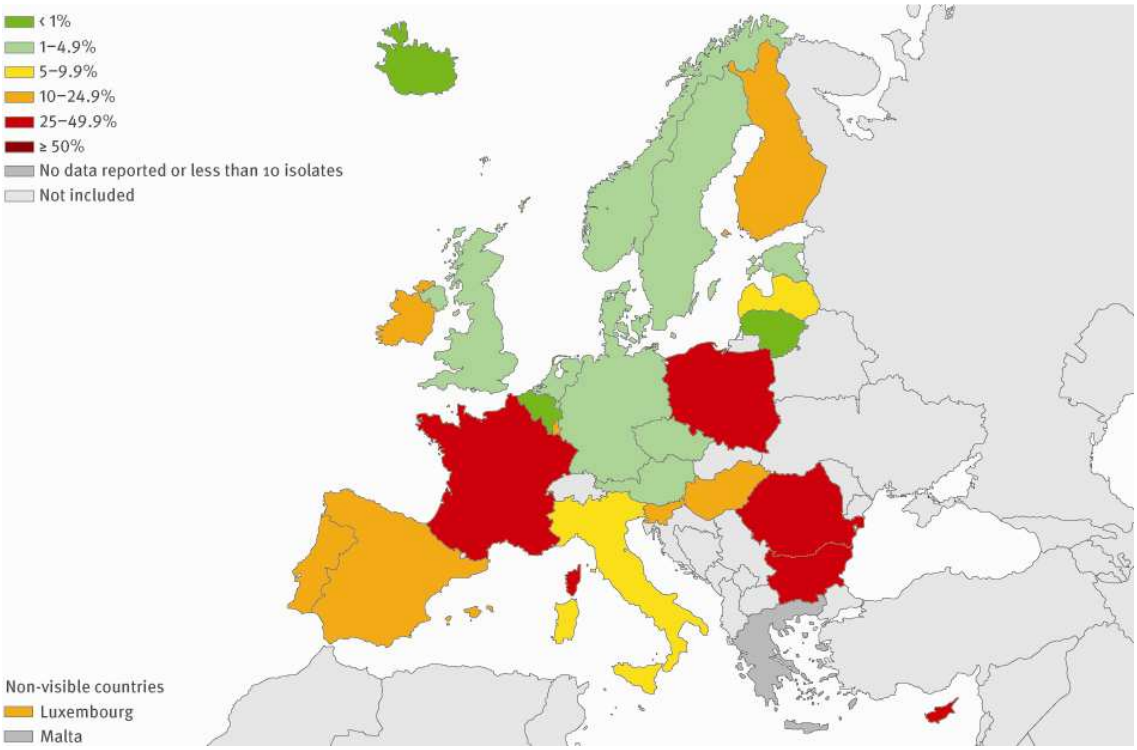

Figure 5.1 *Streptococcus pneumoniae*: proportion of invasive isolates non susceptible to penicillin (PNSP) in 2009. Only countries reporting 10 or more isolates are included.

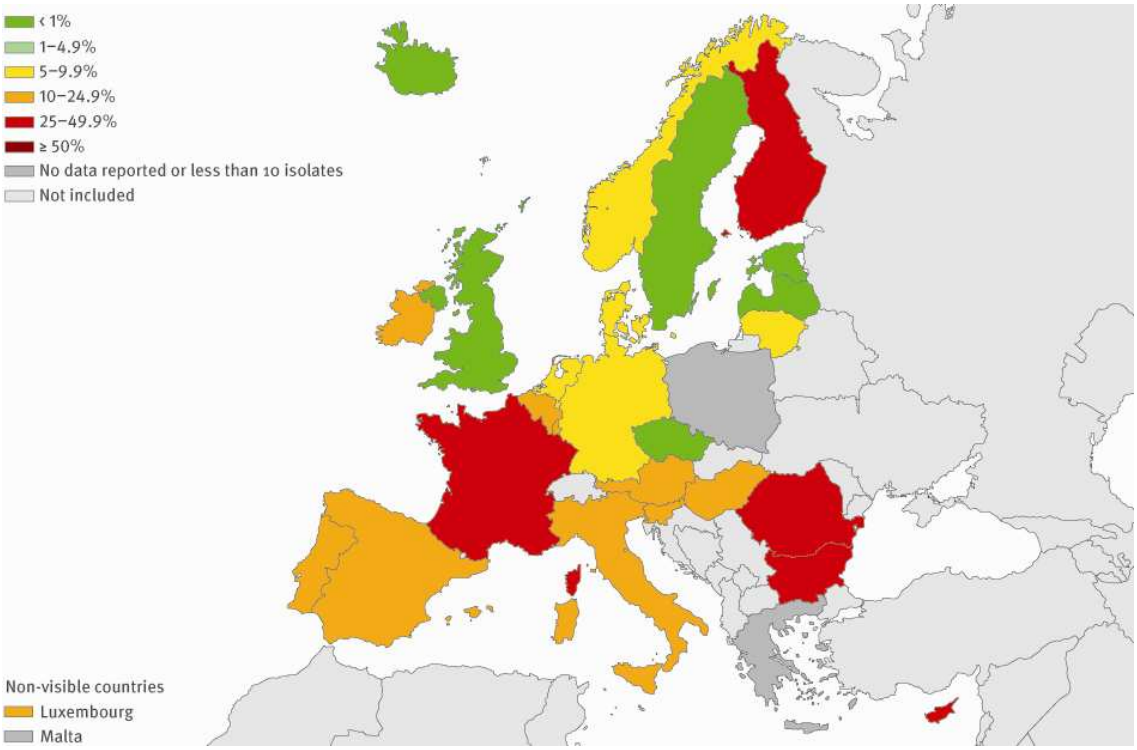

Figure 5.2 *Streptococcus pneumoniae*: proportion of invasive isolates non susceptible to macrolides in 2009. Only countries reporting 10 or more isolates are included.

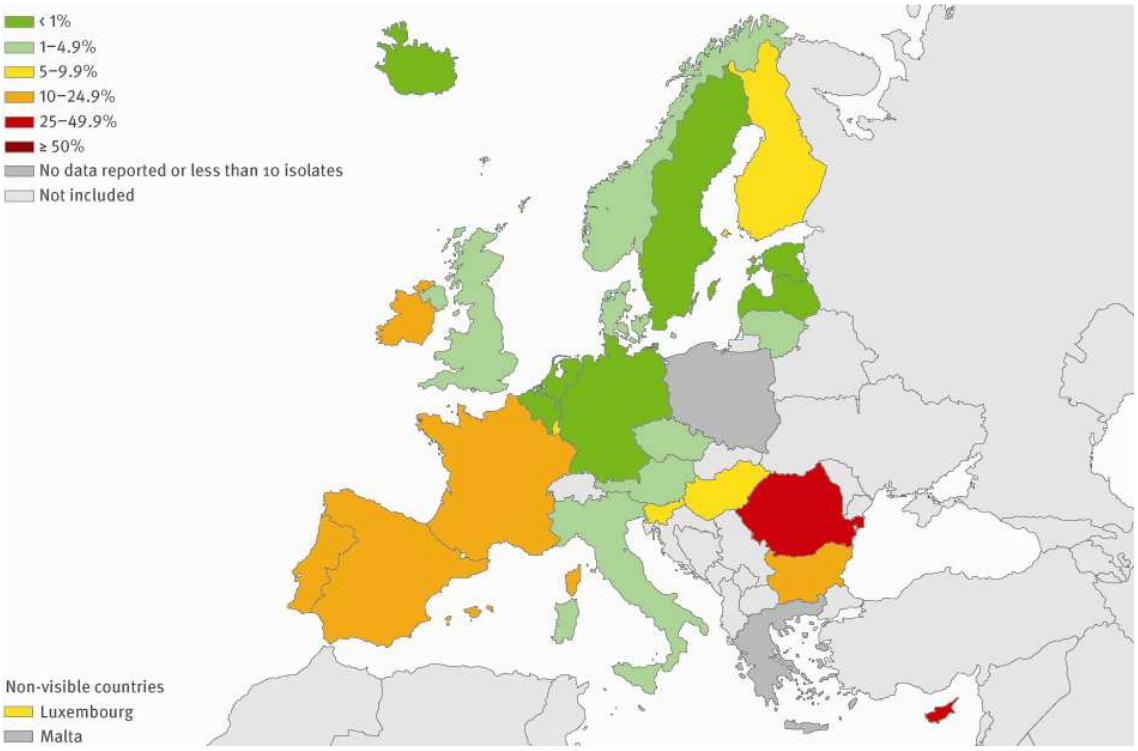

Figure 5.3 *Streptococcus pneumoniae*: proportion of invasive isolates with dual non susceptibility to penicillin and macrolides in 2009. Only countries reporting 10 or more isolates are included.

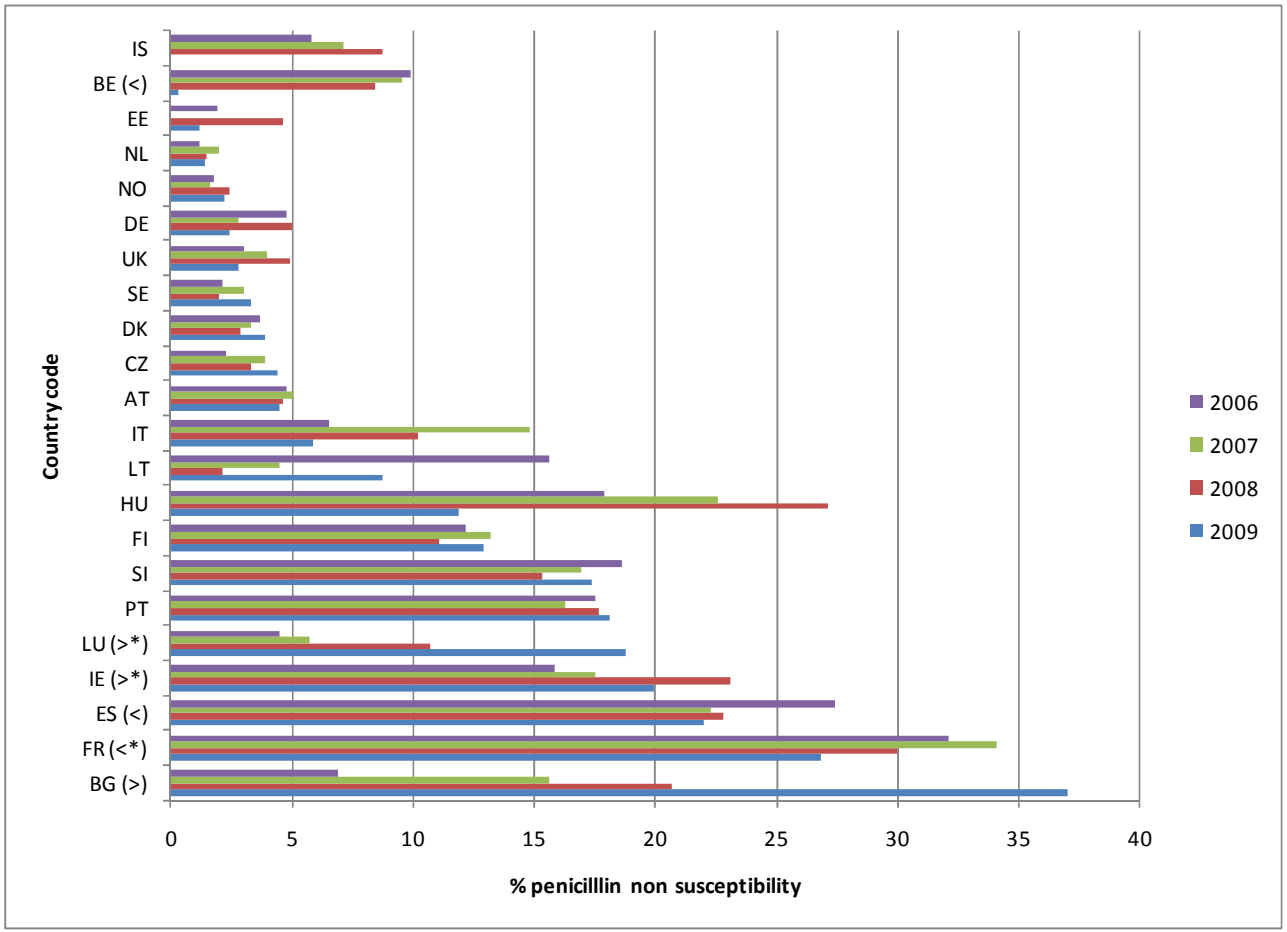

Figure 5.4 *Streptococcus pneumoniae*: trend of penicillin non susceptibility by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

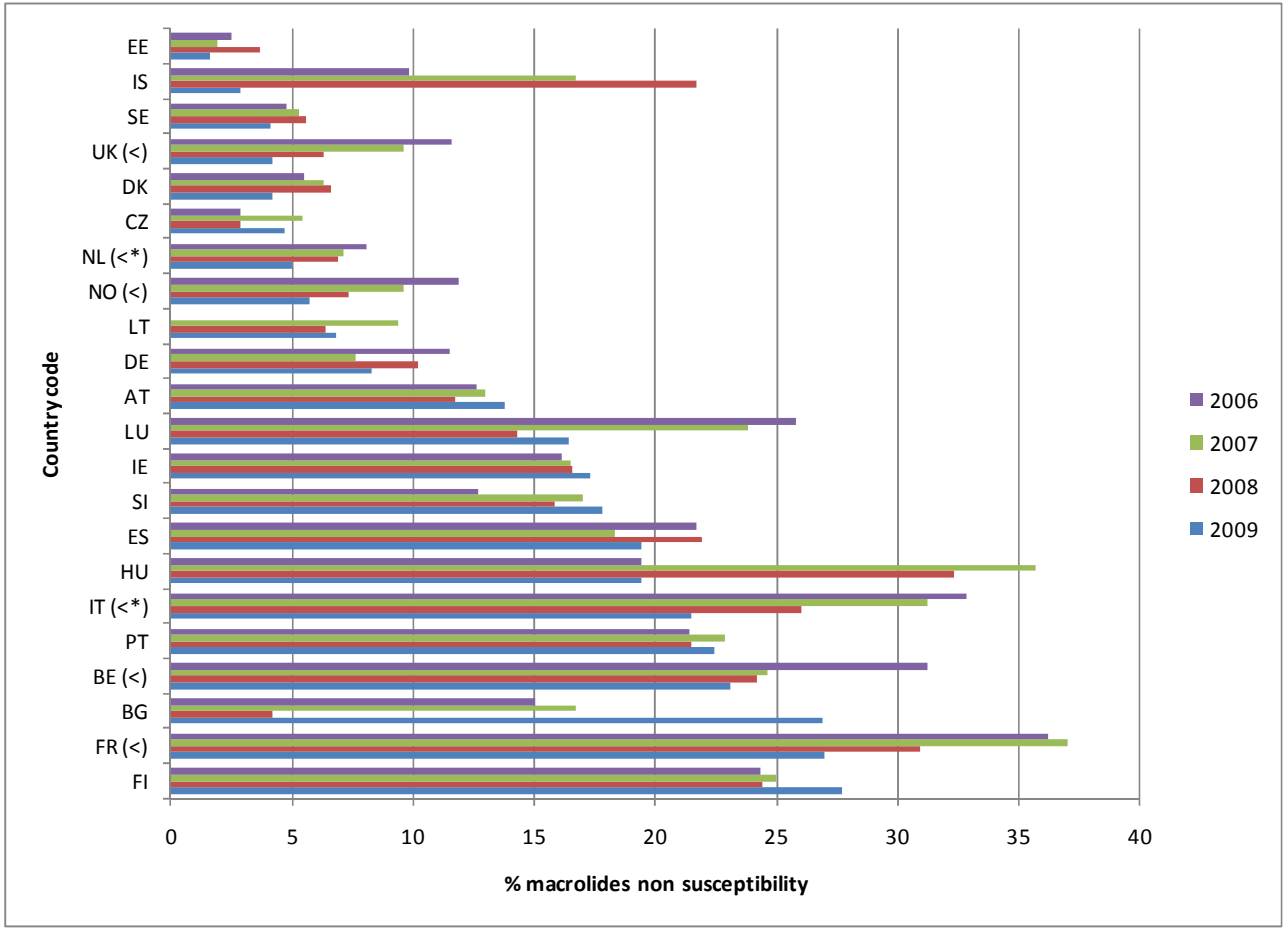

Figure 5.5 *Streptococcus pneumoniae*: trend of macrolides non susceptibility by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

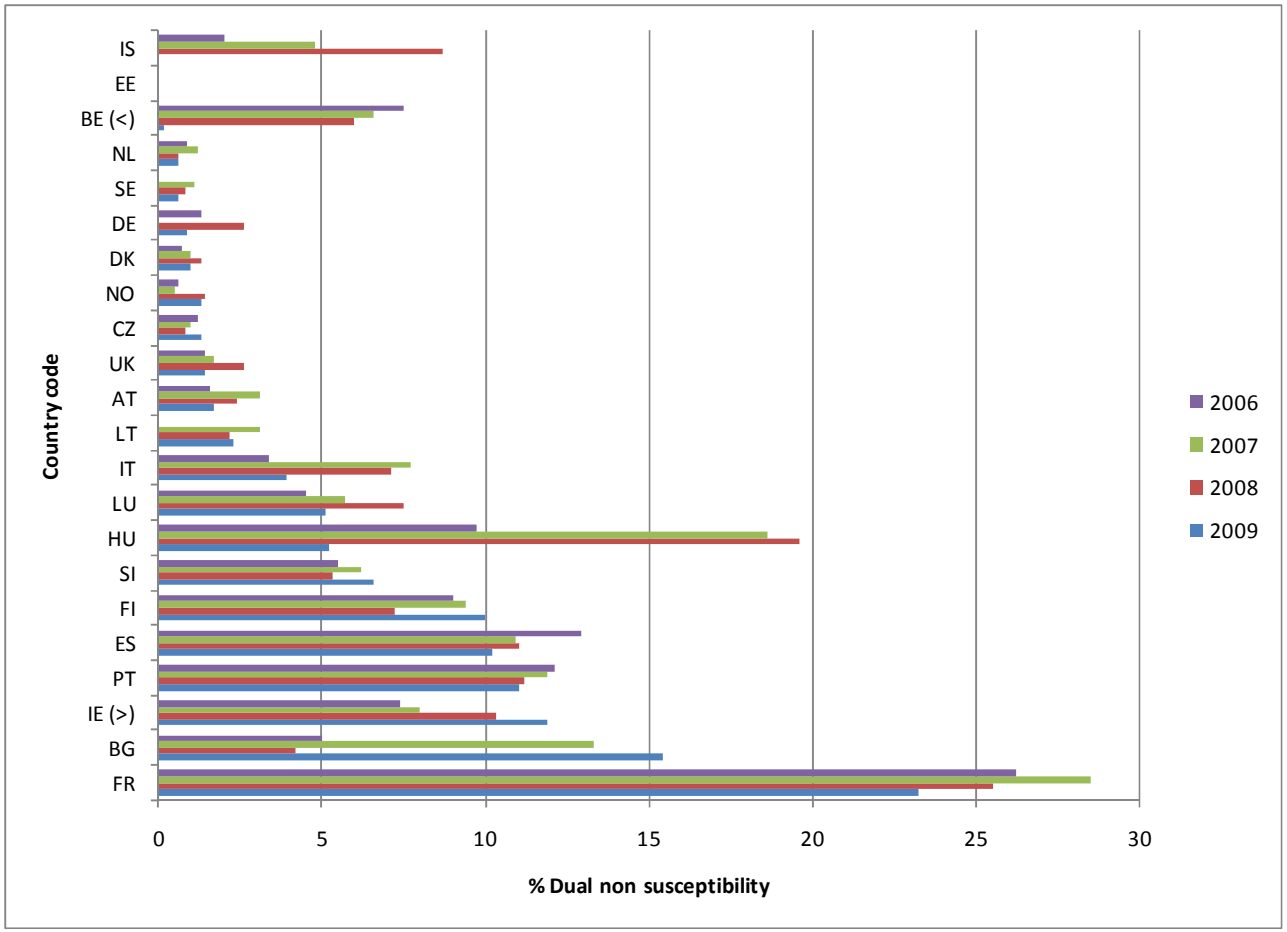

Figure 5.6 *Streptococcus pneumoniae*: trend of dual non susceptibility (penicillin and macrolides) by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively.

Table 5.1 The number of invasive *S. pneumoniae* isolates and the proportion of penicillin non-susceptible (PNSP), penicillin resistant (PRSP), macrolide non-susceptible (MNSP), single penicillin (PEN), single macrolides (MACR) and dual non susceptible (DUAL) isolates, including 95% confidence intervals (95CI)reported per country in 2009.

Country	N. isolates tested for (PEN/MACR/both)	%PNSP (95CI)	%PRSP (95CI)	%MNSP (95CI)	%single PEN (95CI)	%single MACR (95CI)	%DUAL (95CI)*
AT	352/319/294	4.5 (3-7)	2.6 (1-5)	13.8 (10-18)	2.7 (1-5)	12.9 (9-17)	1.7 (1-4)
BE	1885/1885/1885	0.3 (0-1)	0.2 (0-1)	23.1 (21-25)	0.1 (0-0)	23.0 (21-25)	0.2 (0-0)
BG	27/26/26	37.0 (19-58)	22.2 (9-42)	26.9 (12-48)	23.1 (9-44)	11.5 (2-30)	15.4 (4-35)
CY	11/11/11	36.4 (11-69)	18.2 (2-52)	36.4 (11-69)	9.1 (0-41)	9.1 (0-41)	27.3 (6-61)
CZ	297/297/297	4.4 (2-7)	0.7 (0-2)	4.7 (3-8)	3.0 (1-6)	3.4 (2-6)	1.3 (0-3)
DE	328/339/323	2.4 (1-5)	0.6 (0-2)	8.3 (6-12)	1.5 (1-4)	7.4 (5-11)	0.9 (0-3)
DK	996/996/996	3.9 (3-5)	3.9 (3-5)	4.2 (3-6)	2.9 (2-4)	3.2 (2-5)	1.0 (0-2)
EE	82/63/63	1.2 (0-7)	0.0 (0-4)	1.6 (0-9)	1.6 (0-9)	1.6 (0-9)	0.0 (0-6)
ES	708/697/697	22.0 (19-25)	8.3 (6-11)	19.4 (16-23)	11.8 (9-14)	9.2 (7-12)	10.2 (8-13)
FI	652/679/643	12.9 (10-16)	1.7 (1-3)	27.7 (24-31)	2.8 (2-4)	17.9 (15-21)	10.0 (8-13)
FR	826/826/826	26.8 (24-30)	5.9 (4-8)	27.0 (24-30)	3.5 (2-5)	3.8 (3-5)	23.2 (20-26)
HU	143/134/134	11.9 (7-18)	2.8 (1-7)	19.4 (13-27)	5.2 (2-10)	14.2 (9-21)	5.2 (2-10)
IE	356/336/336	19.9 (16-24)	6.2 (4-9)	17.3 (13-22)	7.7 (5-11)	5.4 (3-8)	11.9 (9-16)
IS	35/35/35	0.0 (0-10)	0.0 (0-10)	2.9 (0-15)	0.0 (0-10)	2.9 (0-15)	0.0 (0-10)
IT	202/191/178	5.9 (3-10)	3.0 (1-6)	21.5 (16-28)	1.7 (0-5)	16.3 (11-23)	3.9 (2-8)
LT	46/44/44	8.7 (2-21)	6.5 (1-18)	6.8 (1-19)	6.8 (1-19)	4.5 (1-15)	2.3 (0-12)
LU	64/61/59	18.8 (10-30)	10.9 (5-21)	16.4 (8-28)	6.8 (2-16)	11.9 (5-23)	5.1 (1-14)
LV	30/30/30	0.0 (0-12)	0.0 (0-12)	3.3 (0-17)	0.0 (0-12)	3.3 (0-17)	0.0 (0-12)
MT	7/8/7	14.3 (0-58)	0.0 (0-41)	12.5 (0-53)	0.0 (0-41)	0.0 (0-41)	14.3 (0-58)
NL	572/679/505	1.4 (1-3)	0.2 (0-1)	5.0 (3-7)	0.6 (0-2)	4.8 (3-7)	0.6 (0-2)
NO	554/545/545	2.2 (1-4)	0.4 (0-1)	5.7 (4-8)	0.9 (0-2)	4.4 (3-6)	1.3 (1-3)
PL	57/7/6	29.8 (18-43)	29.8 (18-43)	71.4 (29-96)	0.0 (0-46)	0.0 (0-46)	66.7 (22-96)
PT	237/237/237	18.1 (13-24)	18.1 (13-24)	22.4 (17-28)	7.2 (4-11)	11.4 (8-16)	11.0 (7-16)
RO	17/16/16	29.4 (10-56)	11.8 (1-36)	25.0 (7-52)	6.3 (0-30)	0.0 (0-21)	25.0 (7-52)
SE	1058/1008/1005	3.3 (2-5)	2.5 (2-4)	4.1 (3-5)	2.8 (2-4)	3.5 (2-5)	0.6 (0-1)
SI	213/213/213	17.4 (13-23)	7.0 (4-11)	17.8 (13-24)	10.8 (7-16)	11.3 (7-16)	6.6 (4-11)
UK	1300/1252/1166	2.8 (2-4)	1.0 (1-2)	4.2 (3-5)	1.5 (1-2)	2.7 (2-4)	1.4 (1-2)

** Dual non susceptibility was defined as being non-susceptible to penicillin and macrolides.*

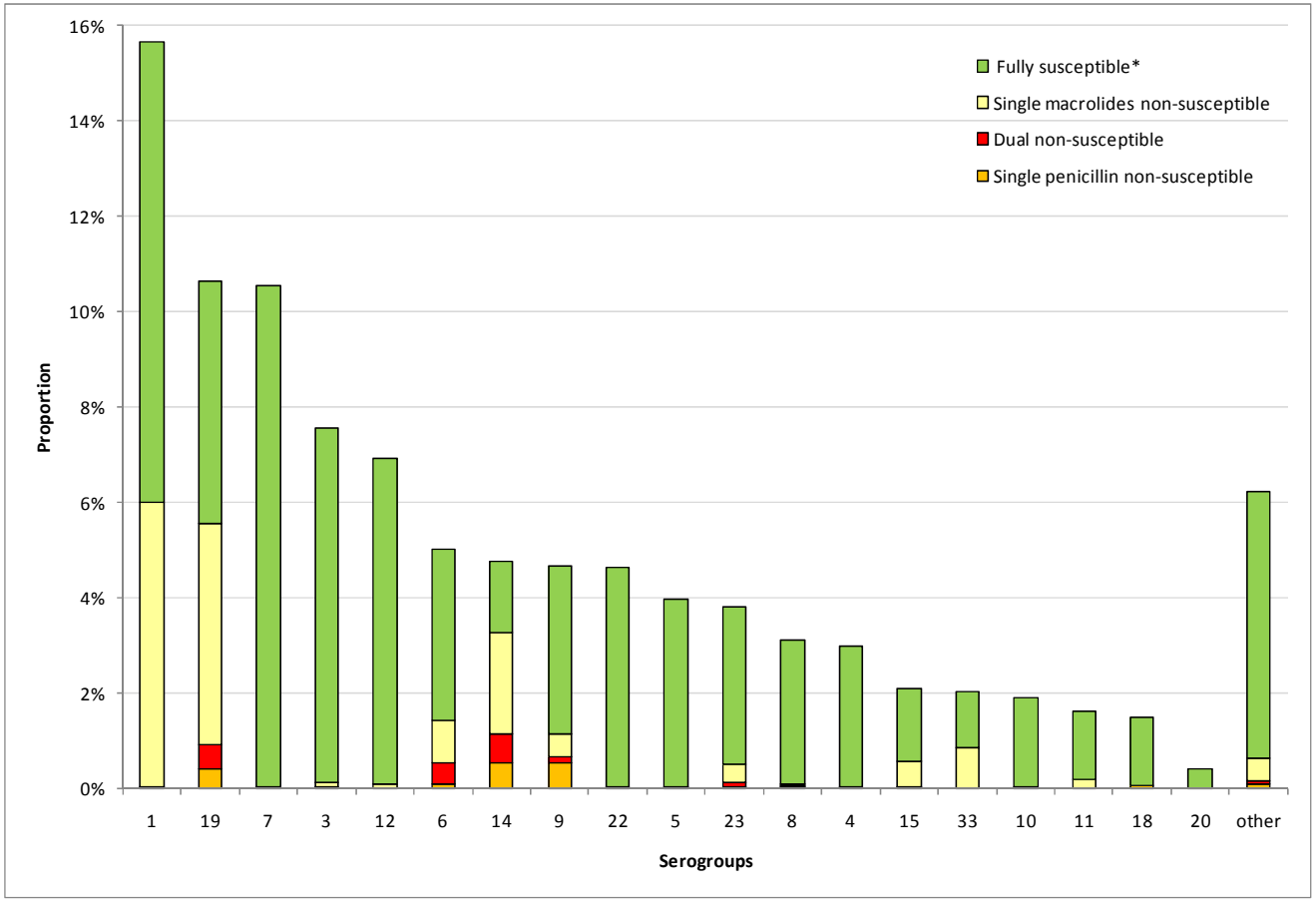

Figure 5.7 The distribution of serogroups and the resistance profile of *S. pneumonia* isolates per serogroups in 2009. Only countries that reported serogroups information for more than 30 isolates were included in the figure.

*Susceptible to at least penicillin and macrolides.

Table 5.2 Distribution of single penicillin, single macrolides and dual penicillin-macrolides non-susceptibility, among the most common serogroups reported per country in 2009. Only countries reporting more that 30 isolates were presented.

Serogroups	Belgium				Czech republic				Iceland				Ireland				Slovenia				United Kingdom			
	Number	%single PEN	%single MACR	% DUAL	Number	%single PEN	%single MACR	% DUAL	Number	%single PEN	%single MACR	% DUAL	Number	%single PEN	%single MACR	% DUAL	Number	%single PEN	%single MACR	% DUAL	Number	%single PEN	%single MACR	% DUAL
1	368	0	48	0	28	0	0	0	1	0	0	0	13	0	0	0	25	4	0	0	28	0	0	0
3	125	0	1	0	24	0	4	0	1	0	0	0	18	0	6	0	33	0	0	0	23	4	0	0
4	42	0	0	0	20	0	5	0	0	0	0	0	11	0	0	0	9	0	0	0	6	0	0	0
5	115	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
6	71	0	30	1	18	0	6	0	3	0	0	0	22	5	5	45	16	13	13	13	18	0	6	0
7	202	0	0	0	24	0	0	0	4	0	0	0	32	0	3	0	9	0	0	0	41	0	0	0
8	42	0	2	0	14	0	0	0	0	0	0	0	9	0	0	0	1	0	0	0	26	4	0	4
9	49	0	22	0	28	25	4	0	9	0	0	0	20	15	0	20	21	29	5	0	11	0	9	0
10	40	0	3	0	11	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	2	0	0	0
11	24	0	17	0	2	0	0	0	1	0	0	0	5	0	20	0	3	0	0	0	13	0	8	0
12	193	0	2	0	0	0	0	0	0	0	0	0	4	0	0	0	1	0	0	0	7	0	0	0
14	37	0	65	3	26	4	8	12	6	0	17	0	33	27	36	24	28	21	61	18	11	0	64	9
15	38	0	39	0	3	0	0	0	0	0	0	0	11	0	9	0	3	0	0	0	7	0	0	14
18	16	0	0	0	6	0	0	0	0	0	0	0	16	13	0	0	5	0	0	0	1	0	0	0
19	201	1	64	0	16	6	25	0	4	0	0	0	32	6	3	28	21	29	5	24	41	2	7	0
20	5	0	0	0	3	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
22	88	0	0	0	7	0	0	0	0	0	0	0	15	0	0	0	2	0	0	0	25	4	0	0
23	49	0	20	0	23	0	0	4	2	0	0	0	20	0	0	5	11	0	9	9	8	13	0	0
33	36	0	64	0	1	0	0	0	1	0	0	0	7	0	0	0	6	0	17	0	9	0	11	0
other	139	0	9	0	12	0	0	0	0	0	0	0	10	20	0	10	9	0	11	11	14	7	0	0
Total/average	**1880**	**0**	**23**	**0**	**266**	**3**	**4**	**2**	**33**	**0**	**3**	**0**	**283**	**7**	**6**	**12**	**206**	**10**	**12**	**7**	**291**	**2**	**5**	**1**

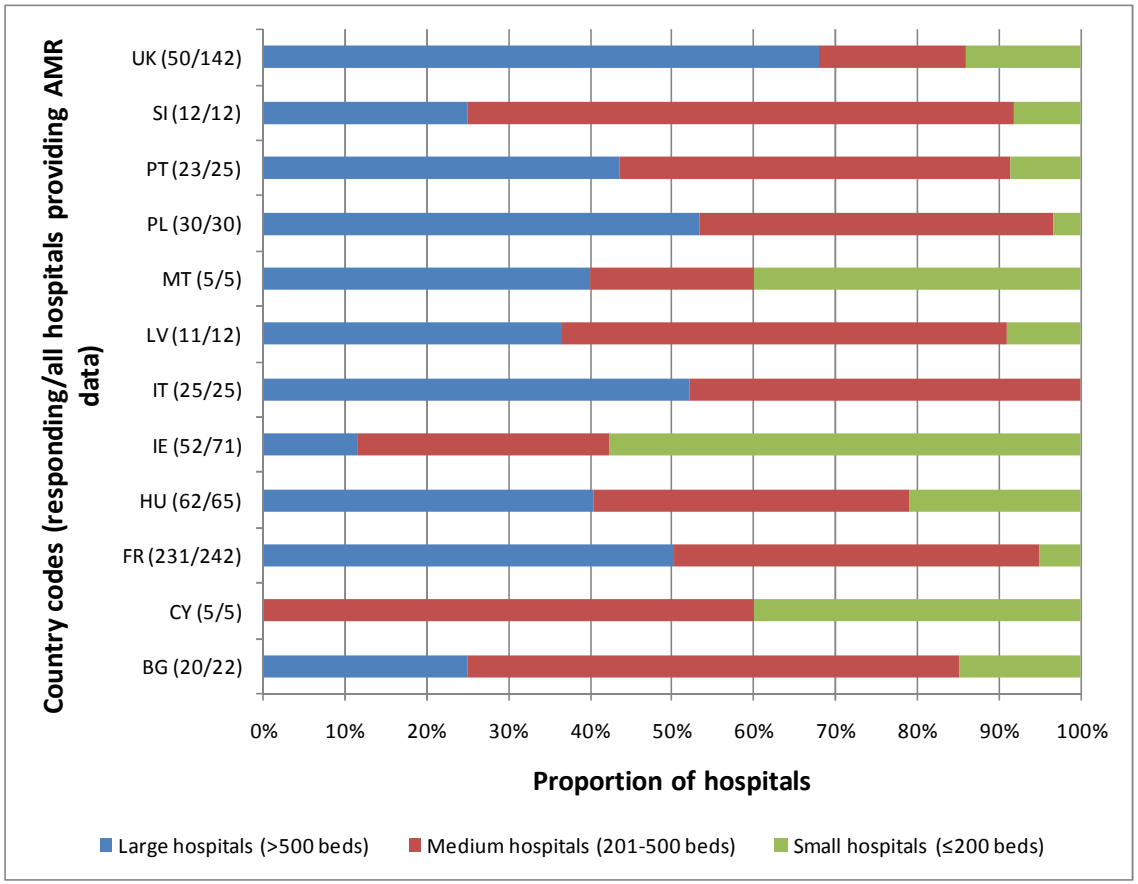

Figure 4.2. The proportion of small, medium and large hospitals per country, based on the number of beds, for all hospital reporting both antimicrobial resistance data and denominator data in 2009

Table 4.3. Laboratory denominator information for 2009

Country code	Labs reporting (denominator/AMR data)	N. of hospitals*	Total number of blood culture sets	Number of blood culture sets per 1000 patients days
BG	(18/20)	20	19855	7.5
CY	(5/5)	5	12991	37.6
HU	(23/27)	54	14549	1.5
IE	(37/43)	51	184985	47.8
IT	(25/25)	25	125577	26.8
LV	(10/12)	10	7859	6.2
MT	(1/1)	5	4899	9.1
PL	(30/30)	30	82521	21.6
PT	(21/22)	23	158902	50.7
SI	(9/9)	12	46040	24.2
UK	(10/72)	10	95017	45.2

*Number of hospitals served by laboratories reporting denominator data

Staphylococcus aureus

Clinical and epidemiological importance

Staphylococcus aureus is a gram-positive bacterium that colonizes the skin of about 30% of healthy humans. Although mainly a harmless coloniser, *S. aureus* can cause severe infection. Its oxacillin-resistant form (meticillin-resistant *S. aureus*, MRSA) is the most important cause of antibiotic-resistant health care-associated infections worldwide. Since health care-associated MRSA infections add to the number of infections caused by meticillin-susceptible *S. aureus*, a high incidence of MRSA adds to the overall burden of infections caused by this species in hospitals. Moreover, infections with MRSA may result in prolonged hospital stay and in higher mortality rates, owing mainly to the increased toxicity and limited effectiveness of alternative treatment regimens. MRSA is currently the most commonly identified antibiotic-resistant pathogen in hospitals in many parts of the world, including Europe, the Americas, North Africa and the Middle- and Far-East.

Resistance mechanisms

S. aureus acquires resistance to meticillin and all other beta-lactam antibiotics through expression of the exogenous *mecA* gene, that codes for a variant penicillin binding protein PBP2' (PBP2a) with low affinity to beta-lactams, thus preventing the drug induced inhibition of cell wall synthesis. The level of meticillin resistance (defined by its minimum inhibitory concentration, MIC) depends on the amount of PBP2' production, which is influenced by various genetic factors. Resistance levels of *mecA*-positive strains can thus range from phenotypically susceptible to highly resistant. Upon challenge with meticillin, a population of a heterogeneously resistant MRSA strain may quickly be outgrown by a subpopulation of highly resistant variants.

Results

Beta-lactams

- Twenty-eight countries reported 30680 isolates of which 5965 were identified as meticillin resistant *Staphylococcus aureus* (MRSA).
- Proportion of MRSA was: below 1% in two countries, between 1-5% in five countries, between 5-10% in two countries, between 10-25% in nine countries, between 25-50% in nine countries and above 50% in one country (Fig 5.8, Tab 5.3).

- Trends in the 2006-2009 period have been calculated for 28 countries. Significant decreasing trends have been observed for eight countries. In six of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.9). In 2009, these eight countries reported proportion of MRSA: between 5-10% in two cases (Austria and Latvia), between 10-25% in two cases (Bulgaria and France) and between 25-50% in four cases (Greece, Ireland, Romania and UK).
- One country (Czech Republic) reported a significant increasing trend of MRSA proportion (Fig 5.9) which, in 2009, was 15%.

Rifampin

- Twenty-five countries reported 21190 isolates of which 203 were identified as resistant to rifampin. Seventeen countries reported at least one resistant isolate. Most resistant isolates (159) were also MRSA. The proportion of rifampin resistance was 3.7% among the MRSA isolates and 0.3% among the MSSA isolates.
- Proportion of resistance was: below 1% in seventeen countries, between 1-5% in six countries, between 5-10% in one country, between 10-25% in one country (Tab 5.3).

Conclusions

The proportion of MRSA is stabilizing or decreasing in most European countries. Eight countries reported decreasing trends while only one reported an increasing trend. The countries showing a more evident and sustained decrease of MRSA proportion are: Austria, France, Ireland, Latvia and UK. Although these signals provide grounds for optimism, MRSA occurrence should remain a priority in the public health agenda since the proportion of MRSA is still above 25% in ten out of twenty-eight countries, mainly belonging to the southern Europe and the British Isles areas.

Rifampin, which is recommended in combination with other antimicrobials to treat various staphylococcal infections, has still very low proportions of resistance in most European countries.

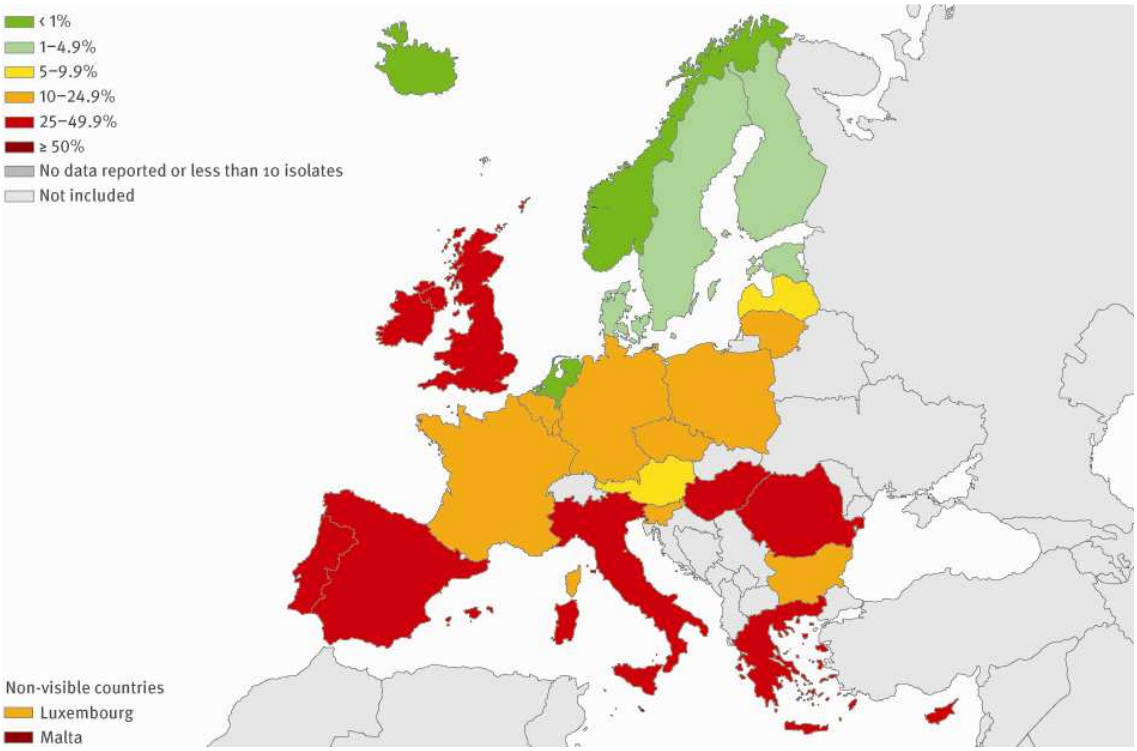

Figure 5.8 *Staphylococcus aureus*: proportion of invasive isolates resistant to meticillin (MRSA) in 2009. Only countries reporting 10 or more isolates are included.

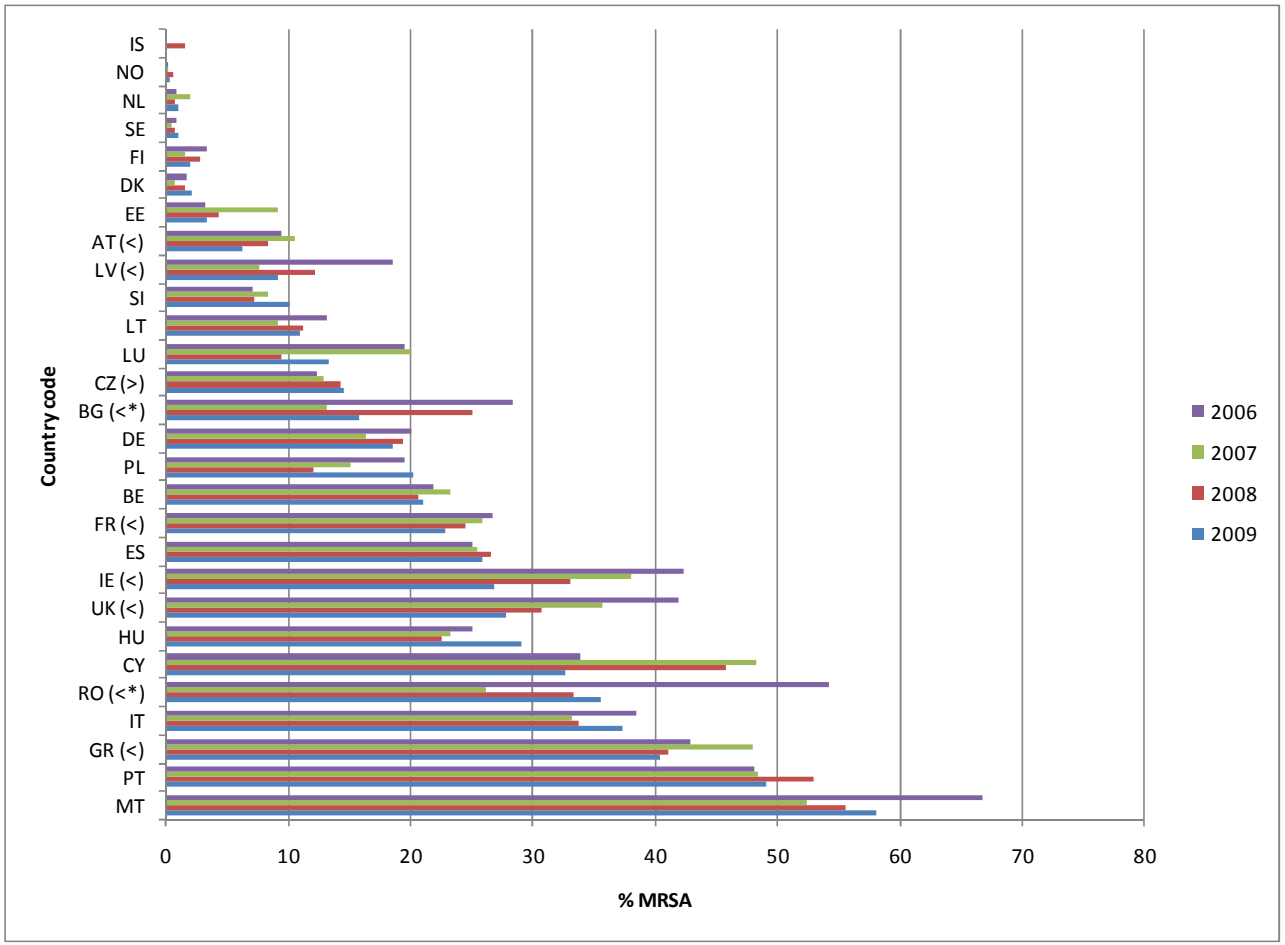

Figure 5.9 *Staphylococcus aureus*: trend of meticillin-resistance (MRSA) by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

Table 5.3 - The number of invasive *S.aureus* isolates and the proportion resistant to meticillin (MRSA) and rifampin (RIF) including 95% confidence intervals (95CI) reported per country in 2009.

	Meticillin		Rifampin	
Country	**N**	**% MRSA (95CI)**	**N**	**% RIF (95CI)**
AT	1739	6.3 (5-8)	1677	0.2 (0-1)
BE	949	21.1 (19-24)	0	. (.-.)
BG	221	15.8 (11-21)	137	2.9 (1-7)
CY	89	32.6 (23-43)	89	0.0 (0-4)
CZ	1695	14.6 (13-16)	716	1.3 (1-2)
DE	1885	18.5 (17-20)	1152	0.5 (0-1)
DK	1395	2.1 (1-3)	1395	0.1 (0-1)
EE	213	3.3 (1-7)	63	0.0 (0-6)
ES	1715	25.9 (24-28)	1614	0.5 (0-1)
FI	978	1.9 (1-3)	934	0.0 (0-0)
FR	4720	22.8 (22-24)	4519	1.2 (1-2)
GR	996	40.4 (37-43)	0	. (.-.)
HU	1068	29.0 (26-32)	287	2.4 (1-5)
IE	1261	26.8 (24-29)	1051	0.4 (0-1)
IS	59	0.0 (0-6)	0	. (.-.)
IT	978	37.4 (34-41)	908	5.5 (4-7)
LT	254	11.0 (7-16)	164	0.0 (0-2)
LU	113	13.3 (8-21)	72	0.0 (0-5)
LV	186	9.1 (5-14)	159	0.6 (0-3)
MT	86	58.1 (47-69)	86	0.0 (0-4)
NL	1035	1.0 (0-2)	861	0.0 (0-0)
NO	907	0.3 (0-1)	262	0.0 (0-1)
PL	506	20.2 (17-24)	259	1.2 (0-3)
PT	1824	49.1 (47-51)	828	3.5 (2-5)
RO	45	35.6 (22-51)	45	15.6 (6-29)
SE	2456	1.0 (1-1)	1608	0.1 (0-0)
SI	424	10.1 (7-13)	395	0.5 (0-2)
UK	2883	27.8 (26-29)	1909	0.6 (0-1)

Enterococci

Clinical and epidemiological importance

Enterococci belong to the normal bacterial flora of the gastrointestinal tract of humans, other mammals, birds and reptiles. Enterococci are regarded harmless commensals, and are even believed to have positive effects on a number of gastrointestinal and systemic conditions. However, when the commensal relationship with the host is disrupted, enterococci can cause invasive diseases. Recently, the recognition of "high risk clones" as those of the polyclonal sub-cluster CC17 in *Enterococcus faecium*, suggests that some particular strains can act as true pathogens, and not only as opportunistic commensals. Enterococci can cause a variety of clinical syndromes including endocarditis, bacteraemia, meningitis, wound and urinary tract infections and are associated with peritonitis and intra-abdominal abscesses. In the USA, three to four nosocomial bloodstream infections per 10,000 hospital discharges are caused by enterococci, and contribute to patient mortality as well as additional hospital stay.

The vast majority (around 80%) of clinical enterococcal infections in humans are caused by *Enterococcus faecalis*, whereas *E. faecium* accounts for the majority of the remaining 20%. Epidemiological data collected over the last two decades have documented the emergence of enterococci, and in particular *E. faecium*, as important nosocomial pathogens, which is seen as the expansion of a major hospital adapted polyclonal sub-cluster CC17, but also in *E. faecalis* CC2 and CC9, which are also isolated from farm animals. The emergence of particular clones and clonal complexes of *E. faecalis* and *E. faecium* was paralleled by increases in glycopeptide and high-level aminoglycoside resistance. These two antimicrobial groups represent the few remaining therapeutic options for treatment of human infections caused by *E. faecium* when resistance has emerged against penicillins. Besides the fact that infections caused by resistant enterococci are difficult to treat, they are highly tenacious and thus easily disseminate in the hospital setting.

Resistance mechanisms

Enterococci are intrinsically resistant to a broad range of antibiotics including cephalosporins, sulphonamides and low concentrations of aminoglycosides. Patient safety in

hospitals is challenged by the ability of enterococci to acquire additional resistance through the transfer of plasmids and transposons and recombination or mutation.

Beta-lactam antibiotics: By nature, enterococci have low susceptibility to many beta-lactam antibiotics – a consequence of their low-affinity PBPs. Two possible mechanisms of resistance of enterococci to beta-lactams have been reported; i) the production of beta-lactamase which is an extremely rare finding and ii) the overproduction and modification of penicillin-binding proteins (PBPs, particularly PBP5) that causes high level penicillin resistance in *E. faecium*. Complete penicillin resistance in *E. faecalis* is currently absent; therefore, the first choice for treatment of infections caused by this microorganism is still an aminopenicillin such as ampicillin. In *E. faecium*, ampicillin-resistance has increased significantly during the last years due to the wide dissemination of ampicillin-resistant strains belonging to the polyclonal sub-cluster CC17.

Aminoglycosides: In addition to the intrinsic mechanism of low-level resistance, which causes a low uptake of the drug, enterococci have acquired genes conferring high-level resistance to aminoglycosides. High-level resistance to streptomycin can be mediated by single mutations within a protein of the 30S ribosomal subunit, the target of aminoglycoside activity. In addition, different aminoglycoside-modifying enzymes have been identified, targeting 8 different aminoglycosides.

Glycopeptides: Vancomycin resistance in enterococci was first encountered in France and England but showed the most dramatic increase in the United States and was attributed to the widespread use of vancomycin in US hospitals. Whereas vancomycin consumption was less pronounced in Europe, a closely related glycopeptide, avoparcin, was widely used as growth promoter in animal husbandry from the late-1970s until it was banned in the EU by 1998. Glycopeptide resistance is due to the synthesis of modified cell wall precursors that show a decreased affinity for glycopeptides. Five phenotypes have been identified of which two have clinical relevance; i) VanA with high-level resistance to both vancomycin and teicoplanin, and ii) VanB with a variable level of resistance to only vancomycin. The VanA and VanB phenotypes, mostly found among *E. faecalis* and *E. faecium,* may be transferred by plasmids and conjugative transposition.

Results *E. faecalis*

High-level aminoglycosides

- Twenty-six countries reported 6950 isolates of which 2484 were high-level resistant to aminoglycosides.
- Three countries reported resistance proportions above 50 % (Cyprus, Greece and Hungary), and the majority of countries (18 of 26) reported resistant proportions between 50% and 30%. Among 5 countries reporting proportions below 30%, the lowest proportions were reported by Sweden (18.6 %), France (18.0 %) and Iceland (15.4%) (Figure 5.10 and Table 5.4).
- Nineteen of 26 countries reported more than 20 isolates per year since 2006 and were included in the trend analysis for the period 2006-2009. During the past four years, a significant increase was observed for 3 of 19 countries. Only for Cyprus the increasing trend was significant when considering data from laboratories reporting consistently for all four years (Figure 5.11).
- Two countries (Germany and Ireland) reported significant decreasing trends of high-level aminoglycosides resistance. The decreasing trend was significant only for Ireland when considering data from laboratories reporting consistently for all four years (Figure 5.11).

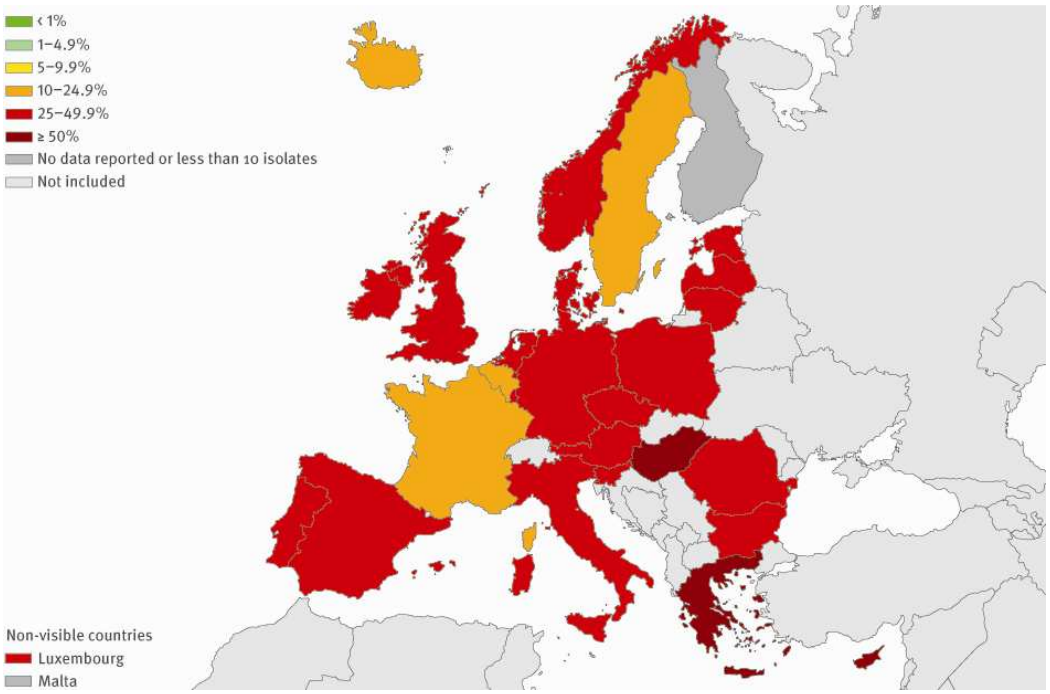

Figure 5.10. *Enterococcus faecalis*: proportion of invasive isolates with high-level resistance to aminoglycosides in 2009. Only countries reporting 10 or more isolates are included.

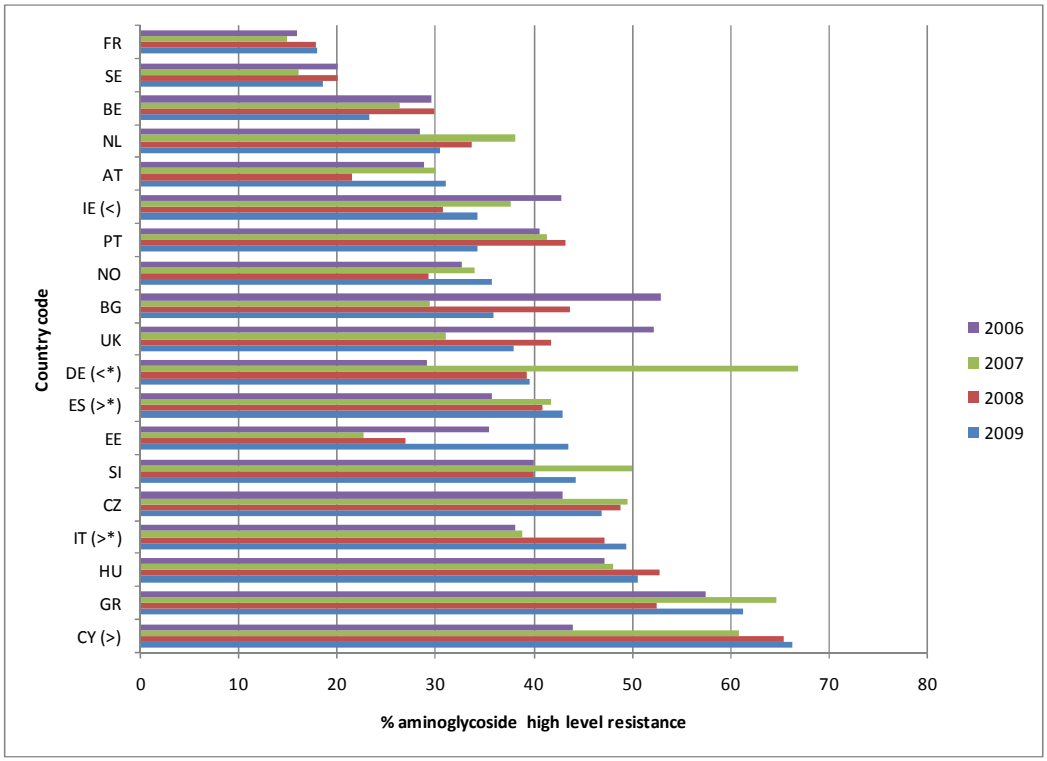

Figure 5.11. *Enterococcus faecalis*: trends of high-level aminoglycoside resistance by country 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

Results *E. faecium*

Vancomycin

- Twenty-eight countries reported 4945 isolates of which 451 were resistant to vancomycin. Only two countries (Malta and Romania) reported less than 10 isolates (thus not shown in figure 5.12).
- Three countries reported resistance proportions above 25% (Ireland, Luxembourg, and Greece) and five countries reported resistant proportions between 10% and 25%, while the majority of countries (18 of 26) reported resistant proportions below 10%. Several countries reported even below 1% (Bulgaria, Estonia, Finland, France, Norway and Sweden) (Figure 5.12 and Table 5.4).
- Nineteen of 28 countries reported more than 20 isolates per year since 2006 and were included in the trend analysis for the period 2006-2009. During the past four years, a significant increase was observed only for Austria; however the increasing trend was not significant when considering data from laboratories reporting consistently for all four years (Figure 5.13).
- Four countries (Greece, Germany, Italy and France) reported significant decreasing trends of vancomycin resistance. Considering data from laboratories reporting consistently for all four years, the decreasing trend was significant for Greece and Italy (Figure 5.13).

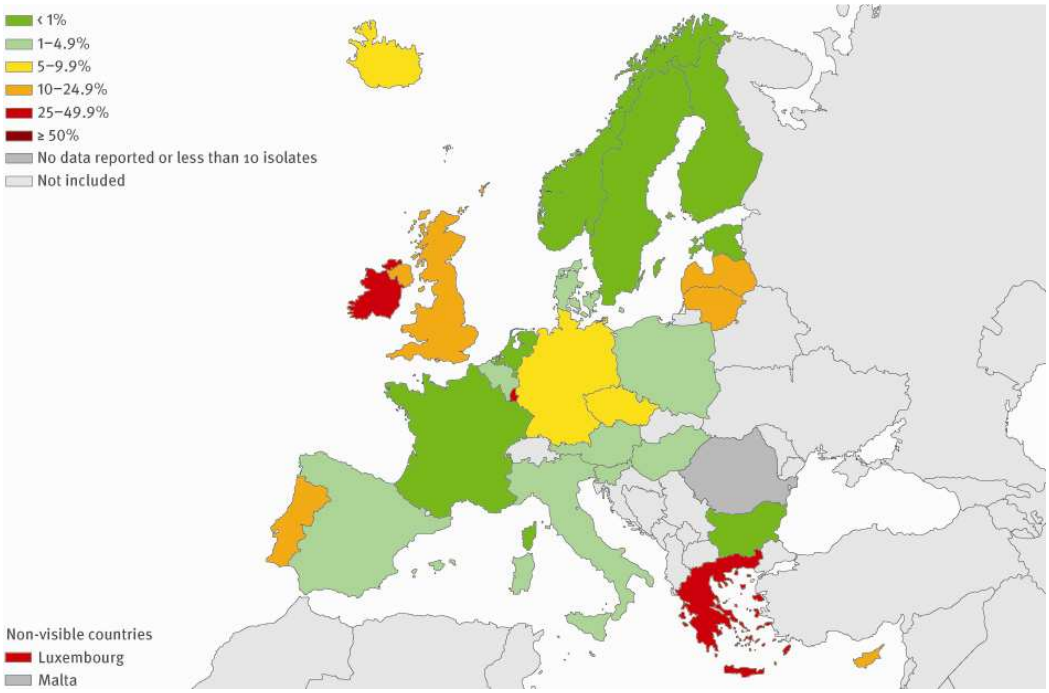

Figure 5.12. *Enterococcus faecium*: proportion of invasive isolates resistant to vancomycin in 2009. Only countries reporting 10 isolates or more are included.

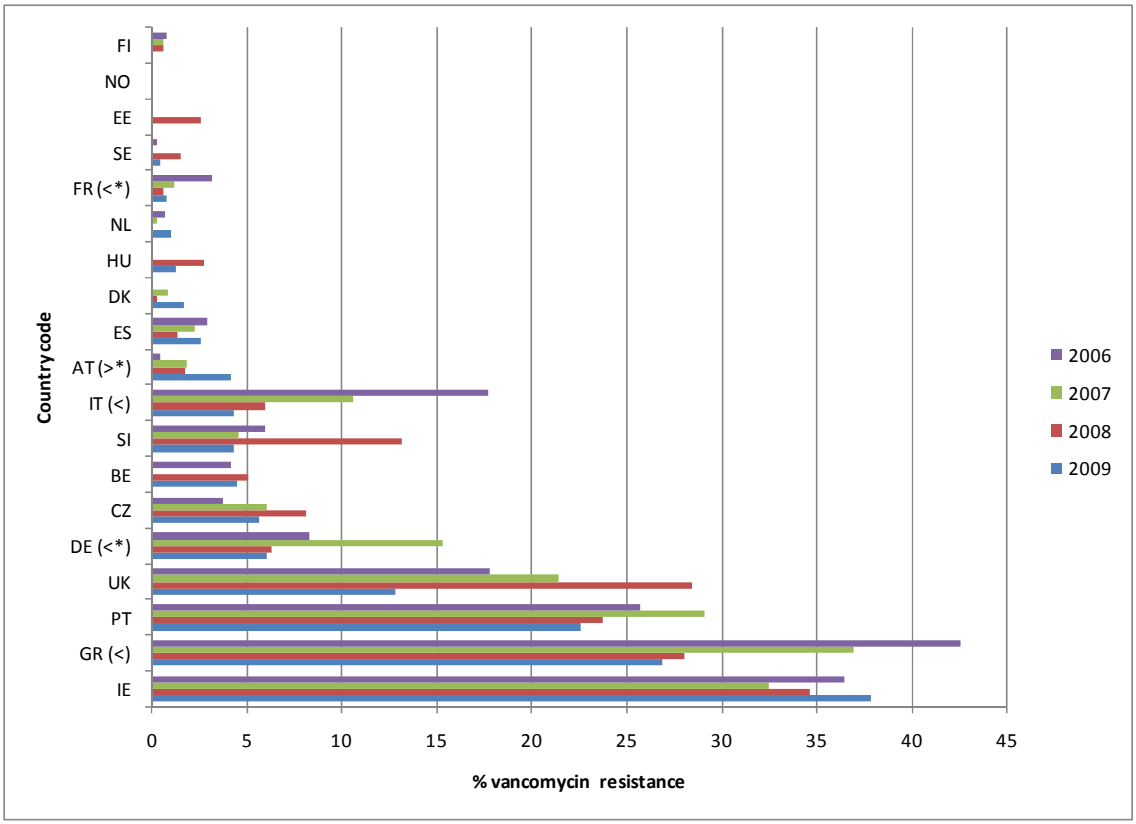

Figure 5.13. *Enterococcus faecium*: trends of vancomycin resistance by country 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

Conclusions

High-level aminoglycoside resistance in *E. faecalis* seems stable in Europe but at a relatively high level. The majority of countries reported proportions of resistant isolates between 30% and 50%, however a consistent decrease was reported by Germany and Ireland.

The occurrence of vancomycin resistance in *E. faecium* seems also to stabilize in Europe, although fluctuations may arise from hospitals outbreaks e.g. related to the continuing dissemination of strains belonging to the polyclonal sub-cluster CC17. This may however not reflect the overall trend or the situation in hospitals which have remained unaffected.

In some countries (Greece, Germany, Italy and France) the efforts to control glycopeptide resistant Enterococci seem to be successful and resulting in a continuous decrease of proportions of resistant isolates, and several countries reported resistant proportions below 1%.

Table 5.4 The number of invasive *E. faecalis* and *E. faecium* isolates, and the proportion high level aminoglycoside resistant *E. faecalis*, and vancomycin resistant *E. faecium* (%R) including 95% confidence intervals (95CI) reported per country in 2009.

	High level aminoglycoside resistant *E. faecalis*		Vancomycin resistant *E. faecium*	
Country	N	% R (95CI)	N	% R (95CI)
AT	252	31.0 (25-37)	310	4.2 (2-7)
BE	150	23.3 (17-31)	67	4.5 (1-13)
BG	53	35.8 (23-50)	26	0.0 (0-13)
CY	65	66.2 (53-77)	15	13.3 (2-40)
CZ	626	46.8 (43-51)	209	5.7 (3-10)
DE	450	39.6 (35-44)	408	6.1 (4-9)
DK	52	32.7 (20-47)	348	1.7 (1-4)
EE	23	43.5 (23-66)	40	0.0 (0-9)
ES	735	42.9 (39-47)	342	2.6 (1-5)
FI	0	. (.-.)	243	0.0 (0-2)
FR	1298	18.0 (16-20)	591	0.8 (0-2)
GR	547	61.2 (57-65)	435	26.9 (23-31)
HU	368	50.5 (45-56)	76	1.3 (0-7)
IE	260	34.2 (28-40)	386	37.8 (33-43)
IS	26	15.4 (4-35)	25	8.0 (1-26)
IT	136	49.3 (41-58)	188	4.3 (2-8)
LT	31	48.4 (30-67)	19	10.5 (1-33)
LU	32	28.1 (14-47)	14	35.7 (13-65)
LV	26	38.5 (20-59)	22	18.2 (5-40)
MT	0	. (.-.)	6	0.0 (0-46)
NL	190	30.5 (24-38)	207	1.0 (0-3)
NO	252	35.7 (30-42)	101	0.0 (0-4)
PL	163	38.7 (31-47)	85	1.2 (0-6)
PT	420	34.3 (30-39)	217	22.6 (17-29)
RO	17	35.3 (14-62)	9	11.1 (0-48)
SE	597	18.6 (16-22)	221	0.5 (0-2)
SI	115	44.3 (35-54)	69	4.3 (1-12)
UK	66	37.9 (26-51)	266	12.8 (9-17)

Escherichia coli

Clinical and epidemiological importance

Escherichia coli is the most frequent gram-negative rod isolated from blood cultures in clinical settings. It is the most frequent cause of bacteremia, community and hospital-acquired urinary tract infections, is associated with spontaneous and surgical peritonitis and with skin and soft tissue infections due to multiple microorganisms, causes neonatal meningitis and is one of the most important food-borne pathogens worldwide.

Resistance mechanisms

Beta-lactamases hydrolyse the beta-lactam ring of beta-lactam antibiotics, which is crucial for inhibition of PBPs in bacteria. In *E. coli* resistance to broad-spectrum penicillins such as ampicillin or amoxicillin is usually conferred by plasmid coded beta-lactamases mainly of the TEM type and to a lesser extend of the SHV type, whereby TEM-1 accounts for up to 60% of aminopenicillin resistance. In 1982 the first ESBL was identified during a hospital outbreak of *Klebsiella pneumoniae* in Germany. It was soon understood that single or multiple amino acid substitutions in the basic structure of SHV or TEM enzymes can alter their spectrum of activity and enhance their hydrolyzing ability to include third generation cephalosporins (in this report referring to: cefotaxime, ceftriaxone, ceftazidime) and monobactams. Most ESBLs can be inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam. More than 200 ESBL variants are known to date. Most of them belong to four enzyme families TEM, SHV, CTX-M and OXA (an overview of identified ESBL types is given on http://www.lahey.org/studies/). Until 2000, over 90% of ESBL resistance was mediated through TEM or VHS variants. In the late 1980's, new ESBLs of the CTX-M family emerged first in South America and during early 2000's attained global importance. In contrast to conventional TEM, and SHV ESBLs, most CTX-Ms display a higher hydrolysing ability against cefotaxime than ceftazidime (hence their name). An important part of this global success is due to the wide dissemination of particular plasmids or bacterial clones producing ESBL (e.g. CTX-M15). Other enzymes affecting the susceptibility to third generation cephalosporins include plasmid encoded variants from the chromosomal AmpC beta-lactamases. CMY-2 is the most widespread enzyme belonging to this group, which is still less common in *E. coli* in Europe but frequent in the USA. An important threat that will require close surveillance in the future is the development of carbapenem-resistance in *E coli,* mediated by metallo-betalactamases (as VIM or IMP enzymes, or

the emerging NDM enzyme) and serin-beta-lactamases (as KPC enzymes), providing resistance to virtually all available beta-lactam agents.

Fluoroquinolones interact with DNA gyrase and topoisomerase IV which are enzymes that regulate conformational changes in the bacterial chromosome during replication and transcription. This interaction leads to irreversible inhibition of the enzyme activity followed by DNA fragmentation and eventually to cell death. Resistance to fluoroquinolones arises through stepwise mutations in the coding regions of the gyrase subunits (*gyrA* and *gyrB*) and DNA topoisomerase IV (*parC*). Accumulation of mutations in several of these genes increases the MIC in a stepwise manner. Low-level resistance to fluoroquinolones may also arise through changes in outer membrane porins or from upregulation of efflux pumps, resulting in lower outer membrane permeability and higher efflux, respectively. In recent years, several plasmid-mediated quinolone resistance mechanisms have also been identified, including the Qnr proteins, which protect DNA topoisomerases from quinolone binding, the AAC6'-Ib-cr enzyme which inactivates some fluoroquinolones by acetylation, and the QepA efflux pump which effluxes hydrophilic quinolones. These mechansims are of concern because of transferability and their frequent association with CTX-M and CMY-type enzymes inactivating third generation cephalosporins.

Aminoglycosides block protein synthesis by binding to the ribosomes, which are involved in the translation of RNA into proteins, and are also able to damage the outer membrane of gram-negative rods. Resistance to aminoglycosides can be due to targeted modification (methylation) of the large ribosomal subunit which excludes aminoglycoside molecules, or by aminoglycoside modifying enzymes that acetylate, adenylate or phosphorylate their target molecules and thereby neutralize the biologic effect of aminoglycosides.

Results

Aminopenicillins

- Twenty-eight countries reported 47318 isolates of which 25254 were resistant to aminopenicillins.
- The majority of countries (19 of 28) reported resistance proportions from 50 % to 66.5 %. Among nine countries reporting proportions below 50%, the lowest proportions were reported by Norway (37.2 %), Finland (36.4 %) and Sweden (33.2 %) (Table 5.5). Trends for the period 2006-2009 were calculated for 28 countries. During the past four years, a significant increase was observed in 10 of 28 countries. In seven of these countries the

trends were significant also when considering only data from laboratories reporting consistently for all four years (Figure 5.17). Among the 10 countries with increasing trends, four countries reported proportion of resistance to aminopenicillin higher than 60 % even though these countries were already at relatively high levels, five countries reported between 60% and 50%, and one country reported below 50%.

- Two countries (Austria and Estonia) reported significant decreasing trends of resistance to aminopenicillin although both countries were already at relatively low levels (Figure 5.17).

Third generation cephalosporins

- Twenty-eight countries reported 49720 isolates of which 3657 were resistant to third generation cephalosporins.
- Nine of 28 countries reported 3rd generation cephalosporins resistance higher than 10% and the proportions ranged up to 19.2%. Among the 19 countries reporting less than 10% resistance, the lowest proportions were reported by Iceland (1.8%), Estonia (2.2%) and Norway (2.3%) (Table 5.5 and Figure 5.14).
- Trends for the period the 2006-2009 were calculated for 28 countries. During the past four years, a significant increase was observed in more than half (16 of 28) countries. In 15 of these countries, the trends were significant also when considering only data from laboratories reporting consistently for all four years (Figure 5.18). Among the 16 countries with increasing trends for 2006-2009, five countries reported proportions of resistance to 3rd generation cephalosporins above 10%, nine countries reported between 10 and 5%, and two countries reported below 5 %.
- Only one country reported a decreasing trend in resistance to 3rd third generation cephalosporins, however the trends was not significant when considering only data from laboratories reporting consistently for all four years.

<u>Extended-Spectrum Beta-Lactamase (ESBL)</u>

- Among *E. coli* isolates resistant to third generation cephalosporins, a large proportion has been ascertained as ESBL positive by the participating laboratories in 2009. Eleven of twelve countries reported proportions of ESBL production between 85% and 100% among isolates resistant to third generation cephalosporins (Table 5.6).

Fluoroquinolones

- Twenty-eight countries reported 48054 isolates of which 9488 were resistant to fluoroquinolones.
- The majority of countries (16 of 28) reported resistant proportions higher than 20 %, ranging up to 43.4%. Seven countries reported between 20% and 10%. Among five countries reporting below 10%, the lowest proportions of resistant isolated were reported by Iceland (7.1%), Sweden (8.2 %) and Estonia (8.3%) (Table 5.5 and Figure 5.15).
- Trends in the period 2006-2009 were calculated for 28 countries. A significant increase was observed for seven countries. In six of these countries the trends were significant also when considering only data from laboratories reporting consistently all four years (Figure 5.19).
- Among the seven countries with increasing trends for 2006-2009, two countries reported proportions of resistance to fluoroquinolones above 30%, two countries reported between 30% and 20%, and three countries reported below 20%.
- Only two countries (Austria and Germany) reported significant decreasing trends in resistance to fluoroquinolones (Figure 5.19).

Aminoglycosides

- Twenty-eight countries reported 50699 isolates of which 3983 were resistant to aminoglycosides.
- Eleven of 28 countries reported proportions of resistance higher than 10%, ranging up to 21.4%. Eleven countries reported between 10% and 5%. Among six countries reporting below 5%, the lowest proportions of resistant isolated were reported by Norway (3.3%), Finland (2.8%) and Sweden (2.5 %) (Table 5.5 and Figure 5.20).
- Trends in the period 2006-2009 were calculated for 28 countries. A significant increase in the proportion of isolates resistant to aminoglycosides was observed for 10 countries. Among these, five countries reported proportions higher than 10%, one country reported between 10% and 5%, and four countries reported below 5%.
- For two countries (Austria and Romania) the proportions of resistance to aminoglycosides significantly decreased over the last four years. For Poland the trend was significant also when considering only data from laboratories consistently reporting for all four years (Figure 5.20).

Combined resistance (aminopenicillins, third generation cephalosporins, fluoroquinolones and aminoglycosides)

- In 2009, 28 countries reported 42898 isolates tested for aminopenicillins, third generation cephalosporins, fluoroquinolones and aminoglycosides. Fifty-seven percent of these isolates were resistant to one or more of the four considered antibiotic classes.
- In nine countries, the proportion of multi resistant isolates (resistance to third generation cephalosporins, fluoroquinolones and aminoglycosides) was higher than 5% , between 5% and 1% in 14 countries, and below one percent in four countries (Table 5.7 and Figure 5.21).
- Trends in the period 2006-2009 were calculated for 28 countries. A significant increase in proportions of multi resistant isolates was observed for 12 countries. In 10 of these countries the trends were significant also when considering only data from laboratories reporting consistently for all four years (Figure 5.21). Among the 12 countries with significantly increasing proportions of multi resistant isolates, four countries reported above 5%, eight countries reported between 5% and 1%, whereas no countries reported below 1%. No country had a significantly decreasing trend of proportions of multi resistance in E. coli in 2006- 2009.
- The most frequent resistance phenotypes in *E. coli* were single aminopenicillin resistance (33.3%), followed by dual resistance to aminopenicillins and fluoroquinolones (8.7%). Combined resistance to all 4 antimicrobials was reported for 3.3% of the isolates and combined resistance to aminopenicillins, fluoroquinolones and aminoglycosides was 3.2% (Table 5.7).

Table 5.5 - The number of invasive *E. coli* isolates and the proportion aminopenicillins, third generation cephalosporins, fluoroquinolones, aminoglycosides and multi-resistance (%R) including 95% confidence intervals (95CI) reported per country in 2009.

	Aminopenicillins		Fluoroquinolones		Third gen. cephalosporins		Aminoglycosides		Multi-resistance*	
Country	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)
AT	2605	48.6 (47-51)	2616	20.5 (19-22)	2597	7.5 (7-9)	2619	6.1 (5-7)	2588	2.2 (2-3)
BE	1607	55.9 (53-58)	1504	19.9 (18-22)	1596	6.5 (5-8)	1195	7.3 (6-9)	1175	1.6 (1-3)
BG	181	66.3 (59-73)	193	28.0 (22-35)	193	19.2 (14-25)	194	18.0 (13-24)	192	7.3 (4-12)
CY	136	66.2 (58-74)	136	43.4 (35-52)	136	14.0 (9-21)	136	9.6 (5-16)	136	5.1 (2-10)
CZ	2759	60.6 (59-62)	2758	23.2 (22-25)	2759	9.8 (9-11)	2742	9.0 (8-10)	2741	3.0 (2-4)
DE	2158	56.1 (54-58)	2786	23.4 (22-25)	2759	8.2 (7-9)	2796	8.4 (7-9)	2745	3.2 (3-4)
DK	3531	42.9 (41-45)	3406	13.2 (12-14)	2705	6.2 (5-7)	3530	4.4 (4-5)	2634	1.4 (1-2)
EE	319	38.2 (33-44)	302	8.3 (5-12)	319	2.2 (1-4)	320	3.8 (2-6)	301	0.7 (0-2)
ES	3821	64.7 (63-66)	3810	31.5 (30-33)	3821	11.3 (10-12)	3820	13.0 (12-14)	3809	4.5 (4-5)
FI	1906	36.4 (34-39)	2223	9.2 (8-10)	2176	2.8 (2-4)	2033	2.8 (2-4)	1984	1.5 (1-2)
FR	8436	55.5 (54-57)	8353	18.6 (18-19)	8449	6.7 (6-7)	8448	8.2 (8-9)	8350	3.0 (3-3)
GR	1694	50.9 (48-53)	1806	23.4 (21-25)	1815	10.1 (9-12)	1828	14.5 (13-16)	1806	6.4 (5-8)
HU	1047	59.8 (57-63)	1046	30.5 (28-33)	1052	12.9 (11-15)	1056	15.9 (14-18)	1042	9.8 (8-12)
IE	1987	66.5 (64-69)	2005	21.7 (20-24)	1986	6.5 (5-8)	2009	8.8 (8-10)	1976	2.4 (2-3)
IS	109	49.5 (40-59)	99	7.1 (3-14)	110	1.8 (0-6)	110	7.3 (3-14)	99	0.0 (0-4)
IT	764	63.4 (60-67)	863	36.2 (33-39)	687	17.0 (14-20)	863	12.5 (10-15)	687	5.5 (4-8)
LT	291	58.4 (53-64)	295	14.6 (11-19)	293	7.8 (5-12)	297	15.2 (11-20)	291	3.8 (2-7)
LU	298	57.0 (51-63)	300	25.7 (21-31)	300	8.3 (5-12)	300	9.3 (6-13)	300	4.3 (2-7)
LV	86	43.0 (32-54)	85	23.5 (15-34)	86	11.6 (6-20)	86	12.8 (7-22)	85	5.9 (2-13)
MT	159	54.7 (47-63)	159	30.8 (24-39)	159	15.1 (10-22)	159	21.4 (15-29)	159	12.6 (8-19)
NL	2359	45.1 (43-47)	2377	11.0 (10-12)	2368	4.3 (3-5)	2389	4.4 (4-5)	2339	1.4 (1-2)
NO	1845	37.2 (35-39)	1830	8.7 (7-10)	1846	2.3 (2-3)	1841	3.3 (3-4)	1827	0.7 (0-1)
PL	487	64.7 (60-69)	567	23.1 (20-27)	584	9.1 (7-12)	595	7.1 (5-9)	546	1.8 (1-3)
PT	1919	58.3 (56-61)	1973	27.7 (26-30)	1912	9.2 (8-11)	2038	10.8 (9-12)	1885	5.5 (4-7)
RO	12	83.3 (52-98)	53	22.6 (12-36)	53	17.0 (8-30)	80	12.5 (6-22)	53	15.1 (7-28)
SE	2195	33.2 (31-35)	1596	8.2 (7-10)	4233	2.8 (2-3)	4121	2.5 (2-3)	1529	0.7 (0-1)
SI	783	53.8 (50-57)	783	18.9 (16-22)	783	6.6 (5-9)	783	10.5 (8-13)	783	4.6 (3-6)
UK	3824	61.6 (60-63)	4130	18.0 (17-19)	3943	9.4 (8-10)	4311	7.4 (7-8)	3616	4.0 (3-5)

**Multi resistance was defined as being resistant to third generation cephalosporins, fluoroquinolones and aminoglycosides*

Table 5.6 The number of invasive *E. coli* isolates resistant to third generation cephalosporins (n. CREC) and the proportion of ESBL positive (%ESBL) among these isolates, as ascertained by the participating laboratories in 2009.

Country (n. laboratories)	n. CREC	%ESBL
AT (24)	86	94.2
BE (4)	14	92.9
BG (12)	37	94.6
CY (5)	19	100
CZ (42)	270	85.6
ES (33)	432	90.7
FR (38)	316	64.9
IE (25)	117	85.5
LT (8)	23	100
NL (6)	40	90
PT (15)	122	91.8
SI (7)	52	92.3

Only data from laboratories consistently reporting the ESBL test results for all isolates identified as resistant to third generation cephalosporins and from countries with at least 10 of such isolates were selected for the analysis.

Table 5.7 Overall resistance and resistance combinations among invasive *Escherichia coli* isolates tested against aminopenicillins, fluoroquinolones, third generation cephalosporins and aminoglycosides (n= 42898) in Europe, 2009.

Resistance pattern	Number	% of total
fully susceptible	18622	43.4
Single resistance (to indicated drug classes)		
Aminopen	14292	33.3
Fluoroq	908	2.1
Aminogl	86	0.2
Resistance to two classes of antimicrobial drugs		
aminopen+fluoroq	3718	8.7
aminopen+3rd gen ceph	640	1.5
aminopen+aminogl	555	1.3
fluoroq+aminogl	40	0.1
Resistance to three classes of antimicrobial drugs		
aminopen+fluoroq+aminogl	1387	3.2
aminopen+3rd gen ceph+fluoroq	1123	2.6
aminopen+3rd gen ceph+aminogl	132	0.3
Resistance to four classes of antimicrobial drugs		
aminopen+3rd gen ceph+fluoroq+aminogl	1395	3.3

Conclusions

The remarkable and constant Europe wide decline of antimicrobial susceptibility in *E. coli* observed during recent years continued in 2009. Resistance in *E. coli* shows increasing trends in several countries and for both multi resistance and for single antimicrobials under surveillance.

The highest proportions of resistance in *E. coli* were reported for aminopenicillins ranging up to 66.5%. Irrespective of the high level of resistance, proportions continue to increase in several countries, including those already presenting resistance well above 50%.

The proportion of 3rd generation cephalosporins resistance reported for *E. coli* has increased significantly during the last four years in more than half of the reporting countries. Among isolates resistant to 3rd generation cephalosporins, a high proportion (85% - 100%) was identified as ESBL positive. These data indicate that ESBL production is highly prevalent in 3rd generation cephalosporin resistant *E. coli* in European hospitals.

Fluoroquinolone resistance in *E. coli* continues the increase as in previous years. Although the number of countries showing an increasing trend over the last 4 years was reduced compared to 2008, the situation becomes progressively dire and more than half of the countries are reporting resistance proportions higher than 20%.

Ten countries had significant increases in the proportion of isolates resistant to aminoglycosides and 5 of these countries reported proportions higher than 10%. This indicates that aminoglycosides resistance is increasing even among the countries already reporting higher levels of resistance.

The most frequent resistance phenotypes in *E. coli* were single aminopenicillin resistance (33.3%), followed by dual resistance to aminopenicillins and fluoroquinolones (8.7%). Combined resistance to all 4 antimicrobials was reported for 3.3% of the isolates and combined resistance to aminopenicillins, fluoroquinolones and aminoglycosides was 3.2%. Resistance to third generation cephalosporins was associated with resistance to aminoglycosides in almost half of the cases and to fluoroquinolones in approx 75% of the cases. These results indicate that the loss of antimicrobial susceptibility in *E. coli* requires close surveillance.

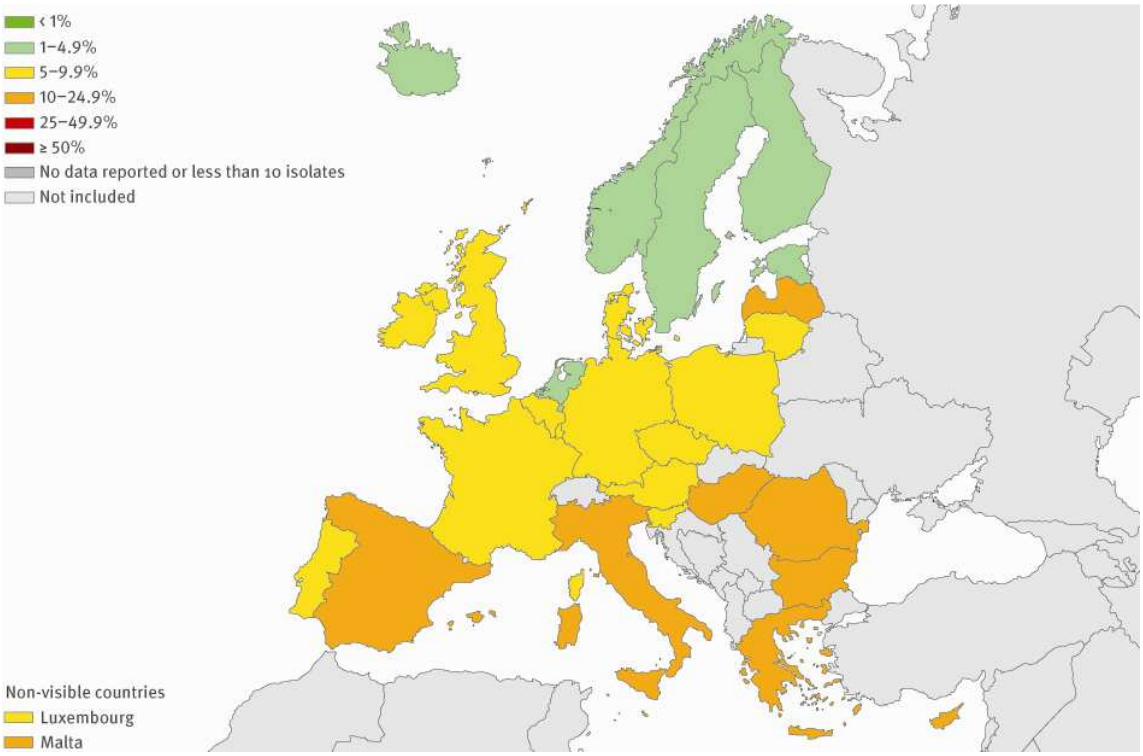

Figure 5.14. *Escherichia coli*: Proportion of third generation cephalosporin resistance in 2009. Only countries reporting 10 isolates or more are included.

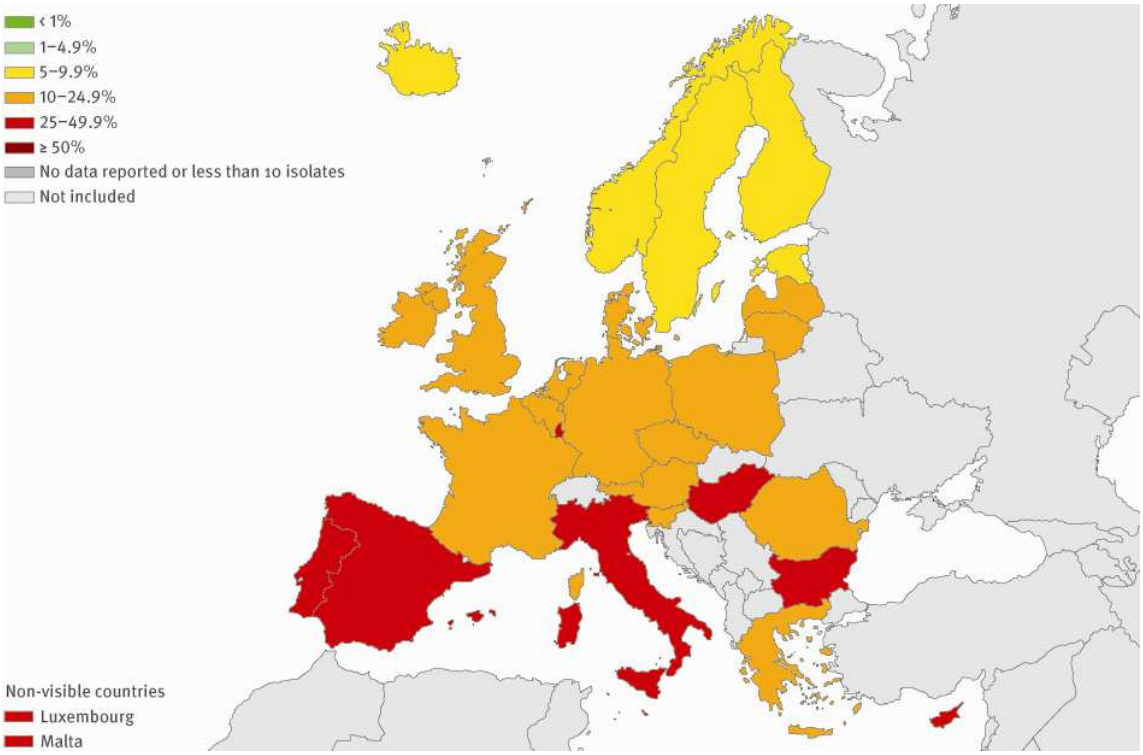

Figure 5.15. *Escherichia coli*: Proportion of invasive isolates with resistance to fluoroquinolones in 2009. Only countries reporting 10 isolates or more are included

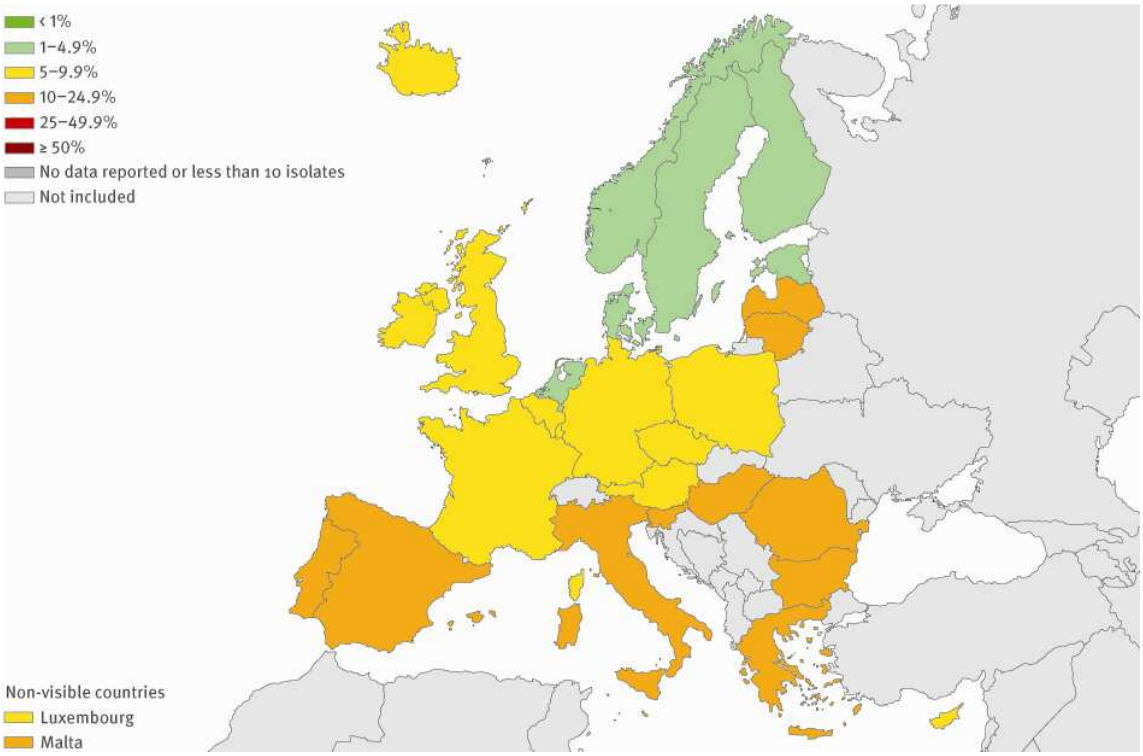

Figure 5.16. *Escherichia coli*: Proportion of invasive isolates with resistance to aminoglycosides in 2009. Only countries reporting 10 isolates or more are included

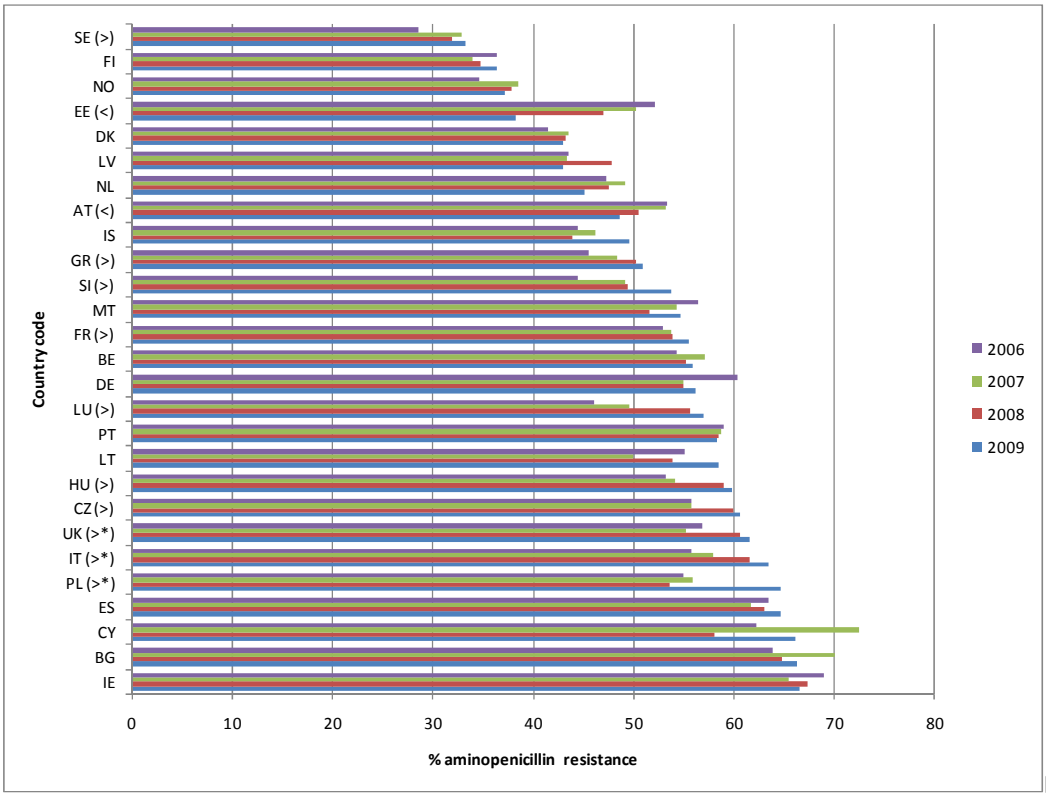

Figure 5.17. *Escherichia coli*: trends of aminopenicillin resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

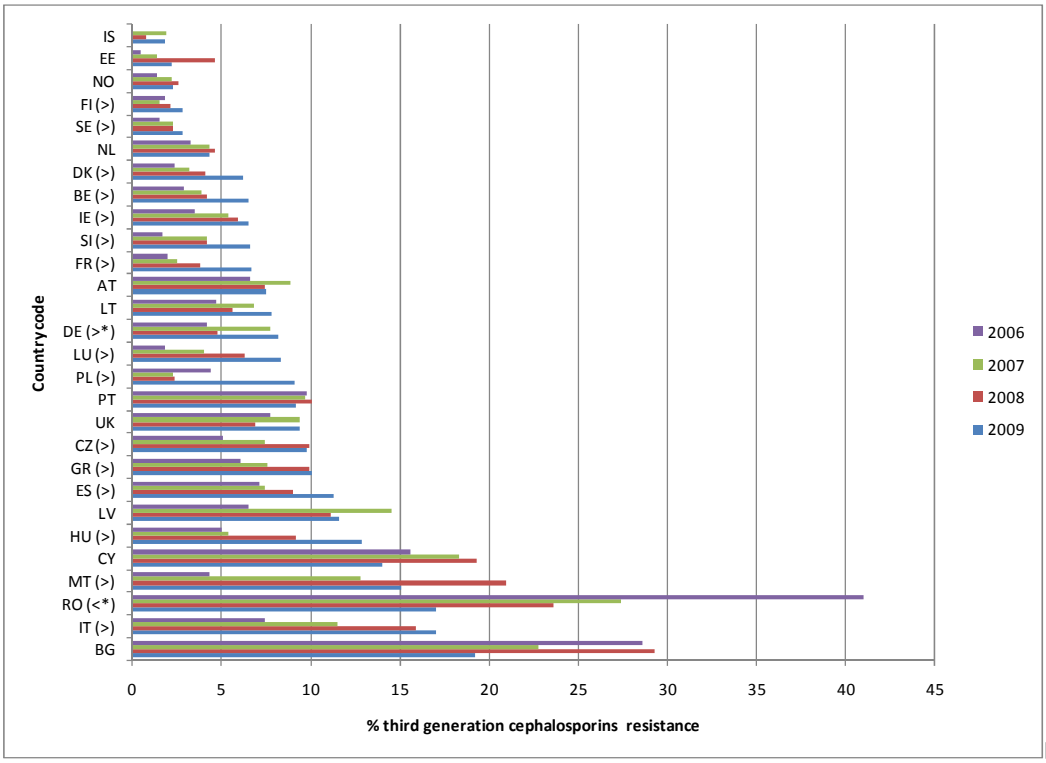

Figure 5.18. *Escherichia coli*: trends of third generation cephalosporin resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

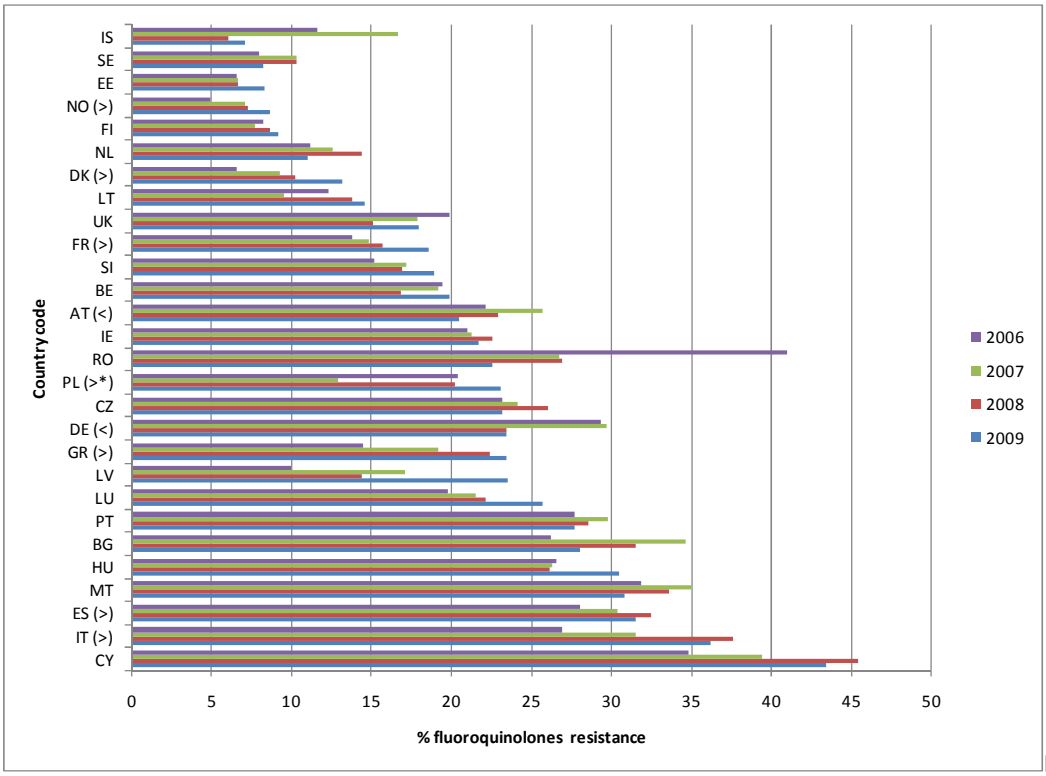

Figure 5.19. *Escherichia coli*: trends of fluoroquinolone resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

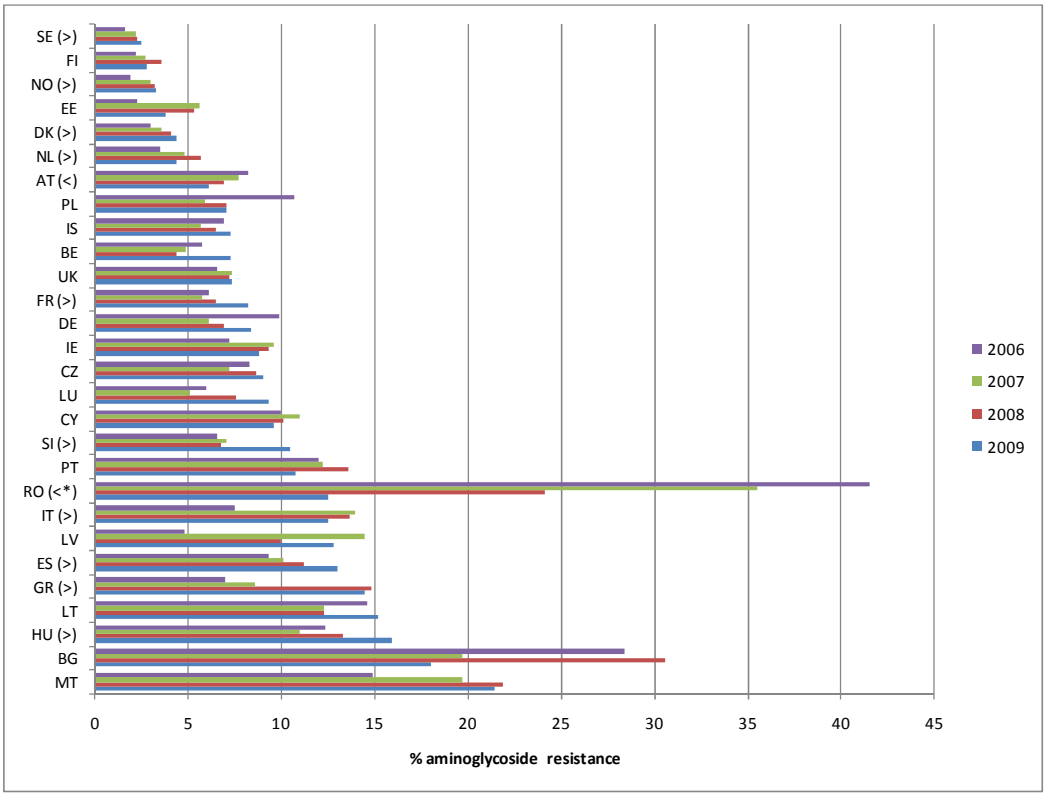

Figure 5.20. *Escherichia coli*: trends of aminoglycoside resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

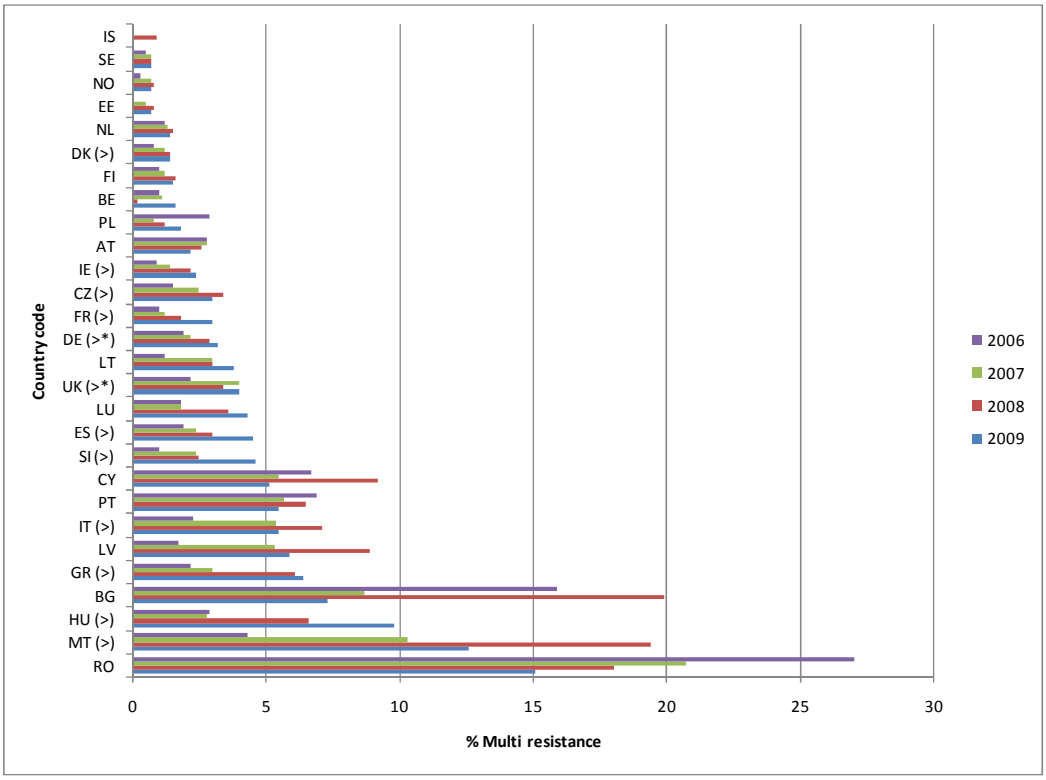

Figure 5.21. *Escherichia coli*: trends of combined resistance (resistant to fluoroquinolones, 3rd gen. cephalosporins and aminoglycosides) by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

Klebsiella pneumoniae

Clinical and epidemiological importance

Bacteria of the genus *Klebsiella* are frequent colonizers of the gastrointestinal tract in humans but may also be found on skin, in the oro-pharynx and upper airways in hospitalized individuals. *Klebsiella pneumoniae* is associated with opportunistic infections in individuals with impaired immune defenses, such as diabetics, alcoholics, and hospitalized patients with indwelling devices. The most common sites of infection are the urinary and the respiratory tract. Organisms can spread rapidly, from the gastrointestinal tract of patients and via the hands of hospital personnel to other patients, leading to nosocomial outbreaks. *K. pneumoniae* is the second most frequent cause of gram-negative blood stream infections after **Escherichia coli**. The mortality rates of pneumonia caused by *K. pneumonia*e can be high even when appropriate antibiotic treatment is given, however also depends on the severity of the underlying condition.

Resistance mechanisms

Similar to *E. coli*, *K. pneumoniae* can be resistant to multiple antibiotics, and resistance traits are frequently acquired through plasmids. However, in contrast to *E. coli, K. pneumoniae* has a chromosomally encoded SHV beta-lactamase and is thus intrinsically resistant against aminopenicillins. Moreover, this organism readily acquires plasmid-mediated resistance determinants. Many novel ESBL variants were initially identified in *K. pneumoniae* and were only subsequently found in *E. coli*. Since the resistance mechanisms do not significantly differ from those described for *E. coli*, readers should referred to the *E. coli* chapter for further details. Carbapenems have been widely used in many countries due to the increasing rate of ESBLs producing Enterobacteriaceae with a consequent impact on the emergence of resistance to these antibiotics, especially in *K. pneumoniae*. KPC carbapenemase producing clones of *K. pneumoniae* have been observed in USA, Greece, and Israel while plasmids encoding the VIM metallo-carbapenemase are frequent in *K. pneumoniae* in Greece. Recently, a new type of plasmidic carbapenemase, the New Delhi metallo-β-lactamase 1 (NDM-1), has been observed in patients returning from the Indian subcontinent.

Results

Third generation cephalosporins

- Twenty-eight countries reported 11665 isolates of which 3214 were resistant to third generation cephalosporins.
- Proportion of resistance was: below 1% in two countries, between 1-5% in three countries, between 5-10% in three countries, between 10-25% in seven countries, between 25-50% in seven countries and above 50% in six countries (Fig 5.22, Tab 5.8).
- Trends in the 2006-2009 period have been calculated for 24 countries. A significant increase has been observed for nine countries. In eight of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.26). In 2009, the nine countries with increasing trends reported proportion of resistance to third generation cephalosporins: between 10-25% in three cases (Denmark, Estonia and France), between 25-50% in three cases (Hungary, Luxembourg and Portugal) and above 50% in three cases (Czech Republic, Greece and Latvia).
- Two countries (Malta and United Kingdom) reported significant decreasing trends of proportions of resistance to third generation cephalosporins (Fig 5.26) which, in 2009, were 0% and 7%, respectively.

Extended-Spectrum Beta-Lactamase (ESBL)

- Sixteen countries have been included in the analysis of ESBL proportion for *K. pneumoniae*. Only data from laboratories consistently reporting the ESBL test results for all isolates identified as resistant to third generation cephalosporins and from countries with at least 10 of such isolates were selected for the analysis.
- The proportion of *K. pneumonia* isolates resistant to third generation cephalosporins and ESBL producers, as ascertained by the participating laboratories, ranged between 73% and 100%. Proportion of ESBL producers was: between 70-80% in three countries, between 80-90% in four countries and above 90% in nine countries (Tab 5.9).

Fluoroquinolones

- Twenty-eight countries reported 11344 isolates of which 3203 were resistant to fluoroquinolones.

- Proportion of resistance was: below 1% in one country, between 1-5% in four countries, between 5-10% in three countries, between 10-25% in ten countries, between 25-50% in eight countries and above 50% in two countries (Fig 5.23, Tab 5.8).
- Trends in the 2006-2009 period have been calculated for 23 countries. A significant increase has been observed for eleven countries. In ten of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.27). In 2009, the eleven countries with increasing trends reported proportion of resistance to fluoroquinolones: between 10-25% in four cases (Denmark, Estonia, France and Spain), between 25-50% in five cases (Bulgaria, Cyprus, Hungary, Lithuania and Portugal) and above 50% in two cases (Czech Republic and Greece).
- Two countries (Ireland and United Kingdom) reported significant decreasing trends of proportions of resistance to fluoroquinolones (Fig 5.27) which, in 2009, were 11% and 6%, respectively.

Aminoglycosides

- Twenty-eight countries reported 11922 isolates of which 2787 were resistant to aminoglycosides.
- Proportion of resistance was: below 1% in three countries, between 1-5% in four countries, between 5-10% in five countries, between 10-25% in seven countries, between 25-50% in six countries and above 50% in three countries (Fig 5.24, Tab 5.8).
- Trends in the 2006-2009 period have been calculated for 24 countries. A significant increase has been observed for eleven countries. In ten of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.28). In 2009, the eleven countries with increasing trends reported proportion of resistance to aminoglycosides: between 1-5% in one case (Norway), between 5-10% in one case (Denmark), between 10-25% in four cases (Estonia, France, Luxemburg and Portugal), between 25-50% in three cases (Czech Republic, Hungary and Latvia) and above 50% in two cases (Greece and Lithuania).
- Two countries (Malta and United Kingdom) reported significant decreasing trends of proportions of resistance to aminoglycosides (Fig 5.28) which, in 2009, were 0% and 6%, respectively.

Combined resistance (third generation cephalosporins, fluoroquinolones and aminoglycosides)

- Twenty-eight countries reported 10952 isolates tested for third generation cephalosporins, fluoroquinolones and aminoglycosides. In 2009, 35% of isolates were resistant to one or more of the three considered antibiotic classes. The most frequent pattern of resistance was the multi resistance (R to all three antibiotic classes) (19%). More than 90% of the isolates resistant to third generation cephalosporins were also resistant to either fluoroquinolones or aminoglycosides and 2/3 were resistant to both these classes (Tab 5.10).
- Proportion of multi resistance was: below 1% in four countries, between 1-5% in six countries, between 5-10% in four countries, between 10-25% in nine countries, between 25-50% in four countries and above 50% in one country (Tab 5.8).
- Trends in the 2006-2009 period have been calculated for 23 countries. A significant increase of multi resistance has been observed for thirteen countries. In ten of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.29). In 2009, the thirteen countries with increasing trends reported proportion of multi resistance: below 1% in one case (Sweden), between 1-5% in two cases (Norway and Spain), between 5-10% in two cases (Denmark and Estonia), between 10-25% in three cases (Latvia, France and Portugal), between 25-50% in four cases (Bulgaria, Czech Republic, Hungary and Lithuania) and above 50% in one case (Greece).
- One country (United Kingdom) reported a significant decreasing trend of proportions of multi resistance (Fig 5.29) which, in 2009, was 3%.

Carbapenems

- Twenty-seven countries reported 10573 isolates of which 738 were resistant to carbapenems. Most of the resistant isolates (708) were identified in Greece; other 12 countries reported at least one resistant isolate.
- Proportion of resistance was: 43.5% in Greece, 17.0% in Cyprus, 1.3% in Italy, 1.2% in Belgium and below 1% in the other twenty-three reporting countries (Fig 5.25).

Conclusions

The antimicrobial resistance of *K. pneumoniae* is a worrisome problem in Europe. In 2009, many countries reported high proportions and increasing trends of resistance to third generation cephalosporins, fluoroquinolones and aminoglycosides. Greece and Cyprus have also reported high proportions of resistance to carbapenems; proportions of resistance to these agents above 1% have been observed in Belgium and Italy.

Two third of the countries have reported resistance to third generation cephalosporins and fluoroquinolones above 10%. Proportions of multi resistance (R to third generation cephalosporins, fluoroquinolones and aminoglycosides) above 10% have been reported by half of the countries.

The majority of isolates resistant to third generation cephalosporins were resistant also to fluoroquinolones and aminoglycosides. Increasing trends of resistance to specific antibiotic classes and of multi resistance have been observed also in northern European countries, like Denmark and Norway, with a traditionally prudent approach to the antibiotic use.

Only United Kingdom has shown a consistent reduction of resistance proportion of *K. pneumoniae* for all the antibiotic classes under surveillance. Decreasing trends for specific antibiotic classes were also observed for Ireland and Malta.

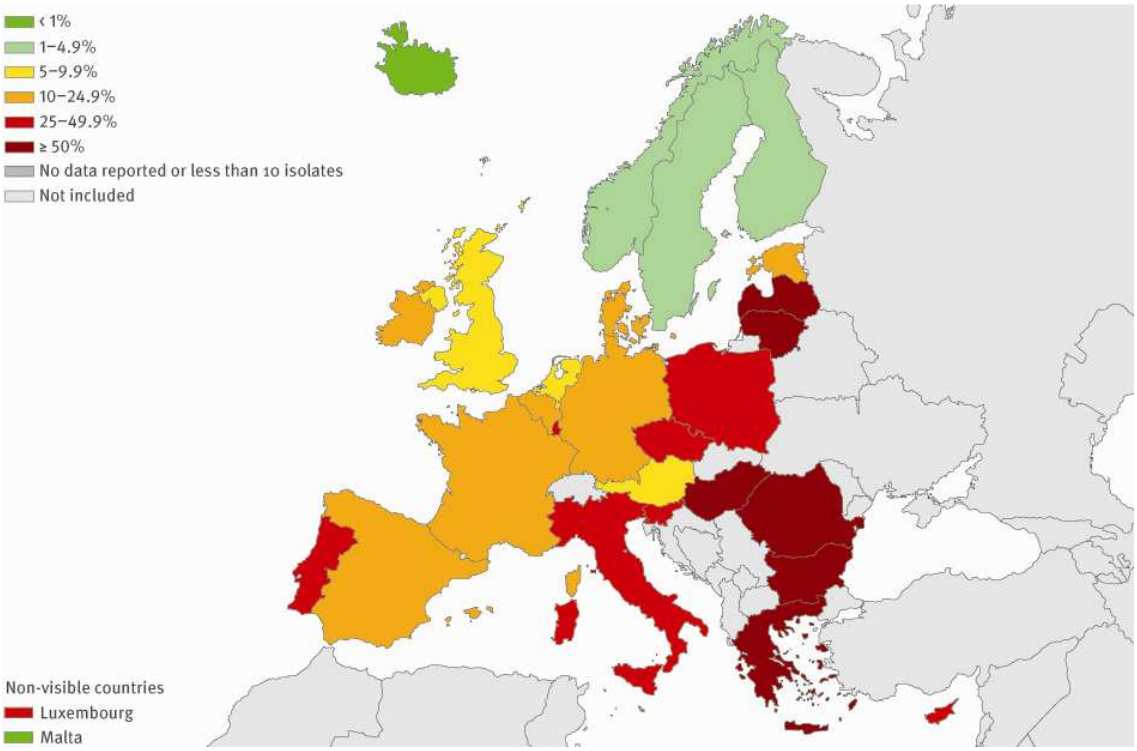

Figure 5.22 *Klebsiella pneumoniae*: proportion of invasive isolates resistant to 3rd generation cephalosporins in 2009. Only countries reporting 10 or more isolates are included

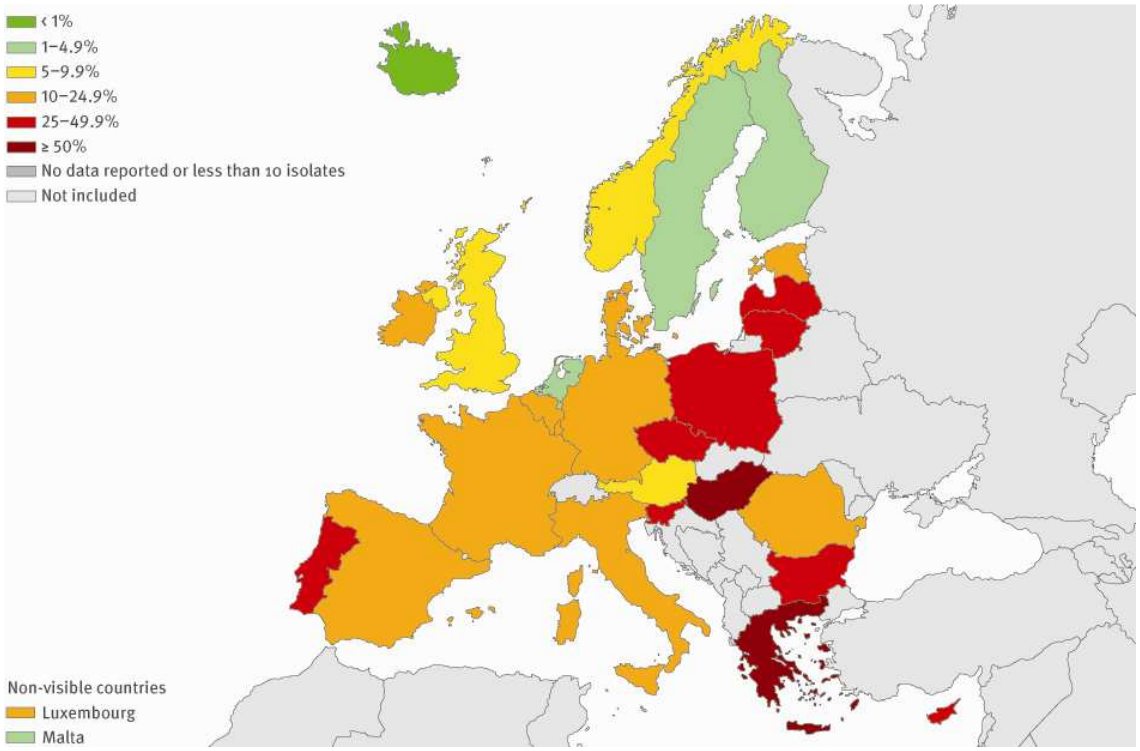

Figure 5.23 *Klebsiella pneumoniae*: proportion of invasive isolates resistant to fluoroquinolones in 2009. Only countries reporting 10 or more isolates are included

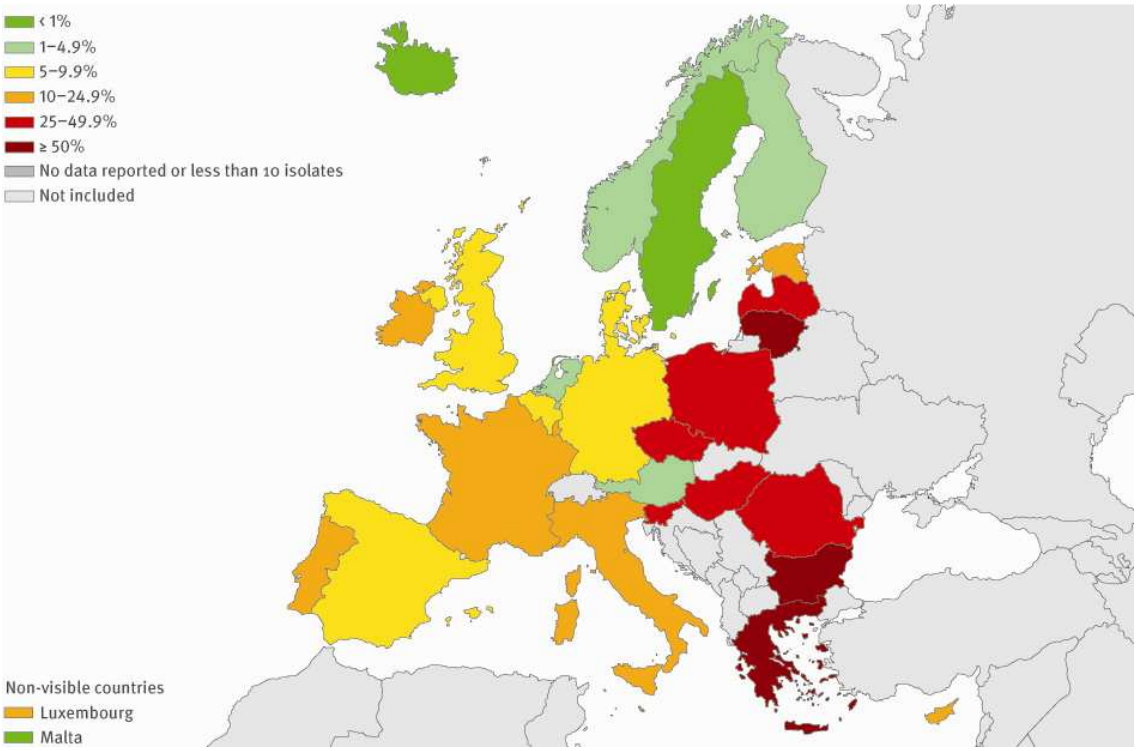

Figure 5.24 *Klebsiella pneumoniae*: proportion of invasive isolates resistant to aminoglycosides in 2009. Only countries reporting 10 or more isolates are included

Figure 5.25 *Klebsiella pneumoniae*: proportion of invasive isolates resistant to carbapenems in 2009. Only countries reporting 10 or more isolates are included

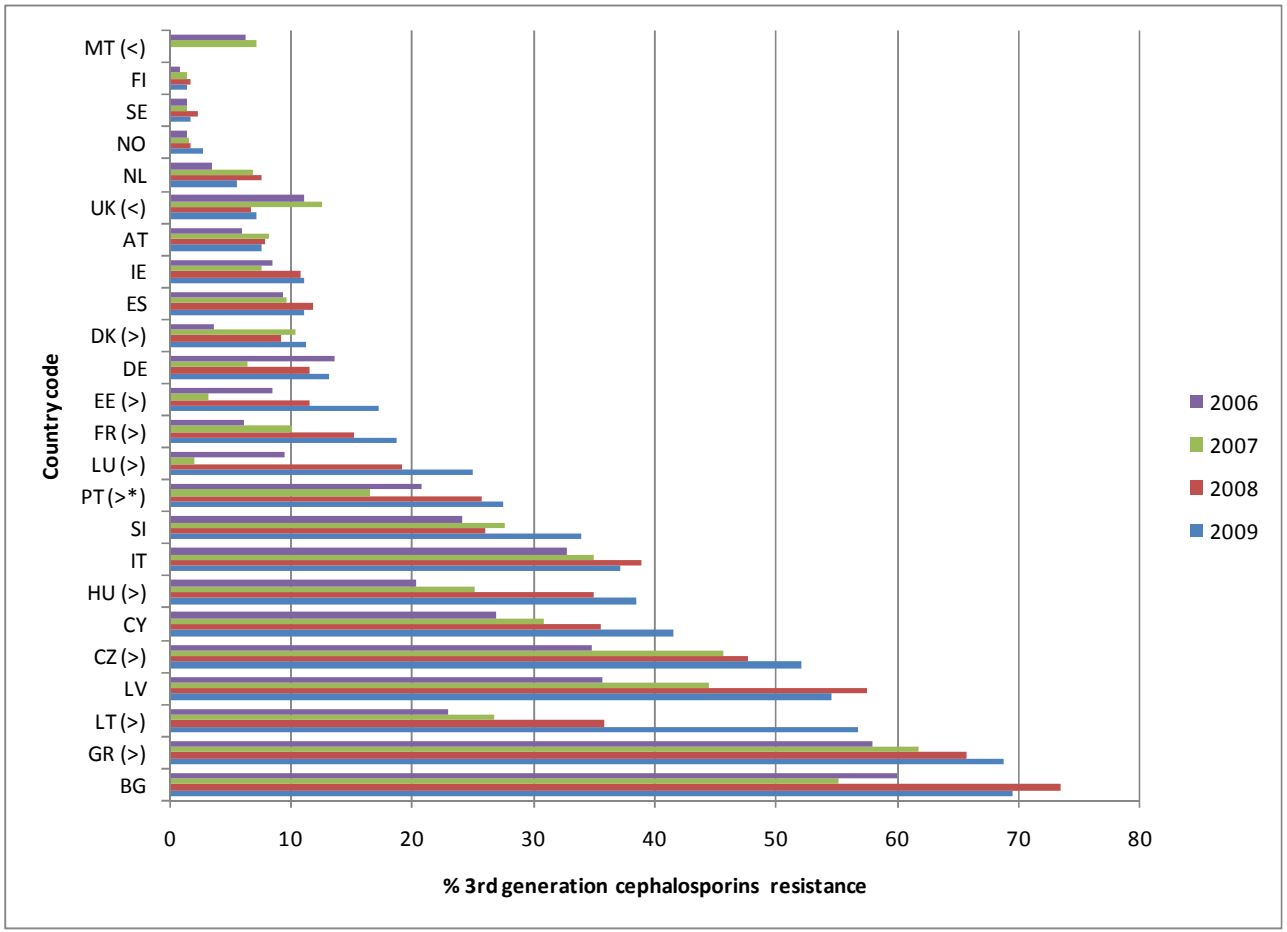

Figure 5.26 *Klebsiella pneumoniae*: trend of 3rd generation cephalosporins resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

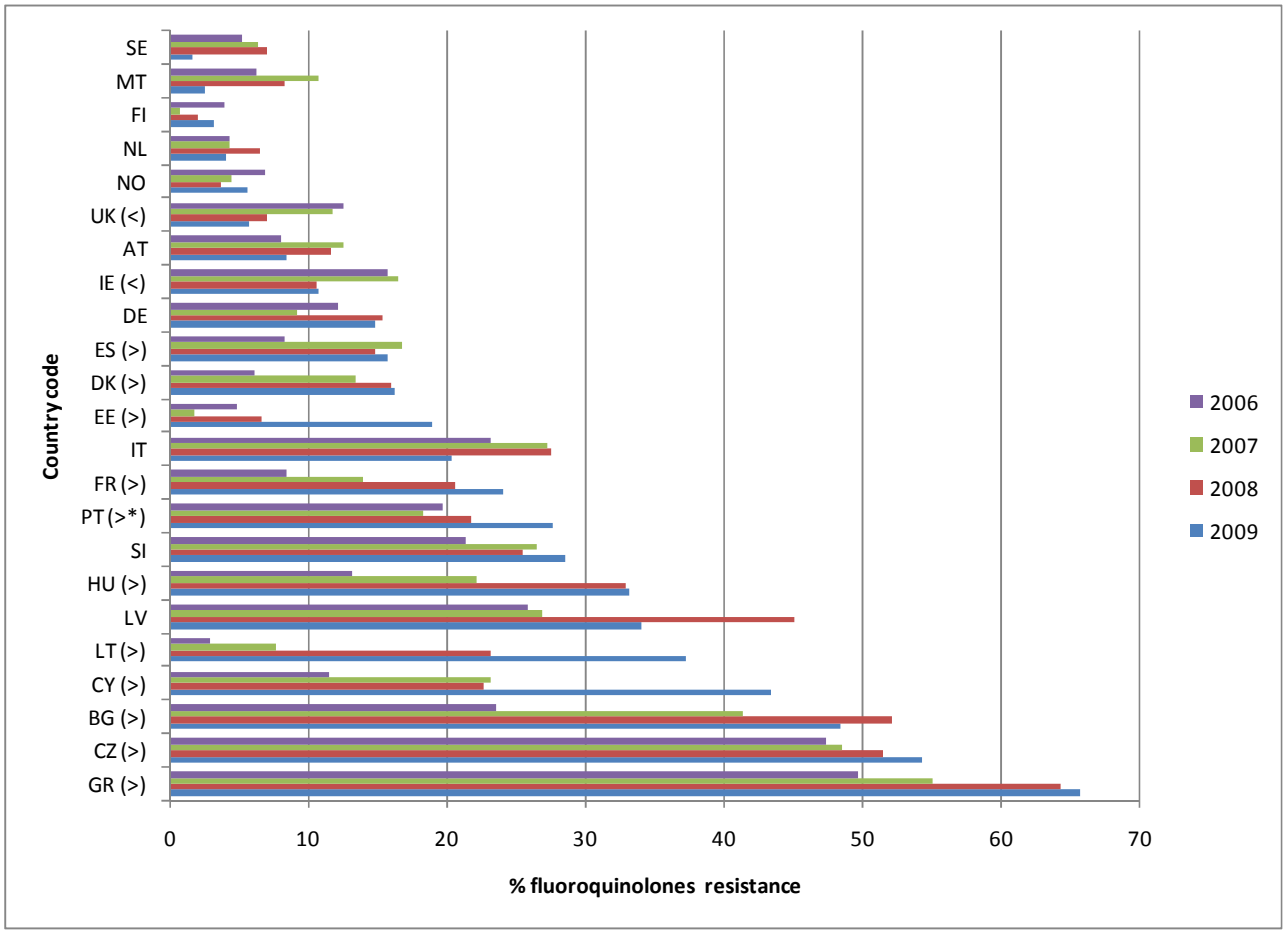

Figure 5.27 *Klebsiella pneumoniae*: trend of fluoroquinolones resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

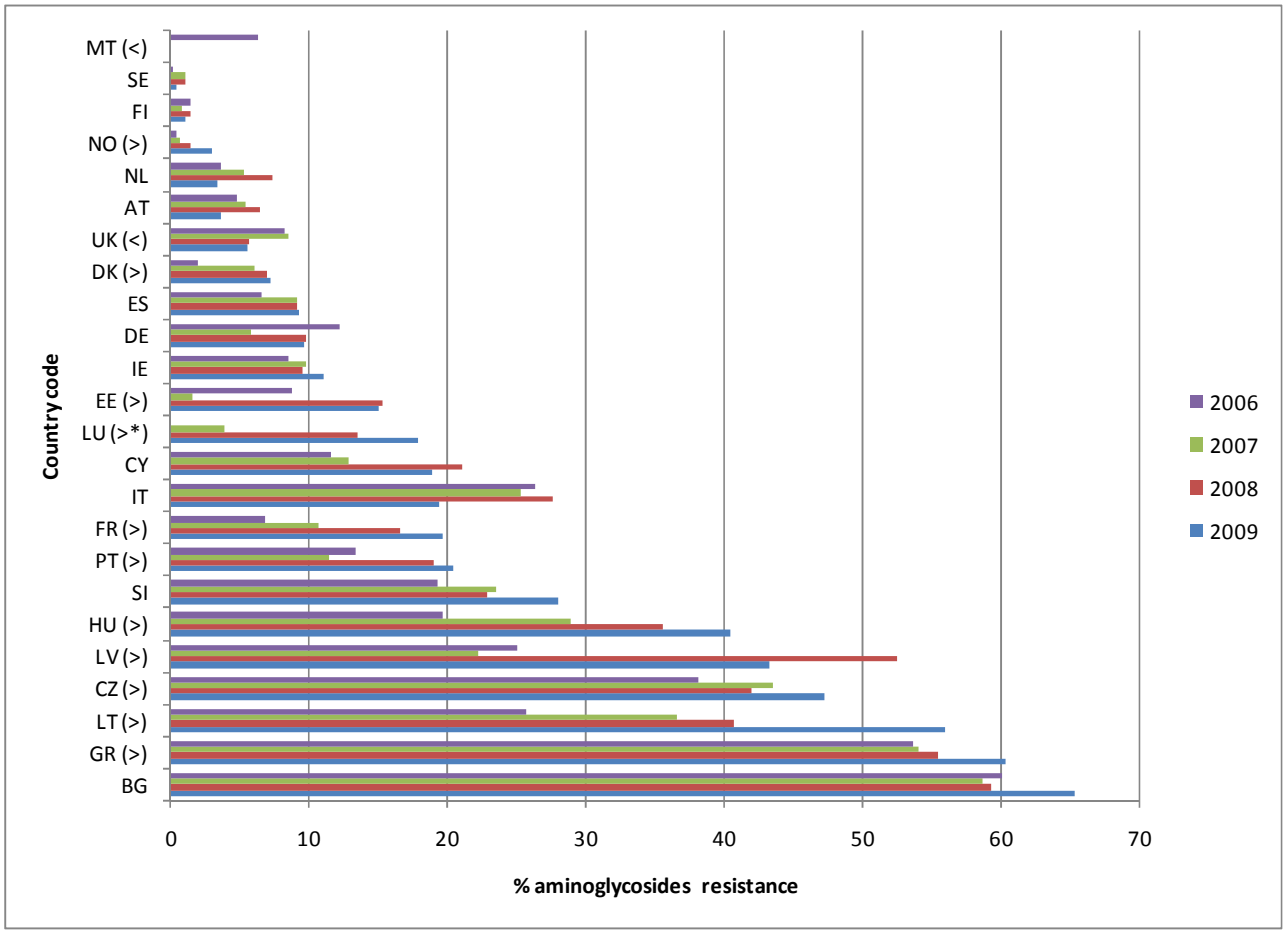

Figure 5.28 *Klebsiella pneumoniae*: trend of aminoglycosides resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

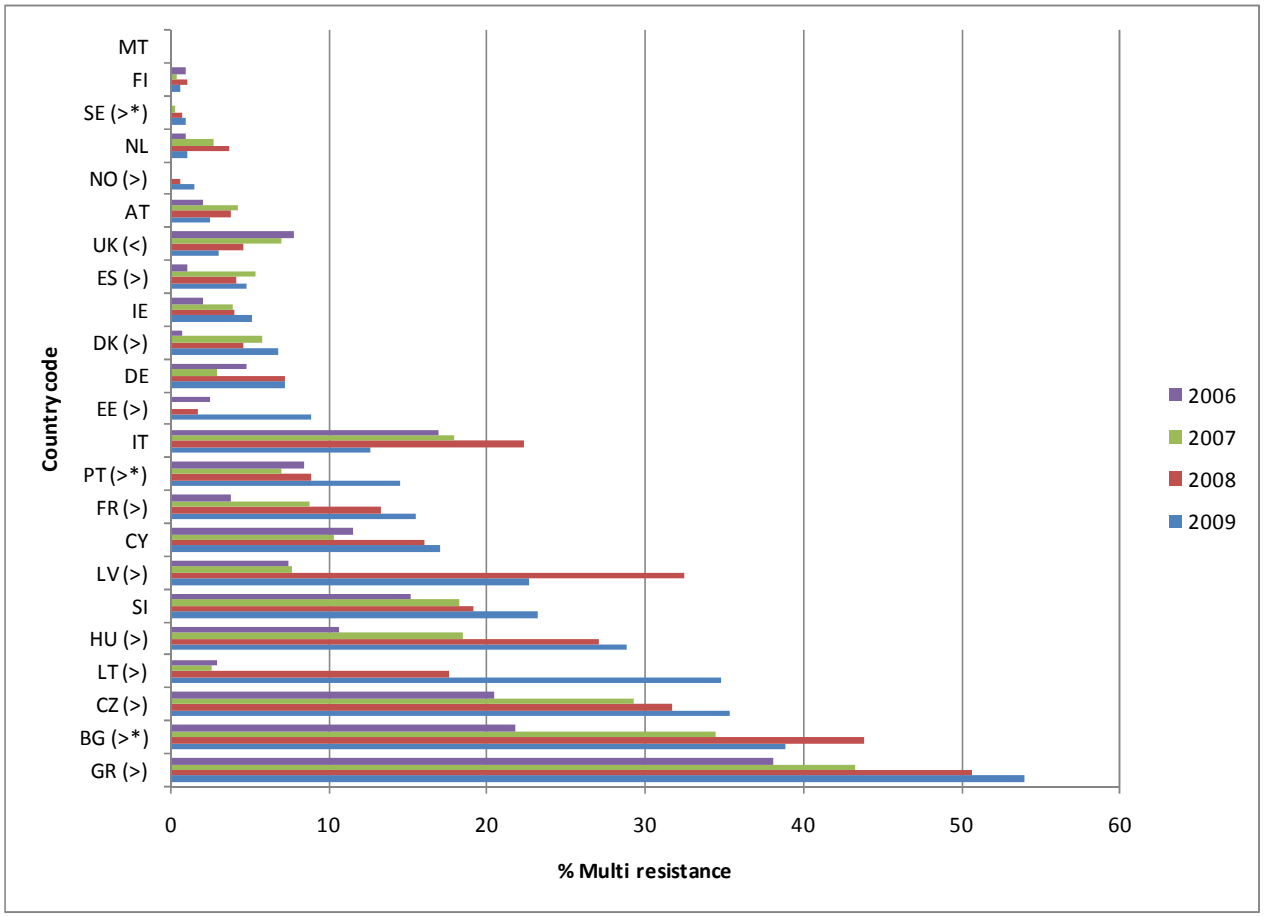

Figure 5.29 *Klebsiella pneumoniae*: trend of multi resistance (third generation cephalosporins, fluoroquinolones and aminoglycosides) by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

Table 5.8 The number of invasive *K. pneumoniae* isolates and the proportion of fluoroquinolones, third generation cephalosporins, aminoglycosides and multi-resistance (%R) including 95% confidence intervals (95CI)reported per country in 2009.

	Fluoroquinolones		Third gen. cephalosporins		Aminoglycosides		Multi-resistance*	
Country	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)
AT	604	8.4 (6-11)	615	7.6 (6-10)	618	3.6 (2-5)	599	2.5 (1-4)
BE	142	13.4 (8-20)	142	14.8 (9-22)	142	9.9 (5-16)	142	2.8 (1-7)
BG	95	48.4 (38-59)	95	69.5 (59-79)	95	65.3 (55-75)	95	38.9 (29-49)
CY	53	43.4 (30-58)	53	41.5 (28-56)	53	18.9 (9-32)	53	17.0 (8-30)
CZ	1415	54.3 (52-57)	1415	52.1 (49-55)	1394	47.2 (45-50)	1394	35.3 (33-38)
DE	479	14.8 (12-18)	471	13.2 (10-17)	478	9.6 (7-13)	470	7.2 (5-10)
DK	791	16.2 (14-19)	623	11.2 (9-14)	822	7.2 (6-9)	600	6.8 (5-9)
EE	58	19.0 (10-31)	58	17.2 (9-29)	60	15.0 (7-27)	56	8.9 (3-20)
ES	627	15.8 (13-19)	628	11.1 (9-14)	628	9.2 (7-12)	627	4.8 (3-7)
FI	375	3.2 (2-6)	367	1.4 (0-3)	349	1.1 (0-3)	341	0.6 (0-2)
FR	1352	24.0 (22-26)	1378	18.7 (17-21)	1378	19.6 (18-22)	1352	15.5 (14-17)
GR	1626	65.6 (63-68)	1634	68.7 (66-71)	1644	60.3 (58-63)	1625	54.0 (52-56)
HU	355	33.2 (28-38)	359	38.4 (33-44)	361	40.4 (35-46)	354	28.8 (24-34)
IE	315	10.8 (8-15)	314	11.1 (8-15)	316	11.1 (8-15)	313	5.1 (3-8)
IS	26	0.0 (0-13)	27	0.0 (0-13)	27	0.0 (0-13)	26	0.0 (0-13)
IT	299	20.4 (16-25)	272	37.1 (31-43)	309	19.4 (15-24)	254	12.6 (9-17)
LT	67	37.3 (26-50)	67	56.7 (44-69)	68	55.9 (43-68)	66	34.8 (24-48)
LU	28	21.4 (8-41)	28	25.0 (11-45)	28	17.9 (6-37)	28	14.3 (4-33)
LV	44	34.1 (20-50)	44	54.5 (39-70)	44	43.2 (28-59)	44	22.7 (11-38)
MT	38	2.6 (0-14)	38	0.0 (0-9)	38	0.0 (0-9)	38	0.0 (0-9)
NL	393	4.1 (2-7)	400	5.5 (3-8)	397	3.3 (2-6)	380	1.1 (0-3)
NO	392	5.6 (4-8)	396	2.8 (1-5)	394	3.0 (2-5)	391	1.5 (1-3)
PL	139	32.4 (25-41)	143	49.0 (41-57)	150	29.3 (22-37)	133	15.0 (9-22)
PT	537	27.6 (24-32)	556	27.5 (24-31)	564	20.4 (17-24)	532	14.5 (12-18)
RO	18	11.1 (1-35)	18	55.6 (31-78)	26	30.8 (14-52)	18	11.1 (1-35)
SE	248	1.6 (0-4)	701	1.7 (1-3)	676	0.4 (0-1)	235	0.9 (0-3)
SI	168	28.6 (22-36)	168	33.9 (27-42)	168	28.0 (21-35)	168	23.2 (17-30)
UK	660	5.8 (4-8)	655	7.2 (5-9)	695	5.5 (4-7)	618	3.1 (2-5)

**Multi resistance was defined as being resistant to third generation cephalosporins, fluoroquinolone and aminoglycosides.*

Table 5.9 The number of invasive *K. pneumoniae* isolates resistant to third generation cephalosporins (n. CRKP) and the proportion of ESBL positive (%ESBL) among these isolates, as ascertained by the participating laboratories in 2009.

Country (n. laboratories)	n. CRKP	%ESBL
AT (16)	40	87.5
BG (10)	66	98.5
CY (4)	22	86.4
CZ (44)	737	79.9
EE (4)	10	100
ES (20)	70	85.7
FR (27)	89	80.9
IE (12)	32	78.1
IT (7)	47	91.5
LT (9)	38	97.4
LV (5)	17	94.1
NL (4)	11	72.7
PL (12)	70	92.9
PT (11)	92	90.2
RO (3)	10	100
SI (7)	57	96.5

Only data from laboratories consistently reporting the ESBL test results for all isolates identified as resistant to third generation cephalosporins and from countries with at least 10 of such isolates were selected for the analysis.

Table 5.10 Overall resistance and resistance combinations among invasive *Klebsiella pneumoniae* isolates tested against fluoroquinolones, third generation cephalosporins and aminoglycosides (n= 10952) in Europe, 2009.

Resistance pattern	Number	% of total
fully susceptible	7108	64.9
Single resistance (to indicated drug classes)		
fluoroq	409	3.7
3rd gen ceph	241	2.2
aminogl	135	1.2
Resistance to two classes of antimicrobial drugs		
3rd gen ceph+fluoroq	477	4.4
3rd gen ceph+aminogl	320	2.9
fluoroq+aminogl	150	1.4
Resistance to three classes of antimicrobial drugs		
3rd gen ceph+fluoroq+aminogl	2112	19.3

Pseudomonas aeruginosa

Clinical and epidemiological importance

Pseudomonas aeruginosa is a non-fermenting gram-negative bacterium that is ubiquitously present in aquatic environments in nature. It is an opportunistic pathogen for plants, animals and humans, and is a major and dreaded cause of infection among hospitalized patients with localized or systemic impairment of immune defenses, being a common cause of hospital-acquired pneumonia (including ventilator-associated pneumonia), bloodstream and urinary tract infections. Because of its ubiquitous presence, its enormous versatility and intrinsic tolerance to many detergents, disinfectants and antimicrobial compounds, it is difficult to control *P. aeruginosa* in hospitals and institutional environments. Moreover, *P. aeruginosa* is a frequent cause for skin infections such as folliculitis and otitis externa in recreational and competitive swimmers. In patients with cystic fibrosis, *P. aeruginosa* causes the most important bacterial complication leading to chronic colonization and intermittent exacerbations ranging from bronchiolitis to acute respiratory distress syndrome. Finally, *P. aeruginosa* is a common pathogen found in burns units and in these locations it is almost impossible to eradicate colonizing strains by classical infection control procedures.

Resistance mechanism

P. aeruginosa is intrinsically resistant to the majority of antimicrobial compounds due to its selective ability to exclude various molecules from penetrating its outer membrane. The antibiotic classes that remain active include the fluoroquinolones, the aminoglycosides, some beta-lactams (piperacillin, ceftazidime, carbapenems) and colistin. Acquired resistance in *P. aeruginosa* is caused by one or more of several mechanisms: i) mutational modification of antibiotic targets, such as gyrase, topoisomerase or ribosomal proteins, which confer resistance to fluoroquinolones or aminoglycosides, respectively; ii) mutational derepression of the chromosomally coded AmpC beta-lactamase; iii) mutational loss of outer membrane proteins preventing the uptake of antimicrobial substances such as carbapenems; iv) mutational upregulation of efflux systems, that can confer resistance to beta-lactams, fluoroquinolones, and aminoglycosides; and v) acquisition of plasmid-mediated resistance genes coding for various beta-lactamases and aminoglycoside modifying enzymes that can confer resistance to various beta-lactams including carbapenems (e. g. metallo-beta-lactamases) and aminoglycosides.

Results

Piperacillin±tazobactam

- Twenty-eight countries reported 8028 isolates of which 1306 were resistant to piperacillin±tazobactam.
- Proportion of resistance was: between 1-5% in six countries, between 5-10% in four countries, between 10-25% in twelve countries and between 25-50% in six countries (Fig 5.30, Tab 5.11).
- Trends in the 2006-2009 period have been calculated for 21 countries. A significant increase has been observed for France and Hungary that, in 2009, reported a proportion of resistance to piperacillin±tazobactam of 21% and 19%, respectively. A significant decreasing trend has been observed for Greece (proportion of resistance in 2009 was 33%) (Fig 5.35).

Ceftazidime

- Twenty-eight countries reported 7937 isolates of which 1171 were resistant to ceftazidime; one country (Romania) reported less than 10 isolates and, therefore, it was not included in the map.
- Proportion of resistance was: between 1-5% in four countries, between 5-10% in eight countries, between 10-25% in twelve countries and between 25-50% in three countries (Fig 5.31, Tab 5.11).
- Trends in the 2006-2009 period have been calculated for 21 countries. A significant increase has been observed for France and Hungary that, in 2009, reported a proportion of resistance to ceftazidime of 17% and 12%, respectively. A significant decreasing trend has been observed for Portugal (proportion of resistance in 2009 was 13%) but the trend significance disappeared when considering only data from laboratories consistently reporting all four years (Fig 5.36).

Fluoroquinolones

- Twenty-eight countries reported 8253 isolates of which 1884 were resistant to fluoroquinolones.
- Proportion of resistance was: between 1-5% in one country, between 5-10% in five countries, between 10-25% in thirteen countries and between 25-50% in nine countries (Fig 5.32, Tab 5.11).

- Trends in the 2006-2009 period have been calculated for 20 countries. A significant increase has been observed for Hungary (proportion of resistance in 2009 was 27%). A significant decreasing trend has been observed for Germany, Ireland and Norway that, in 2009, reported a proportion of resistance to fluoroquinolones of 17%, 9% and 3%, respectively. In two of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.37).

Aminoglycosides

- Twenty-eight countries reported 8223 isolates of which 1437 were resistant to aminoglycosides.
- Proportion of resistance was: below 1% in four countries, between 1-5% in four countries, between 5-10% in six countries, between 10-25% in seven countries and between 25-50% in seven countries (Fig 5.33, Tab 5.11).
- Trends in the 2006-2009 period have been calculated for 21 countries. Three countries (Hungary, Malta and Spain) reported a significant increasing trend (Fig 5.37) with proportions of resistance to aminoglycosides which, in 2009, were 29%, 21% and 19%, respectively.
- Significant decreasing trends have been observed for five countries. In three of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.38). In 2009, these five countries reported proportion of resistance to aminoglycosides: between 1-5% in one case (United Kingdom), between 5-10% in one case (Germany), between 10-25% in one case (Portugal) and between 25-50% in two cases (Greece and Poland).

Carbapenems

- Twenty-eight countries reported 8129 isolates of which 1541 were resistant to carbapenems.
- Proportion of resistance was: below 1% in one country, between 1-5% in two countries, between 5-10% in nine countries, between 10-25% in ten countries, between 25-50% in five countries and above 50% in one country (Fig 5.34, Tab 5.11).
- Trends in the 2006-2009 period have been calculated for 20 countries. Three countries (France, Hungary and Italy) reported a significant increasing trend with proportions of resistance to carbapenems which, in 2009, were 17%, 27% and 31%, respectively. In two of

these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.39).

- Significant decreasing trends have been observed for Austria, Czech Republic and Germany with proportions of resistance to carbapenems which, in 2009, were 9%, 29% and 11%, respectively. In one of these countries the trend is significant also when considering only data from laboratories consistently reporting all four years (Fig 5.39).

Combined resistance (piperacillin±tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems)

- Twenty-eight countries reported 8376 isolates tested for at least three antibiotic classes among piperacillin±tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems. In 2009, 33% of isolates were resistant to one or more of the five considered antibiotic classes while 16% were resistant to 3 or more antibiotic classes. The most frequent pattern was the resistance to all five antibiotic classes (5%) (Tab 5.12).
- Proportion of multi resistance (R to at least three of the five considered antibiotic classes) was: below 1% in one country, between 1-5% in seven countries, between 5-10% in four countries, between 10-25% in eleven countries and between 25-50% in five countries (Tab 5.11).
- Trends in the 2006-2009 period have been calculated for 21 countries. Significant increasing trends of multi resistance have been observed for four countries. In three of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.40). In 2009, these four countries (France, Hungary, Malta and Spain) reported proportion of multi resistance between 10-25%.
- Significant decreasing trends of multi resistance have been observed for four countries. In two of these countries the trends are significant also when considering only data from laboratories consistently reporting all four years (Fig 5.40). In 2009, these four countries reported proportion of multi resistance: between 1-5% in one case (Ireland), between 5-10% in one case (Germany), between 10-25% in one case (Portugal) and between 25-50% in one case (Greece).

Conclusions

High proportions of resistance of *P. aeruginosa* to antimicrobials have been reported by many countries especially in Southern and Eastern Europe. The combined resistance is also frequent with 16% of isolates resistant to at least 3 antibiotic classes (multi resistance) and with 5% of isolates resistant to all five antibiotic classes under surveillance. Despite the high proportions of resistance, the situation appears to be generally stable in Europe with few countries reporting significant increasing or decreasing trends of resistance. The countries with increasing trends of resistance to specific antibiotic classes or of multi resistance are: France, Hungary, Italy, Malta and Spain. In 2009, Greece reported the highest proportion of multi resistance (40%) although a significant decreasing trend was observed from 2006 to 2009.

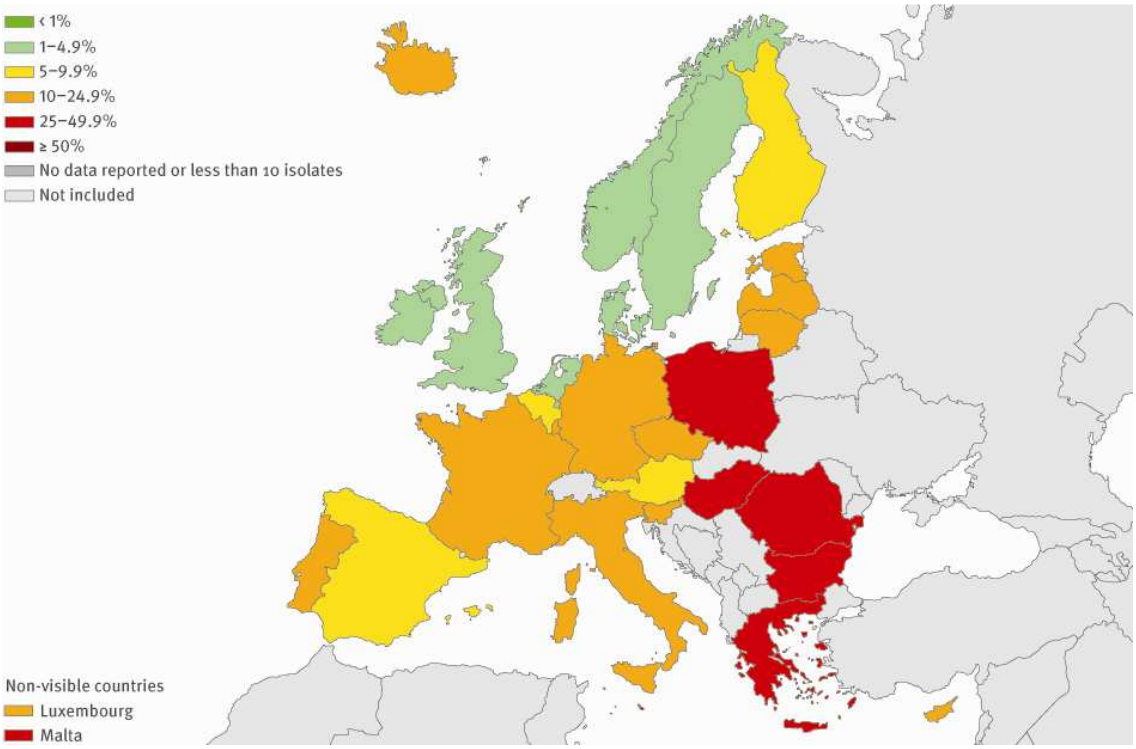

Figure 5.30 *Pseudomonas aeruginosa*: proportion of invasive isolates resistant to piperacillin±tazobactam in 2009. Only countries reporting 10 or more isolates are included

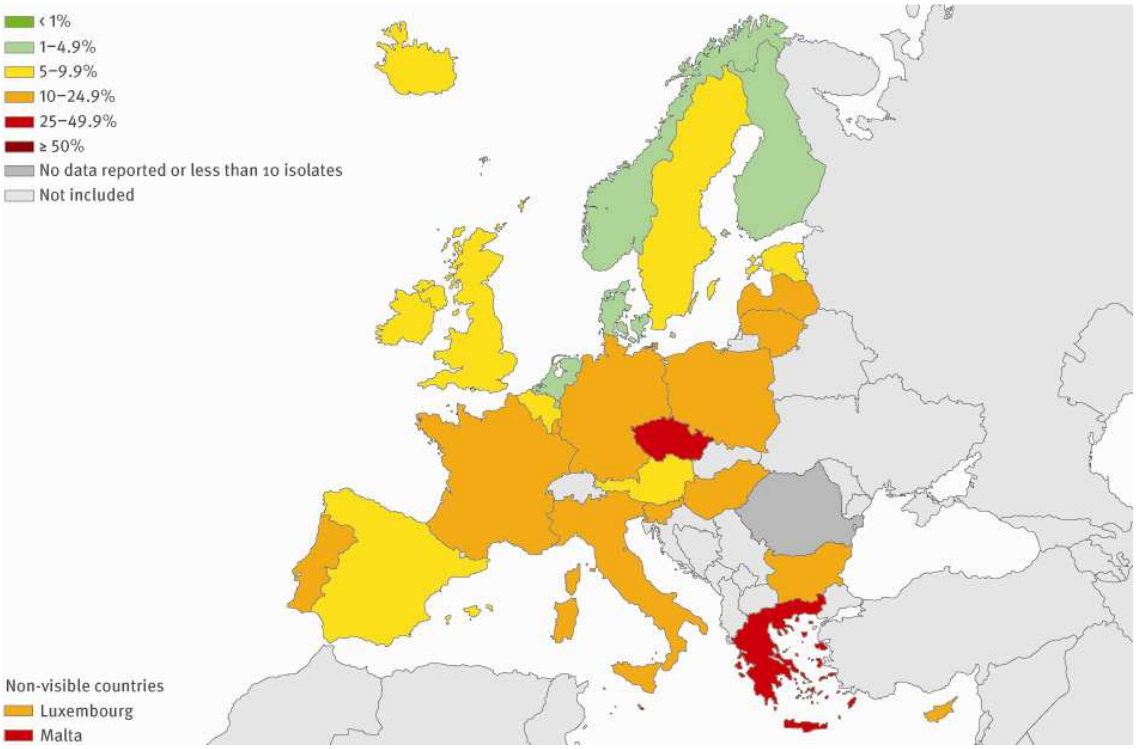

Figure 5.31 *Pseudomonas aeruginosa*: proportion of invasive isolates resistant to ceftazidime in 2009. Only countries reporting 10 or more isolates are included

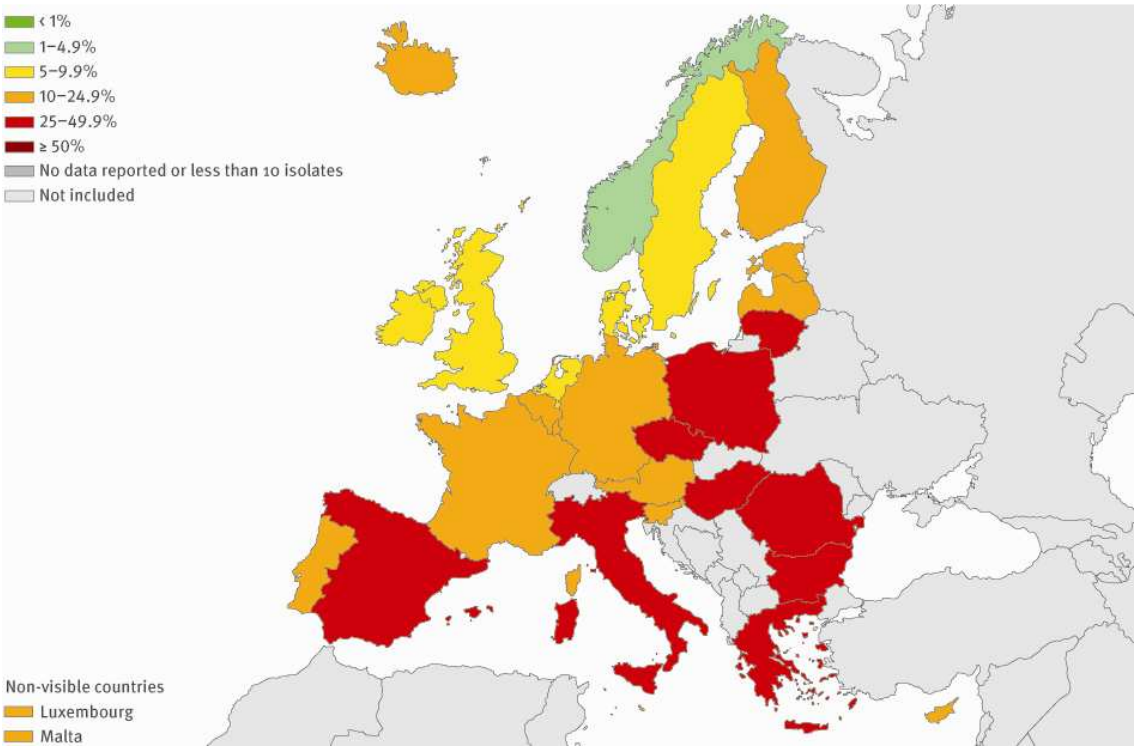

Figure 5.32 *Pseudomonas aeruginosa*: proportion of invasive isolates resistant to fluoroquinolones in 2009. Only countries reporting 10 or more isolates are included

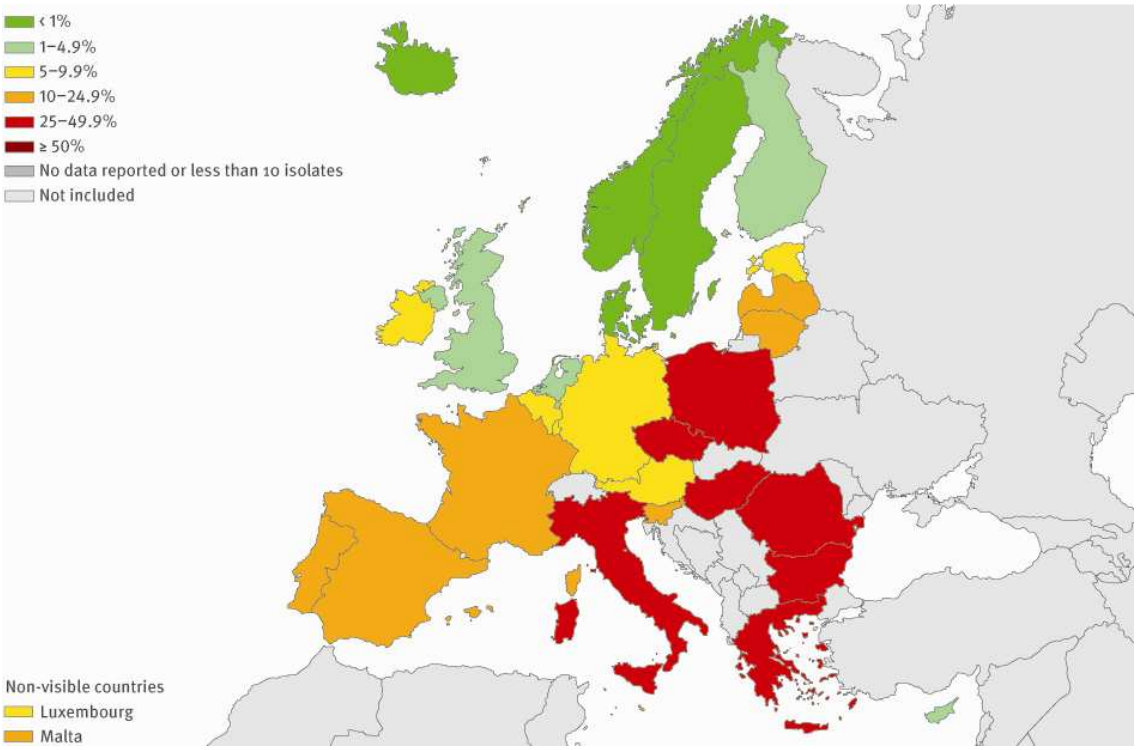

Figure 5.33 *Pseudomonas aeruginosa*: proportion of invasive isolates resistant to aminoglycosides in 2009. Only countries reporting 10 or more isolates are included

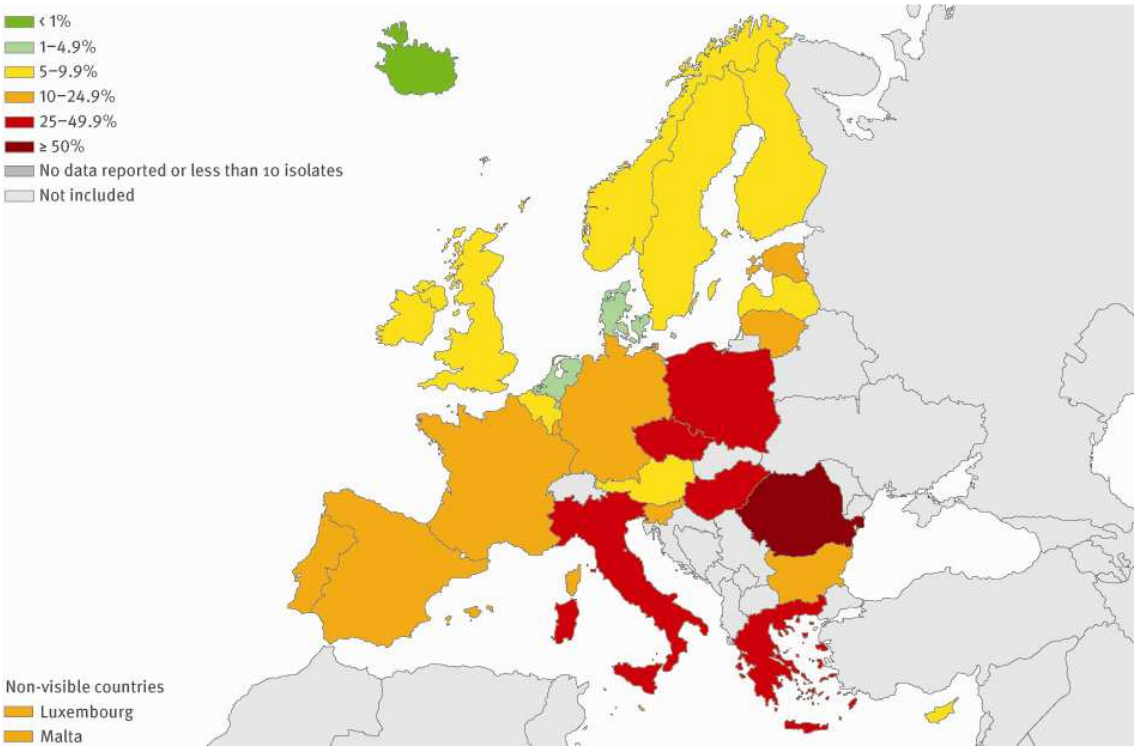

Figure 5.34 *Pseudomonas aeruginosa*: proportion of invasive isolates resistant to carbapenems in 2009. Only countries reporting 10 or more isolates are included

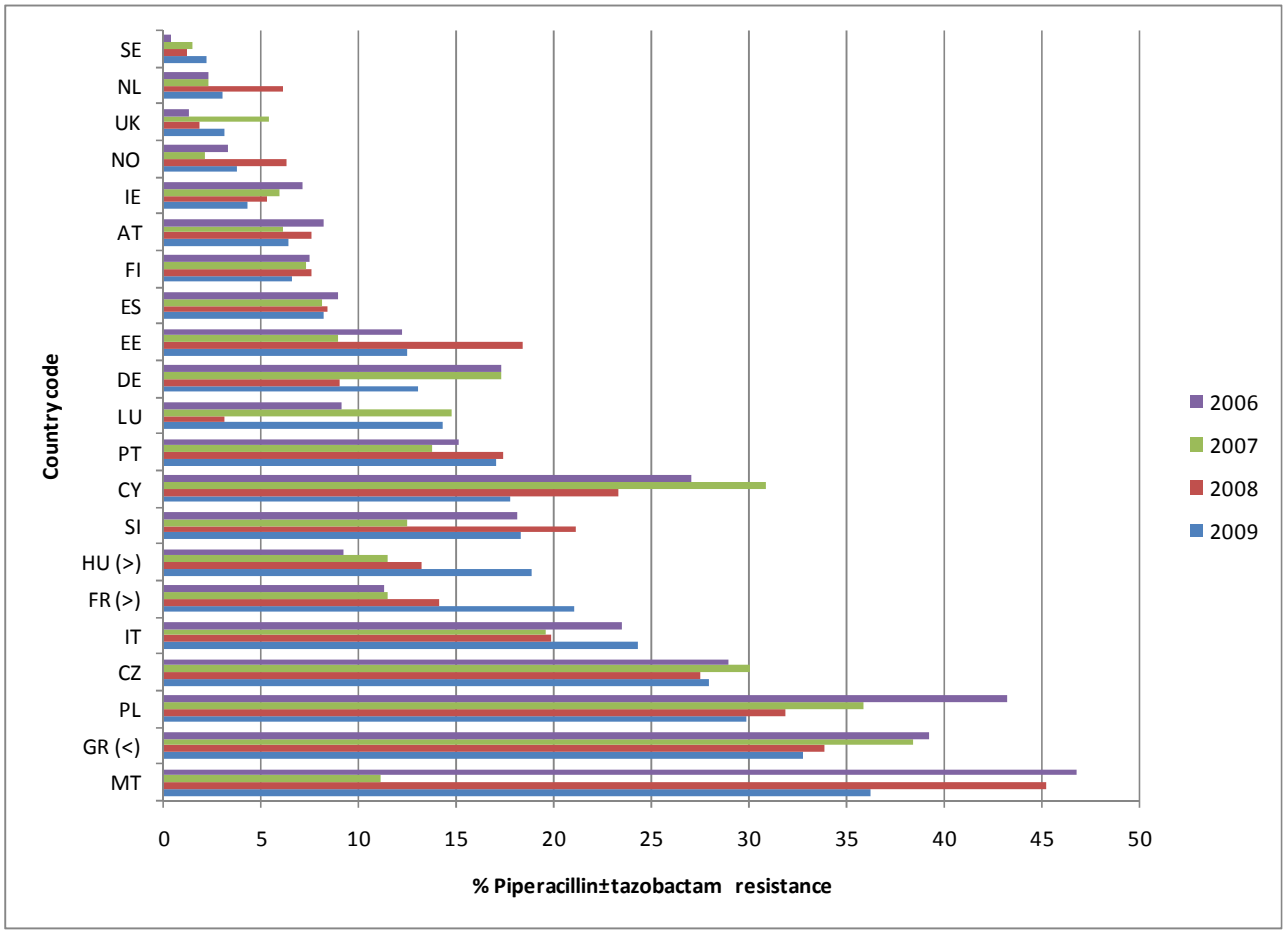

Figure 5.35 *Pseudomonas aeruginosa*: trend of piperacillin±tazobactam resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

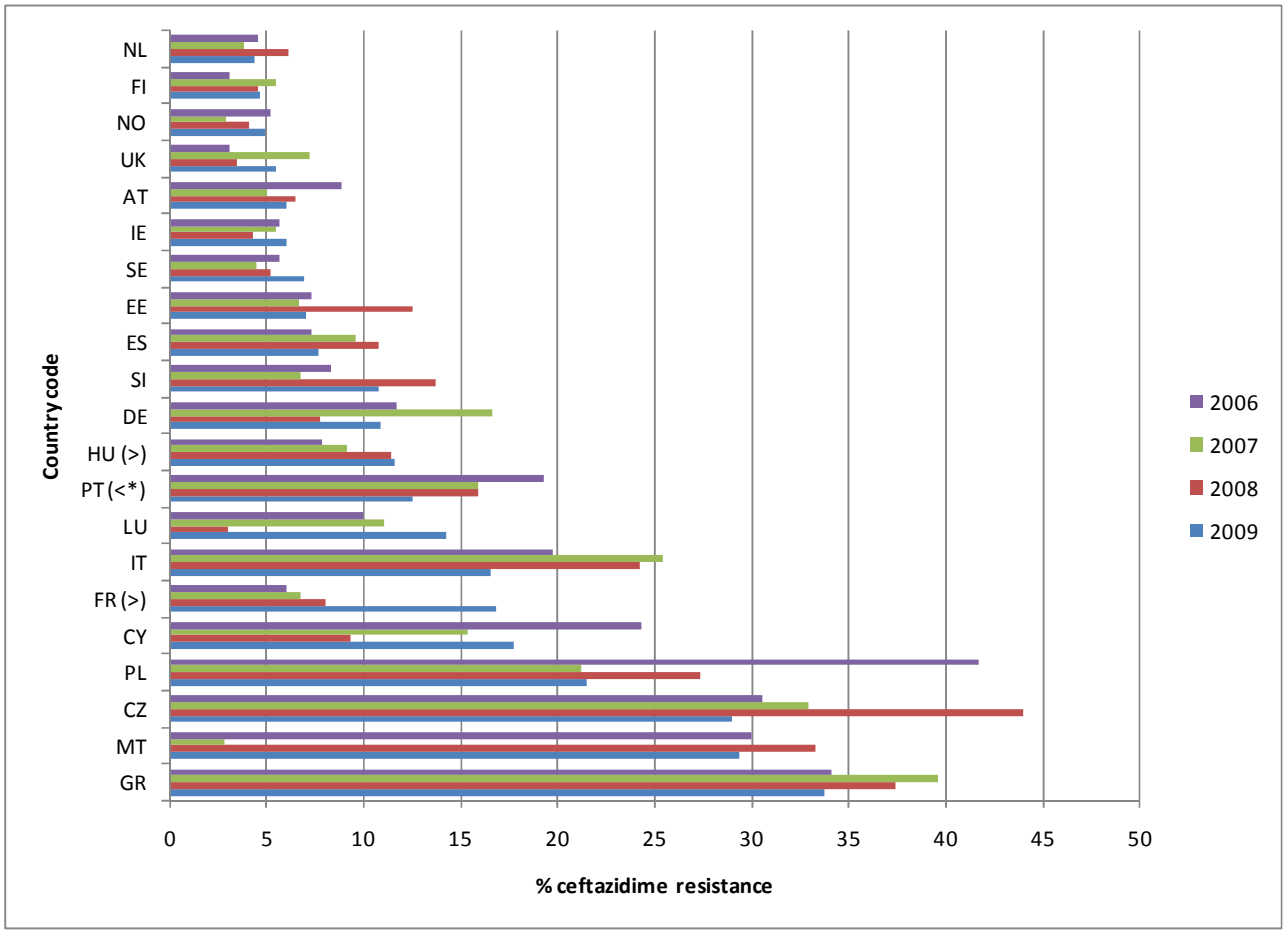

Figure 5.36 *Pseudomonas aeruginosa*: trend of ceftazidime resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

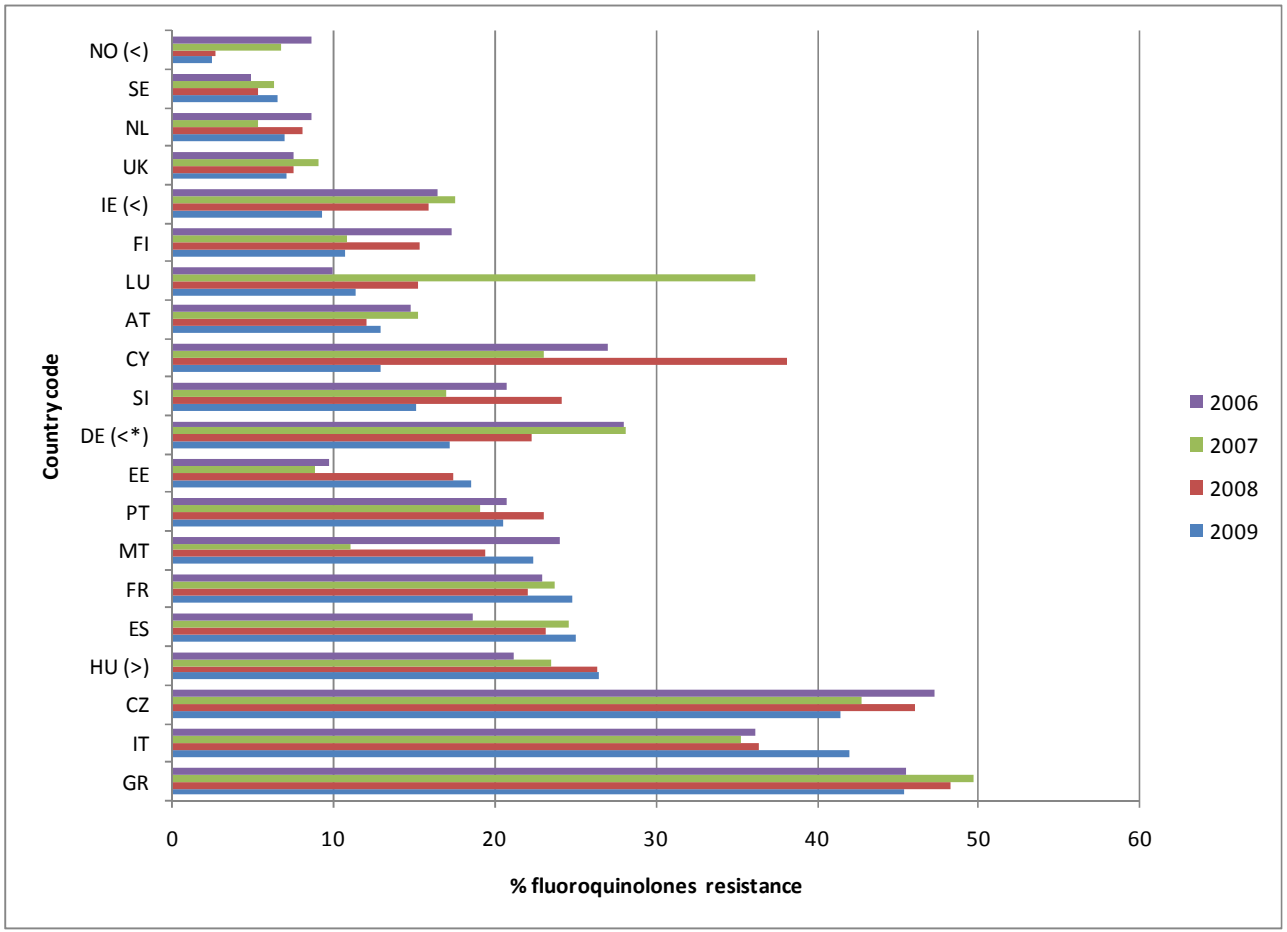

Figure 5.37 *Pseudomonas aeruginosa*: trend of fluoroquinolones resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

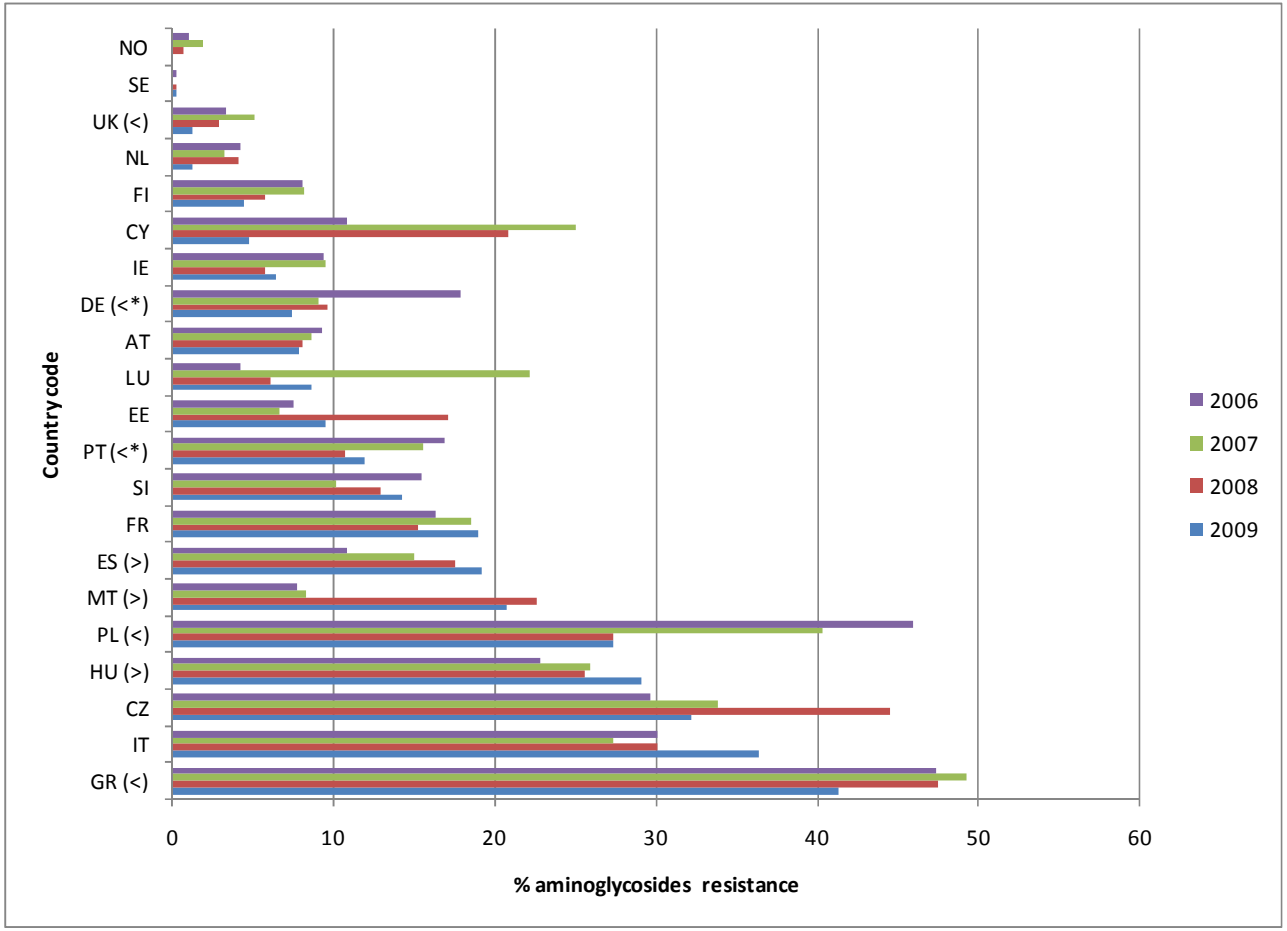

Figure 5.38 *Pseudomonas aeruginosa*: trend of aminoglycosides resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

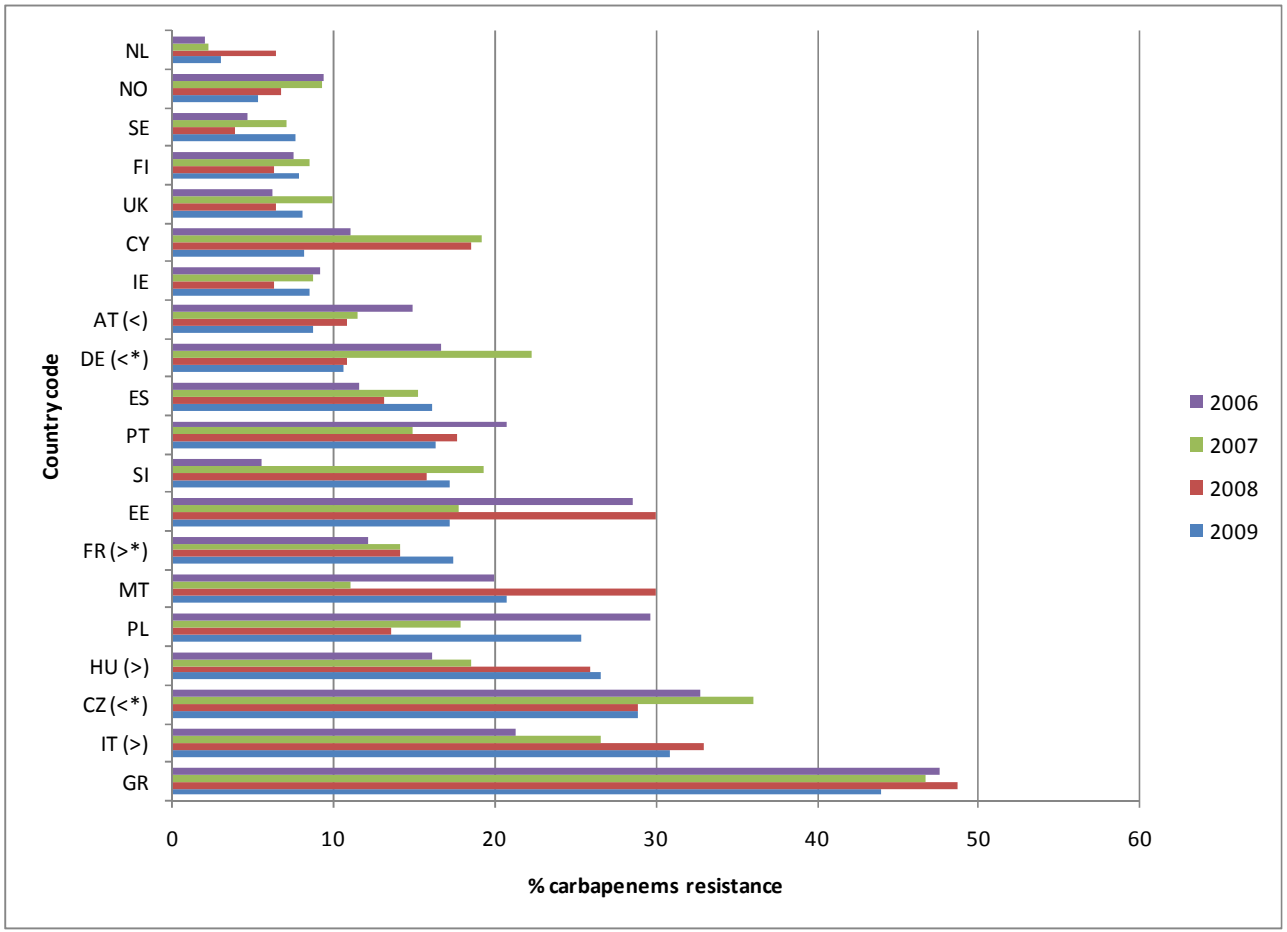

Figure 5.39 *Pseudomonas aeruginosa*: trend of carbapenems resistance by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

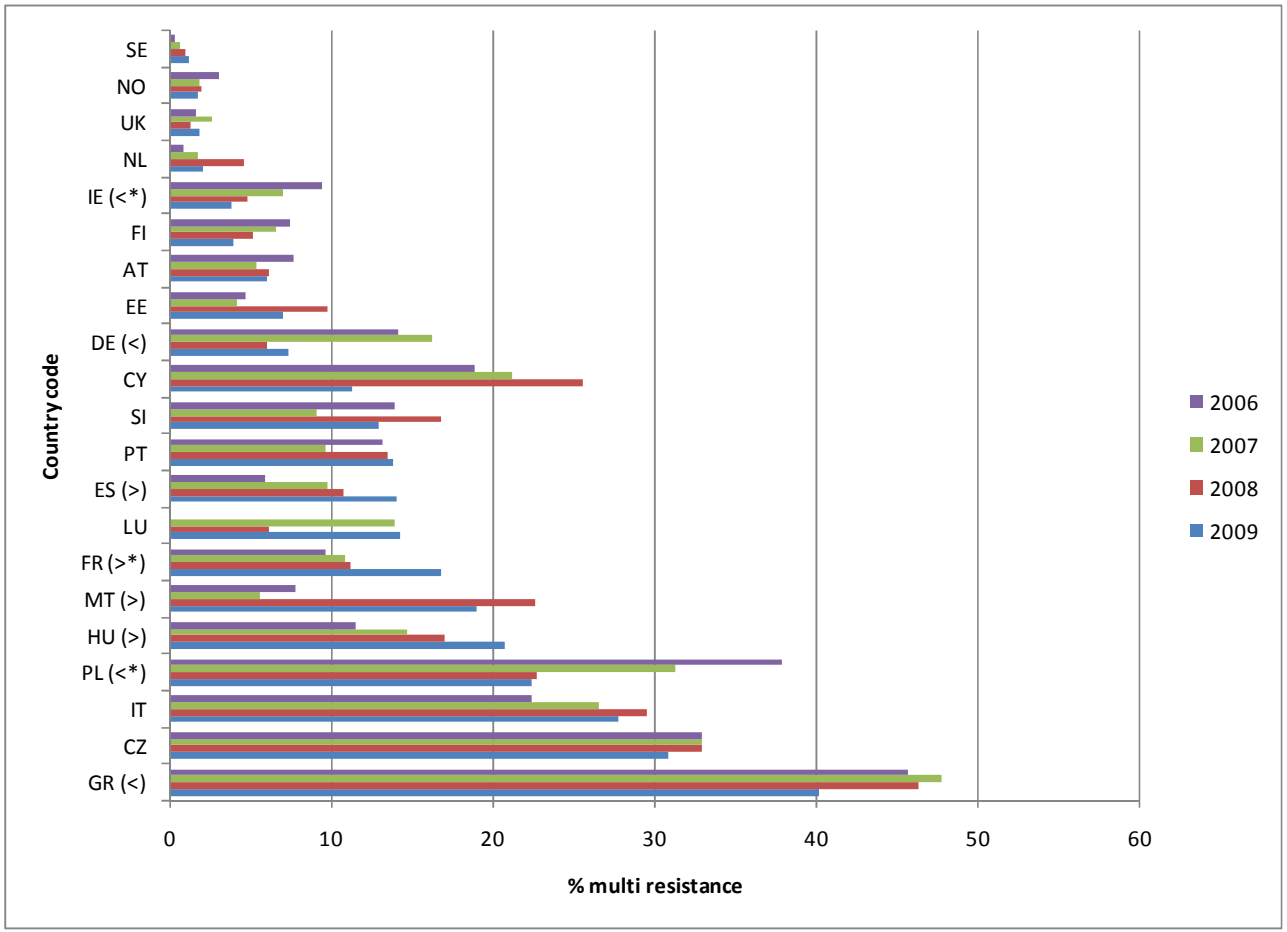

Figure 5.40 *Pseudomonas aeruginosa*: trend of multi resistance (R to three or more antibiotic classes among piperacillin±tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems) by country, 2006-2009. Only the countries that reported 20 isolates or more per year were included. The symbols **>** and **<** indicate significant increasing and decreasing trend, respectively. The asterisks indicate significant trends in the overall data that were not supported by data from laboratories consistently reporting for all four years.

Table 5.11 The number of invasive *P. aeruginosa* isolates and the proportion of piperacillin±tazobactam, fluoroquinolones, ceftazidime, aminoglycosides, carbapenems and multi-resistance (%R) including 95% confidence intervals (95CI)reported per country in 2009.

	Piperacillin±tazobactam		Fluoroquinolones		Ceftazidime		Aminoglycosides		Carbapenems		Multi resistance*	
Country	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)	N	%R (95CI)
AT	512	6.4 (4-9)	512	12.9 (10-16)	483	6.0 (4-9)	509	7.9 (6-11)	513	8.8 (6-12)	516	6.0 (4-8)
BE	104	6.7 (3-13)	134	15.7 (10-23)	134	6.0 (3-11)	105	9.5 (5-17)	134	9.0 (5-15)	134	5.2 (2-10)
BG	36	33.3 (19-51)	36	33.3 (19-51)	35	22.9 (10-40)	33	33.3 (18-52)	34	23.5 (11-41)	36	27.8 (14-45)
CY	62	17.7 (9-30)	62	12.9 (6-24)	62	17.7 (9-30)	62	4.8 (1-13)	61	8.2 (3-18)	62	11.3 (5-22)
CZ	574	27.9 (24-32)	575	41.4 (37-46)	575	29.0 (25-33)	575	32.2 (28-36)	575	28.9 (25-33)	575	30.8 (27-35)
DE	284	13.0 (9-18)	285	17.2 (13-22)	284	10.9 (8-15)	284	7.4 (5-11)	283	10.6 (7-15)	285	7.4 (5-11)
DK	373	1.9 (1-4)	411	5.4 (3-8)	311	3.5 (2-6)	428	0.7 (0-2)	347	2.9 (1-5)	415	1.7 (1-3)
EE	40	12.5 (4-27)	43	18.6 (8-33)	43	7.0 (1-19)	42	9.5 (3-23)	29	17.2 (6-36)	43	7.0 (1-19)
ES	539	8.2 (6-11)	544	25.0 (21-29)	544	7.7 (6-10)	543	19.2 (16-23)	540	16.1 (13-19)	544	14.0 (11-17)
FI	229	6.6 (4-11)	233	10.7 (7-15)	233	4.7 (2-8)	224	4.5 (2-8)	229	7.9 (5-12)	233	3.9 (2-7)
FR	1156	21.0 (19-23)	1204	24.8 (22-27)	1085	16.8 (15-19)	1159	19.0 (17-21)	1219	17.4 (15-20)	1220	16.8 (15-19)
GR	1084	32.7 (30-36)	1080	45.4 (42-48)	1074	33.7 (31-37)	1090	41.3 (38-44)	1095	44.0 (41-47)	1077	40.2 (37-43)
HU	505	18.8 (15-22)	513	26.5 (23-31)	516	11.6 (9-15)	516	29.1 (25-33)	516	26.6 (23-31)	516	20.7 (17-24)
IE	235	4.3 (2-8)	236	9.3 (6-14)	232	6.0 (3-10)	235	6.4 (4-10)	224	8.5 (5-13)	236	3.8 (2-7)
IS	16	12.5 (2-38)	16	12.5 (2-38)	16	6.3 (0-30)	16	0.0 (0-21)	16	0.0 (0-21)	16	0.0 (0-21)
IT	185	24.3 (18-31)	193	42.0 (35-49)	164	16.5 (11-23)	151	36.4 (29-45)	188	30.9 (24-38)	195	27.7 (22-35)
LT	20	20.0 (6-44)	21	33.3 (15-57)	21	14.3 (3-36)	21	19.0 (5-42)	21	19.0 (5-42)	21	19.0 (5-42)
LU	35	14.3 (5-30)	35	11.4 (3-27)	35	14.3 (5-30)	35	8.6 (2-23)	33	15.2 (5-32)	35	14.3 (5-30)
LV	18	16.7 (4-41)	17	11.8 (1-36)	18	16.7 (4-41)	18	22.2 (6-48)	14	7.1 (0-34)	18	11.1 (1-35)
MT	58	36.2 (24-50)	58	22.4 (13-35)	58	29.3 (18-43)	58	20.7 (11-33)	58	20.7 (11-33)	58	19.0 (10-31)
NL	233	3.0 (1-6)	230	7.0 (4-11)	228	4.4 (2-8)	230	1.3 (0-4)	234	3.0 (1-6)	234	2.1 (1-5)
NO	161	3.7 (1-8)	162	2.5 (1-6)	164	4.9 (2-9)	165	0.0 (0-2)	166	5.4 (3-10)	165	1.8 (0-5)
PL	151	29.8 (23-38)	149	25.5 (19-33)	121	21.5 (15-30)	143	27.3 (20-35)	142	25.4 (18-33)	152	22.4 (16-30)
PT	535	17.0 (14-20)	517	20.5 (17-24)	536	12.5 (10-16)	536	11.9 (9-15)	520	16.3 (13-20)	536	13.8 (11-17)
RO	11	36.4 (11-69)	11	36.4 (11-69)	8	37.5 (9-76)	11	45.5 (17-77)	11	54.5 (23-83)	11	36.4 (11-69)
SE	224	2.2 (1-5)	259	6.6 (4-10)	317	6.9 (4-10)	321	0.3 (0-2)	326	7.7 (5-11)	330	1.2 (0-3)
SI	93	18.3 (11-28)	93	15.1 (8-24)	93	10.8 (5-19)	91	14.3 (8-23)	93	17.2 (10-26)	93	12.9 (7-21)
UK	555	3.1 (2-5)	624	7.1 (5-9)	547	5.5 (4-8)	622	1.3 (1-3)	508	8.1 (6-11)	620	1.9 (1-3)

**Multi resistance was defined as being resistant to three or more antibiotic classes among piperacillin±tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems.*

Table 5.12 Overall resistance and resistance combinations among invasive *Pseudomonas aeruginosa* isolates tested against at least three antibiotic classes among piperacillin±tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems (n= 8376) in Europe, 2009.

Resistance pattern	Number	% of total
fully susceptible (to the tested drugs)	5588	66.7
Resistance to one class of antimicrobial drugs		
Fluoroq	350	4.2
Carbap	309	3.7
Aminogl	101	1.2
Ceftaz	83	1
pip(±taz)	72	0.9
Resistance to two classes of antimicrobial drugs		
fluoroq+aminogl	185	2.2
pip(±taz)+ceftaz	147	1.8
fluoroq+carbap	93	1.1
pip(±taz)+fluoroq	27	0.3
pip(±taz)+carbap	26	0.3
pip(±taz)+aminogl	21	0.3
aminogl+carbap	16	0.2
ceftaz+carbap	16	0.2
fluoroq+ceftaz	11	0.1
ceftaz+aminogl	5	0.1
Resistance to three classes of antimicrobial drugs		
fluoroq+aminogl+carbap	165	2
pip(±taz)+fluoroq+aminogl	83	1
pip(±taz)+ceftaz+carbap	62	0.7
pip(±taz)+fluoroq+ceftaz	39	0.5
fluoroq+ceftaz+aminogl	36	0.4
pip(±taz)+fluoroq+carbap	19	0.2
fluoroq+ceftaz+carbap	16	0.2
pip(±taz)+aminogl+carbap	12	0.1
pip(±taz)+ceftaz+aminogl	9	0.1
ceftaz+aminogl+carbap	9	0.1
Resistance to four classes of antimicrobial drugs		
pip(±taz)+fluoroq+aminogl+carbap	139	1.7
fluoroq+ceftaz+aminogl+carbap	100	1.2
pip(±taz)+fluoroq+ceftaz+aminogl	96	1.1
pip(±taz)+fluoroq+ceftaz+carbap	94	1.1
pip(±taz)+ceftaz+aminogl+carbap	18	0.2
Resistance to five classes of antimicrobial drugs		
pip(±taz)+fluoroq+ceftaz+aminogl+carbap	429	5.1

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Annex 1. Technical Notes

1.1. Technical Notes for chapter 4

Number of blood culture sets

The total number of blood culture sets was defined as the number of blood samples, not the number of patients sampled.

Patient-days.

If patient-days were not available at hospital level, these were calculated by:

Number of beds * (*Annual occupancy* / 100) * 365

Type of hospitals

Since hospital categorisation was always intricate, more specific definitions from WHO has been implemented to make the categorisation of hospitals easier.

Primary level, often referred to as a district hospital or first-level referral: A hospital with few specialities, mainly internal medicine, obstetrics-gynecology, pediatrics, and general surgery, or only general practice; limited laboratory services are available for general, but not for specialized pathological analysis; the bed capacity ranges from 30 to 200 beds.

Secondary level, often referred to as provincial hospital: A hospital highly differentiated by function with five to ten clinical specialities; bed capacity ranging from 200-800 beds.

Tertiary level, often referred to as central, regional or tertiary-level hospital: A hospital with highly specialized staff and technical equipment, e.g., cardiology, ICU and specialized imaging units; clinical

services are highly differentiated by function; the hospital may have teaching activities; bed capacity ranges from 300 to 1,500 beds.A fourth category was for hospitals with a single specialty.

Averaged variables

Annual occupancy rate and length of stay were averaged per country. In these totals only laboratory/hospital questionnaires were included that provided information on all variables needed for the specific formula.

1.2. Technical Notes for chapter 5

Resistance trend analysis

Resistance trends were calculated for the last four years. To determine significant trends over time, the Cochrane Armitage test was used, excluding countries reporting less than 20 isolates per year. To exclude possible biases in the trend analyses, a sensitivity analysis was done, per country, to determine the sensitivity of the trend analysis for using the complete dataset, versus a subset from laboratories reporting all four years. In the graphs, trends were indicated in the following way:

i) using <* if decreasing or >* if increasing when significant trends were only identified in the complete dataset,
ii) using < if decreasing or > if increasing when a significant trend was detected in both the subset and the complete dataset.

European maps showing resistance levels

To be included in the maps of Europe displaying the resistance proportions per country, for all drug-bug combinations under surveillance by EARS-Net, a country had to report results for at least 10 isolates.

Annex 2. Country Summary Sheets

Explanation to the country summary sheets

General information about EARS-Net participating laboratories and hospitals

Table 1 gives the number of laboratories and isolates reported by year and by pathogen under EARS-Net surveillance for the period 2002 to 2009.

Antibiotic resistance 2002-2009

Table 2 provides information on the proportion of invasive bacterial isolates non-susceptible (I+R) or resistant (R) to the antibiotics or antibiotic classes mentioned in the EARSS protocols. When interpreting the results in Table 2, always check the number of isolates provided in Table 1.

Demographic characteristics

Table 3 gives the proportional distribution of the isolates reported by source, gender, age, and hospital department, and the proportion of resistance within the different groups, for the period 2008-2009.

The abbreviations used in this table stand for; PNSP = penicillin non-susceptible *S. pneumoniae,* MRSA = meticillin resistant *S. aureus*, FREC = fluoroquinolone resistant *E. coli*, VRE = vancomycin resistant *E. faecalis* or *E. faecium*, CRKP = 3rd generation cephalosporin resistant *K. pneumoniae*, and CRPA = carbapenem resistant *P. aeruginosa*. If the number of isolates in a certain category accounts for less than 0.5% of the total number of isolates, the % total is set at 0 and the % resistance is not shown.

PNSP at laboratory level / MRSA, FREC and CRKP at hospital level

Figures 1, 2, 3 and **4** show the local variation in the proportions of PNSP by laboratory and of MRSA, FREC and CRKP by hospital. These figures are based on data from 2008 and 2009, only including the laboratories and hospitals that reported at least 5 isolates in these 2 years. The total number of laboratories or hospitals, the minimum, maximum, median, 1st and 3rd quartile of the proportion of resistance is displayed in a box in the figures.