European Antimicrobial Resistance Surveillance Network

(EARS-Net Belgium)

Report 2017

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Data up to and including 2016

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility test
BE	Belgium
CLSI	Clinical and Laboratory Standards Institute (USA)
CP	Carbapenemase-production
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control (Stockholm, Sweden)
ESBL	Extended-Spectrum Beta-Lactamase
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing (EU)
HAI	Healthcare-associated infection
I/R	Intermediary resistant / Resistant
KUL	Katholieke Universiteit Leuven
MIC	Minimum inhibitory concentration
MRSA	Meticillin-resistant *Staphylococcus aureus*
NRC	National Reference Center
NSIH	National Surveillance of Infections in Hospitals (Belgium)
PPS	Point prevalence survey of healthcare-associated infections and antimicrobial use
R	Resistant
S/I/R	Susceptible / Intermediary resistant/ Resistant
WIV-ISP	Wetenschappelijk Instituut Volksgezondheid - Institut Scientifique de Santé Publique – Scientific Institute of Public Health

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Executive summary

Background

Antimicrobial resistance is the ability of a microorganism to resist the effects of medication intended to be used against them. Resistance arises in three ways: natural (*intrinsic*) resistance in certain types of bacteria, or acquired via either genetic mutation, or by horizontal gene transfer. The European Antimicrobial Resistance Surveillance Network (EARS-Net) monitors the evolution of *acquired* antimicrobial resistance at the European level. The Scientific Institute of Public Health (WIV-ISP) is the nominated focal contact point for Belgium (BE), and collects data from the clinical laboratories through its national surveillance EARS-Net BE.

Results

In 2016, 31 clinical laboratories voluntarily reported results on Antimicrobial susceptibility tests (AST) in 8 pathogens isolated from blood or cerebrospinal fluid samples. For *Streptococcus pneumonia* isolates, national AST data for 2016 from 97 laboratories was provided by the National Reference Center (NRC) at the Catholic University of Leuven (KUL).

We observed an increase in antimicrobial resistance for *Escherichia coli* to third-generation cephalosporins and to fluoroquinolones, leading to countrywide resistance percentages in 2016 of 10.5% and 24.5%, respectively. An increasing trend could also be observed for antimicrobial resistance of *Klebsiella pneumoniae* to third-generation cephalosporins and to carbapenems, resulting in mean resistance percentages of 22.9% and 2.4%, respectively. Extended-spectrum beta-lactamase (ESBL) was detected in 83.5% of the additionally tested samples that were resistant to third-generation cephalosporins.

Pseudomonas aeruginosa showed resistance to almost all antimicrobial groups. The predominant resistance in *P. aeruginosa* was to fluoroquinolones (14.5%), followed by resistance to aminoglycosides (11.0%), piperacillin-tazobactam (9.8%) and carbapenems (9.6%). The highest resistance levels in *Acinetobacter species* were observed to fluoroquinolones (7.7%), followed by aminoglycosides (5.1%) and carbapenems (2.6%).

Apart from macrolides (15.9% intermediary resistant + resistant strains), non-susceptibility was rare in *Streptococcus pneumoniae:* 0.4% of the samples were non-susceptible to penicillins or to third-generation cephalosporins, and 0.2% was non-susceptible to fluoroquinolones. Over the period 2012-2016, we observed a strong decreasing trend for non-susceptibility to macrolides.

In 2016, 12.2% of all tested *Staphylococcus aureus* samples were non-susceptible to meticillin (MRSA) and 12.7% were resistant to fluoroquinolones. For both antimicrobial

groups, we could observe a decreasing trend between 2012 and 2016. For MRSA, the decreasing trend up till 2015 did not continue in 2016.

No *E. faecalis* isolates were found resistant to vancomycin and 1.7% of *E. faecium* isolates tested resistant to this antibiotic. Resistance to linezolid remained almost non-existent in both Enterococci, with none of the *E. faecalis*, and 0.5% of *E. faecium* isolates testing resistant. High-level gentamicin resistance was common in both *Enterococcus faecalis* (19.8%) and *Enterococcus faecium* (19.7%).

Conclusions

Klebsiella pneumoniae isolates have continuously lost susceptibility for third-generation cephalosporins over the last 5 years, resulting in a mean resistance percentage of 22.9% in 2016. Resistance of these isolates to carbapenems is emerging, with 2.4% resistance in 2016. A majority of third-generation cephalosporins resistant *E. coli* and *K. pneumoniae* isolates produced ESBL. Resistance of *P. aeruginosa* to ceftazidime, carbapenems, aminoglycosides and fluoroquinolones was common in 2016. The decreasing trend between 2012 and 2015 for meticillin non-susceptibility of *S. aureus* (MRSA) stabilized in 2016 around 12.2%. No *E. faecalis* isolates and 1.7% of *E. faecium* isolates tested resistant to vancomycin. Resistance to linezolid remained very low in both Enterococci.

For upcoming data calls, we encourage laboratories to submit quantitative results on AST (next to the current final interpretations of susceptibility), to improve standardization of national results on AMR. We also encourage harmonization of case and data definitions used for national AMR surveillance in Belgium, to reduce workload and increase internal validation.

Nederlandstalige samenvatting

Achtergrond

Antimicrobiële resistentie (AMR) ontstaat wanneer een micro-organisme de werking van een antimicrobieel medicijn, dat ter genezing wordt toegediend, kan weerstaan. Resistentie bestaat op drie manieren: natuurlijke (intrinsieke) resistentie in welbepaalde bacteriën, of verworven resistentie hetzij via een genetische mutatie of door horizontale genenoverdracht. Het Europees Antimicrobiële Resistentie Surveillance Network (EARS-Net) volgt de evolutie op van verworven antimicrobiële resistentie op Europees niveau. Het Wetenschappelijk Instituut voor Volksgezondheid (WIV-ISP) is het aangewezen centrum voor België (BE) en verzamelt gegevens uit de klinische laboratoria met behulp van de nationale surveillance EARS-Net BE.

Resultaten

In 2016 meldden 31 klinische laboratoria op een vrijwillige basis gegevens van antimicrobiële gevoeligheidstesten op 8 pathogenen geisoleerd uit bloed- of cerebrospinaal vochtstalen. Voor *Streptococcus pneumoniae* isolaten werden deze gegevens bekomen van 97 labo's van het Nationaal Referentie centrum (NRC) van de Katholieke Universiteit Leuven (KUL).

We observeerden een toename van de antimicrobiële resistentie voor *Escherichia coli* ten opzichte van derde generatie cefalosporinen en fluorochinolonen, met gemiddelde resistentiepercentages van respectievelijk 10,5% en 24,5%. Een stijgende trend werd ook waargenomen voor antimicrobiële resistentie van *Klebsiella pneumoniae* tegen derde generatie cephalosporinen en carbapenems, wat resulteerde in gemiddelde resistentiepercentages in 2016 van respectievelijk 22,9% en 2,4%. Extended-spectrum beta-lactamase (ESBL) werd gedetecteerd bij 83.5% van de geteste stalen die resistent waren voor derde generatie cephalosporines.

Resistentie werd waargenomen bij *Pseudomonas aeruginosa* tegenover bijna alle antimicrobiële groepen. De meest voorkomende resistentie was die tegen fluorochinolonen (14,5%), gevolgd door resistentie tegen aminoglycosiden (11,0%), piperacillin-tazobactam (9,8%) en carbapenem (9,6%). De hoogste resistentieniveaus in Acinetobacter-soorten werden waargenomen tegen fluorochinolonen (7,7%), gevolgd door de aminoglycosides (5.1%) en carbapenems (2,6%).

Naast macroliden (% intermediaire resistente + resistente stammen = 15,9) was niet-gevoeligheid zeldzaam bij *Streptococcus pneumoniae*: 0,4% van de stalen waren niet gevoelig voor penicillines of derde generatie cefalosporinen, en 0,2% was niet gevoelig

voor fluoroquinolonen. In de periode 2012-2016 werd een sterk dalende trend voor niet-gevoeligheid tegen macroliden waargenomen.

In 2016 waren 12,2% van geteste *Staphylococcus aureus* isolaten niet gevoelig voor meticilline (MRSA) en 12,7% was resistent tegen fluoroquinolonen. Voor beide antimicrobiële groepen konden we tussen 2012 en 2016 een dalende trend waarnemen. Voor MRSA werd de afname tot 2015 weliswaar niet verdergezet in 2016.

Er werd geen vancomycine resistentie gevonden bij *E. Faecalis,* van de geteste *E. faecium* isolaten was 1.7% resistent tegen dit antibioticum. Resistentie tegen linezolide bleef vrijwel onbestaand in beide enterokokken, met geen van de *E. faecalis*, en 0,5% van *E. faecium* isolaten die resistent testten. Gentamicine-resistentie kwam vaak voor bij zowel *Enterococcus faecalis* (19,8%) en *Enterococcus faecium* (19,7%).

Conclusies

K. pneumoniae isolaten verloren op continue basis gevoeligheid voor cephalosporinen van de derde generatie gedurende de laatste 5 jaar, met een gemiddeld resistentiepercentage van 22,9% in 2016. Resistentie van deze isolaten voor carbapenems is opkomend, met een gemiddeld resistentiepercentages van 2,4% in 2016. Een meerderheid van de derde generatie cephalosporine-resistente *E. coli* en *K. pneumoniae* isolaten produceerde ESBL. Resistentie van *P. aeruginosa* isolaten voor ceftazidime, carbapenem, aminoglycosiden en fluoroquinolonen was algemeen in 2016. De dalende trend voor niet-gevoeligheid tegenover meticilline van *S. aureus* (MRSA) tussen 2012 en 2015 stabiliseerde in 2016 op ongeveer 12,2%. Geen *E. faecalis* isolaten en 1,7% van de geteste *E. faecium* isolaten waren resistent tegen vancomycine. Resistentie tegen linezolide bleef zeer laag in beide enterokokken.

Voor aanstaande EARS-Net BE dataverzamelingen raden wij laboratoria aan om de kwantitatieve resultaten van de gevoeligheidstesten in te dienen (naast de interpretaties van de gevoeligheid), en dit om de standaardisering van de nationale resultaten van AMR te verbeteren. Wij moedigen ook de harmonisatie van case- en data-definities die worden gebruikt voor nationaal AMR-toezicht in België aan, om de werklast te verminderen en de interne validatie te verhogen.

Résumé en français

Contexte

La résistance aux antimicrobiens désigne la capacité des micro-organismes à résister à des traitements utilisés à leur encontre. La résistance peut revêtir trois formes : résistance naturelle (intrinsèque) de certains types de bactérie, résistance acquise par mutation génétique et résistance acquise par transmission horizontale de gènes. Le réseau européen de surveillance de la résistance aux antimicrobiens (European Antimicrobial Resistance Surveillance Network – EARS-Net) contrôle l'évolution de la résistance acquise aux antimicrobiens à l'échelle européenne. L'Institut Scientifique de Santé Publique (WIV-ISP), désigné centre de référence pour la Belgique (BE), recueille des données à ce sujet auprès des laboratoires cliniques par la surveillance nationale EARS-Net BE.

Résultats

En 2016, 31 laboratoires cliniques belges ont déclaré, à titre volontaire, des données sur la résistance antimicrobienne de huit pathogènes à EARS-Net BE. Pour *Streptococcus pneumoniae*, des données nationales de 97 laboratoires étaient contribuées par le Centre National de Référence de la Katholieke Universiteit Leuven (KUL).

Nous avons ainsi observé une hausse de la résistance d'*Escherichia coli* aux céphalosporines de troisième génération et aux fluoroquinolones, avec des pourcentages de résistance de respectivement 10,5 % et 24,5 % à l'échelle du pays. Une tendance croissante a également été enregistrée pour la résistance de *Klebsiella pneumoniae* aux céphalosporines de troisième génération et aux carbapénèmes, atteignant une moyenne de respectivement 22,9 % et 2,4 %. La bêta-lactamase à spectre étendu (BLSE) a été détectée dans 83,5% des échantillons testés résistants aux céphalosporines de troisième génération.

Des niveaux élevés de résistance ont été observés chez *Pseudomonas aeruginosa* par rapport à presque tous les groupes antimicrobiens. La principale résistance observée était dirigée contre les fluoroquinolones (14,5 %), suivies par les aminoglycosides (11,0 %), le pipéracilline/tazobactam (9,8 %) et les carbapénèmes (9,6%). Les plus hauts niveaux de résistance du genre *Acinetobacter* ont été recensés contre les fluoroquinolones (7,7 %), suivies par les aminoglycosides (5,1 %) et les carbapénèmes (2,6 %).

Hormis son opposition aux macrolides (15,9 % si l'on additionne résistance intermédiaire et résistance de haut niveau), *Streptococcus pneumoniae* présentait peu de résistances : 0,4 % des échantillons n'étaient pas sensibles aux pénicillines ou céphalosporines de troisième génération et 0,2 % n'étaient pas sensibles aux fluoroquinolones. Au cours de la période 2012-2016, on a observé une tendance à la baisse pour la non-sensibilité aux macrolides.

En 2016, 12,2 % de l'ensemble des échantillons de *Staphylococcus aureus* testés présentaient une résistance à la meticillin (SARM) et 12,7 % aux fluoroquinolones. Pour les deux groupes d'antimicrobiens, une tendance à la baisse a été remarquée entre 2012 et 2016. Toutefois, pour les SARM, la tendance décroissante jusqu'en 2015 ne s'est pas poursuivie en 2016.

Aucun isolat d'*E. faecalis* n'était résistant à la vancomycine, et 1,7% des isolats d'*E. faecium* ont été testés résistants à cet antibiotique. La résistance au linézolide est restée presque inexistante chez les deux entérocoques. Aucun isolat d'*E. faecalis*, et 0,5% des isolats d'*E. faecium* étaient résistant au linézolide. La résistance à la gentamicine reste fréquente tant chez *Enterococcus faecalis* (19,8 %) que chez *Enterococcus faecium* (19,7 %).

Conclusions

Pendant la période 2012-16, *Klebsiella pneumoniae* est devenu de moins en moins sensible aux céphalosporines de troisième génération, conduisant à des pourcentages moyens de résistance 22,9 %. La résistance de *Klebsiella pneumoniae* aux carbapénèmes est en hausse, avec un pourcentage moyen de résistance de 2,4 % en 2016. La majorité des isolats *E. coli* et *K. pneumoniae* résistants aux céphalosporines de troisième génération ont produit des BLSE. Les niveaux de résistance de *P. aeruginosa* restaient persistants en 2016 pour les produits suivants: la ceftazidime, les carbapénèmes, les aminoglycosides, et les fluoroquinolones. La tendance à la baisse enregistrée entre 2012 et 2015 pour la non-sensibilité de *S. aureus* à la meticillin (MRSA) s'est stabilisée autour de 12,2 % en 2016. Aucun isolat d'*E. faecalis* et 1,7% des isolats d'*E. faecium* ont été testés résistants à la vancomycine. La résistance au linézolide est restée très faible chez les deux entérocoques.

Pour les prochains appels de données, nous recommandons aux laboratoires de soumettre des résultats quantitatifs des tests de sensibilité (en plus des interprétations de sensibilité) afin d'améliorer la standardisation des résultats nationaux d'AMR. Nous encourageons également l'harmonisation des définitions de cas et de données utilisées par les surveillances nationales de la AMR en Belgique, afin de réduire la charge de travail et d'augmenter la validation interne.

1. Methods

1.1. Antimicrobial resistance surveillance in Europe

The results presented in this report are based on antimicrobial resistance data from invasive isolates, reported to the Scientific Institute of Public Health (WIV-ISP) by Belgian clinical laboratories in 2017 (data referring to samples taken in 2016). The WIV-ISP collects these data in collaboration with and on request of the European Antimicrobial Resistance Surveillance Network (EARS-Net), coordinated by the European Centre for Disease Prevention and Control (ECDC).

EARS-Net was founded in 1999 by the Dutch National Institute for Public Health and the Environment (RIVM), and was transferred to ECDC in 2010. The aim of this surveillance is to follow up trends of antimicrobial resistance (AMR) in bacteria isolated from severe clinical infections in the European Union (EU).[1] It is the main EU epidemiologic surveillance system for AMR, and data reported from the network serve as important indicators on the occurrence and spread of resistance in European countries. EARS-Net performs AMR surveillance for following bacterial pathogens: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa* and *Acinetobacter species*. The Point prevalence survey (PPS) of Healthcare- associated infections (HAI) and antimicrobial use in acute care hospitals organised by ECDC and performed for Belgium by WIV-ISP in 2011, confirmed that these pathogens are the predominant bacteria associated with hospital acquired infections in Belgium.[2] The ECDC results indicated the importance of the same pathogens at a European level.[3] EARS-Net data are based on invasive isolates only (blood or cerebrospinal fluid), to prevent potential inconsistencies in the data analysis.[1]

WIV-ISP coordinates the Belgian branch of EARS-Net, through close collaboration with the hospital and national reference laboratories, whose time and effort should be acknowledged. This report describes the results from the Belgian data-collection (EARS-Net BE).

1.2. The EARS-Net BE surveillance

Table 1 describes the microorganism and antimicrobial group combinations under EARS-Net BE surveillance. Rationale and modalities for data collection can be found in the latest version of the EARS-Net BE protocol, dated July 2017.[4] This protocol describes in detail the case definitions, inclusion criteria, data definitions, submitting and reporting procedures, data management and validation. Case definitions and inclusion criteria follow those of the EU protocol.[5] Laboratories retrieve their annual surveillance data by extraction from the local (laboratory) database and sending the data file to WIV-ISP. The result of the Antimicrobial susceptibility test (AST) that is reported and used in the calculation of the reported resistance percentages is based on the final interpretation of the laboratory.

Participation of the laboratories to this surveillance is voluntary. Statistical reports from 2012-2016 can be downloaded from the NSIH website.[6]

Results presented in this report are derived from surveillance data for 2016 submitted by participating laboratories to the WIV-ISP, except for the results on *S. pneumoniae.* Those were provided by the national surveillance of *Pneumococci*, organised by the National Reference Centre (NRC) of the Catholic University Leuven (KUL). The NRC receives its isolates from laboratories for microbiology across Belgium; its AST results are interpreted following Clinical and Laboratory Standards Institute (CLSI) guidelines.[7]

EARS-Net data are collected up to the individual AST level (e.g. Meropenem, Imipenem, (Table 1, 2nd column)). De-duplication of annual laboratory data goes as follows: for each laboratory and pathogen: (1) All tests results are aggregated within the same isolate, prioritizing test results according to the resistance result (R>I>S), (2) then for each patient, results on the first occurring specimen within the study year are kept. In case of multiple samples on the same date for the same patient, cerebrospinal fluid samples are prioritized over blood cultures.

In accordance with EARS-Net reporting, results are aggregated at the level of antimicrobial group (e.g. Carbapenems, (Table 1, 1st column)). Depending on the pathogen-antimicrobial test combination, either resistance rates (%R) or non-susceptibility rates (%IR, intermediary resistant + resistant) are reported. Intermediate results may arise because of the possibility of using different antibiotic dosages in clinical practice. They may also result from imperfections in the AST. Non-susceptibility (%IR) is reported for *S. pneumoniae* (for all tested antimicrobial groups) and for *S. aureus (*concerning the antimicrobial group MRSA only). All other reported rates are resistance rates (%R). Because ECDC defines resistance of Gram-negative bacteria to aminoglycosides excluding Amikacin, a separate group “Aminoglycosides including Amikacin” was created.

2016 was the first year for which supplementary information on confirmation tests for selected pathogens was collected and reported by EARS-Net BE. These are tests for detection of mecA-gene (polymerase chain reaction) and agglutination of penicillin-binding protein 2a (for *S. aureus* isolates), for detection of extended-spectrum beta-lactamase (ESBL, for *E. coli* and *K. pneumoniae isolates*) and for detection of carbapenemase-production (CP, for *E. coli, K. pneumoniae, P. aeruginosa and Acinetobacter spp* isolates).

For the first time, we also present resistance data on the subgroup of isolates originating from hospitalized patients only. This allows comparison of results of the EARS-Net BE surveillance with those of other AMR surveillances carried out by WIV-ISP and only restricting on hospitalized patients. The selection of this subgroup is done prior to the de-duplication of the data as described above.

Tables reporting the national results of antimicrobial resistance in 2016 contain several indicators. For every antimicrobial group, the number of reporting hospital laboratories, the number of resistant isolates and the total number of investigated isolates are reported. Apart from the overall mean resistance percentage (Database mean), we also report the

mean of the laboratory mean resistances and the median resistance. By adding the standard deviations and the most important percentiles (p10, p25, p75 and p90), we obtain insight into the spread of the data, the potential influence of extreme values (outliers) and the influence of low sample sizes. We can get an idea about the effect of outlying values by comparing the median (p50) with the database mean. If these values differ a lot, this might indicate the presence of outliers. In that case, the median value will give a more accurate estimation of the resistance level of the pathogen to the respective antimicrobial than the mean, because its value is less sensitive to extreme values. Also, breakpoints for the interpretation of a particular AST that differ substantially between guidelines, for example European Committee on Antimicrobial Susceptibility Testing (EUCAST) versus CLSI, will lead to a larger variability of resistance rates (or standard deviation) for this AST if laboratories contributing results used a mixture of above guidelines. Results for the subgroup of hospitalized patients are also added to this table.

Table 1 | Microorganism and antimicrobial group combinations under EARS-Net BE surveillance (European Antimicrobial Resistance Surveillance – Network Belgium, 2016)

ANTIMICROBIAL GROUP	ANTIMICROBIAL TESTS
E. faecalis/ E. faecium	
High-level aminoglycoside resistance	Gentamicin-High
Aminopenicillins	Amoxicillin, Ampicillin
Glycopeptides	Vancomycin, Teicoplanin
Oxazolidones	Linezolid
E. coli/ K. pneumoniae	
Aminopenicillins (only for E. coli)	Amoxicillin, Ampicillin
Fluoroquinolones	Ciprofloxacin, Ofloxacin, Levofloxacin, Moxifloxacin, Norfloxacin
Third-gen. Cephalosporins	Cefotaxime, Ceftriaxone, Ceftazidime
Third-gen. Cephalosporins, ESBL+	Cefotaxime, Ceftriaxone, Ceftazidime, Extended Spectrum Beta Lactamase
Aminoglycosides, excl. Amikacin	Netilmicin, Gentamicin, Tobramycin
Aminoglycosides, incl. Amikacin	Netilmicin, Gentamicin, Tobramycin, Amikacin
Carbapenems	Imipenem, Meropenem
Carbapenems, CP+	Imipenem, Meropenem, Carbapenemase
Polymyxins	Colistin, Polymyxin B
P. aeruginosa	
Piperacillin-tazobactam	Piperacillin/Tazobactam
Ceftazidime	Ceftazidime
Fluoroquinolones	Ciprofloxacin, Levofloxacin
Aminoglycosides, excl. Amikacin	Netilmicin, Gentamicin, Tobramycin
Aminoglycosides, incl. Amikacin	Netilmicin, Gentamicin, Tobramycin, Amikacin
Carbapenems	Imipenem, Meropenem
Polymyxins	Colistin, Polymyxin B
Acinetobacter spp.	
Fluoroquinolones	Ciprofloxacin, Levofloxacin
Aminoglycosides, excl. Amikacin	Netilmicin, Gentamicin, Tobramycin
Aminoglycosides, incl. Amikacin	Netilmicin, Gentamicin, Tobramycin, Amikacin
Carbapenems	Imipenem, Meropenem
Carbapenems, CP+	Imipenem, Meropenem, Carbapenemase
Polymyxins	Colistin, Polymyxin B
S. pneumoniae	
Penicillins	Penicillin, Oxacillin
Macrolides	Erythromycin, Clarithromycin, Azithromycin
Fluoroquinolones	Levofloxacin, Norfloxacin, Moxifloxacin
Third-gen. Cephalosporins	Cefotaxime, Ceftriaxone
S. aureus	
MRSA	Meticillin, Oxacillin, Cefoxitin, Flucloxacillin, Cloxacillin, Dicloxacillin
Fluoroquinolones	Ciprofloxacin, Levofloxacin, Ofloxacin, Norfloxacin
Rifampicin	Rifampicin
Linezolid	Linezolid

Gen: generation, ESBL: extended spectrum beta-lactamase, CP+: carbapenemase-production, *S. aureus: Staphylococcus aureus, S. pneumoniae: Streptococcus pneumoniae, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, E. faecalis: Enterococcus faecalis, E. faecium: Enterococcus faecium, P. aeruginosa: Pseudomonas aeruginosa, MRSA: methicillin-resistant Staphylococcus aureus*

Tables reporting mean resistance rates to principal antimicrobial groups between 2012 and 2016 contain following indicators: database mean resistance percentages, together with the number of resistant isolates and the total number of isolates for the respective antimicrobial test, and a column reporting the trend over the last five years. This trend is calculated using the Pearson Chi-squared test for trends, and is reported as either positive (+) or negative (-), or not present. The number of reported '+' or '-'signs indicates the level of significance of the test result: +++ or --- indicate p≤0.001, ++ or -- indicate p≤0.01, + or – indicate p≤0.05, (+) or (-) indicate p≤0.10.

All data management, analysis, and statistical tests were performed using STATA 14.2 (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP).

2. Results

2.1. Participation

Table 2 displays the number of clinical laboratories reporting at least one isolate to EARS-Net BE from 2007 until 2016. Although all laboratories for microbiology performing antimicrobial susceptibility testing in Belgium were invited in 2017 to submit results for 2016, only hospital laboratories reported results. This is probably due to the nature of the isolates being merely invasive. Participation rates are therefore calculated with regard to hospital laboratories only, except for *S. pneumoniae*, for which the participation rate is calculated with respect to all laboratories for microbiology in Belgium.

Table 2 | Number of clinical laboratories reporting at least one isolate for the European Antimicrobial Resistance Surveillance Network (EARS-Net), Belgium 2007-2016 (% participation)

Year	*S. Pneumoniae*	*S. aureus*	*E. coli*	Enterococci	*K. pneumoniae*	*P. aeruginosa*	*Acinetobacter spp.*
2007	34/149 (23%)	34/108 (31%)	17/108 (16%)	20/108 (19%)	-	-	-
2008	97/149 (65%)	38/107 (36%)	16/107 (15%)	19/107 (18%)	-	-	-
2009	98/149 (66%)	34/108 (31%)	18/108 (17%)	14/108 (13%)	8/108 (7%)	8/108 (7%)	-
2010	94/149 (63%)	40/108 (37%)	23/108 (21%)	22/108 (20%)	14/108 (13%)	15/108 (14%)	-
2011	89/148 (60%)	50/107 (47%)	43/107 (40%)	46/107 (43%)	44/107 (41%)	43/107 (40%)	-
2012	93/147 (63%)	44/107 (41%)	41/107 (38%)	41/107 (38%)	41/107 (38%)	40/107 (37%)	-
2013	92/148 (62%)	41/106 (39%)	41/106 (39%)	39/106 (37%)	41/106 (39%)	40/106 (38%)	2/106 (2%)
2014	96/146 (66%)	27/105 (26%)	27/105 (26%)	25/105 (24%)	26/105 (25%)	27/105 (26%)	3/105 (3%)
2015	89/142 (63%)	25/102 (24%)	25/102 (24%)	25/102 (24%)	24/102 (23%)	25/102 (24%)	8/102 (8%)
2016	97/139 (70%)	31/102 (30%)	31/102 (30%)	30/102 (29%)	28/102 (27%)	31/102 (30%)	18/102 (18%)

Percentages were calculated by dividing the number of reporting laboratories by the total number of laboratories (*S. Pneumoniae*) and total number of hospital laboratories (all other isolate types) in Belgium during that particular year. (Source of the data on annual number of (hospital) laboratories in Belgium: WIV-ISP, Department of Quality of Medical Laboratories); *S. aureus: Staphylococcus aureus, S. pneumoniae: Streptococcus pneumoniae, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, E. faecalis: Enterococcus faecalis*, *E. faecium: Enterococcus faecium*, *P. aeruginosa: Pseudomonas aeruginosa*

The total number of hospital laboratories for microbiology in Belgium decreased from 109 in 2007 to 102 in 2016. The number of laboratories participating to EARS-Net BE declined between 2011 and 2015, but slightly increased again in 2016. In 2016, about one third of the Belgian hospital laboratories participated to EARS-Net. The participation rate for *Acinetobacter spp*. was lower (18%). This may be due to a lower prevalence of the species in the included invasive isolates, or due to the more recent inclusion of the pathogen in the

surveillance. Thanks to the exhaustiveness of the national surveillance of pneumococcal infections as organized by the National Reference Center (NRC) at the Catholic University of Leuven (KUL), the participation rate in 2016 for *S. pneumoniae* was much higher, and reached 70% (97 out of 139 clinical laboratories in BE).

Table 3 displays the number of hospital laboratories reporting to EARS-Net per region and per hospital type in Belgium in 2016. The spread of the laboratories across the country was satisfactory; participation rates fluctuate around 30% in all three regions. All hospital types, except for the specialized military hospital, are represented in the surveillance.

Table 3 | Number of hospital laboratories reporting to EARS-Net BE 2016 per region and per hospital type

	Participating hospital labs (n)	Total number of hospital labs (N)	Participation percentage
Regions			
Brussels Capital Region	3	10	30%
Flanders	18	56	32%
Walloon region	10	36	28%
Hospital types [a]			
Primary	20	79	25%
Secondary	8	15	53%
Tertiary	3	7	43%
Specialized	0	1	0%
TOTAL	**31**	**102**	**30%**

[a] See annex 2 for information on the classification of hospital types

2.2. *Escherichia coli*

2.2.1. Clinical importance and epidemiology

Despite being part of the normal intestinal microbiota (flora) in humans and many animals, *E. coli* is a frequent cause of bloodstream and urinary tract infections. It is also associated with intra-abdominal infections, neonatal meningitis, and foodborne infections.[1,8,9] In Belgium, as well as in Europe, *E. coli* is the most frequently isolated pathogen in HAI, accounting for 19.6% of all Belgian HAI identified by the PPS of 2011, and for 15.9% of all HAI in Europe.[2,3] This high prevalence, in combination with a wide range of antimicrobial resistance mechanisms in *E. coli*, makes access to effective antimicrobial treatment essential to reduce the health related and economic burden.[1]

2.2.2. National results: resistance rates in Belgium, 2016

Table 4 displays the mean resistance rates of *E. coli* to principal antimicrobial groups in Belgium in 2016, both for all reported isolates and for the subgroup of hospitalized patients. Out of 22 clinical laboratories reporting their use of AST guidelines, 57.0% applied the EUCAST breakpoints.

The mean percentage of aminopenicillin resistance was 58.0%. Mean resistance percentages of 10.5% and 24.5% were observed for third-generation cephalosporins and for fluoroquinolones respectively. Among the additionally tested isolates that were resistant to third-generation cephalosporins, 77.7% tested positive for ESBL. ESBL hydrolyze extended-spectrum cephalosporins with an oxyimino side chain. ESBL are often plasmid encoded. Plasmids responsible for ESBL-production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing organisms are limited.[10,11]

Mean resistance to aminoglycosides, excluding the more susceptible antibiotic amikacin, reached 8.4% in 2016. Mean resistance of the total group of aminoglycosides, including amikacin, was 7.8%. Resistance to polymyxins (0.4%) remained low. Out of 3845 isolates, only two were resistant to carbapenems (0.1%), and none of those two isolates produced carbapenemase (0.0% CP+). For 2016 and for the first time, the EARS-Net BE report includes resistance rates for subgroup of hospitalized patients (Table 2 lower part). Isolates from hospitalized patients (75.3% of all patients) showed similar resistance rates to the selected antimicrobials in *E. coli*.

Table 4 | *Escherichia coli*: Number of laboratories contributing results on ASTs, mean resistance rates to principal antimicrobial groups for all isolates and for the subgroup of hospitalized patients, EARS-Net BE 2016

Antimicrobial group	Labs (N)	R samples (N)	Total samples (N)	Mean %R (database)	Mean %R (lab means)	Std. Dev.	p10	p25	p50	p75	p90
All isolates											
Aminopenicillins	31	2167	3736	58.0	58.2	5.1	52.5	54.1	57.7	61.5	65.0
Third-generation cephalosporins	31	392	3737	10.5	11.3	6.3	5.7	6.9	9.6	13.9	15.4
Third-generation cephalosporins, ESBL+	14	150	193	77.7	74.0	25.1	50.0	68.8	81.2	88.9	91.4
Carbapenems	31	2	3845	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Carbapenems, CP+	6	0	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aminoglycosides - Amikacin	28	294	3499	8.4	8.2	3.1	4.0	6.5	7.8	10.3	12.7
Aminoglycosides +Amikacin	31	299	3856	7.8	7.6	4.0	1.3	5.8	7.3	10.3	12.6
Fluoroquinolones	31	946	3854	24.5	25.1	6.2	18.2	19.2	25.6	28.8	30.8
Polymyxins	13	7	1784	0.4	0.3	0.5	0.0	0.0	0.0	0.5	1.1
Subgroup: Isolates from hospitalized patients											
Aminopenicillins	29	1536	2640	58.2	59.3	6.6	52.6	53.8	58.3	62.1	68.5
Third-generation cephalosporins	29	282	2647	10.6	11.2	4.9	4.2	7.1	10.2	15.2	17.2
Third-generation cephalosporins, ESBL+	13	94	123	76.4	75.4	26.5	50.0	75.0	85.7	87.5	100.0
Carbapenems	29	1	2638	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Aminoglycosides -Amikacin	27	212	2529	8.4	8.7	3.9	3.5	5.8	7.6	10.7	15.3
Fluoroquinolones	29	657	2648	24.8	25.7	6.4	17.2	22.2	25.3	29.0	36.0
Polymyxins	11	4	1176	0.3	0.3	0.6	0.0	0.0	0.0	0.6	0.6

ESBL: extended spectrum beta-lactamase, CP+: Carbapenemase-production, N: total number, R: resistance, p: percentile, AST: Antimicrobial susceptibility test, Std. Dev.: Standard deviation

2.2.3. Trends in resistance 2012-2016

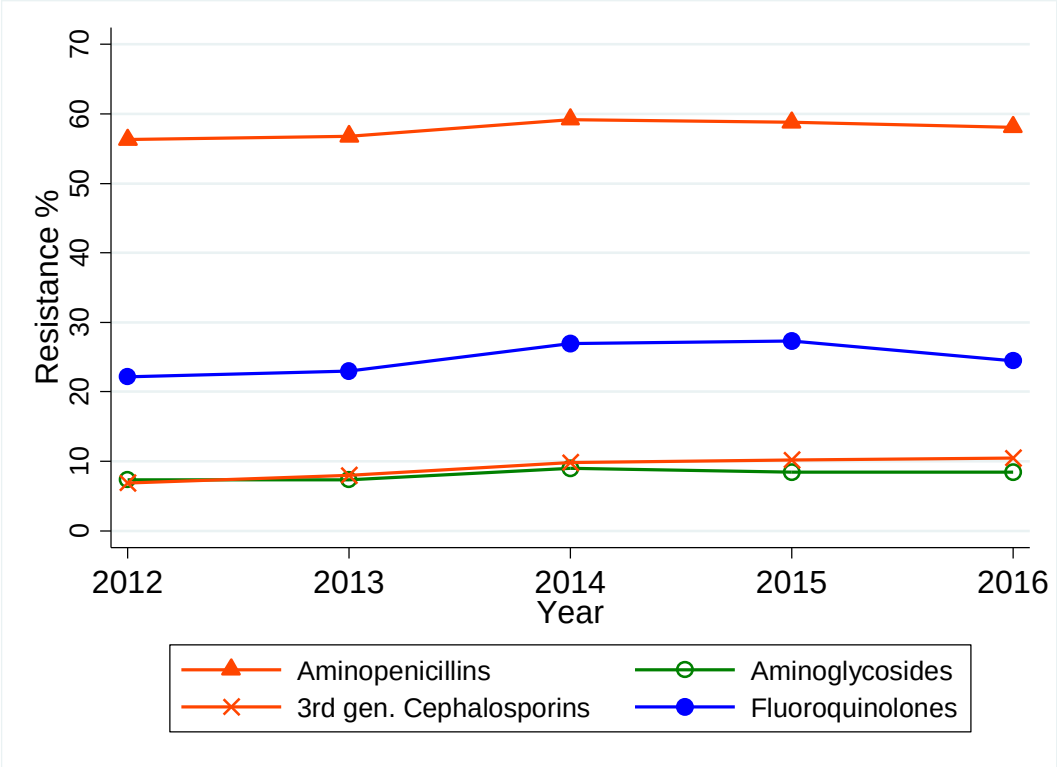
Table 5 and figure 1 display trends from 2012 until 2016 of mean resistance rates of *E. coli* to principal antimicrobial groups. No significant trend was noted for aminopenicillin resistance between 2012 and 2016. We could however observe a significant increase in antimicrobial resistance to third-gen. cephalosporins and to fluoroquinolones. Resistance to third-generation cephalosporins increased from 6.9% in 2012 to 10.5% in 2016. The increasing trend for fluoroquinolone resistance from 22.2% in 2012 up to 27.3% in 2015 did not continue in 2016 (24.5%). A moderate increasing trend could be observed for aminoglycosides, increasing from 7.3% in 2012 up to 8.4% in 2016.

Table 5 | *Escherichia coli*: Mean resistance rates to principal antimicrobial groups, EARS-Net BE 2012-2016

Antimicrobial group	2012 %R (#R/N)	2013 %R (#R/N)	2014 %R (#R/N)	2015 %R (#R/N)	2016 %R (#R/N)	TREND[a]
Aminopenicillins	56.3 (2193/3898)	56.8 (2470/4350)	59.2 (1655/2795)	58.8 (1762/2995)	58.1 (2155/3708)	
Third-generation Cephalosporins	6.9 (284/4097)	8.0 (324/4051)	9.8 (269/2741)	10.2 (298/2910)	10.5 (390/3709)	+++
Carbapenems	0.0 (1/4119)	0.1 (2/4246)	0.0 (1/2511)	0.0 (0/2591)	0.1 (3/3817)	
Aminoglycosides -Amikacin	7.3 (219/3010)	7.3 (243/3309)	9.0 (178/1974)	8.4 (218/2587)	8.4 (294/3499)	+
Fluoroquinolones	22.2 (779/3515)	23.0 (944/4113)	26.9 (682/2535)	27.3 (788/2885)	24.5 (946/3853)	++
Polymyxins	0.0 (0/257)	0.6 (7/1082)	0.2 (1/490)	1.3 (12/914)	0.4 (7/1784)	

[a] Pearson Chi-squared test for trends: 'plus' signs indicate an increasing trend, 'minus' signs indicate decreasing trend. (+++ or --- indicate p<=0.001, ++ or -- indicate p<=0.01, + or – indicate p<=0.05, (+) or (-) indicate p<=0.10) R: resistance, N: total number, #: number

Figure 1 | *Escherichia coli*: Trends in resistance (%R) to aminopenicillins, fluoroquinolones, third-generation cephalosporins and to aminoglycosides (-amikacin), EARS-Net BE 2012-2016



Note: Y-axis is not extended beyond 70% resistance, to reduce overlap in trend lines and improve visibility

2.3. *Klebsiella pneumoniae*

2.3.1. Clinical importance and epidemiology

The majority of *K. pneumoniae* infections is healthcare-associated and can spread rapidly between patients and via the hands of hospital personnel, leading to nosocomial outbreaks.[1] Infections typically affect the urinary tract, lower respiratory tract and the bloodstream of hospitalized, often more susceptible patients. But healthy subjects can also suffer from severe invasive infections by some hypervirulent strains. *K. pneumoniae* frequently acquires its resistance traits through plasmids.[10] In Belgium, the PPS performed in 2011 showed that *Klebsiella spp.* infections accounted for 5.1% of all HAI.[2] In Europe, *Klebsiella spp* .was documented in 8.7% of all HAI.[3]

2.3.2. National results: resistance rates in Belgium, 2016

Table 6 displays the mean resistance rates of *K. pneumoniae* to principal antimicrobial groups in Belgium in 2016, both for all reported isolates and for the subgroup of hospitalized patients. Out of 21 clinical laboratories reporting their use of AST guidelines, 55.3% applied EUCAST breakpoints.

Mean resistance percentages to third-generation cephalosporins and to carbapenems were 22.9% and 2.4% resp. Out of the 115 additionally tested samples that were resistant to third-generation cephalosporins, 83.5% were ESBL-positive. Out of the 11 additionally tested carbapenem-resistant isolates, 10 (90.9%) tested carbapenemase positive. Carbapenemases are enzymes that can hydrolyze most beta-lactams, including carbapenems, leading to limited treatment options retaining antimicrobial activity against *K. pneumoniae*.

Resistance to fluoroquinolones (23.6%) and to aminoglycosides (13.8%), regardless of inclusion of amikacin, was also common. Resistance to polymyxins (0.6%) remained low. *K. pneumoniae* isolates from the subgroup of hospitalized patients (76.9 % of all patients) often showed higher resistance rates to the selected antimicrobials: third-generation cephalosporins (+3.3%), carbapenems (+0.3%), aminoglycosides other than amikacin (+1.6%) and fluoroquinolones (+1.7%), while other resistance rates were similar.

Table 6 | *Klebsiella pneumoniae*: Number of laboratories contributing results on ASTs, mean resistance rates to principal antimicrobial groups, EARS-Net BE 2016

Antimicrobial group	Labs (N)	R samples (N)	Total samples (N)	Mean %R (data-base)	Mean %R (lab means)	Std. Dev.	p10	p25	p50	p75	p90
All isolates											
Third-generation cephalosporins	28	153	669	22.9	18.9	12.5	0.0	10.6	20.0	30.2	34.8
Third-generation cephalosporins (ESBL+)	14	96	115	83.5	85.5	23.2	42.9	85.7	100	100	100
Carbapenems	28	16	669	2.4	1.4	3.3	0.0	0.0	0.0	0.0	6.3
Carbapenems, (CP+)	6	10	11	90.9	66.7	57.7	0.0	0.0	100	100	100
Aminoglycosides -Amikacin	26	88	637	13.8	11.0	10.1	0.0	0.0	9.4	17.4	22.3
Aminoglycosides +Amikacin	28	92	669	13.8	10.7	10.6	0.0	0.0	8.5	17.0	23.1
Fluoroquinolones	28	158	669	23.6	22.4	12.1	9.1	15.9	20.4	27.0	44.4
Polymyxins	13	2	347	0.6	0.2	0.6	0.0	0.0	0.0	0.0	0.9
Subgroup: Isolates from hospitalized patients											
Third-generation cephalosporins	27	124	474	26.2	23.0	16.1	0.0	8.7	22.2	35.4	40.0
Third-generation cephalosporins (ESBL+)	13	76	89	85.4	88.4	20.4	63.6	87.3	100.0	100	100
Carbapenems	27	13	474	2.7	1.9	4.9	0.0	0.0	0.0	0.0	8.5
Carbapenems, (CP+)	5	7	8	87.5	50.0	70.7	0.0	0.0	50.0	100	100
Aminoglycosides -Amikacin	25	70	454	15.4	13.1	12.4	0.0	0.0	10.0	22.2	26.8
Fluoroquinolones	27	120	474	25.3	24.4	13.8	9.1	16.7	23.1	33.3	43.8
Polymyxins	12	1	228	0.4	0.1	0.3	0.0	0.0	0.0	0.0	0.0

ESBL: extended-spectrum beta-lactamase, CP+: Carbapenemase-production, N: total number, R: resistance, p: percentile, AST: Antimicrobial susceptibility test, Std. Dev.: Standard deviation

2.3.3. Trends in resistance 2012-2016

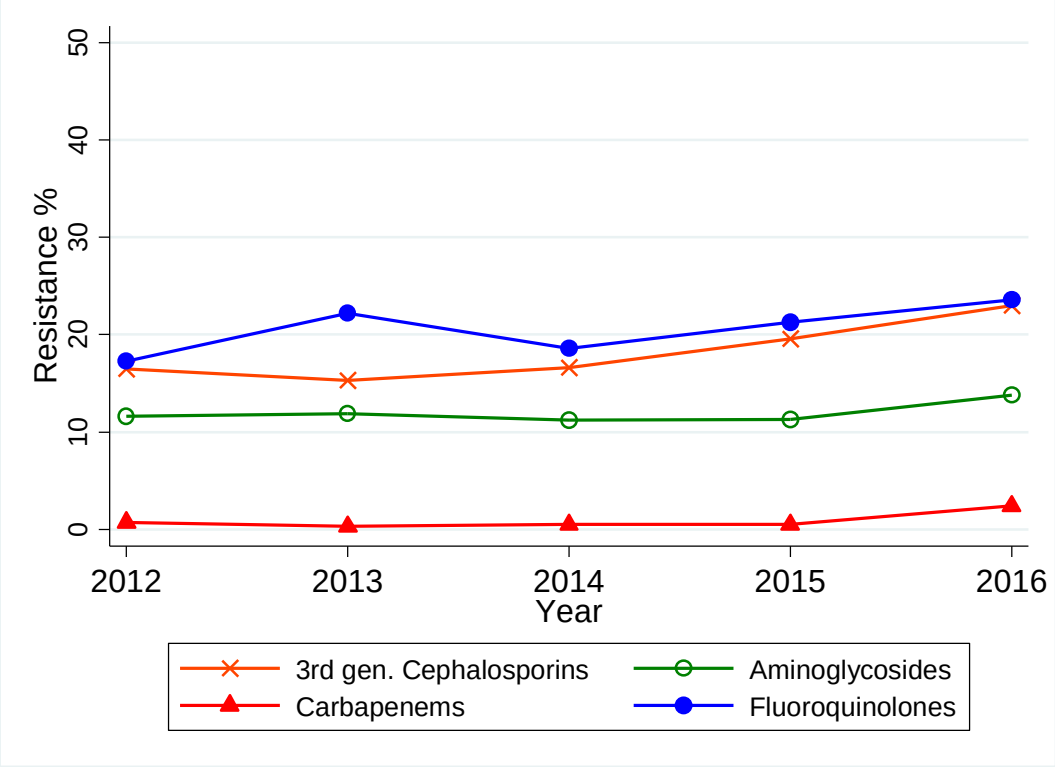
Table 7 and figure 2 display trends from 2012 until 2016 of mean resistance rates of *K. pneumoniae* to principal antimicrobial groups. A statistically significant increasing trend could be observed for antimicrobial resistance to third-generation cephalosporins and to carbapenems. Resistance to third-generation cephalosporins increased from 16.5% in 2012 to 22.9% in 2016. Resistance to carbapenems increased from 0.7% in 2012 to 2.4% in 2016. A moderate increasing trend could also be observed for fluoroquinolones, increasing from 17.3% in 2012 up to 23.6% in 2016. Resistance to aminoglycosides and polymyxins remained fairly stable over the past 5 years.

Table 7 | *Klebsiella pneumoniae*: Mean resistance rates to principal antimicrobial groups, EARS-Net BE 2012-2016

Antimicrobial group	2012 %R (#R/N)	2013 %R (#R/N)	2014 %R (#R/N)	2015 %R (#R/N)	2016 %R (#R/N)	TREND[a]
Third-generation Cephalosporins	16.5 (89/540)	15.3 (91/594)	16.6 (79/477)	19.6 (88/448)	22.9 (153/669)	++
Carbapenems	0.7 (4/545)	0.3 (2/618)	0.5 (2/417)	0.5 (2/389)	2.4 (16/666)	++
Aminoglycosides -Amikacin	11.6 (48/414)	11.9 (58/486)	11.2 (37/331)	11.3 (45/398)	13.8 (88/637)	
Fluoroquinolones	17.3 (92/532)	22.2 (142/639)	18.6 (92/495)	21.3 (90/422)	23.6 (158/669)	(+)
Polymyxins	0.0 (0/34)	0.6 (1/164)	1.9 (2/105)	4.6 (6/131)	0.6 (2/347)	

[a] Pearson Chi-squared test for trends: 'plus' signs indicate an increasing trend, 'minus' signs indicate decreasing trend. (+++ or --- indicate p<=0.001, ++ or -- indicate p<=0.01, + or – indicate p<=0.05, (+) or (-) indicate p<=0.10) R: resistance, N: total number, #: number

Figure 2 | *Klebsiella pneumoniae*: Trends in resistance to carbapenems, fluoroquinolones, third-generation cephalosporins and aminoglycosides (-amikacin), EARS-Net BE 2012-2016



Note: Y-axis is not extended beyond 50% resistance, to reduce overlap in trend lines and improve visibility

2.4. *Pseudomonas aeruginosa*

2.4.1. Clinical importance and epidemiology

P. aeruginosa is an opportunistic pathogen. It is a major cause of infection in hospitalized patients who suffer from impairment of their immune defenses. Hospital-acquired pneumonia, bloodstream and urinary tract infections are some of the commonly caused opportunistic *P. aeruginosa* infections. Because of its ubiquity in aquatic environments, its enormous versatility and its intrinsic tolerance to many disinfectants and antimicrobials, adequate control of *P. aeruginosa* in hospitals remains difficult.[1]

The PPS study estimated that about 9.5% of HAI were caused by *P. aeruginosa,* making it the third most often isolated pathogen from HAI in Belgium.[2] In Europe, 8.9% of all HAI were caused by *P. aeruginosa.*[3] These levels of resistance are a concern, as *P. aeruginosa* is intrinsically resistant to a number of antimicrobial groups and any additionally acquired resistance could severely limit the therapeutic options.[1]

2.4.2. National results: resistance rates in Belgium, 2016

Table 8 displays the mean resistance rates of *P. aeruginosa* to principal antimicrobial groups in Belgium in 2016, both for all reported isolates and for the subgroup of hospitalized patients only. Out of 22 clinical laboratories reporting their use of AST guidelines, 59.3% applied EUCAST breakpoints.

P. aeruginosa showed resistance to almost all tested antimicrobial groups. The most often occurring resistance of *P. aeruginosa* in Belgium was to fluoroquinolones (14.5%), followed by resistance to aminoglycosides (11.0%), piperacillin-tazobactam (9.8%) and to carbapenems (9.6%). Out of the 20 additionally tested carbapenem-resistant isolates, 10% produced carbapenemase.

Isolates from the subgroup of hospitalized patients showed similar resistance rates to the selected antimicrobials in *P. aeruginosa*. Nearly 82% of all patients from which *P. aeruginosa* was isolated, were hospitalized.

Table 8 | *Pseudomonas aeruginosa*: Number of laboratories contributing results on ASTs, mean resistance rates to principal antimicrobial groups, EARS-Net BE 2016

Antimicrobial group	Labs (N)	R samples (N)	Total samples (N)	Mean %R (data-base)	Mean %R (lab means)	Std. Dev.	p10	p25	p50	p75	p90
All isolates											
Piperacillin-tazobactam	30	31	318	9.8	7.8	9.6	0.0	0.0	4.3	14.3	20.7
Ceftazidime	30	25	320	7.8	6.1	8.5	0.0	0.0	0.0	12.5	20.7
Carbapenems	31	35	365	9.6	6.7	8.3	0.0	0.0	0.0	14.3	19.6
Carbapenems, CP+	6	2	20	10.0	13.3	23.0	0.0	0.0	0.0	40.0	40.0
Aminoglycosides -Amikacin	28	36	327	11.0	8.6	10.1	0.0	0.0	5.3	14.3	21.0
Aminoglycosides +Amikacin	31	39	365	10.7	9.0	9.8	0.0	0.0	7.1	16.7	20.8
Fluoroquinolones	31	53	366	14.5	13.4	15.0	0.0	0.0	12.5	20.8	25.0
Polymyxins	17	2	240	0.8	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup: Isolates from hospitalized patients											
Piperacillin-tazobactam	28	22	235	9.4	8.1	8.9	0.0	0.0	4.6	18.0	20.0
Ceftazidime	28	17	235	7.2	6.3	8.8	0.0	0.0	0.0	13.1	20.0
Carbapenems	29	26	264	9.9	7.0	9.3	0.0	0.0	0.0	14.3	20.0
Carbapenems, CP+	5	0	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aminoglycosides -Amikacin	27	26	248	10.5	7.8	9.3	0.0	0.0	0.0	16.7	22.2
Fluoroquinolones	29	38	265	14.3	12.2	16.2	0.0	0.0	9.1	18.5	26.7
Polymyxins	15	2	181	1.1	0.4	1.7	0.0	0.0	0.0	0.0	0.0

CP+: Carbapenemase-production, N: total number, R: resistance, p:percentile, AST: Antimicrobial susceptibility test, Std. Dev.: Standard deviation

2.4.3. Trends in resistance 2012-2016

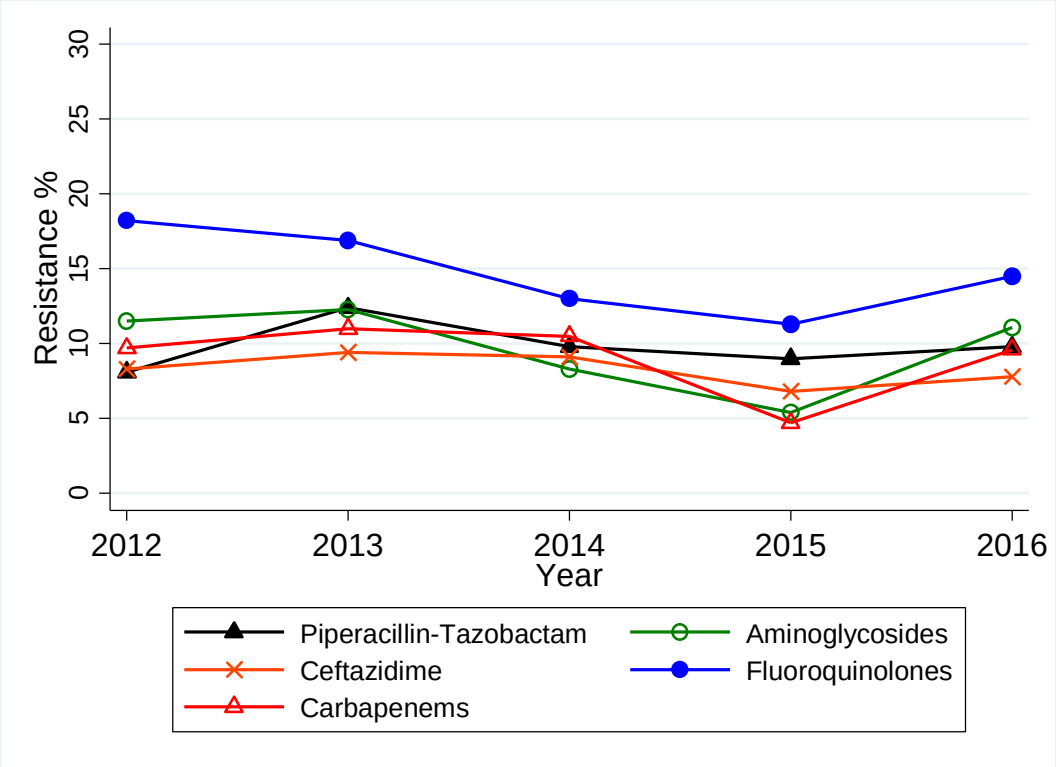
Table 9 and figure 3 display trends from 2012 until 2016 of mean resistance rates of *P. aeruginosa* to principal antimicrobial groups. The decreasing trend for resistance to fluoroquinolones that could be observed between 2012 and 2015, declining from 18.2% down to 11.3%, did not continue in 2016 (14.5%). Resistance to aminoglycosides declined from 11.5% in 2012 down to 5.4% in 2015, to increase again to 11.1% in 2016. We could however observe that the median percentage resistance in 2016 (p50, table 8) equals zero for many antimicrobial groups, which indicates that only a minority of laboratories reported resistant samples. This leads to a high influence of outlying results and higher fluctuations of the resistance percentages over the years. Higher sample sizes, and thus a higher participation rate to the surveillance, will be needed to overcome this interpretation problem.

Table 9 | *Pseudomonas aeruginosa*: Mean resistance rates to principal antimicrobial groups, EARS-Net BE 2012-2016

Antimicrobial group	2012 %R (#R/N)	2013 %R (#R/N)	2014 %R (#R/N)	2015 %R (#R/N)	2016 %R (#R/N)	TREND[a]
Piperacillin-Tazobactam	8.1 (26/319)	12.4 (50/403)	9.8 (28/286)	9.0 (24/268)	9.8 (31/318)	
Ceftazidime	8.3 (27/326)	9.4 (43/459)	9.1 (28/309)	6.8 (17/249)	7.8 (25/320)	
Carbapenems	9.7 (38/391)	11.0 (57/518)	10.5 (35/334)	4.7 (13/278)	9.6 (35/365)	
Aminoglycosides -Amikacin	11.5 (33/286)	12.3 (50/407)	8.3 (21/253)	5.4 (13/241)	11.1 (36/324)	
Fluoroquinolones	18.2 (60/329)	16.9 (82/486)	13.0 (39/301)	11.3 (32/284)	14.5 (53/366)	(-)
Polymyxins	0.0 (0/113)	0.4 (1/230)	0.0 (0/74)	0.8 (1/134)	0.8 (2/237)	

[a] Pearson Chi-squared test for trends: 'plus' signs indicate an increasing trend, 'minus' signs indicate decreasing trend. (+++ or --- indicate p<=0.001, ++ or -- indicate p<=0.01, + or – indicate p<=0.05, (+) or (-) indicate p<=0.10) R: resistance, N: total number, #: number

Figure 3 | *Pseudomonas aeruginosa*: Trends in resistance to piperacillin-tazobactam, ceftazidime, fluoroquinolones, aminoglycosides (-amikacin) and carbapenems, EARS-Net BE 2012-2016



Note: Y-axis is not extended beyond 30% resistance, to reduce overlap in trend lines and improve visibility

2.5. *Acinetobacter species*

2.5.1. Clinical importance and epidemiology

Acinetobacter species are Gram-negative opportunistic pathogens. Nosocomial pneumoniae (often ventilator-associated), bloodstream and urinary tract infections, surgical site infections and other types of wound infection are often caused by species belonging to the *A. baumannii* group. Advanced age, immune suppression, mechanical ventilation and extended hospital stay are some of the risk factors for *Acinetobacter* infections.[1]

The 2011 PPS study estimated that 0.1% of HAI in Belgium were caused by *Acinetobacter spp.*[2] In Europe, 3.6% of all HAI was caused by *Acinetobacter spp.*[3]

2.5.2. National results: resistance rates in Belgium, 2016

Table 10 displays the mean resistance rates of *Acinetobacter spp.* to principal antimicrobial groups in Belgium in 2016, both for all isolates and for the subgroup of hospitalized patients. Out of 13 clinical laboratories reporting their use of AST guidelines, 30.5% applied EUCAST breakpoints.

Highest resistance levels were observed for fluoroquinolones (7.7%), followed by aminoglycosides (including amikacin) (5.1%) and carbapenems (2.6%). Resistance to aminoglycosides other than amikacin (1.5%) and to polymyxins (0.0%) was low.

Resistance rates in the subgroup of hospitalized patients (67.2% of all patients) did not differ much from those in the whole study population, with the exception of resistance to fluoroquinolones being 3.7% higher in isolates originating from hospitalized patients only. However, small sample sizes for this pathogen limit the drawing of meaningful conclusions based on these results.

2.5.3. Trends in resistance, Belgium, 2013-2016

EARS-Net BE started surveillance of *Acinetobacter spp.* in 2013. No significant increasing or decreasing trends could be observed between 2013 and 2016 [12], because the combination of low sample sizes and low resistance levels in this pathogen hampered the detection of trends. The number of hospital laboratories reporting resistance levels of *Acinetobacter spp.* has increased over the past 4 years. Still, we can observe that for several antimicrobial groups, the median resistance percentage is zero, indicating that only a minority of laboratories provided resistant samples.

Table 10 | *Acinetobacter species*: Number of laboratories contributing results on ASTs, mean resistance rates to principal antimicrobial groups, EARS-Net BE 2016

Antimicrobial group	Labs (N)	R samples (N)	Total samples (N)	Mean %R (database)	Mean %R (lab means)	Std. Dev.	p10	p25	p50	p75	p90
All isolates											
Carbapenems	18	2	78	2.6	1.5	4.3	0.0	0.0	0.0	0.0	12.5
Carbapenems, CP+	5	0	0	.	.	.	.	.	.	.	.
Aminoglycosides - Amikacin	15	1	66	1.5	1.7	6.5	0.0	0.0	0.0	0.0	0.0
Aminoglycosides +Amikacin	17	4	78	5.1	5.3	10.6	0.0	0.0	0.0	0.0	25.0
Fluoroquinolones	18	6	78	7.7	9.5	17.3	0.0	0.0	0.0	12.5	50.0
Polymyxins	7	0	37	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup: Isolates from hospitalized patients											
Carbapenems	16	1	44	2.3	1.0	4.2	0.0	0.0	0.0	0.0	0.0
Aminoglycosides - Amikacin	14	1	41	2.4	2.4	8.9	0.0	0.0	0.0	0.0	0.0
Fluoroquinolones	16	5	44	11.4	15.3	28.9	0.0	0.0	0.0	22.2	50.0

CP+: Carbapenemase-production, N: total number, R: resistance, p: percentile, AST: Antimicrobial susceptibility test, Std. Dev.: Standard Deviation

2.6. *Streptococcus pneumoniae*

2.6.1. Clinical importance and epidemiology

S. pneumoniae can cause upper airway infections, such as sinusitis and otitis media, but also bloodstream infections, pneumonia and meningitis. High morbidity and mortality rates are therefore common in infections with this pathogen. It typically affects non-vaccinated infants, elderly people and immunocompromised patients.[1]

2.6.2. National results: resistance rates in Belgium, 2016

Table 11 displays the mean resistance rates of *S. pneumoniae* to principal antimicrobial groups in Belgium in 2016. Data for *S. pneumoniae* were provided by the National Reference Centre (NRC) of the Catholic University Leuven. 97 Belgian laboratories sent their samples to the NRC.[7] For *S. pneumoniae*, non-susceptibility rates are reported (%IR). Interpretation of susceptibility results was done following CLSI guidelines. Except for macrolides (%IR=15.9), non-susceptibility was rare in *S. pneumoniae:* 0.4% of the samples were non-susceptible to penicillins or to third-generation cephalosporins, and 0.2% was non-susceptible to fluoroquinolones.[7]

Table 11 | *Streptococcus pneumoniae*: Number of laboratories contributing results on ASTs, mean non-susceptibility rates (%IR) to principal antimicrobial groups, EARS-Net BE 2016

Antimicrobial group	Labs (N)	IR samples (N)	Total samples (N)	Mean %IR (database)	Mean %IR (lab means)	p10	p25	p50	p75	p90
Penicillins	97	5	1327	0.4	1.2	0.0	0.0	0.0	0.0	0.0
Macrolides	97	211	1327	15.9	20.0	0.0	0.0	12.5	25.0	50.0
Fluoroquinolones	97	2	1327	0.2	0.1	0.0	0.0	0.0	0.0	0.0
Third-generation cephalosporins	97	5	1324	0.4	1.3	0.0	0.0	0.0	0.0	0.0

N: total number, IR: non-susceptibility (intermediate resistant/resistant), p: percentile, AST: Antimicrobial susceptibility test

2.6.3. Trends in resistance 2012-2016

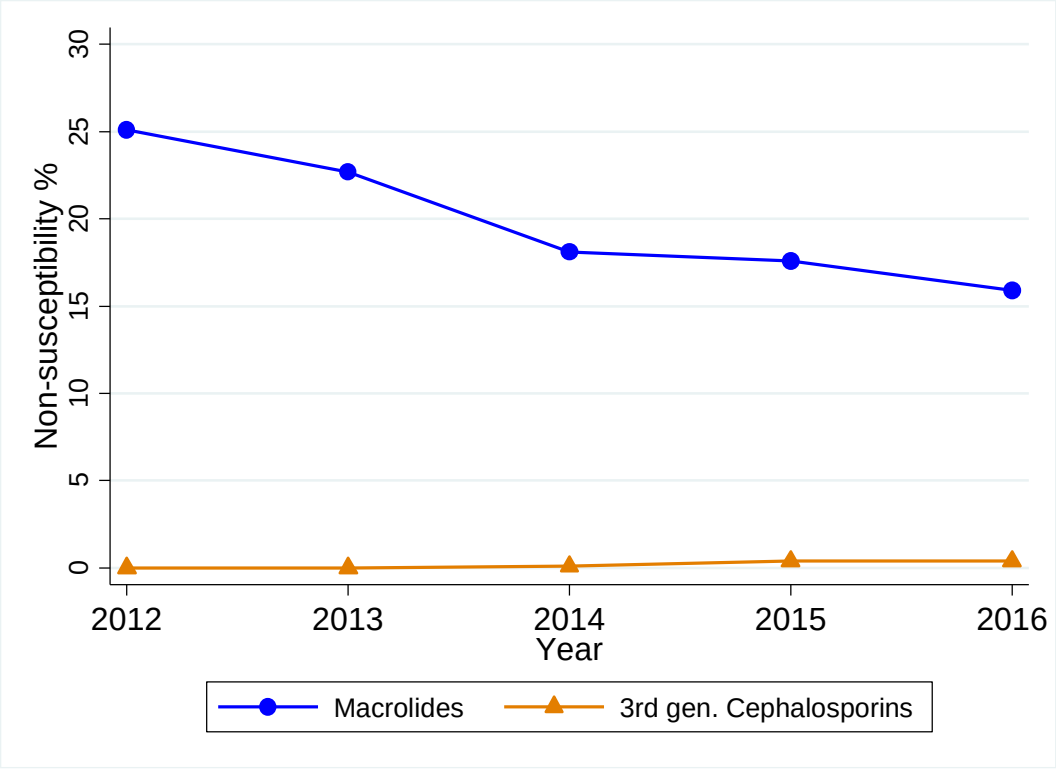
Table 12 and figure 4 display mean resistance rates and trends from 2012 until 2016 for *S. pneumoniae* to principal antimicrobial groups. Over the past 5 years, we could observe a highly significant decreasing trend for non-susceptibility to macrolides. Resistance to macrolides decreased from 25.1% in 2012 to 15.9% in 2016. Non-susceptibility rates for third-gen. cephalosporins underwent an increasing trend that is statistically significant but without clinical relevance, given the very low number of resistant isolates. No meaningful trends could be observed for penicillins and fluoroquinolones.

Table 12 | *Streptococcus pneumoniae*: Mean non-susceptibility rates to principal antimicrobial groups, EARS-Net BE 2012-2016

Antimicrobial group	2012 %IR (#R/N)	2013 %IR (#R/N)	2014 %IR (#R/N)	2015 %IR (#R/N)	2016 %IR (#R/N)	TREND[a]
Penicillins	1.1 (18/1658)	1.2 (18/1536)	0.7 (7/1018)	2.1 (34/1592)	0.4 (5/1327)	
Macrolides	25.1 (418/1662)	22.7 (358/1574)	18.1 (184/1018)	17.6 (284/1611)	15.9 (211/1327)	---
Fluoroquinolones	0.4 (6/1653)	0.4 (6/1580)	0.1 (1/1020)	0.2 (3/1600)	0.2 (2/1327)	
Third-generation Cephalosporins	0.0 (0/1618)	0.0 (0/1460)	0.1 (1/986)	0.4 (6/1577)	0.4 (5/1324)	++

[a] Pearson Chi-squared test for trends: 'plus' signs indicate an increasing trend, 'minus' signs indicate decreasing trend. (+++ or --- indicate p<=0.001, ++ or -- indicate p<=0.01, + or – indicate p<=0.05, (+) or (-) indicate p<=0.10) IR: non-susceptibility (intermediate resistant/resistant), N: total number, #: number

Figure 4 | *Streptococcus pneumoniae*: Trends in non-susceptibility to third-generation cephalosporins and macrolides, EARS-Net BE 2012-2016



Note: Y-axis is not extended beyond 30% resistance, to reduce overlap in trend lines and improve visibility

2.7. *Staphylococcus aureus*

2.7.1. Clinical importance and epidemiology

S. aureus is an opportunistic bacterium. Meticillin-resistant *S. aureus* (MRSA) has been the most important cause of healthcare-associated infections worldwide. MRSA causes prolonged hospital stays and higher mortality, increasing the clinical and economic burden in hospitals.[1]

The 2011 PPS study estimated that 10.7% of HAI were caused by *S. aureus,* making it the 2nd most often isolated pathogen from HAI in Belgium.[2] In Europe, 12.3% of all HAI were caused by *S. aureus.*[3]

2.7.2. National results: resistance rates in Belgium, 2016

Table 13 displays the mean resistance rates of *S. aureus* to principal antimicrobial groups in Belgium in 2016, both for all reported isolates and for the subgroup of hospitalized patients only. Out of 22 clinical laboratories reporting their use of AST guidelines, 61.3% applied EUCAST breakpoints.

In 2016, 12.2% of included *S. aureus* samples were MRSA. MRSA rates of laboratories reporting additional results on detection of mecA-gene and penicillin-binding protein 2a did not differ from regular MRSA rates.[6] Resistance (%R) to fluoroquinolones was 12.7%. Resistance (%R) levels for rifampicin (0.6%), linezolid (0.1%) and vancomycin (0.0%) were low.

Isolates from the subgroup of hospitalized patients (77.4 % of all patients) showed similar resistance rates to the selected antimicrobials in *S. aureus*, although non-susceptibility to meticillin was 1.8% lower in isolates originating from hospitalized patients.

2.7.3. Trends in resistance 2012-2016

Table 14 and figure 5 display trends from 2012 until 2016 of mean resistance rates of *S. aureus* to principal antimicrobial groups. We could observe a significantly decreasing trend for non-susceptibility to meticillin (MRSA) and resistance to fluoroquinolones between 2012 and 2016, but, for MRSA, the decreasing trend until 2015 did not continue in 2016. Non-susceptibility to meticillin decreased (16.5% to 12.2%), but remains substantial. Resistance to fluoroquinolones decreased from 21.7% to 12.7%. While these trends are calculated based on database means, it must be noted that median resistance percentage to meticillin were substantially lower in 2016 than the mean (median=8.3% versus mean=12.2%). This

can be explained by the presence of outliers. A similar difference between mean and median could be observed for fluoroquinolone resistance.

Table 13 | *Staphylococcus aureus*: Number of laboratories contributing results on ASTs, mean resistance rates to principal antimicrobial groups, EARS-Net BE 2016

Antimicrobial group	Labs (N)	R samples (N)	Total samples (N)	Mean %R (database)	Mean %R (lab means)	Std. Dev.	p10	p25	p50	p75	p90
All isolates											
MRSA (IR)	31	166	1364	12.2	11.7	10.7	0.0	3.2	8.3	15.0	28.3
Rifampicin	27	6	1031	0.6	0.5	1.3	0.0	0.0	0.0	0.0	2.5
Fluoroquinolones	31	167	1319	12.7	12.4	9.6	2.7	6.4	10.0	16.7	25.0
Linezolid	27	1	1040	0.1	0.1	0.7	0.0	0.0	0.0	0.0	0.0
Vancomycin	28	0	1118	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup: Isolates from hospitalized patients											
MRSA (IR)	29	100	962	10.4	10.3	10.0	0.0	1.4	9.5	13.9	25.0
Rifampicin	25	3	728	0.4	0.4	1.6	0.0	0.0	0.0	0.0	0.0
Fluoroquinolones	29	117	924	12.7	12.5	9.9	0.0	6.4	10.9	16.7	25.6
Linezolid	25	1	760	0.1	0.2	0.8	0.0	0.0	0.0	0.0	0.0
Vancomycin	26	0	827	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

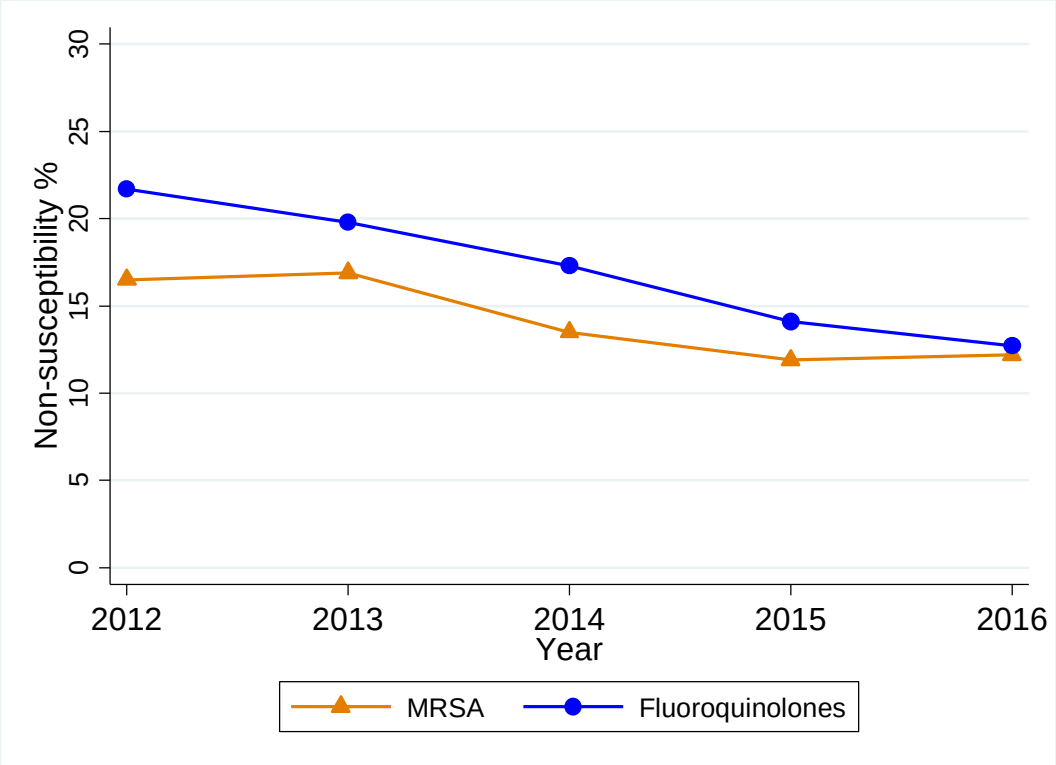
MRSA: *Meticillin-resistant Staphylococcus aureus,* IR: non-susceptibility (intermediate resistant/resistant), N: total number, R: resistant, p: percentile, AST: Antimicrobial susceptibility test, Std. Dev.: Standard Deviation

Table 14 | *Staphylococcus aureus*: Mean resistance rates to principal antimicrobial groups, EARS-Net BE 2012-2016

Antimicrobial group	2012 %R (#R/N)	2013 %R (#R/N)	2014 %R (#R/N)	2015 %R (#R/N)	2016 %R (#R/N)	TREND[a]
MRSA (IR)	16.5 (260/1575)	16.9 (272/1612)	13.5 (130/960)	11.9 (123/1033)	12.2 (167/1364)	---
Rifampicin	0.7 (7/979)	0.3 (4/1193)	0.5 (2/437)	0.2 (1/646)	0.6 (6/1031)	
Fluoroquinolones	21.7 (267/1231)	19.8 (287/1448)	17.3 (142/821)	14.1 (135/956)	12.7 (167/1314)	---
Linezolid	0.2 (2/1150)	0.0 (0/1243)	0.1 (1/712)	0.1 (1/855)	0.1 (1/1034)	
Vancomycin	0.1 (1/754)	0.0 (0/1055)	0.0 (0/367)	0.0 (0/845)	0.0 (0/1118)	

[a] Pearson Chi-squared test for trends: 'plus' signs indicate an increasing trend, 'minus' signs indicate decreasing trend. (+++ or --- indicate p<=0.001, ++ or -- indicate p<=0.01, + or – indicate p<=0.05, (+) or (-) indicate p<=0.10) IR: non-susceptibility (intermediate resistant/resistant), R: resistance, N: total number, #: number

Figure 5 | *Staphylococcus aureus*: Trend in non-susceptibility (%IR) to meticillin (MRSA), and trend in resistance (%R) to fluoroquinolones, EARS-Net BE 2012-2016



Note: Y-axis is not extended beyond 30% resistance, to reduce overlap in trend lines and improve visibility

2.8. Enterococci

2.8.1. Clinical importance and epidemiology

Enterococci are regarded as harmless commensals of our gastrointestinal tract. When this commensal relationship with the host is disrupted, they can however cause invasive diseases, such as endocarditis, bloodstream infections and urinary tract infections. Most clinical enterococcus infections are caused by *E. faecalis* and *E. faecium.*[1]

The 2011 PPS study estimated that 7.8% of HAI were caused by Enterococci, making it the 5th most often isolated pathogen from HAI in Belgium.[2] In Europe, 9.6% of all HAI was caused by Enterococci.[3]

2.8.2. National results: resistance rates in Belgium, 2016

Table 15 and 16 display the mean resistance rates of *E. faecalis* and *E. faecium* to principal antimicrobial groups in Belgium in 2016, both for all reported isolates and for the subgroup of hospitalized patients only. Out of 21 clinical laboratories reporting the use of AST guidelines, 56.8% applied EUCAST breakpoints in *E. faecalis*. Regarding *E. faecium*, 67.7% of 18 laboratories applied EUCAST breakpoints.

High-level gentamicin resistance was common in both *E. faecalis* (19.8%) and *E. faecium* (19.7%). While 30 hospital laboratories reported results on AST in Enterococci, high-level gentamicin resistance was only reported by 23 laboratories in *E. faecalis*, and by 19 laboratories in *E. faecium*. We could also observe large standard deviations, large differences between the database mean resistance percentages, the mean of the laboratory means and the median for this antimicrobial. This can be explained by the use of different methods as well as guidelines for interpretation of ASTs (EUCAST versus CLSI) by the laboratories. For example, isolates with a gentamicin minimum inhibitory concentration (MIC) >128 mg/L are considered resistant according to recent EUCAST guidelines,[13] while they are considered resistant if the MIC > 500 mg/L according to CLSI guidelines.[14]

Resistance to other antimicrobials under surveillance was low. *E. faecalis* showed no resistance to vancomycin and linezolid. Resistance to these antibiotics also remained low in *E. faecium*, with 1.7% of the isolates testing resistant to vancomycin, and 0.5% testing resistant to linezolid.

The hospitalization rate was higher in *E. faecium* (90.2%) than in *E. faecalis* (84.4%). Isolates from this subgroup showed similar resistance rates to the selected antimicrobials, except for high-level gentamicin resistance. This type of resistance was higher in hospitalized patients in both *E. faecalis* (+2.8%) and *E. faecium* (+5.9%).

Table 15 | *Enterococcus faecalis*: Number of laboratories contributing results on ASTs, mean resistance rates to principal antimicrobial groups, EARS-Net BE 2016

Antimicrobial group	Labs (N)	R samples (N)	Total samples (N)	Mean %R (database)	Mean %R (lab means)	Std. Dev.	p10	p25	p50	p75	p90
All isolates											
High-level Gentamicin resistance	23	65	328	19.8	21.1	19.2	0.0	8.3	12.5	33.3	50.0
Vancomycin	30	0	463	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aminopenicillins	30	2	461	0.4	0.4	1.7	0.0	0.0	0.0	0.0	0.0
Teicoplanin	24	1	364	0.3	0.4	2.0	0.0	0.0	0.0	0.0	0.0
Linezolid	23	0	330	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup: Isolates from hospitalized patients											
High-level Gentamicin resistance	21	53	234	22.6	23.9	24.8	0.0	6.3	14.3	40.0	50.0
Vancomycin	28	0	343	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aminopenicillins	28	1	342	0.3	0.4	1.9	0.0	0.0	0.0	0.0	0.0
Teicoplanin	22	1	261	0.4	0.5	2.1	0.0	0.0	0.0	0.0	0.0
Linezolid	22	0	255	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

N: total number, R: resistance, p: percentile, AST: Antimicrobial susceptibility test, Std. Dev.: Standard Deviation

Table 16 | *Enterococcus faecium*: Number of laboratories contributing results on ASTs, mean resistance rates to principal antimicrobial groups, EARS-Net BE 2016

Antimicrobial group	Labs (N)	R samples (N)	Total samples (N)	Mean %R (database)	Mean %R (lab means)	Std. Dev.	p10	p25	p50	p75	p90
All isolates											
High-level Gentamicin resistance	19	42	213	19.7	29.6	29.9	0.0	0.0	17.6	50.0	83.3
Vancomycin	27	5	289	1.7	0.5	1.4	0.0	0.0	0.0	0.0	2.9
Aminopenicillins	27	246	287	85.7	85.9	15.3	66.7	75.0	88.9	100.0	100.0
Teicoplanin	22	3	243	1.2	0.3	1.2	0.0	0.0	0.0	0.0	0.0
Linezolid	22	1	205	0.5	0.1	0.6	0.0	0.0	0.0	0.0	0.0
Subgroup: Isolates from hospitalized patients											
High-level Gentamicin resistance	17	30	117	25.6	31.1	31.5	0.0	0.0	33.3	50.0	83.3
Vancomycin	25	2	186	1.1	0.3	1.1	0.0	0.0	0.0	0.0	0.0
Aminopenicillins	25	155	184	84.2	86.0	16.8	66.7	75.0	90.0	100.0	100.0
Teicoplanin	20	0	145	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Linezolid	20	1	137	0.7	0.2	0.9	0.0	0.0	0.0	0.0	0.0

N: total number, R: resistance, p: percentile, AST: Antimicrobial susceptibility test, Std. Dev.: Standard Deviation

2.8.3. Trends in resistance 2012-2016

Tables 17 and 18 display trends from 2012 until 2016 of mean resistance rates of Enterococci to principal antimicrobial groups. We observed a decreasing trend for both pathogens regarding high-level gentamicin resistance between 2012 and 2016, although this trend should be interpreted with caution, as there is a lot of variability in the data. The low resistance levels to vancomycin and linezolid of both Enterococci remained stable over the years.

Table 17 | *Enterococcus faecalis*: Mean resistance rates to principal antimicrobial groups, EARS-Net BE 2012-2016

Antimicrobial group	2012 %R (#R/N)	2013 %R (#R/N)	2014 %R (#R/N)	2015 %R (#R/N)	2016 %R (#R/N)	TREND[a]
High-level Gentamicin	24.7 (100/405)	27.6 (110/398)	21.7 (35/161)	12.5 (36/287)	19.8 (65/328)	--
Vancomycin	0.2 (1/531)	0.4 (2/498)	0.3 (1/352)	0.2 (1/420)	0.0 (0/463)	
Aminopenicillins	1.4 (7/493)	2.6 (15/573)	0.3 (1/342)	0.5 (2/424)	0.4 (2/461)	--
Teicoplanin	0.6 (2/338)	0.3 (1/358)	0.4 (1/220)	0.4 (1/248)	0.3 (1/364)	
Linezolid	0.0 (0/363)	0.3 (1/309)	0.0 (0/187)	0.0 (0/258)	0.0 (0/330)	

[a] Pearson Chi-squared test for trends: 'plus' signs indicate an increasing trend, 'minus' signs indicate decreasing trend. (+++ or --- indicate p<=0.001, ++ or-- indicate p<=0.01, + or – indicate p<=0.05, (+) or (-) indicate p<=0.10) R: resistance, N: total number, #: number

Table 18 | *Enterococcus faecium*: Mean resistance rates to principal antimicrobial groups, EARS-Net BE 2012-2016

Antimicrobial group	2012 %R (#R/N)	2013 %R (#R/N)	2014 %R (#R/N)	2015 %R (#R/N)	2016 %R (#R/N)	TREND[a]
High-level Gentamicin	38.8 (66/170)	29.1 (48/165)	29.7 (30/101)	31.4 (38/121)	19.7 (42/213)	--
Vancomycin	1.4 (3/215)	1.7 (4/235)	3.1 (6/192)	1.6 (3/189)	1.7 (5/289)	
Aminopenicillins	77.9 (155/199)	73.8 (254/344)	84.7 (161/190)	82.3 (153/186)	85.7 (246/287)	
Teicoplanin	2.9 (4/137)	3.5 (5/144)	1.8 (2/110)	1.7 (2/117)	1.2 (3/243)	
Linezolid	0.0 (0/163)	0.7 (1/144)	0.0 (0/89)	0.0 (0/105)	0.5 (1/205)	

[a] Pearson Chi-squared test for trends: 'plus' signs indicate an increasing trend, 'minus' signs indicate decreasing trend. (+++ or --- indicate p<=0.001, ++ or -- indicate p<=0.01, + or – indicate p<=0.05, (+) or (-) indicate p<=0.10) R: resistance, N: total number, #: number

3. Discussion and conclusions

In *E. coli*, resistance to third-generation cephalosporins increased from 6.9% in 2012 to 10.5% in 2016. The increasing trend for fluoroquinolone resistance in this pathogen from 22.2% in 2012 up to 27.3% in 2015 did not continue in 2016 (24.5%). A moderate increasing trend could also be observed for aminoglycosides, increasing from 7.3% in 2012 up to 8.4% in 2016.

Noteworthy is the further increase of resistance in *Klebsiella pneumoniae* to third-generation cephalosporins and to carbapenems, resulting in mean resistance percentages of 22.9% and 2.4%, respectively. This is in line with similar observations in other countries.[1] Laboratories that submitted results on the detection of carbapenemase, collected for the first time in 2016, reported a majority [91%(10/11)] of carbapenem-resistant *K. pneumoniae* isolates being carbapenemase-positive. However, the low number of tested isolates and laboratories submitting results indicates that this percentage should be interpreted with caution. Carbapenemase can hydrolyze most beta-lactams, meaning that treatment options are limited to few alternative (often more toxic) agents retaining activity, such as colistin, tigecycline, fosfomycin and gentamicin.[1] Carbapenems have been widely used in many countries due to the increasing rate of ESBL-producing *Enterobacteriaceae*, resulting in the emergence of resistance. Information on carbapenemase in the EU remains limited, but evidence suggests that it continues to spread across European member states.[1] The percentage of resistance to colistin, often required when beta-lactams are no longer active, remains low.

P. aeruginosa showed resistance to almost all antimicrobial groups. This is a concern, as *P. aeruginosa* is intrinsically resistant to a number of antimicrobial groups and any additionally acquired resistance could severely limit the therapeutic options.[1] None of the observed decreasing resistance trends in this pathogen were statistically significant. The actual number of laboratories reporting results on *P. aeruginosa* isolates might be too low however to detect accurate trends for these levels of resistance. A similar problem concerning low sample sizes could be observed for *Acinetobacter spp*. An increase in participation level of Belgian hospital laboratories reporting to EARS-Net can overcome this problem, and should therefore be encouraged. As long as the sample sizes for these pathogens are low, the results should be interpreted with caution, and the analysis of trends remains difficult.

The decreasing trend between 2012 and 2015 for meticillin non-susceptibility of *S. aureus* (MRSA) stabilized in 2016 around 12.2%, which means MRSA is still common. Resistance of *S. aureus* to fluoroquinolones decreased from 21.7% in 2012 down to 12.7% in 2016.

E. faecalis showed no resistance to vancomycin. While vancomycin resistance also remained low for *E. faecium* (1.7%) in Belgium, a significant increase in vancomycin resistance was observed for this pathogen in 12 of 26 participating EU countries in 2015. The population-weighted mean in the EU reached 8.3% that year, indicating a potential change in epidemiology of vancomycin-resistant *E. faecium* in Europe.[1] Resistance to

linezolid remained very low in both Enterococci, with none of the *E. faecalis*, and 0.5% of *E. faecium* isolates testing resistant.

The following limitations have to be taken into account when interpreting this report's results. The use of different guidelines (e.g. EUCAST versus CLSI) between laboratories reporting to EARS-Net BE results in increased variability in the reported resistance levels.[13,15] In order to reduce this variability and to increase the precision of our estimates, we encourage the reporting of the quantitative AST data (e.g. actual MIC values or Disc diffusion test values) in addition to the reporting of S/I/R categories.

We also recommend a further standardisation of data definitions and harmonisation with other national surveillances on AMR within BE. This is strongly needed to increase the internal validation of surveillance results, and to reduce the workload in both the hospitals and also at the WIV-ISP. Because laboratories submit data to EARS-Net BE on a voluntary basis, such harmonisation can also lead to increased participation to this surveillance.

Laboratories in Belgium report to EARS-Net by annual extraction of electronic data from their laboratory database. Such electronic surveillance asks for careful standardization of the info that encodes antimicrobial tests, the results of these, and characteristics of isolates and patients. Because errors in this standardisation process will lead to erroneous results, we have implemented from this year on so-called *laboratory-specific codebooks*, which document how each laboratory's specific nomenclature corresponds with EARS-Net BE codification. By including these codebooks in the annual laboratory report, it is clear that their validation by the laboratory is an essential step in obtaining correct results. From next year on, we intend to extend these codebooks with variables encoding patient and unit types for specific specialties (for example intensive care), such that results for more specific patient types can be reported.

A limitation of the EARS-Net surveillance that is harder to assess, is the potential for differential sampling in the hospitals. Differential sampling can occur if blood cultures are typically solely performed after an unsuccessful empirical treatment.[1] It is essentially against current guidelines to start empirical treatment for a suspected blood stream infection without taking a blood culture first,[16] so the potential bias resulting from differential sampling in this surveillance will probably be small. Theoretically, differential sampling would lead to an overestimation of the resistance percentage since isolates with acquired resistance have more chance to be included. The resulting selection bias may differ from hospital to hospital and from ward to ward, depending among others on clinical activities and local susceptibility profiles.[1] Some measures to reduce this type of bias are already taken: EARS-Net not only includes samples from hospitalized, but also from non-hospitalized patients. Those patients are often hospitalized at a later stage, but in the de-duplication phase of data management, only the first sample originating from a patient is retained. In order to assess the magnitude of bias due to differential sampling, insight is needed into the timing of blood/CSF culture sampling and how it relates to timing of antimicrobial use.

Finally, surveillance of AMR in itself does not control infection. It is only one of the necessary measures required to tackle resistance. Reduced and more appropriate use of antimicrobial agents, infection prevention and control, and development of new effective antimicrobial agents or alternatives for treatment are as important. The problem of AMR calls for concerted efforts at all health care levels, both nationally as at a European level.[1]

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Annex

Glossary of hospital types

The categorization of hospital types is based on the definitions provided by ECDC[17]:

Primary

Often referred to as ‘district hospital’ or ‘first-level referral’

- Few specialties (mainly internal medicine, obstetrics-gynecology, pediatrics, general surgery or only general practice)
- Limited laboratory services are available for general, but not for specialized pathological analysis
- Often corresponds to general hospital without teaching function

Secondary

Often referred to as ‘provincial hospital’

- Hospital is highly differentiated by function with five to ten clinical specialties, such as hematology, oncology, nephrology, ICU
- Takes some referrals from other (primary) hospitals
- Often corresponds to general hospital with teaching function

Tertiary

- Often referred to as ‘central’, ‘regional’ or ‘tertiary-level’ hospital
- Highly specialized staff and technical equipment (ICU, hematology, transplantation, cardio-thoracic surgery, neurosurgery)
- Clinical services are highly differentiated by function
- Specialized imaging units
- Provides regional services and regularly takes referrals from other (primary and secondary) hospitals
- Often a university hospital or associated to a university

Specialized hospital

- Single clinical specialty, possibly with sub-specialties
- Highly specialized staff and technical equipment