EUROPEAN ANTIMICROBIAL RESISTANCE SURVEILLANCE FOR BELGIUM (EARS-BE)

Report 2018

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healthy all life long

WHO WE ARE

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SCIENSANO can count on more than 700 staff members who commit themselves, day after day, to achieving our motto: Healthy all life long. As our name suggests, science and health are central to our mission. Sciensano's strength and uniqueness lie within the holistic and multidisciplinary approach to health. More particularly we focus on the close and indissoluble interconnection between human and animal health and their environment (the "One health" concept). By combining different research perspectives within this framework, Sciensano contributes in a unique way to everybody's health.

For this, Sciensano builds on the more than 100 years of scientific expertise of the former Veterinary and Agrochemical Research Centre (CODA-CERVA) and the ex-Scientific Institute of Public Health (WIV-ISP).

Sciensano

Epidemiology and public health - Healthcare-associated infections and antimicrobial resistance

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EXECUTIVE SUMMARY

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Background

Antimicrobial resistance (AMR) is the ability of a microorganism to resist the action of one or more medications intended to be used against them. The European Antimicrobial Resistance Surveillance Network (EARS-Net) monitors the evolution of *acquired* antimicrobial resistance in invasive bacterial isolates at the European level. Sciensano is the focal contact point for Belgium (BE), and collects data from the clinical laboratories through its national surveillance EARS-BE. EARS-BE differs from EARS-Net by the additional collection of data on Antimicrobial Susceptibility Tests (ASTs) on isolates from urine (next to blood and cerebrospinal fluid (CSF)). EARS-BE 2018 data on blood/CSF isolates were submitted in August 2019 to ECDC for inclusion in the Annual European report on antimicrobial resistance[1]; the ECDC report's results for Belgium correspond directly to the results presented here.

Material and Methods

Surveillance results are collected retrospectively (previous year) on a voluntary basis. Laboratories retrieve their annual surveillance data by extraction from the local database and send the data file to Sciensano. The results of the AST used to calculate the reported resistance percentages is based on the final interpretation of the laboratory. The detailed methodology of EARS-BE 2018 can be found in the latest version of the EARS-BE protocol[2].

Results

In 2018, 32 (31 hospital labs) and 34 (21 hospital labs) laboratories voluntarily reported results on AST on isolates from blood/CSF and for urine samples, respectively. For *Streptococcus pneumoniae* isolated from blood/CSF, additional national AST data from 88 laboratories were provided by the National Reference Centre (NRC) at the Catholic University of Leuven (KUL), among which 25 laboratories submitted results in both databases. The use of EUCAST breakpoints has increased over the years. In 2018, more than 90% of the participating laboratories used EUCAST for both sample types.

Blood/CSF samples

In 2018, 8.9% of all tested *Staphylococcus aureus* samples were resistant to methicillin (MRSA) and 10.2% were resistant to fluoroquinolones. For both antimicrobial groups, we could observe a decreasing trend between 2014 and 2018. For MRSA, the decreasing trend up to 2017 did not continue in 2018.
In *Streptococcus pneumoniae* isolates, according to the NRC database, except from macrolides for which 15.2% of tested isolates were resistant in 2018, resistance was rare : no resistance was detected against third-generation cephalosporins, 0.1% were resistant to fluoroquinolones and 0.1% of the samples were non-susceptible to penicillins.
In general in enterococci, higher resistance levels were observed in *Enterococcus faecium* compared to *Enterococcus faecalis*. 0.3% of *E. faecalis* and 1.8% of *E. faecium* isolates were found resistant to vancomycin. Resistance to linezolid remained very low in both enterococci, with 0.7% of the *E. faecalis*, and 0.4% of *E. faecium* isolates tested as resistant.
Over the past two years, an increasing trend has been observed in the amoxicillin-clavulanic acid resistance for both *Escherichia coli* and *Klebsiella pneumoniae*, leading to countrywide resistance percentages in 2018 of 39.4% and 33.4%, respectively. We observed a significant decrease in antimicrobial resistance for *E. coli* to fluoroquinolones from 26.9% in 2014 to 21.8% in 2018 and to aminoglycosides from 9.0% in 2014 to 7.4% in 2018. By contrast, a moderate increase in fluoroquinolone resistance was noted for *K. pneumoniae*.

Extended-spectrum beta-lactamase (ESBL) production was common for both pathogens and detected in 94.6% and 82.9% of the additionally tested samples that were resistant to third-generation cephalosporins for *E.coli* and *K. pneumoniae* respectively. Moreover, multi-drug resistance was not rare in both these pathogens with respectively 9.3% and 10.1% of *E. coli* and *K.pneumoniae* isolates being resistant to at least three antimicrobial groups under surveillance (aminopenicillins (only for *E. coli*), fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides and carbapenems).

Pseudomonas aeruginosa showed resistance to almost all antimicrobial groups under surveillance. The predominant resistance in *P. aeruginosa* was to fluoroquinolones (14%), followed by resistance to piperacillin-tazobactam (10%), aminoglycosides (8.4%), ceftazidime (7.5%) and carbapenems (7.4%). 5.5% of *P. aeruginosa* isolates were resistant to at least three antimicrobial groups under surveillance (piperacillin-tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems).

The highest resistance levels in *Acinetobacter species* were observed to fluoroquinolones (12.7%), followed by aminoglycosides (7.4%) and carbapenems (3.8%). However, given the low number of isolates included in this study, it remains difficult to obtain precise estimation of resistance prevalence within this species at a national level.

Urine samples

In 2018, EARS-BE also collected for the second year results from urine isolates allowing the extension of the study to non-hospital settings and a larger panel of infections. The overall resistance level for urine isolates from hospital settings followed more or less those of blood/CSF isolates. Combined AMR levels of urine isolates from hospital laboratories in 2018 were only slightly lower than blood/CSF for *E. coli* and *K. pneumoniae*, while those of *P. aeruginosa* were very similar between the two sample types. When restricting the analysis to the group of hospitalized patients, differences for *E. coli* and *K. pneumoniae* largely disappeared, while for *P. aeruginosa*, higher rates of resistance are observed in urine compared to blood/CSF samples. In general lower resistance rates were detected in urine samples from non-hospital settings in comparison to those from hospital laboratories. Of note, for some pathogens and antibiotics, consistent higher resistance rates were detected in urine isolates from non-hospital settings in comparison to hospital-settings both in 2017 and 2018 : trimethoprim-sulfamethoxazole (37.3% vs 32.8% in 2018) and fosfomycin (30.0% vs 25.9% in 2018) resistance in *Proteus mirabilis* isolates as well as fluoroquinolone resistance (16.9% vs 15.1% in 2018) in *P. aeruginosa* urine isolates.

Conclusions

The EARS-BE project aims at long-term standardised monitoring of AMR for Belgium and as such contributes to monitor the situation in Europe. By including non-invasive urine samples, the surveillance gives an opportunity to extend the surveillance setting to the community beyond the acute hospital health care sector. In order to further improve this surveillance, harmonization with other national surveillances as well as additional incentives for non-participating laboratories and partners involved in this surveillance are needed.

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ABBREVIATIONS

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AMC	Amoxicillin-clavulanic acid
AMC_UC	Amoxicillin-clavulanic acid breakpoint: for uncomplicated urinary tract infections
AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility test
BE	Belgium
CLSI	Clinical and Laboratory Standards Institute (USA)
CP	Carbapenemase-production
CSF	Cerebrospinal fluid
ColRE	colistin resistance *Escherichia coli*
CRE	Carbapenem-resistant enterobacteriaceae
Dbm	Database Mean
EARS-BE	European Antimicrobial Resistance Surveillance Network for Belgium
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control (Stockholm, Sweden)
EEA	European Economic Area
ESBL	Extended-Spectrum Beta-Lactamase
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing (EU)
H	Hospital settings
HABSI	Hospital-Acquired Bloodstream Infections
HAI	Healthcare-associated infection
I/R	Intermediary resistant / Resistant
KUL	Katholieke Universiteit Leuven
MIC	Minimum inhibitory concentration
Mlm	Mean of the laboratory mean resistances
MRSA	Methicillin-resistant *Staphylococcus aureus*
NH	Non-hospital settings
NRC	National Reference Centre
NSIH	National Surveillance of Infections in Hospitals (Belgium)
PBP	Penicillin Binding Protein
PEN_MENI	penicillin meningitis
PEN_NMEN	penicillin non-meningitis
PPS	Point prevalence survey of healthcare-associated infections and antimicrobial use
RIVM	Dutch National Institute for Public Health and the Environment
VRE	Vancomycin Resistant Enterococci

INTRODUCTION

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Acquired antimicrobial resistance (AMR) occurs when a particular microorganism develops the ability to resist the activity of one or several antimicrobial agents to which it used to be susceptible. AMR is now one of the most serious health threats around the world. The main factors leading to the emergence and spread of AMR are

(1) mis- and over-use of antibiotics exerting selective pressure on bacterial population, killing susceptible bacteria but allowing resistant microorganisms to survive and multiply.

(2) poor infection prevention and control practices allowing the transmission of antimicrobial-resistant microorganisms between humans, animals and the environment.

The European Antimicrobial Resistance Surveillance Network (EARS-Net), founded in 1998 by the Dutch National Institute for Public Health and the Environment (RIVM) and coordinated by the European Centre for Disease Prevention and Control (ECDC) since 2010, is the main surveillance system for AMR in bacteria that cause serious infections in the European Union (EU).

The objectives of EARS-Net[3] are to :

- collect comparable, representative and accurate AMR data
- analyse temporal and spatial trends of AMR in Europe
- provide timely AMR data for policy decisions
- encourage the implementation, maintenance and improvement of national AMR surveillance programmes; and
- support national systems in their efforts to improve diagnostic accuracy by offering an annual external quality assessment.

Sciensano coordinates the Belgian branch of EARS-Net (EARS-BE), through close collaboration with the hospital and dedicated national reference laboratories, whose time and efforts should be acknowledged.

This report describes the results from the Belgian data collection (EARS-BE) for 2018.

METHODS

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EARS-Net performs AMR surveillance for the following bacterial pathogens: *Staphylococcus (S.) aureus*, *Streptococcus (S.) pneumoniae*, *Enterococcus (E.) faecalis*, *Enterococcus (E.) faecium*, *Escherichia (E.) coli*, *Klebsiella (K.) pneumoniae*, *Pseudomonas (P.) aeruginosa* and *Acinetobacter* species. In order to prevent potential inconsistencies in the data analysis, EARS-Net data are based on invasive isolates only (blood or cerebrospinal fluid)[3].

EARS-BE differs from EARS-Net in three major points :
- (1) the additional collection of antimicrobial susceptibility tests (AST) on isolates collected from urine samples (next to blood/CSF samples);
- (2) the inclusion of the pathogen *Proteus (P.) mirabilis*, a frequent causative pathogen of community-acquired urinary tract infections[4];
- (3) the distinction between all *Acinetobacter species* and the pathogen *Acinetobacter (A.) baumannii* (complex), the predominant species of the genus comprised in the ESKAPE pathogens commonly associated with antimicrobial resistance[5].

Table 1 describes the microorganism and antimicrobial group combinations under EARS-BE surveillance. Rationale and modalities for data collection can be found in the **EARS-BE protocol for 2018 data**, dated from December 2018[2]. This protocol describes in detail case definitions and inclusion criteria, data definitions, submitting and reporting procedures, data management and validation.

Participation to this surveillance is voluntary. Laboratories retrieve their annual surveillance data by extraction from the local (laboratory) database and sending the data file to Sciensano. 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. EARS-BE encourages the use of European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, but results based on other interpretive criteria are accepted for the analysis.

In addition to results received from individual laboratories, for invasive *S. pneumoniae* isolates, EARS-BE also collects data from the national surveillance of Pneumococci, organised by the National Reference Centre (NRC) of the Catholic University Leuven (KUL). The NRC receives isolates from microbiology laboratories across Belgium. The results derived from these two different sources (individual laboratories and NRC) are presented in this report for *S. pneumoniae*, while only the results obtained from the NRC are submitted to ECDC for inclusion in the EARS-Net report.

EARS-BE data are collected up to the individual AST level (e.g. Oxacillin, Penicillin, (Table 1, 3rd column)). For each laboratory, sample type (blood/CSF versus urine) and pathogen, de-duplication of annual laboratory data proceeds as follows:
- (1) All tests results are aggregated within the same isolate, patient and AST, prioritizing test results according to the resistant, intermediate, or susceptible result (R>I>S);
- (2) In case of multiple samples on the same date for the same patient, results from CSF samples are prioritized over blood cultures;
- (3) For each patient, results on the first occurring specimen within the study year are kept.

In accordance with EARS-Net reporting, results are then aggregated at the level of antimicrobial group (e.g. Penicillins (Table 1, 2nd column)). Resistance rates (%R) are reported, except for penicillin within *S. pneumoniae* isolates, for which non-susceptibility ((%IR, intermediary resistant (currently susceptible with increased exposure) + resistant) is reported.

Next to the AST, we collect since three years supplementary information on confirmation tests for selected pathogens. These are tests for detection of extended-spectrum beta-lactamase (ESBL, for *E. coli*, *K. pneumoniae and P. mirabilis isolates*) and for detection of carbapenemase production (CP, for *E. coli, K. pneumoniae, P. aeruginosa and Acinetobacter spp* isolates).

The **EARS-BE statistical report 2018**[6] shows the complete reference data as collected and compiled by Sciensano. A guide for the interpretation of the EARS-BE laboratory report is available online on the NSIH website[7]. This report contains all EARS-BE 2018 results, including indicators on laboratory, samples and patients characteristics, as well as antimicrobial resistance results for included pathogens and sample types. For each antimicrobial group, the number of reporting laboratories, the percentage of tested isolates, the percentage of tests interpreted according to EUCAST and the percentage of resistance are reported.

Furthermore, the statistical report presents results for isolates obtained from blood/CSF side-by-side with those from urine samples, and this for following sets of inclusion criteria and subgroups:
(1) general EARS-BE inclusion criteria, as defined in the surveillance protocol;
(2) (1), but for hospital laboratories only;
(3) (1), but for hospital laboratories and EUCAST-interpreted ASTs only;
(4) (1), but for non-hospital laboratories only;
(5) (1), but for hospitalized patients only.

This **EARS-BE descriptive report 2018** describes the main results obtained from the 2018 data collection. The first chapter describes the participation data for both sample types (blood/CSF and urine). Following chapters display the resistance rates obtained for the main antibiotics groups for each included pathogen. In each section, the first part summarizes the national results obtained for invasive samples including 5-year trends for general EARS-BE inclusion criteria. The second part describes data from urinary samples. This part focuses on two comparisons : invasive (blood/CSF) versus urinary samples from hospital laboratories and hospitals versus non-hospital labs data on urinary samples. Two additional parts focuses on colistin resistance within *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates and on combined resistance indicators.

Table 1 • Microorganism, specimen, and main antimicrobial group combinations under EARS-BE surveillance 2018

Specimen source	Antimicrobial group	Antimicrobial test
Streptococcus pneumoniae		
Blood Cerebrospinal fluid	Penicillins	Oxacillin, Penicillin
	Macrolides	Azithromycin, Clarithromycin, Erythromycin
	Fluoroquinolones	Levofloxacin, Moxifloxacin, Norfloxacin
	Third-gen. Cephalosporins	Cefotaxime, Ceftriaxone
Staphylococcus aureus		
Blood	MRSA	Cefoxitin, Cloxacillin, Dicloxacillin, Flucloxacillin,, Methicillin, Oxacillin
	Fluoroquinolones	Ciprofloxacin, Levofloxacin, Norfloxacin, Ofloxacin
	Rifampicin	Rifampicin
	Oxazolidinones	Linezolid
	Glycopeptides	Vancomycin
Enterococcus faecalis & *Enterococcus faecium*		
Blood Urine	Aminopenicillins	Ampicillin, Amoxicillin
	Co-trimoxazole	Trimethoprim-sulfamethoxazole
	Fosfomycin	Fosfomycin
	Glycopeptides	Teicoplanin
	Glycopeptides	Vancomycin
	High-level aminoglycoside resistance	Gentamicin-High
	Nitrofurans	Nitrofurantoin (Urine samples only)
	Oxazolidinones	Linezolid
Escherichia coli & *Klebsiella pneumoniae*		
Blood Cerebrospinal fluid Urine	Aminopenicillins (only for *E. coli*)	Amoxicillin, Ampicillin
	Carboxypenicillins	Temocillin
	Penicillin + β-lactamase inhibitor	Amoxicillin-clavulanic acid
	Penicillin + β-lactamase inhibitor	Piperacillin-tazobactam
	Aminoglycosides (+Amikacin)	Gentamicin, Tobramycin, (+Amikacin)
	Fluoroquinolones	Ciprofloxacin, Ofloxacin, Levofloxacin, Moxifloxacin, Norfloxacin
	Fosfomycin	Fosfomycin
	Second-gen. Cephalosporins	Cefuroxime
	Third-gen. Cephalosporins (ESBL+)	Cefotaxime, Ceftriaxone, Ceftazidime (,Extended Spectrum β-Lactamase)
	Fourth-gen. Cephalosporins	Cefepime
	Carbapenems (CP+)	Imipenem, Meropenem (Carbapenemases) (+Ertapenem)
	Trimethoprim	Trimethoprim (Urine samples only)
	Co-trimoxazole	Trimethoprim-sulfamethoxazole
	Nitrofurans	Nitrofurantoin (Urine samples only)
	Polymyxins	Polymyxin B, Colistin
	Tetracyclines	Tigecycline
Proteus mirabilis		
Urine	Aminopenicillins	Amoxicillin, Ampicillin
	Carboxypenicillins	Temocillin
	Penicillin + β-lactamase inhibitor	Amoxicillin-clavulanic acid
	Penicillin + β-lactamase inhibitor	Piperacillin-tazobactam
	Aminoglycosides (+Amikacin)	Gentamicin, Tobramycin (+Amikacin)
	Fluoroquinolones	Ciprofloxacin, Ofloxacin, Levofloxacin, Moxifloxacin, Norfloxacin
	Fosfomycine	Fosfomycine
	Second-gen. Cephalosporins	Cefuroxime
	Third-gen. Cephalosporins (ESBL+)	Cefotaxime, Ceftriaxone, Ceftazidime (,Extended Spectrum Beta Lactamase)
	Carbapenems (CP+)	Imipenem, Meropenem (Carbapenemases) (+Ertapenem)
	Trimethoprim	Trimethoprim
	Co-trimoxazole	Trimethoprim-sulfamethoxazole

Specimen source	Antimicrobial group	Antimicrobial test
Pseudomonas aeruginosa		
Blood Cerebrospinal fluid Urine	Acylureidopenicillins	Piperacillin
	Piperacillin-tazobactam	Piperacillin-tazobactam
	Third-gen. Cephalosporins	Ceftazidime
	Forth-gen. Cephalosporins	Cefepime
	Fluoroquinolones	Ciprofloxacin, Levofloxacin
	Aminoglycosides (+Amikacin)	Gentamicin, Tobramycin (+Amikacin)
	Carbapenems (CPE+)	Imipenem, Meropenem (Carbapenemases)
	Polymyxins	Polymyxin B, Colistin
Acinetobacter spp.		
Blood Cerebrospinal fluid	Fluoroquinolones	Ciprofloxacin, Levofloxacin
	Aminoglycosides (+Amikacin)	Gentamicin, Tobramycin (+Amikacin)
	Carbapenems (CPE+)	Imipenem, Meropenem (Carbapenemases)
	Co-trimoxazole	Trimethoprim-sulfamethoxazole
	Polymyxins	Polymyxin B, Colistin

Gen: generation, ESBL: extended spectrum beta-lactamase, CP: carbapenemase producing enterobacteriaceae

RESULTS

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1. Participation

1.1. BLOOD/CSF ISOLATES

Table 2 displays the number of hospital laboratories reporting at least one isolate from blood/CSF to EARS-BE from 2007 to 2018. In addition to these, one non-hospital laboratory submitted results on a single *E coli* blood isolate in 2017 as well as in 2018. In total, twenty-eight labs (87.5%) continued their participation of 2017.

Except for *S. pneumoniae,* for which the participation rate is calculated with respect to all laboratories for microbiology in Belgium, participation rates were calculated with regard to hospital laboratories only.

Table 2 • Number of hospital laboratories reporting at least one blood/CSF isolate for the European Antimicrobial Resistance Surveillance for Belgium (EARS-BE), 2007-2018 (% participation)

Year	*S. aureus*	*S. pneumoniae*	*E. faecalis*	*E. faecium*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*Acinetobacter spp.*
2007	34/108 (31%)	34/149 (23%)	20/108 (19%)	20/108 (19%)	17/108 (16%)	-	-	-
2008	38/107 (36%)	97/149 (65%)	19/107 (18%)	19/107 (18%)	16/107 (15%)	-	-	-
2009	34/108 (31%)	98/149 (66%)	14/108 (13%)	14/108 (13%)	18/108 (17%)	8/108 (7%)	8/108 (7%)	-
2010	40/108 (37%)	94/149 (63%)	22/108 (20%)	22/108 (20%)	23/108 (21%)	14/108 (13%)	15/108 (14%)	-
2011	50/107 (47%)	89/148 (60%)	46/107 (43%)	46/107 (43%)	43/107 (40%)	44/107 (41%)	43/107 (40%)	-
2012	44/107 (41%)	93/147 (63%)	41/107 (38%)	41/107 (38%)	41/107 (38%)	41/107 (38%)	40/107 (37%)	
2013	41/106 (39%)	92/147 (62%)	39/106 (37%)	39/106 (37%)	41/106 (39%)	41/106 (37%)	40/106 (37%)	2/106 (2%)
2014	27/105 (26%)	96/146 (66%)	25/105 (24%)	25/105 (24%)	27/105 (26%)	26/105 (25%)	27/105 (26%)	3/105 (3%)
2015	25/102 (24%)	89/142 (63%)	25/102 (24%)	25/102 (24%)	25/102 (24%)	24/102 (23%)	25/102 (24%)	8/102 (8%)
2016	31/102 (30%)	97/139 (70%)	30/102 (29%)	30/102 (29%)	31/102 (30%)	28/102 (27%)	31/102 (30%)	18/102 (18%)
2017	30/102 (29%)	92/139 (66%)	31/102 (30%)	30/102 (29%)	31/102 (30%)	31/102 (30%)	31/102 (30%)	30/102 (20%)
2018	31/102 (30%)	88/138 (64%)	31/102 (30%)	30/102 (29%)	31/102 (30%)	31/102 (30%)	30/102 (29%)	26/102 (25%)

Percentages were calculated by dividing the number of reporting hospital 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: Sciensano, Department of Quality of Medical Laboratories); *S. pneumoniae: Streptococcus pneumoniae, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis*, *E. faecium: Enterococcus faecium, K. pneumoniae: Klebsiella pneumoniae*, *P. aeruginosa: Pseudomonas aeruginosa*.

The number of laboratories participating to EARS-BE declined between 2011 and 2015, but slightly increased in 2016. Since 2016, the participation rate stabilized and about one third of the Belgian hospital laboratories participate to EARS-BE. Due to the low prevalence of *Acinetobacter spp*. as pathogens responsible of bloodstream infections, the participation rate for this pathogen is a bit lower (25%).

Thanks to the longstanding and very exhaustive national surveillance programme of pneumococcal infections organized by the NRC at the KUL, the participation rate for *S. pneumoniae* in 2019 was much higher and reached 64% (88 out of 138 clinical laboratories in Belgium).

Table 3 displays the number of hospital laboratories reporting to EARS-BE per region and per hospital care type in Belgium in 2018. The participating laboratories were homogeneously distributed in the different regions of the country. However, per hospital care types, secondary hospitals were over-represented (67%) as compared to primary and tertiary hospitals (24% and 29%).

Table 3 • Number of hospital laboratories reporting to EARS-BE 2018 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	19	56	34%
Walloon region	10	36	28%
Hospital types [a]			
Primary	19	79	24%
Secondary	10	15	67%
Tertiary	2	7	29%
Specialized	0	1	0%
TOTAL	**31**	**102**	**30%**

1.2. URINE ISOLATES

In 2017, EARS-BE included for the first time susceptibility data on isolates from urine samples. This strategy aimed at enlarging the coverage of this surveillance to non-hospital-based laboratories and obtaining a larger picture of AMR within clinical samples originating from the community setting. **Table 4** shows the number of hospital and non-hospital laboratories reporting at least one isolate from urine to EARS-BE in 2017 and 2018.

Table 4 • Number of laboratories reporting at least one urine isolate for the European Antimicrobial Resistance Surveillance for Belgium (EARS-BE), 2017-2018 (%participation)

Year	Laboratory type	E. faecalis	E. faecium	E. coli	K. pneumoniae	P. mirabilis	P. aeruginosa
2017	**Hospital**	19/102 (19%)	19/102 (19%)	19/102 (19%)	19/102 (19%)	17/102 (17%)	19/102 (19%)
	Non Hospital	5/37 (14%)	4/37 (11%)	5/37 (14%)	5/37 (14%)	5/37 (14%)	4/37 (11%)
2018	**Hospital**	23/102 (23%)	23/102 (23%)	23/102 (23%)	23/102 (23%)	22/102 (22%)	23/102 (23%)
	Non Hospital	11/37 (30%)	10/37 (27%)	11/37 (30%)	11/37 (30%)	11/37 (30%)	10/37 (27%)

E. faecalis: Enterococcus faecalis, E. faecium: Enterococcus faecium, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. mirabilis: Proteus mirabilis, P. aeruginosa: Pseudomonas aeruginosa

In total, thirty-four labs submitted results for urine samples taken in 2018, 11 of these were not associated to an acute care hospital. The number of participating non-hospital laboratories has doubled between 2017 and 2018. The number of participating hospital laboratories did also slightly increase but remains lower than the number of hospitals submitting results for blood/CSF isolates. Possible explanations could be the optional and the recent inclusion of this sample type in the surveillance requiring the implementation of new extraction procedures. Moreover, the workload to submit these additional data cannot be underestimated, its registration burden being much higher as compared to blood/CSF samples.

The location and distribution of laboratories submitting results on urine isolates is represented in **table 5**. While participating hospital labs are well spread across the three regions of Belgium, the coverage of non-hospital labs is limited. All 5 non-hospital participating laboratories in 2017 were located in the north of the country. The geographical coverage was improved in 2018 by including 6 other non-hospital labs (2 in Flanders and 4 in Wallonia).

Table 5 • Number of laboratories reporting at least one urine isolate to EARS-BE 2017 and 2018 per laboratory type and per region

	2017		2018	
	Hospital labs	Non-hospital labs	Hospital labs	Non-hospital labs
Brussels Capital Region	3	0	3	0
Flanders	8	5	10	7
Walloon region	8	0	7	4
TOTAL	19	5	23	11

1.3. USE OF EUCAST GUIDELINES

In 2018, 29 (91%) labs reported the use of EUCAST guidelines for interpretation of ASTs on blood/CSF isolates. This represents an increase from the 57% and the 75% reported in 2016 and 2017 respectively. A similar increase was observed for labs submitting results on urine isolates: out of the 23 participating hospital labs, 20 (87%) reported the use of EUCAST guidelines. All 11 non-hospital labs submitting results on urine isolates in 2018 reported the use of EUCAST guidelines.

2. *Staphylococcus aureus*

2.1. CLINICAL IMPORTANCE AND EPIDEMIOLOGY

Staphylococcus aureus usually is a commensal bacteria that commonly colonises the skin of healthy humans. However, it can also become an opportunistic pathogen, being a common cause of skin, soft tissue and bone infections. It is also one of the leading cause of bloodstream infections in Europe. The 2017 Belgian Point prevalence survey of healthcare-associated infections and antimicrobial use (PPS) estimated that 8.9% of healthcare-associated infections (HAI) were caused by *S. aureus.* That makes it the 2nd most often isolated pathogen from HAI in Belgium, as it was already the case in the 2011 PPS study[8,9].

Over time, *S. aureus* developed two main resistance mechanisms to β -lactams. First, the production of β-lactamases. Second, the acquisition of the exogenous *mec*A (or less frequently *mec*C) gene which codes for a variant penicillin-binding protein (PBP), PBP2A, with low affinity for methicillin and for most other β-lactam drugs, hence the term "methicillin-resistant" *S. aureus* (MRSA)[10].

2.2. INVASIVE SAMPLES (BLOOD/CSF)

2.2.1. Resistance in 2018 and national trends 2014-2018

Table 6 and **figure 1** display the evolution of the mean resistance rates of *S. aureus* to principal antimicrobial groups in Belgium between 2014 and 2018.

In 2018, 8.9% of *S. aureus* isolates were resistant to methicillin (MRSA) while 10.2% were resistant to fluoroquinolones. Statistically significant decreasing trends were observed between 2014 and 2018 for both antibiotics. However, methicillin and fluoroquinolone resistance remain substantial and seem to stabilize since 2017.
Resistance to vancomycin, linezolid and rifampicin was exceedingly rare (<1%) in 2018 as well as in the whole 2014-2018 period.

Table 6 • *Staphylococcus aureus* : Mean resistance rates to principal antimicrobial groups within blood/CSF isolates, EARS-BE 2014-2018.

Antimicrobial group	% (I)R (#R/N)					Labs in 2018 (N)	Trend[a]
	2014	2015	2016	2017	2018		
EARS-BE 2018, all isolates							
%R MRSA	13.5 (130/960)	11.9 (123/1031)	12.2 (166/1364)	8.5 (129/1510)	8.9 (154/1733)	31	---
%R Fluoroquinolones	17.3 (142/821)	13.8 (131/952)	12.7 (167/1319)	10.3 (148/1431)	10.2 (156/1536)	30	---
%R Vancomycin	0.0 (0/367)	0.0 (0/838)	0.0 (0/1118)	0.1 (1/1225)	0.1 (2/1396)	29	
%R Linezolid	0.1 (1/712)	0.1 (1/848)	0.1 (1/1040)	0.2 (2/1310)	0.0 (0/1382)	28	
%R Rifampicin	0.5 (4/874)	0.2 (2/1266)	0.6 (6/1031)	0.5 (6/1196)	0.4 (5/1256)	26	

[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: nonsusceptibility (intermediate resistant/resistant), R: resistance, N: total number, #: number

Figure 1 • *Staphylococcus aureus* : Evolution of antimicrobial resistance within blood/CSF isolates, EARS- BE 2018 general criteria, 2014-2018.

MRSA = MRSA , FQs = Fluoroquinolones , VAN = Vancomycin , LNZ = Linezolid , RIF = Rifampicin
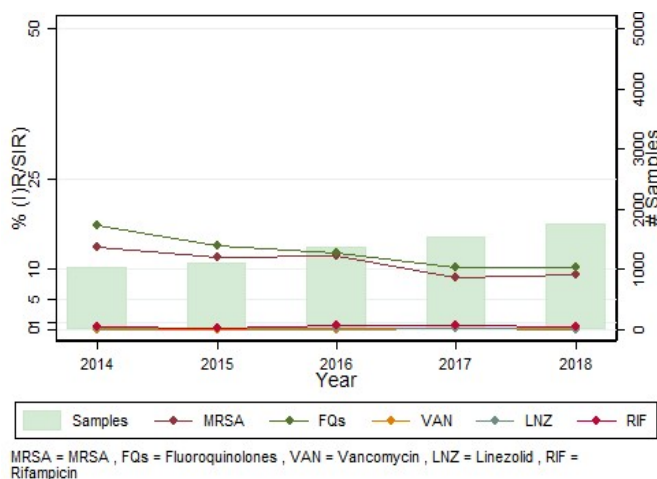
3. *Streptococcus pneumoniae*

3.1. CLINICAL IMPORTANCE AND EPIDEMIOLOGY

Streptococcus pneumoniae is a common cause of infections from upper airway (sinusitis, otitis media) but also pneumonia, bloodstream infections and meningitis. It infects especially young children, elderly people and patients with compromised immune functions.

Aminopenicillins and penicillins are widely used for the treatment of pneumococcal infection. Penicillin resistance results from a complex mutational pathway that involves multiple alterations in several β-lactam target proteins, the penicillin-binding proteins (PBPs)[11]. Acquisition of mosaic PBP results in different degrees of resistance. In the absence of meningitis, infections with intermediate resistant (currently susceptible with increased exposure) isolates are often successfully treated with high doses of benzylpenicillin or of an aminopenicillin.

Resistance to macrolides can occur by modification of the target, the ribosome, either by methylation or by mutations, or by active efflux of the antibiotic[12].

3.2. INVASIVE SAMPLES (BLOOD/CSF)

3.2.1. Resistance in 2018 and national trends 2014-2018

Results for AMR in *S. pneumoniae* are shown in **table 7** for both sources of data : the 2018 AST data of the national surveillance on invasive pneumococcal infections of the NRC (UZ Leuven, KU Leuven) (88 labs submitting results) and the EARS-BE 2018 data collection of *S. pneumoniae* blood/CSF isolates (30 labs submitting results). Among these participating laboratories, 25 submitted results in both databases. **Figure 2** shows the evolution of the mean resistance rates of *S. pneumoniae* to principal antimicrobial groups in Belgium between 2014 and 2018 for the data collected from the NRC. As EARS-BE data for *S. pneumoniae* blood/CSF isolates from individual laboratories are only collected and reported for the last 2 years, trends are not presented for this data collection.

For both databases, more than 95% of pneumococci were isolated from blood cultures; 4.1% and 4.7% being isolated from cerebrospinal fluid respectively for the NRC and the EARS-BE data collection.
For penicillin, non-susceptibility rates are reported (%IR), representing isolates reported by the local laboratories as 'susceptible, increased exposure' (I) or resistant (R) to penicillin.

According to the NRC database, except from macrolides (%R=15.2%), resistance was rare in *S. pneumoniae*: 0.1% of the tested isolates were non-susceptible to penicillins or fluoroquinolones, while no isolate was detected as resistant to third-generation cephalosporins. Decreasing 4-year trends were observed for penicillins (1.4% in 2014) and macrolides (18.1% in 2014).

Non-susceptibility rate against penicillins and resistance rate against 3rd generation cephalosporins obtained from the EARS-BE 2018 data collection were higher (11.9% and 0.8%) than those obtained from the NRC database. On the other hand, resistance to macrolides (15.9%) and to fluoroquinolones (0.5%) were very close to those obtained from the NRC database.

The highest difference between both databases was observed for penicillins non-susceptibilitiy. However, results might not be comparable across all laboratories as clinical breakpoints used to determine penicillin susceptibility in *S. pneumoniae* differ depending on the sites of infection and the dosage used in clinical practice. In order to minimize these discrepancies, we included in the 2018 data collection two indicators for the detection of penicillin susceptibility in *S. pneumoniae* : PEN_MENI (penicillin meningitis) and PEN_NMEN (penicillin non-meningitis); without specifying the breakpoint used. As stated in the EUCAST guidelines, PEN_MENI breakpoint should be 0.06µg/mL while different breakpoints, namely 0.5, 1 or 2µg/mL can be used for the indicator PEN_NMEN according to the dosage. The NRC database was analysed with uniform criteria for non-susceptibility to penicillins : for CSF isolates minimal inhibitory concentration (MIC) > 0.06 µg/mL (PEN_MENI) and for blood isolates MIC > 2µg/mL (PEN_NMEN). Knowledge of breakpoints used by laboratories submitting EARS-BE results on penicillin resistance in *S. pneumoniae* is therefore essential for future data collection in order to allow a comparison with the NRC data. To illustrate, when using the breakpoint of 0.06µg/mL (PEN_MENI) for all isolates, the NRC reports a penicillin resistance rate of 10.9%[13], much closer to the one obtained by the EARS-BE 2018 data collection (11.9%).

Table 7 • *Streptococcus pneumoniae* : Mean resistance rates to principal antimicrobial groups within blood/CSF isolates, EARS-BE 2014-2018.

Antimicrobial group	% (I)R (#R/N)					Labs in 2018 (N)	Trend[a]
	2014	2015	2016	2017	2018		
National Surveillance on invasive pneumococcal infections data							
%IR Penicillins	1.4 (14/1018)	2.1 (33/1582)	0.4 (5/1327)	0.2 (3/1473)	0.1 (1/1526)	88	---
%R 3rd-gen Cephalosporins	0.1 (1/986)	0.1 (1/1566)	0.1 (1/1324)	0.1 (1/1471)	0.0 (0/1526)	88	
%R Macrolides	18.1 (184/1016)	17.4 (278/1602)	15.7 (209/1327)	15.1 (222/1473)	15.2 (232/1526)	88	-
%R Fluoroquinolones	0.1 (1/1018)	0.2 (3/1592)	0.2 (2/1327)	0.2 (3/1473)	0.1 (2/1526)	88	
Lab data collection EARS-BE							
%IR Penicillins				6.9 (34/489)	11.9 (60/502)	26	Nd
%R 3rd-gen Cephalosporins				0.2 (1/450)	0.8 (4/493)	28	Nd
%R Macrolides				12.1 (62/514)	15.9 (93/584)	28	Nd
%R Fluoroquinolones				0.4 (2/531)	0.5 (3/617)	28	Nd

[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); nd : not determined; IR: nonsusceptibility (intermediate resistant/resistant), R: resistance, N: total number, #: number

Figure 2 • *Streptococcus pneumoniae* : Evolution of antimicrobial resistance within blood/CSF isolates, EARS- BE 2018 general criteria, 2014-2018 : data collected from the national reference centre of pneumococci.

PENs = Penicillins , 3GCs = 3rd-gen Cephalosporins , MLs = Macrolides , FQs = Fluoroquinolones
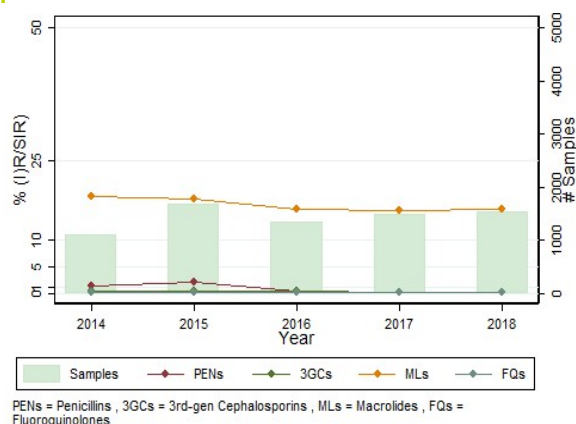
4. Enterococci

4.1. CLINICAL IMPORTANCE AND EPIDEMIOLOGY

Enterococci are part of the normal intestinal flora of humans and animals. However, when this commensal relationship is disrupted, they can cause a large variety of invasive diseases such as urinary tract infections, bloodstream infections and endocarditis. The genus *Enterococcus* includes more than 17 species, but the vast majority of clinical enterococcal infections in humans are caused by *E. faecalis* and *E. faecium*[1].

The 2017 PPS study estimated that 4.8% of HAI were caused by *E. faecalis*, making it the 4th most often isolated pathogen from HAI in Belgium. *E. faecium* was less commonly isolated and reported to cause 1.2% of HAI in Belgium[8].

Enterococci are intrinsically resistant to a broad range of antimicrobial agents including cephalosporins, sulphonamides and low concentrations of aminoglycosides. Due to the expression of low affinity penicillin-binding proteins, they exhibit decreased susceptibility to many beta-lactam agents. However, there is commonly *in vitro* synergy between cell-wall active agents (penicillins or glycopeptides) and aminoglycosides. Some enterococci have developed high resistance to aminoglycosides, causing loss of any synergy with this class of antibiotic[14]. The increasing trend of enterococci vancomycin resistance consists in a great cause of concern at the European level[3]. This glycopeptide resistance is mostly mediated through two phenotypes: VanA vancomycin resistant enterococci (VRE) is high-level resistant to vancomycin (and displays a variable level of resistance to teicoplanin) while VanB VRE exhibits a variable level of resistance, in most cases, to vancomycin only[14].

4.2. INVASIVE SAMPLES (BLOOD/CSF)

4.2.1. Resistance in 2018 and national trends 2014-2018

Table 8, **figures 3** and **4** display the evolution of the mean resistance rates to principal antimicrobial groups in Belgium between 2014 and 2018 for *E. faecalis* and *E. faecium*.

Glycopeptides resistance rates remain low but are higher in *E. faecium* (1.8% for vancomycin and 1.5% for teicoplanin) than in *E. faecalis*, (0.3% for vancomycin and 0.2% for teicoplanin). For both enterococci, no statistically significant trend was detected for glycopeptides resistance at the Belgian level. However, the rate of vancomycin resistance reported in 2017 for *E. faecium* was much higher (5.5%) than in 2018 (1.8%). This previous higher rate might be indicative of possible outbreaks in some hospitals as shown from the percentiles of the distribution of lab means[15]. When comparing the medians for 2017 and 2018, which are less sensitive to extreme values than the mean, these are both equal to 0.0%.

While no *E. faecalis* isolate resistant to aminopenicillins was reported in 2018, the prevalence of aminopenicillin resistance was high in *E. faecium* isolates (84.7%). This difference between both species is explained by the higher intrinsic resistance to penicillins of *E. faecium* isolates[16].

High-level gentamicin resistance is common both in *E. faecalis* (12.3%) and *E. faecium* (20.2%) but this resistance rate has decreased since 2014 (-9.4% for *E. faecalis* and -9.5% for *E. faecium*). Of note, the higher high-level gentamicin resistance rate detected for *E. faecium* in comparison to *E. faecalis* is in agreement with the observations at the European level[1].

While no resistance to linezolid was detected from 2014 to 2016 for *E. faecalis*, 1.1% of the tested isolates were resistant in 2017 and 0.7% in 2018. Concerning *E. faecium* isolates, linezolid resistance (0.4%) was attributed to one single isolate from an hospitalized patient.

Table 8 • *Enterococcus faecalis* & *Enterococcus faecium*: Mean resistance rates to principal antimicrobial groups within blood/CSF isolates, EARS-BE 2014-2018.

Antimicrobial group	% R (#R/N)					Labs in 2018 (N)	Trend[a]
	2014	2015	2016	2017	2018		
Enterococcus faecalis, EARS-BE 2018 general criteria							
%R Aminopenicillins	0.3 (1/342)	0.5 (2/424)	0.4 (2/461)	0.4 (2/550)	0.0 (0/607)	31	
%R Gentamicin high-level	21.7 (35/161)	12.5 (36/287)	19.8 (65/328)	16.1 (50/310)	12.3 (48/390)	22	(-)
%R Vancomycin	0.3 (1/352)	0.2 (1/420)	0.0 (0/463)	0.7 (4/556)	0.3 (2/598)	30	
%R Teicoplanin	0.4 (1/220)	0.4 (1/248)	0.3 (1/364)	0.0 (0/377)	0.2 (1/475)	26	
%R Linezolid	0.0 (0/187)	0.0 (0/257)	0.0 (0/330)	1.1 (4/350)	0.7 (3/428)	23	+
Enterococcus faecium, EARS-BE 2018 general criteria							
%R Aminopenicillins	84.7 (161/190)	81.2 (151/186)	85.7 (246/287)	88.5 (363/410)	84.7 (370/437)	30	
%R Gentamicin high-level	29.7 (30/101)	29.8 (36/121)	19.7 (42/213)	25.0 (67/268)	20.2 (50/247)	21	(-)
%R Vancomycin	3.1 (6/192)	1.1 (2/189)	1.7 (5/289)	5.5 (23/417)	1.8 (8/436)	29	
%R Teicoplanin	1.8 (2/110)	0.9 (1/116)	1.2 (3/243)	6.5 (20/306)	1.5 (5/343)	24	
%R Linezolid	0.0 (0/89)	0.0 (0/104)	0.5 (1/205)	2.3 (7/300)	0.4 (1/278)	23	

[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 • *Enterococcus faecalis*: Evolution of antimicrobial resistance within blood/CSF isolates, EARS- BE 2018 general criteria, 2014-2018.

AMPs = Aminopenicillins , GEH = Gentamicin high-level , VAN = Vancomycin , TEC = Teicoplanin , LNZ = Linezolid
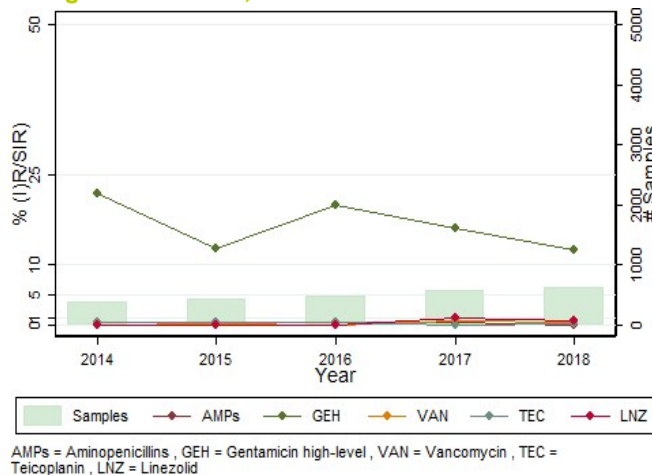
Figure 4 • *Enterococcus faecium*: **Evolution of antimicrobial resistance within blood/CSF isolates, EARS- BE 2018 general criteria, 2014-2018.**

GEH = Gentamicin high-level , VAN = Vancomycin , TEC = Teicoplanin , LNZ = Linezolid
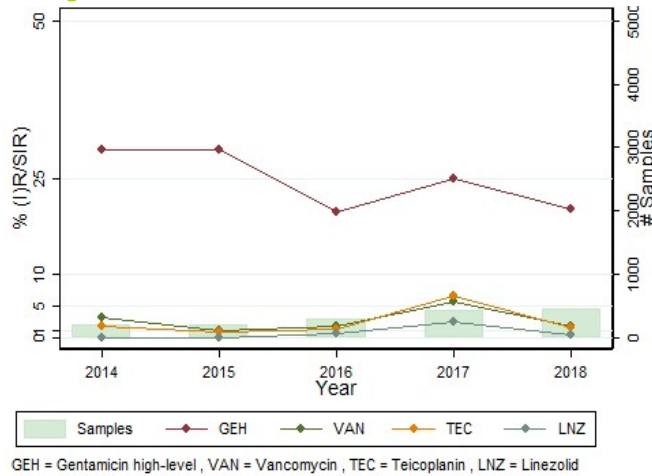
4.3. URINARY SAMPLES

4.3.1. Comparison between invasive and urinary samples from hospital settings

As shown on **figure 5**, resistance of *E. faecalis* (**A**) and *E. faecium* (**B**) urine isolates from hospital labs is similar to the one observed within invasive isolates. Again, higher resistance rates were detected for *E. faecium* than for *E. faecalis*. The resistance rates to vancomycin, teicoplanin and linezolid in urine isolates are low (< 1% for *E. faecalis* and < 2% for *E. faecium*). As shown for invasive isolates, the prevalence of *E. faecium* isolates resistant to aminopenicillins within urine is high (90.0%) while only 13 out of 9218 tested *E. faecalis* urine isolates were reported as resistant to this antibiotic class (0.1%).

Figure 5 • Comparison of EARS-BE 2018 antimicrobial resistance (%R (#R/N)) within hospital labs between blood/CSF and URINE isolates for *Enterococcus faecalis* **(A) and** *Enterococcus faecium* **(B)**

AMPs = Aminopenicillins , GEH = Gentamicin high-level , VAN = Vancomycin , TEC = Teicoplanin , LNZ = Linezolid
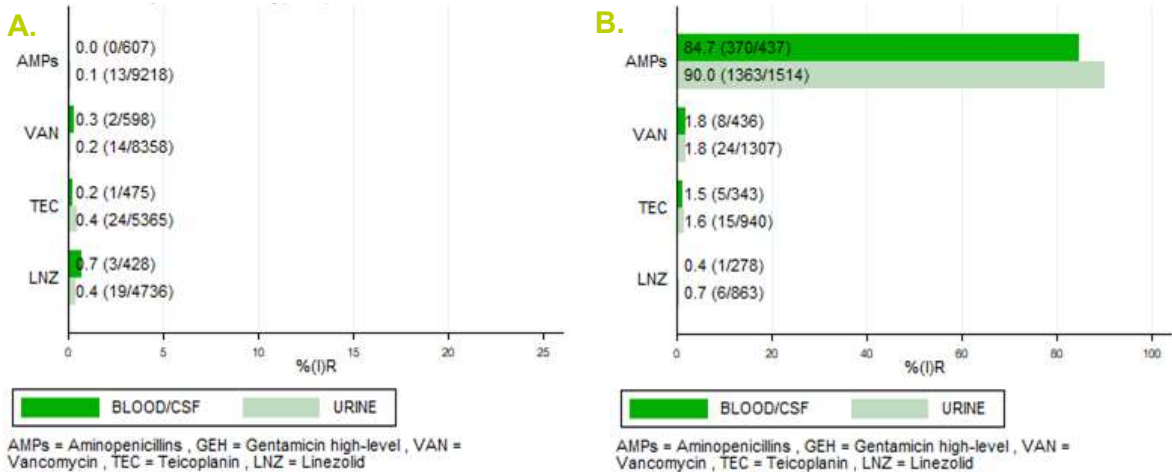
4.3.2. Comparison between hospital and non-hospital settings

Figure 6 displays the mean resistance rates to principal antimicrobial groups within urine isolates for *E. faecalis* (**A**) and *E. faecium* (**B**) for both the subgroups of isolates from hospital and non-hospital settings in 2018. As resistance data within urinary samples were only collected for the second year, we don't report any trend in this report.

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Figure 6 • Comparison of EARS-BE 2018 antimicrobial resistance (%R (#R/N)) within URINE isolates between HOSPITAL and NON-HOSPITAL settings for *Enterococcus faecalis* (A) and *Enterococcus faecium* (B)
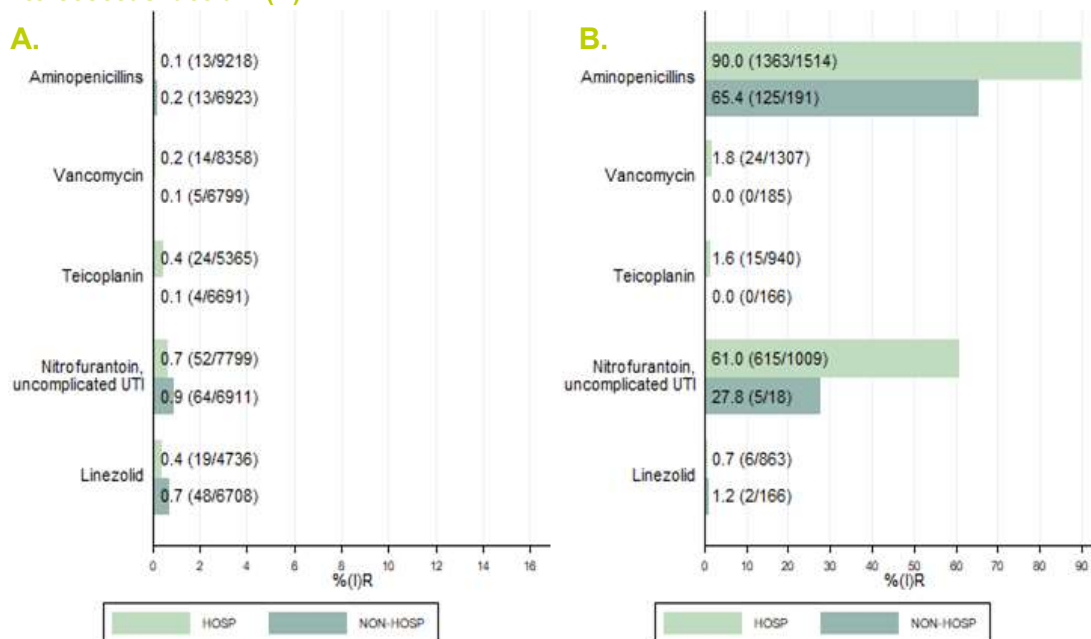
HOSP: hospital labs; NON-HOSP : non-hospital labs; #: number; R: resistance, N: total number.

5. *Escherichia coli*

5.1. CLINICAL IMPORTANCE AND EPIDEMIOLOGY

Escherichia coli is a part of the normal intestinal microbiota in humans and animals, but is also a common cause of bloodstream and urinary tract infections. It can also be associated with intra-abdominal infections and neonatal meningitis[1].

E. coli is the most frequently isolated pathogen in HAI accounting for 17.8% of all Belgian HAI identified by the PPS of 2017, as it was already the case in 2011[8,9]. Moreover, among hospital-acquired bloodstream infections (HABSI) from urinary origin (21% of all HABSI) reported in Belgium in 2018, 49% were caused by *E. coli*[17].

Pathogenic *E. coli* strains may become a larger threat if they possess or acquire certain antimicrobial resistance mechanisms. The main ones of concern are the extended-spectrum β-lactamase (ESBL)-producing strains and the carbapenem-resistant *Enterobacteriaceae* (CRE) strains. ESBLs are enzymes that confer resistance to most β-lactam antibiotics, including 3rd-generation cephalosporins. Carbapenems usually resist the action of ESBLs and might remain as one of the few treatment options for severe infections. However, the emergence of carbapenem resistance, mediated by the production of carbapenemases may confer resistance to virtually all available β-lactam antibiotics. Moreover, these carbapenemase genes are often located on transmissible plasmids in combination with several other resistance genes, leading to multi-drug resistance[1,18].

5.2. INVASIVE SAMPLES (BLOOD/CSF)

5.2.1. Resistance in 2018 and national trends 2014-2018

As shown in **table 9** and **figure 7**, in 2018, the highest Belgian mean resistance rate for *E. coli* isolates was reported for aminopenicillins (55.8%), followed by amoxicillin-clavulanic acid (39.4%), fluoroquinolones (21.8%), 3rd-generation cephalosporins (9.0%), piperacillin-tazobactam (8.0%), and

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aminoglycosides (7.4%). Resistance to carbapenems was exceptionally reported (0.1%). Among the isolates tested as resistant to 3rd-generation cephalosporins, 94.6% were reported positive for ESBL (14 labs submitting results), while no meaningful carbapenemase production rate could be estimated due to low number of reported tests. Decreasing 5-year trends were detected for aminopenicillins (-3.4%), aminoglycosides (-1.6%) and fluoroquinolones (-5.1%).
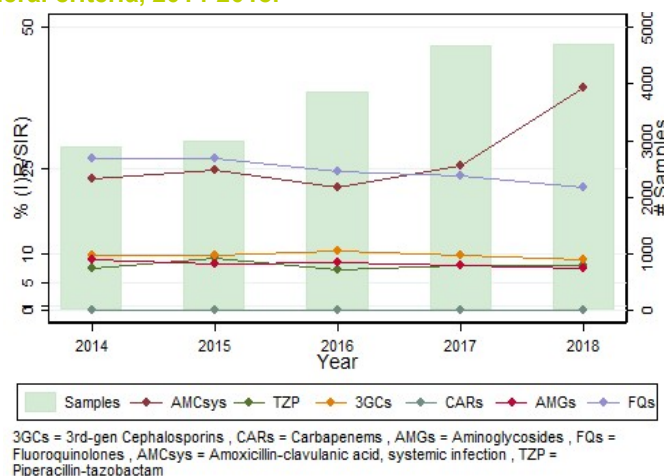
Of note, an important increase in the amoxicillin-clavulanic acid (AMC) resistance rate of *E. coli* invasive isolates was observed this year (+13.9% compared to 2017). This increase could be explained by the increased number of labs using EUCAST guidelines for test interpretation and by the introduction by the company Biomérieux in 2018 of a new AST card (N366) in Vitek systems which follows EUCAST recommendation using a fixed 2 mg/L clavulanate concentration instead of a fixed 2 : 1 ratio of amoxicillin/clavulanic acid for broth microdilution susceptibility testing as per CLSI recommendations. In 2013, a study from the Netherlands determined the influence of such switch of recommended clavulanate concentrations from CLSI to EUCAST on AMC susceptibility rates among clinical *E. coli* and showed that EUCAST methodology resulted in higher AMC *E. coli* resistance rates than CLSI methodology, but correlated better with clinical outcome[19].

Table 9 • *Escherichia coli*: Mean resistance rates to principal antimicrobial groups within blood/CSF isolates, EARS-BE 2014-2018.

Antimicrobial group	% R (#R/N)					Labs in 2018 (N)	Trend[a]
	2014	2015	2016	2017	2018		
EARS-BE 2018, all isolates							
%R Aminopenicillins	59.2 (1655/2795)	58.3 (1741/2989)	58.0 (2167/3736)	57.4 (2681/4669)	55.8 (2480/4445)	31	--
%R Amoxicillin-clavulanic acid, systemic infection	23.3 (224/962)	24.8 (611/2463)	21.8 (753/3458)	25.5 (911/3571)	39.4 (1673/4250)	27	+++
%R Piperacillin-tazobactam	7.4 (88/1189)	9.2 (233/2545)	7.2 (256/3543)	8.1 (314/3903)	8.0 (329/4104)	29	
%R 3rd-gen Cephalosporins	9.8 (269/2741)	9.9 (288/2907)	10.5 (392/3737)	9.7 (455/4670)	9.0 (419/4644)	31	
%R Carbapenems	0.0 (1/2511)	0.0 (0/2903)	0.1 (2/3845)	0.0 (1/4670)	0.1 (3/4641)	31	
%R Aminoglycosides	9.0 (178/1974)	8.2 (212/2583)	8.4 (294/3499)	8.1 (305/3767)	7.4 (283/3822)	27	-
%R Fluoroquinolones	26.9 (682/2535)	26.8 (771/2880)	24.5 (946/3854)	23.8 (1042/4380)	21.8 (918/4211)	30	---

[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: nonsusceptibility (intermediate resistant/resistant), R: resistance, N: total number, #: number

Figure 7 • *Escherichia coli*: Evolution of antimicrobial resistance within blood/CSF isolates, EARS- BE 2018 general criteria, 2014-2018.
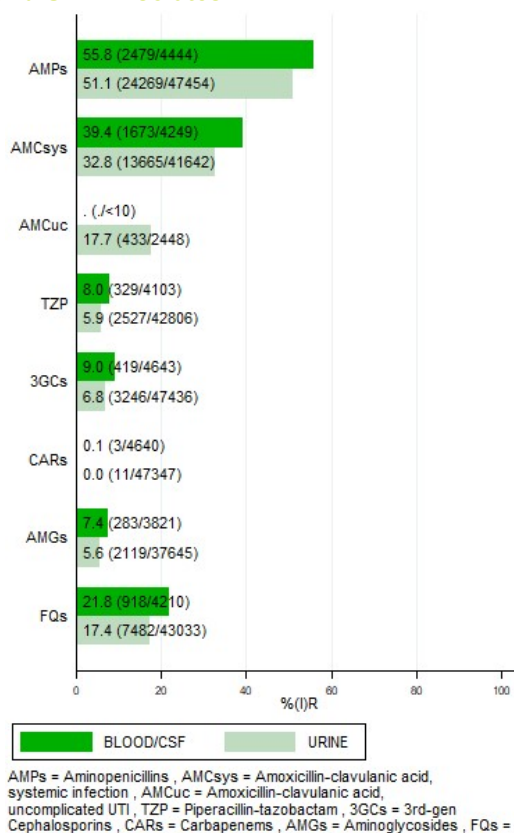

3GCs = 3rd-gen Cephalosporins , CARs = Carbapenems , AMGs = Aminoglycosides , FQs = Fluoroquinolones , AMCsys = Amoxicillin-clavulanic acid, systemic infection , TZP = Piperacillin-tazobactam

5.3. URINARY SAMPLES

5.3.1. Comparison between invasive and urinary samples from hospital settings

Figure 8 displays the resistance percentage of *E. coli* urinary isolates side-by-side to the one of invasive isolates, both obtained from hospital settings. For all antimicrobial groups, the level of resistance detected in urine isolates is slightly lower than in invasive samples. As for invasive samples, the highest Belgian mean resistance rate was reported for aminopenicillins (51.1%), followed by amoxicillin-clavulanic acid (32.8%), fluoroquinolones (17.4%), 3rd-generation cephalosporins (6.8%), piperacillin-tazobactam (5.9%), and aminoglycosides (5.6%). Again, almost no resistance to carbapenems was reported (<0.1%). Among the isolates tested as resistant to 3rd-generation cephalosporins, 93.0% were reported positive for ESBL (14 labs submitting results). Of note, for some urinary samples, the resistance to amoxicillin-clavulanic acid was determined using the cut-off of invasive samples (AMC = 8µg/mL according to EUCAST guidelines[20]). When analysed using the same breakpoints, less resistant isolates are found in urine (32.8%) compared to blood/CSF (39.4%). For other urinary samples, the breakpoint of uncomplicated urinary tract infections was used (AMC_UC = 32µg/mL according to EUCAST guidelines[20]) giving as expected, a lower resistance rate (17.7%).

Figure 8 • *Escherichia coli* : Comparison of antimicrobial resistance (%R (#R/N)) within hospital labs between blood/CSF and URINE isolates.

AMPs = Aminopenicillins , AMCsys = Amoxicillin-clavulanic acid,
systemic infection , AMCuc = Amoxicillin-clavulanic acid,
uncomplicated UTI , TZP = Piperacillin-tazobactam , 3GCs = 3rd-gen
Cephalosporins , CARs = Carbapenems , AMGs = Aminoglycosides , FQs =

5.3.2. Comparison between hospital and non-hospital settings

Figure 9 compares the 2018 antimicrobial resistance rates within *E. coli* urine isolates between hospital and non-hospital settings. Resistance levels in isolates from hospital laboratories were slightly higher than in isolates from non-hospital laboratories for a few antimicrobial groups (aminopenicillins (51.1% for hospitals (H) vs 47.1% for non-hospital settings (NH)), temocillin (4.9% for H vs 1.5% for NH), trimethoprim-sulfamethoxazole (25.3% for H vs 22.4% for NH). For other antimicrobial groups, the rates are similar between both groups (difference ≤ 1%) in 2018 (piperacillin-tazobactam, cefuroxime oral, 3rd generation cephalosporins, aminoglycosides, fluoroquinolones, nitrofurantoin, and fosfomycin).

However, for amoxicillin-clavulanic acid, the resistance rate is higher in non-hospital settings compared to hospitals for both ASTs used (39.8% for NH vs 32.8% for H for AMC and 40.6% for NH vs 17.7% for H for AMC_UC). Unexpectedly, very similar AMC rates are observed in non-hospital settings using the two different breakpoints (AMC and AMC_UC). These results thus have to be taken with caution.

Figure 9 • Comparison of EARS-BE 2018 antimicrobial resistance (%R (#R/N)) within URINE isolates between HOSPITAL and NON-HOSPITAL settings for *Escherichia coli*.
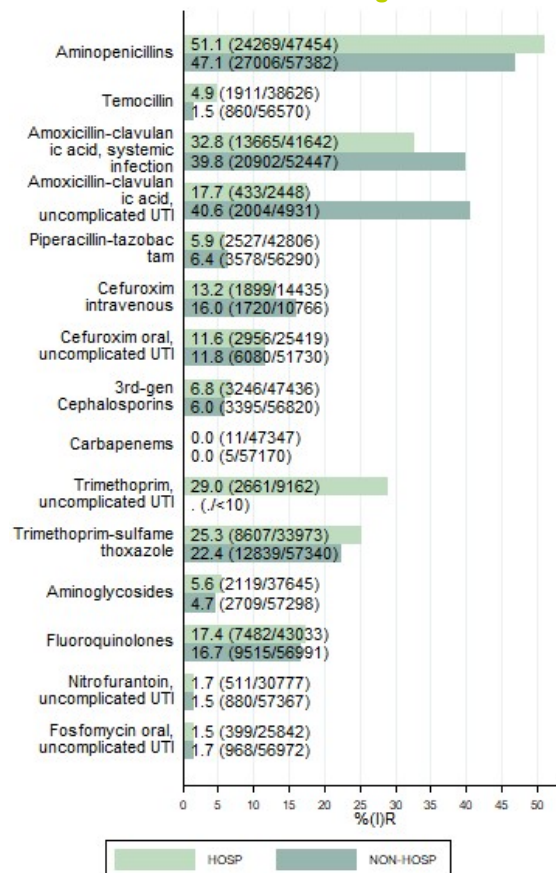
6. *Proteus mirabilis*

6.1. CLINICAL IMPORTANCE AND EPIDEMIOLOGY

P. mirabilis is a commensal bacteria from the gastrointestinal tract. This organism is a common cause of symptomatic urinary tract infections including cystitis and pyelonephritis. *P. mirabilis* particularly infects patients undergoing long-term catheterization. These infections can also cause bacteraemia and progress to potentially life-threatening urosepsis[21].

In 2018 in Belgium, 5% of HABSI from urinary origin were caused by *P. mirabilis*[17]. This microorganism also belongs to the top 10 of most often isolated pathogen from HAI in Belgium, the 2017 PPS study having estimated that 2.2% of HAI were caused by this bacteria placing it at the 9th position[8].

Like many other *Enterobacteriaceae*, *P. mirabilis* can harbour numerous determinants of antimicrobial resistance. Multidrug-resistant (MDR) strains of *P. mirabilis* produce ESBLs or cephalosporinases and rarely carbapenemases[22].

6.2. URINARY SAMPLES

In 2018, AST data on *Proteus mirabilis* from urinary samples were collected for the second year. Among the 33 laboratories submitting AST data for this pathogen this year, 11 were not associated to an hospital.

The highest rates of resistance were reported for aminopenicillins (42.2%), trimethoprim (39.7%), trimethoprim-sulfamethoxazole (35.4%), fluoroquinolones (32.1%) and fosfomycin (28.6%). Resistance rates to aminoglycosides was 13.3%. Resistance against piperacillin-tazobactam (0.9%) and 3rd generation cephalosporins (1.6%) remained low, while no resistance to carbapenems was detected. As for *E.coli*, an important increase in the amoxicillin-clavulanic acid (AMC) resistance rate was observed (+ 6.8%), which could be explained by the increased number of labs using EUCAST breakpoint to determine AMC resistance and by the switch from the CLSI to EUCAST methodology.

More than half (54.4%) of the *P. mirabilis* isolates reported to EARS-BE for 2018 were resistant to at least one of the antimicrobial groups under regular surveillance (aminopenicillins, fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides and carbapenems) and more than a quarter (27.4%) were resistant to at least two of the same antimicrobial groups.

6.2.1. Comparison between hospital and non-hospital settings

As shown on **figure 10**, resistance levels within *Proteus mirabilis* urine isolates were similar between isolates from hospital and non-hospital laboratories. Resistance rates were higher in hospital than in non-hospital laboratories for aminopenicillins and aminoglycosides while these were lower in hospital than in non-hospital laboratories for trimethoprim-sulfamethoxazole, fluoroquinolones and fosfomycin.

The results observed for AMC resistance in *P. mirabilis* isolates are similar to these observed for *E. coli*. In 2018, no difference is observed between hospital and non-hospital laboratories when the AST was interpreted using the breakpoint for systemic infections (AMC namely 8µg/mL according to EUCAST guidelines)[20]. Logically, when using the less strict breakpoint for uncomplicated urinary tract infection (AMC_UC namely 32µg/mL according to EUCAST guidelines)[20], resistance rates are lower in hospital settings (AMC_UC = 6.4% vs AMC = 16.2%). However, this difference is not observed in non-hospital settings and even higher resistance percentages are detected using AMC_UC (24.6%, 5 labs submitting results) in comparison to AMC (16.3%, 6 labs submitting results). As with *E. coli*, this difference is unexpected and should therefore be taken with caution.

Figure 10 • Comparison of EARS-BE 2018 antimicrobial resistance (%R (#R/N)) within URINE isolates between HOSPITAL and NON-HOSPITAL settings for *Proteus mirabilis*.
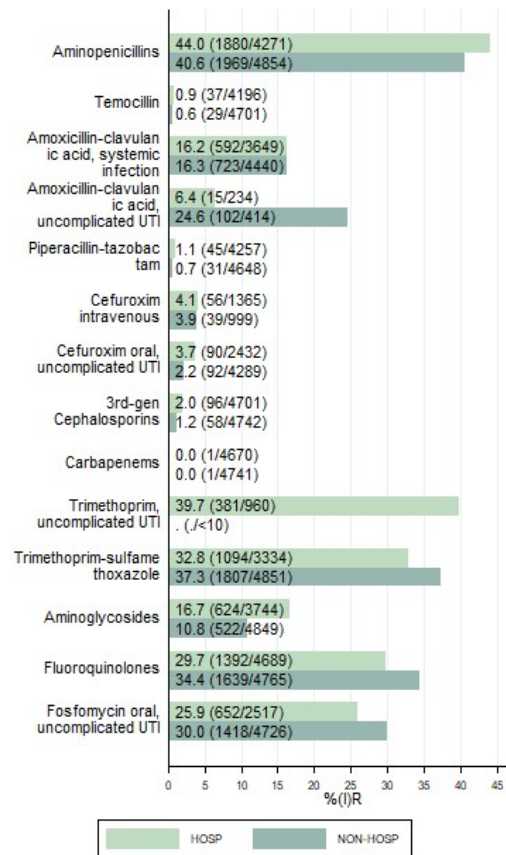
7. *Klebsiella pneumoniae*

7.1. CLINICAL IMPORTANCE AND EPIDEMIOLOGY

Klebsiella pneumoniae is a commensal bacteria frequently found in the gastrointestinal tract, the skin and the respiratory tract of hospitalized patients as well as in natural environment. It is an opportunistic pathogen that can cause severe HAI such as urinary tract, lower respiratory tract, intra-abdominal and bloodstream infections typically affecting debilitated individuals. It can easily spread between patients and via the hands of hospital staff leading to nosocomial outbreaks[23].

In 2017, the Belgian PPS showed that *K. pneumoniae* infections accounted for 4.2% of all HAI. In 2018, this microorganism was reported as causing 8% of HABSI from all sources combined and 13% of HABSI from urinary source[17].

K. pneumoniae has a chromosomally encoded class A β-lactamase and is thus intrinsically resistant to aminopenicillins. Many novel ESBL variants have emerged in this species over the last 30 years and have led to acquired resistance to 3rd generation-cephalosporins, leaving carbapenems as one of the few treatment options. However, a recently increasing threat is carbapenem resistance mediated by a range of carbapenemases, which may confer resistance to virtually all available β-lactams and can be exchanged between *Enterobacteriaceae* species owing to their localisation on transmissible plasmids and on other genetic mobile elements[1,23].

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7.2. INVASIVE SAMPLES (BLOOD/CSF)

7.2.1. Resistance in 2018 and national trends 2014-2018

Table 10 and **figure 11** display trends from 2014 until 2018 of mean resistance rates of *K. pneumoniae* to principal antimicrobial groups under surveillance.

In 2018, the highest resistance rate observed in *K. pneumoniae* isolates was for amoxicillin-clavulanic acid (33.4%). This is the result of a significant increase compared to 2017 (+7.9%). This can be attributed to the switch from CLSI to EUCAST methodology to detect AMC resistance (as also observed for *E. coli* isolates). High resistance rates were also observed for fluoroquinolones (22.6%) and 3rd generation cephalosporins (21.4%). Moderate 5-year increasing trends were detected for antimicrobial resistance to these two last antimicrobial groups (+4% for fluoroquinolones and +4.8% for 3rd generation cephalosporins). Among the 200 3rd generation cephalosporins resistant isolates reported in 2018, 82 were tested for the production of ESBL and 82.9% were reported as positive (12 labs submitting results). Considerable resistance was reported for piperacillin-tazobactam (18.5%) while resistance against carbapenems does occur but remains low in Belgium (1.4%). No meaningful trend was observed for these two last antimicrobials over the 2014-2018 period.

Table 10 • *Klebsiella pneumoniae* **: Mean resistance rates to principal antimicrobial groups within blood/CSF isolates, EARS-BE 2014-2018.**

Antimicrobial group	% R (#R/N)					Labs in 2018 (N)	Trend[a]
	2014	2015	2016	2017	2018		
EARS-BE 2018, all isolates							
%R Amoxicillin-clavulanic acid, systemic infection	26.6 (45/169)	25.6 (91/355)	24.2 (147/607)	25.5 (143/560)	33.4 (249/746)	27	++
%R Piperacillin-tazobactam	17.2 (33/192)	16.4 (63/384)	16.7 (106/635)	17.4 (119/684)	18.5 (152/823)	29	
%R 3rd-gen Cephalosporins	16.6 (79/477)	19.4 (87/448)	22.9 (153/669)	19.3 (155/802)	21.4 (200/935)	31	(+)
%R Carbapenems	0.5 (2/417)	0.5 (2/432)	2.4 (16/669)	1.1 (9/790)	1.4 (13/935)	31	
%R Aminoglycosides	11.2 (37/331)	11.1 (44/397)	13.8 (88/637)	12.5 (79/632)	12.4 (93/747)	27	
%R Fluoroquinolones	18.6 (92/495)	21.1 (89/422)	23.6 (158/669)	23.7 (190/802)	22.6 (211/932)	31	(+)

[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 11 • *Klebsiella pneumoniae* **: Evolution of antimicrobial resistance within blood/CSF isolates, EARS- BE 2018 general criteria, 2014-2018.**

3GCs = 3rd-gen Cephalosporins , CARs = Carbapenems , AMGs = Aminoglycosides , FQs = Fluoroquinolones , AMCsys = Amoxicillin-clavulanic acid, systemic infection , TZP = Piperacillin-tazobactam
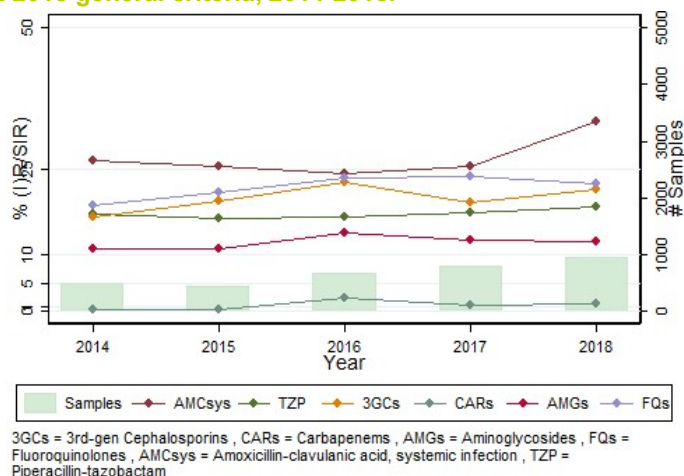
7.3. URINARY SAMPLES

7.3.1. Comparison between invasive and urinary samples from hospital settings

As shown on **Figure 12**, the level of resistance detected in urine *K. pneumoniae* isolates is slightly lower than in invasive samples for all antimicrobial groups under surveillance. As for invasive samples, the highest national mean resistance rate was reported for amoxicillin-clavulanic acid (28.7%), followed by fluoroquinolones (21.3%), 3rd-generation cephalosporins (17.6% of which 92% were reported positive for ESBL), piperacillin-tazobactam (15.6%), and aminoglycosides (10%). Again, low resistance to carbapenems was reported (0.5%). For some laboratories, the resistance to amoxicillin-clavulanic acid of urinary samples was deter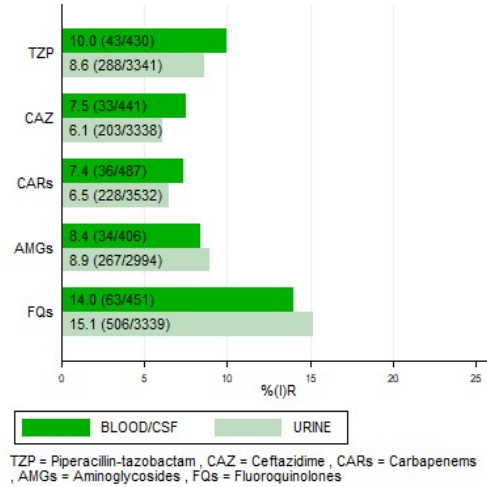mined using the cut-off of systemic infections (AMC) giving a slightly lower resistance percentage in urine (28.7%) compared to blood/CSF (33.4%). For other urinary samples, the breakpoint of uncomplicated urinary tract infections was used (AMC_UC) and the level of resistance is slightly lower (24.3%) using this larger breakpoint (namely 32µg/mL according to EUCAST guidelines)[20].

Figure 12 • *Klebsiella pneumoniae* **: Comparison of EARS-BE 2018 antimicrobial resistance (%R (#R/N)) within hospital labs between blood/CSF and URINE isolates.**

AMCsys = Amoxicillin-clavulanic acid, systemic infection , AMCuc = Amoxicillin-clavulanic acid, uncomplicated UTI , TZP = Piperacillin-tazobactam , 3GCs = 3rd-gen Cephalosporins , CARs = Carbapenems , AMGs = Aminoglycosides , FQs = Fluoroquinolones
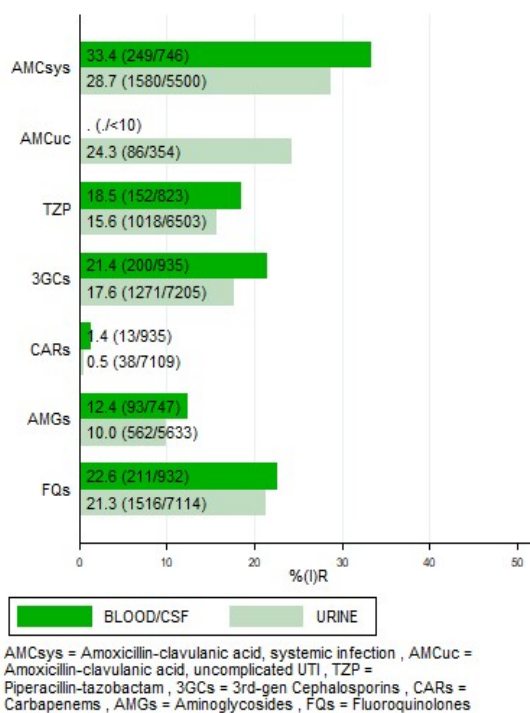
7.3.2. Comparison between hospital and non-hospital settings

Figure 13 displays 2018 mean resistance rates to principal antimicrobial groups within *K. pneumoniae* urine isolates in hospital and non-hospital settings. Except for fosfomycin resistance, resistance levels in non-hospital laboratories were lower than in hospital laboratories for *K. pneumoniae* urine isolates.

In 2018, the resistance of *K. pneumoniae* to amoxicillin-acid clavulanic when using the breakpoint of urinary tract infections is higher in non-hospital settings compared to hospital laboratories (AMC_UC : 31% for NH vs 24.3% for H). However, within non-hospital settings, the resistance percentage observed using this less strict breakpoint (AMC_UC : Resistant if MIC > 32µg/mL according to EUCAST guidelines[20]) is higher than the rate obtained using the breakpoint for systemic infections (AMC : Resistant if MIC > 8µg/mL according to EUCAST[20]). Of note, this unexpected observation was made for all the three pathogen (*K. pneumoniae*, *E. coli* and *P. mirabilis*) for which these indicators (AMC and AMC_UC) were used.

Figure 13 • Comparison of EARS-BE 2018 antimicrobial resistance (%R (#R/N)) within URINE isolates between HOSPITAL and NON-HOSPITAL settings for *Klebsiella pneumoniae*.
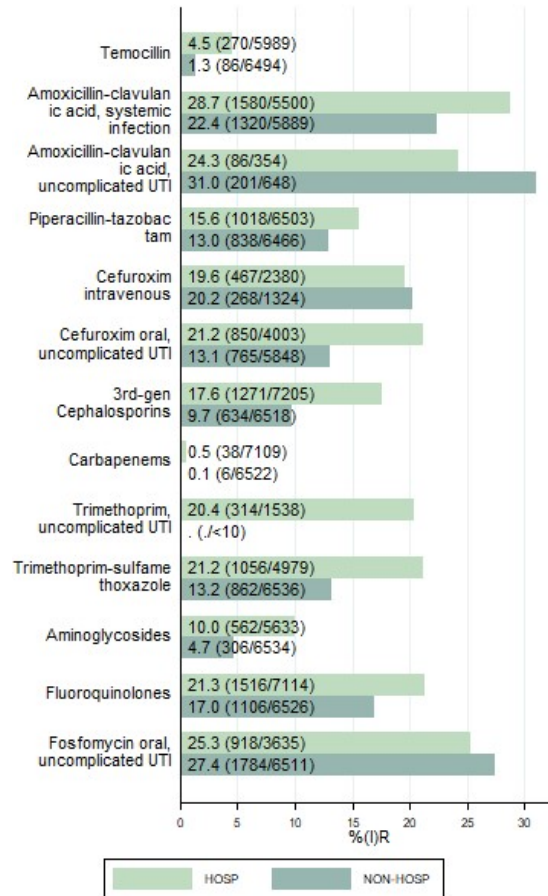
8. *Pseudomonas aeruginosa*

8.1. CLINICAL IMPORTANCE AND EPIDEMIOLOGY

Pseudomonas aeruginosa is commonly found in aquatic environments in nature. It is an opportunistic pathogen and a common cause of healthcare-associated pneumonia (including ventilator-associated pneumonia), bloodstream infections and urinary tract infections[1].

In Belgium, *P. aeruginosa* is the third most often isolated pathogen from all HAI (5.2% of all HAI) as reported in the 2017 Belgian PPS[8]. In 2018, 5% of all HABSI were reported to be caused by this microorganism[17].

One of the most worrisome characteristics of *P. aeruginosa* is its high intrinsic resistance to the majority of antimicrobial agents. In addition, *P. aeruginosa* has a high propensity to develop acquired resistance through one or several mechanisms including modified antimicrobial targets, efflux and reduced permeability as well as degrading enzymes. These different mechanisms of resistance may result either from mutation in chromosomal genes or by acquisition through horizontal gene transfer[24].

8.2. INVASIVE SAMPLES (BLOOD/CSF)

8.2.1. Resistance in 2018 and national trends 2014-2018

As presented in **table 11** and **figure 14**, *Pseudomonas aeruginosa* isolates showed resistance to almost all tested antimicrobial groups under surveillance.

In 2018, the most occurring resistance in *P. aeruginosa* in Belgium was to fluoroquinolones (14.0%), followed by resistance to piperacillin-tazobactam (10.0%), aminoglycosides (8.4%), ceftazidime (7.5%) and carbapenems (7.4%). For none of the antimicrobial groups under surveillance, meaningful increasing or decreasing trends were observed between 2014 and 2018.

Table 11 • *Pseudomonas aeruginosa:* Mean resistance rates to principal antimicrobial groups within blood/CSF isolates, EARS-BE 2014-2018.

Antimicrobial group	% R (#R/N)					Labs in 2018 (N)	Trend[a]
	2014	2015	2016	2017	2018		
EARS-BE 2018, all isolates							
%R Piperacillin-tazobactam	9.8 (56/572)	8.6 (46/534)	9.8 (31/318)	10.5 (46/439)	10.0 (43/430)	28	
%R Ceftazidime	9.1 (56/618)	6.8 (34/498)	7.8 (25/320)	7.2 (31/432)	7.5 (33/441)	28	
%R Carbapenems	10.5 (35/334)	4.3 (12/278)	9.6 (35/365)	8.2 (39/475)	7.4 (36/487)	30	
%R Aminoglycosides	8.3 (21/253)	5.4 (13/241)	11.0 (36/327)	7.7 (29/377)	8.4 (34/406)	27	
%R Fluoroquinolones	13.0 (39/301)	10.9 (31/284)	14.5 (53/366)	10.4 (45/431)	14.0 (63/451)	29	

[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 14 • *Pseudomonas aeruginosa* : Evolution of antimicrobial resistance within blood/CSF isolates, EARS- BE 2018 general criteria, 2014-2018.

CARs = Carbapenems , AMGs = Aminoglycosides , FQs = Fluoroquinolones , TZP = Piperacillin-tazobactam , CAZ = Ceftazidime
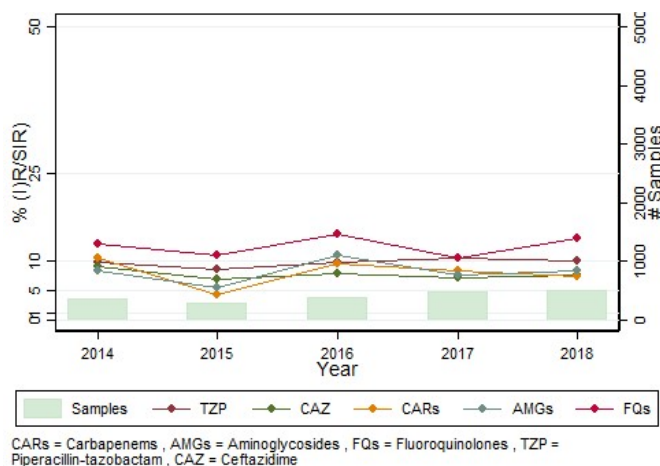
8.3. URINARY SAMPLES

8.3.1. Comparison between invasive and urinary samples from hospital settings

When comparing antimicrobial resistance levels between urine and invasive *P. aeruginosa* isolates in hospital laboratories (**figure 15**), similar levels were observed, with a difference never exceeding 1 to 2% between both groups.

Figure 15 • *Pseudomonas aeruginosa* : Comparison of EARS-BE 2018 antimicrobial resistance (%R (#R/N)) within hospital labs between Blood/CSF and URINE isolates

TZP = Piperacillin-tazobactam , CAZ = Ceftazidime , CARs = Carbapenems , AMGs = Aminoglycosides , FQs = Fluoroquinolones

8.3.2. Comparison between hospital and non-hospital settings

Figure 16 compares 2018 antimicrobial resistance within *P. aeruginosa* urine isolates between hospital and non-hospital settings. Resistance levels in hospital settings were higher than in non-hospital laboratories for all antimicrobials (piperacillin-tazobactam, ceftazidime, carbapenems and aminoglycosides), except for fluoroquinolones.

Figure 16 • Comparison of 2018 antimicrobial resistance (%R (#R/N)) within URINE isolates between HOSPITAL and NON-HOSPITAL settings for *Pseudomonas aeruginosa*.
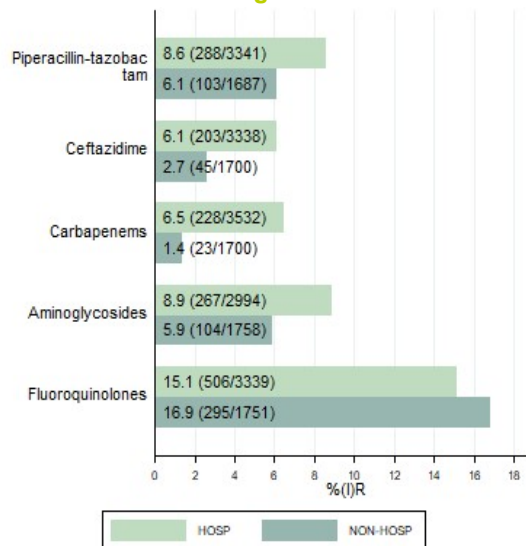
9. *Acinetobacter species*

9.1. CLINICAL IMPORTANCE AND EPIDEMIOLOGY

The *Acinetobacter* genus can be divided into two different groups : the *Acinetobacter baumannii* complex including most of the disease-causing species (*A. baumannii*, *A. pittii* and *A. nosocomialis*) and the less pathogenic *Acinetobacter* non-*baumannii* group[1]. As correct identification of *Acinetobacter* isolates to species level is difficult, the EARS-Net surveillance monitors the antimicrobial resistance of *Acinetobacter spp*. In addition to these results, for the first time in 2018, EARS-BE collected AST results on the subgroup of *Acinetobacter baumannii* species.

Species belonging to the *A. baumannii* group are opportunistic pathogens primarily associated to nosocomial pneumonia (often ventilator-associated), central-line associated bloodstream and urinary tract infections. Immune suppression, advanced age, invasive procedures, burns and traumatic wounds and extended hospital stay are some of the risk factors for *Acinetobacter* infections[1].

In 2017, the Belgian PPS study estimated that 0.4% of HAI in Belgium were caused by *Acinetobacter baumannii* species[8].

9.2. INVASIVE SAMPLES (BLOOD/CSF)

9.2.1. Resistance in 2018 and national trends 2014-2018

Table 12 displays the mean resistance rates of *Acinetobacter spp*. to principal antimicrobial groups in Belgium from 2015 to 2018. **Figure 17** shows a graphical representation of the resistance trend observed in Belgium for all *Acinetobacter spp.* isolates. Of note, trends are very difficult to interpret, due to the selective subset of labs reporting results in the first two years of this surveillance.

In 2018, highest resistance levels were observed for fluoroquinolones (12.7%), followed by aminoglycosides (7.4%) and carbapenems (3.8%).

Among the 134 *Acinetobacter spp* isolates included in the surveillance in 2018, 31 (23.1%) were identified as *Acinetobacter baumannii* species (15 labs reporting results). Resistance levels for this species specifically don't differ much from those obtained for *Acinetobacter spp* isolates (12.9% R to fluoroquinolones and 6.9% R to aminoglycosides), except for the carbapenem resistance which reaches here 6.4%. However, given the small sample sizes for this pathogen, results have to be interpreted with caution.

EARS-BE started surveillance of *Acinetobacter spp.* in 2013 but the total number of isolates reported exceeded 10 only in 2015, and 100 in 2017. Given the low number of isolates, it remains difficult to obtain precise estimation of resistance prevalence on a national level.

Table 12 • *Acinetobacter species*: Mean resistance rates to principal antimicrobial groups within blood/CSF isolates, EARS-BE 2014-2018.

Antimicrobial group	% R (#R/N)					Labs in 2018 (N)	Trend[a]
	2014	2015	2016	2017	2018		
EARS-BE 2018, all isolates							
%R Carbapenems	0.0 (0/24)	2.6 (2/78)	6.9 (9/131)	3.8 (5/132)		26	
%R Aminoglycosides	0.0 (0/16)	1.5 (1/66)	13.1 (13/99)	7.4 (9/122)		25	
%R Fluoroquinolones	0.0 (0/28)	7.7 (6/78)	10.8 (14/130)	12.7 (17/134)		26	+

[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 17 • *Acinetobacter species***: Evolution of antimicrobial resistance within blood/CSF isolates, EARS- BE 2018 general criteria, 2015-2018.**

CARs = Carbapenems , AMGs = Aminoglycosides , FQs = Fluoroquinolones
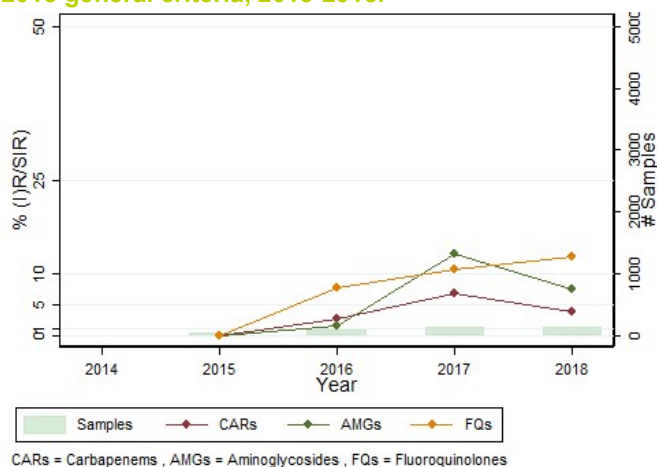
10. Colistin resistance in *E. coli*, *K. pneumoniae* and *P. aeruginosa*

Colistin belongs to a group of antimicrobial agents known as polymyxins. The mode of action involves binding to the outer membrane of Gram-negative organisms, which results in membrane disruption and cell death. Colistin is active against a wide variety of Gram-negative bacteria but not against Gram-positive bacteria, which lack an outer membrane.

Colistin is generally used in human medicine as an agent of last resort against highly resistant bacteria such as carbapenemase-producing *Enterobacteriaceae* or multi-drug resistant *P. aeruginosa*. However, colistin resistance has emerged worldwide. This resistance can be either chromosomally or plasmid-mediated, allowing possible transfer between bacteria[25].

In vitro assessment of antimicrobial susceptibility to colistin by routine methodologies is complex. The technical issues include poor diffusion of polymyxins through agar, which compromises the performance of both disc diffusion and gradient diffusion methods, and the tendency of polymyxins to bind to plastic surfaces. EUCAST only recommends broth microdilution for testing of colistin susceptibility[26].

Due to these technical limitations, only a subset of laboratories submit test results to the EARS-BE surveillance. Even if the number of labs submitting results on colistin resistance increased in 2018 compared to 2017, the test rates stay similar for both years. In addition, the validity of submitted colistin results is uncertain since it is unknown whether the laboratories providing colistin results used recommended testing methods. These limitations make estimation of national colistin resistance using EARS-BE data difficult.

Focusing on hospital laboratories only, colistin test rates on blood/CSF isolates varied from 74.7% in *E. coli* (19 labs reporting), 70.4% in *K. pneumoniae* (20 labs reporting), to 81.3% in *P. aeruginosa* (20 labs reporting). In urine isolates, these rates were 76% in *E. coli* (12 labs reporting), 64.4% in *K. pneumoniae* (12 labs reporting), and 72.8% in *P. aeruginosa* (17 labs reporting).

In *E. coli*, resistance to colistin in 2018 was very low with respectively 0.9% and 0.6% of invasive and urinary isolates tested as resistant. In *K pneumoniae* isolates, resistance was 0.9% in blood/CSF isolates and 1.5% in urine isolates. In *P aeruginosa*, resistance was 0.3% in blood/CSF isolates and 0.7% in urine isolates.

Of importance, in May 2019, the NCR for antibiotic resistant Gram negative bacilli has launched a national surveillance on colistin resistance, coupled with a European survey on carbapenem- and/or colistin-resistant *Enterobacteriaceae* (EURGen-Net survey coordinated by ECDC). The aims of this surveillance are to determine the occurrence, distribution and population dynamics of high-risk carbapenem resistant *E. coli* and *K. pneumoniae* (CRE) and/or colistin-resistant *E. coli* (ColRE) clones and/or transmissible resistance/genetic elements and to identify epidemiological risk factors for infection or colonisation with CRE and/or ColRE.

11. Combined resistance in *E. coli*, *K. pneumoniae* and *P. aeruginosa*

11.1. INVASIVE SAMPLES (BLOOD/CSF)

Table 13 displays the combined mean resistance rates of *E.coli*, *K. pneumoniae* and *P. aeruginosa* to principal antimicrobial groups under surveillance in Belgium from 2014 to 2018.

In 2018, more than half (60.1%) of *E.coli* isolates reported to EARS-BE, more than a quarter (28.7%) of *K. pneumoniae* isolates and 23.8% of *P. aeruginosa* invasive isolates were resistant to at least one of the antimicrobial groups under regular surveillance, and combined resistance to several antimicrobial groups was frequent.

Concerning *E. coli*, more than a fifth of all tested isolates (22.9%) in 2018 showed combined resistance to at least two of the antimicrobial groups under surveillance i.e. aminopenicillins, fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides and carbapenems. For this pathogen, slowly decreasing but statistically significant 5-year trends were observed for some indicators on combined resistance, for example from 27.1% R (2014) to 22.9% R (2018) for resistance to at least two antimicrobial groups and from 12.5% R (2014) to 9.3% R (2018) for resistance to at least three antimicrobial groups under surveillance. The switch from CLSI to EUCAST guidelines cannot explain this decrease as, except for carbapenems, the MICs breakpoints to identify resistant isolates are more strict in EUCAST guidelines compares to CLSI[20,27].

Combined multi-drug resistance was also frequent in *K.pneumoniae* with 10.1% of tested isolates being resistant to at least three antimicrobial groups under surveillance, i.e. fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides, and carbapenems. Of note, 10 out of the 742 tested isolates (1.4%) were resistant to all of these four antibiotic groups.

Multi-drug resistance is slightly less common in *P. aeruginosa*, with 5.5% of isolates being resistant to at least three antimicrobial groups under surveillance, i.e. piperacillin-tazobactam, ceftazidime, carbapenems, aminoglycosides and fluoroquinolones.

Table 13 • *Escherichia coli*, *Klebsiella pneumoniae* **and** *Pseudomonas aeruginosa:* **Combined mean resistance rates to principal antimicrobial groups under surveillance within blood/CSF isolates, EARS-BE 2014-2018, EARS-BE general criteria.**

Antimicrobial group	% R (#R/N)					Labs in 2018 (N)	Trend[a]
	2014	2015	2016	2017	2018		
Escherichia coli **isolates**[b]							
%R ≥ 1/5 antimicrobial groups	63.3 (1222/1929)	62.2 (1546/2485)	61.5 (2139/3478)	60.5 (2273/3759)	60.1 (2174/3619)	28	--
%R ≥ 2/5 antimicrobial groups	27.1 (522/1929)	27.2 (675/2485)	25.0 (871/3478)	24.5 (921/3759)	22.9 (827/3619)	28	---
%R ≥ 3/5 antimicrobial groups	12.5 (241/1929)	10.9 (272/2485)	11.0 (384/3478)	10.6 (399/3759)	9.3 (338/3619)	28	---
%R ≥ 4/5 antimicrobial groups	4.0 (77/1929)	3.4 (85/2485)	3.9 (135/3478)	3.5 (131/3759)	3.0 (108/3619)	28	(-)
%R = 5/5 antimicrobial groups	0.0 (0/1929)	0.0 (0/2485)	0.0 (0/3478)	0.0 (1/3759)	0.0 (1/3619)	28	
Klebsiella pneumoniae **isolates**[c]							
%R ≥ 1/4 antimicrobial groups	25.5 (82/321)	26.7 (101/378)	30.8 (196/637)	30.3 (188/620)	28.7 (213/742)	27	
%R ≥ 2/4 antimicrobial groups	14.9 (48/321)	17.5 (66/378)	20.1 (128/637)	15.6 (97/620)	20.2 (150/742)	27	
%R ≥ 3/4 antimicrobial groups	8.4 (27/321)	9.0 (34/378)	9.7 (62/637)	9.4 (58/620)	10.1 (75/742)	27	
%R = 4/4 antimicrobial groups	0.0 (0/321)	0.3 (1/378)	1.6 (10/637)	0.5 (3/620)	1.4 (10/742)	27	(+)
Pseudomonas aeruginosa **isolates**[d]							
%R ≥ 1/5 antimicrobial groups	20.8 (50/240)	16.8 (34/202)	22.7 (63/278)	19.4 (70/360)	23.8 (87/366)	25	
%R ≥ 2/5 antimicrobial groups	11.3 (27/240)	8.4 (17/202)	14.4 (40/278)	11.1 (40/360)	12.6 (46/366)	25	
%R ≥ 3/5 antimicrobial groups	7.1 (17/240)	4.0 (8/202)	6.5 (18/278)	6.7 (24/360)	5.5 (20/366)	25	
%R ≥ 4/5 antimicrobial groups	2.1 (5/240)	0.5 (1/202)	4.3 (12/278)	4.2 (15/360)	3.3 (12/366)	25	
%R = 5/5 antimicrobial groups	1.3 (3/240)	0.5 (1/202)	3.2 (9/278)	2.8 (10/360)	1.9 (7/366)	25	

[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

[b] Antimicrobial groups under surveillance for *Escherichia coli*: aminopenicillins, fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides and carbapenems

[c] Antimicrobial groups under surveillance for *Klebsiella pneumoniae:* fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides and carbapenems

[d] Antimicrobial groups under surveillance for *Pseudomonas aeruginosa:* piperacillin-tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems.

11.1. URINARY SAMPLES

Results on combined resistance within urinary samples from hospital and non-hospital laboratories are shown in **figure 18**.

Comparing both sample types (blood/CSF vs urine) from hospital laboratories, combined AMR levels in urine isolates were only slightly lower than in blood/CSF for *E. coli* and *K. pneumoniae* while those of *P. aeruginosa* were very similar.

When restricting the analysis to the group of hospitalized patients, levels of combined resistance within urine samples increased; differences between invasive and urinary samples largely disappear for *E. coli*, while for *P. aeruginosa* these became slightly larger in favour of urine isolates.

Note that, according to the EARS-BE protocol, an hospitalized patient is considered as a patient admitted in an acute care hospital ("INPAT") at the moment of sampling. Patients going to the hospital for dialysis, other day hospital care and to emergency room are classified as "other" and not as "INPAT"[2].

Comparing urinary samples from hospital and non-hospital settings, combined AMR levels are lower in isolates from non-hospital laboratories for all three pathogens.

Figure 18 • Combined antimicrobial resistance within blood/CSF and urine isolates of Gram-negative pathogens, following 2018 EARS- BE general criteria.
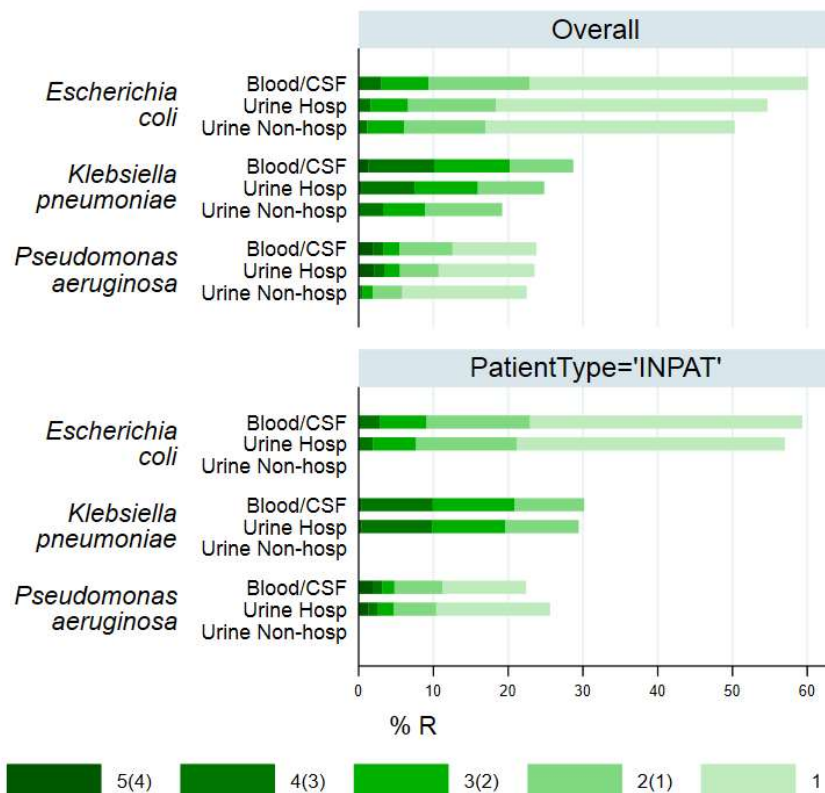
Combined antimicrobial resistance: isolates resistant to 1, 2, 3, 4 or 5 (1,2,3,4 for *Kpneumoniae*) antibiotic groups among those tested for: *Ecoli*: aminopenicillins, 3rd-gen cephalosporins, carbapenems, aminoglycosides, fluoroquinolones; *Kpneumoniae*: 3rd-gen cephalosporins, carbapenems, aminoglycosides, fluoroquinolones; *Paeruginosa*: piperacillin-Tazobactam, ceftazidim, carbapenems, aminoglycosides,

DISCUSSION

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This report presents the 2018 results of the Belgian branch of The European Antimicrobial Resistance Surveillance Network (EARS-BE). In total, 34 Belgian laboratories voluntarily submitted data on invasive and/or urine isolates in 2018. Of note, the number of participating non-hospital laboratories has doubled between 2017 and 2018.

From 2014 to 2018, significant decreasing trends were observed in the resistance to methicillin and fluoroquinolones of invasive *Staphylococcus aureus* isolates. However, these resistance rates remain substantial and seem to stabilize since 2017.

Concerning *Streptococcus pneumoniae,* depending on the database, variations in non-susceptibility (%IR) rates were obtained for penicillin in invasive isolates due to the possible use of different breakpoints. When analyzing the NRC database with a uniform breakpoint of 0.06µg/mL (PEN_MENI cut-off for penicillin meningitis), penicillin non-suscepibility rates were comparable (10.9% for NRC database compared to 11.9% for the EARS-BE data collection). In both databases, *Streptococcus pneumoniae* macrolides resistance was around 16% and decreasing resistance trends were observed between 2014 and 2018 for the NRC data.

For blood/CSF and urine samples and the three pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis)*, an important increase in the amoxicillin-clavulanic acid resistance rate was observed. This could be attributed to the switch of laboratories from CLSI to EUCAST interpretation criteria and especially to the switch from CLSI to EUCAST methodology to detect AMC resistance which resulted from the introduction by the company BioMérieux in 2018 of a new card (N366) to detect AMC susceptibility in Vitek systems. This card now contains a fixed concentration of 2 mg/L clavulanate as per EUCAST recommendations.

Pseudomonas aeruginosa isolates showed resistance to all antibiotic groups under surveillance (fluoroquinolones, piperacillin-tazobactam, aminoglycosides, ceftazidime and carbapenems) with the highest resistance rate detected for fluoroquinolones (14%). Despite the switch from CLSI to EUCAST interpretative guidelines by several laboratories over this period, no meaningful trend was detected for the period between 2014 and 2018.

Given the small sample size for *Acinetobacter spp* (134 isolates for 2018), results have to be interpreted with caution. Resistance levels for *Acinetobacter baumannii* do not differ much from those obtained for the whole *Acinetobacter spp* isolates with highest resistance levels observed for fluoroquinolones (12.9%), followed by aminoglycosides (6.9%) and carbapenems (6.4%).

The validity of colistin susceptibility data is questionable as the current recommended testing methods are rarely performed in routine laboratories.

Antimicrobial susceptibility data in urinary samples were collected for the second time in 2018. In general, resistance rates detected in urine isolates from hospital labs are similar or slightly lower than those observed within invasive isolates. Except from fluoroquinolones resistance within *Proteus mirabilis* and *Pseudomonas aeruginosa* isolates as well as fosfomycin resistance within *Proteus mirabilis* and *Klebsiella pneumoniae* isolates, resistance levels were higher within urine samples from hospital laboratories compared to samples from non-hospital settings. In the 2018 data collection, new test codes have been added for amoxicillin-clavulanic acid susceptibility detection in order to comply with EUCAST guidelines and to cover treatment of urinary tract infections : 'Amoxicillin-clavulanic acid systemic infection (AMC) = 8µg/mL according to EUCAST[20]' and 'Amoxicillin-clavulanic acid uncomplicated urinary tract infection (AMC_UC) = 32µg/mL according to EUCAST[20]'. Both breakpoints were used by non-hospital laboratories to detect the level of AMC resistance of *E. coli*, *P. mirabilis* and *K. pneumoniae* urine isolates. Surprisingly, similar or even higher resistance rates were detected using the less strict AMC_UC breakpoint compared to the AMC cut-off. Even if the correct use of both breakpoints has been verified by e-mail exchange, a misunderstanding or lack of verification by some participating labs could explain this illogical result.

The main aim of this national surveillance for antimicrobial resistance (EARS-BE) is to obtain a large set of homogeneous, representative and comparable AMR results in Belgium in order to participate to the main European surveillance system for AMR (EARS-Net). Through the collection of AMR data from urinary samples, EARS-BE also allows the extension of the study to non-hospital settings and a larger panel of infections.

The EARS-BE surveillance has several strengths. First of all, as based on ECDC methodology, this program allows comparison with results from other European countries through the EARS-Net program. Second, by clearly defining the sample types to include it allows an analysis limited to invasive samples (blood and cerebrospinal fluid). This restriction limits the impact of different sampling frames that would otherwise confound the data analysis if isolates from all anatomical sites were accepted[3]. By including urinary samples since 2017, EARS-BE has enabled to extend the AMR surveillance to non-hospital settings. Given the size of collected data, the results obtained from urinary samples are certainly interesting. Moreover, the relatively good participation of hospital laboratories in 2018 allows fair comparison with results from invasive samples for most antimicrobial groups. Third, data are collected at the antimicrobial susceptibility test level. This standardized data collection allows data manipulation and adjustment to new definitions and avoids possible interpretation mistakes. Of note, this detailed data collection also allows subgroup analyses, for example taking into account the guidelines used to interpret the AST (e.g. EUCAST vs CLSI).   Indeed, the use of different guidelines between years and laboratories results in high variability in the reported resistance rates. However, this complexity of different guidelines has largely disappeared in 2018, given that the vast majority of laboratories currently apply the EUCAST guidelines (above 90% in 2018).

The EARS-BE surveillance program has also several limitations. First, even if the participation of non-hospital labs submitting urinary samples has increased this year, it is still limited (about 25% of all private laboratories did participate in 2018) and there may thus be an underrepresentation in some part of the country (especially in the Brussels area). Second, the retrospective character of the surveillance reduces its utility in clinical and institutional decision making. The surveillance period of one year only allows to describe evolution of mean resistance rates in time but does not allow a fast estimation and follow-up of the burden of resistance and the possible emergence of new resistances in Belgium. Third, EARS surveillance is based on transfer of extracted data that could be limited to the reported results and not all tests results. This could explain sometimes high differences observed in the denominators of each test. Such variations can of course influence the resistance rates detected within this surveillance. Finally, the lack of official regulation, the voluntary character of the participation and the high registration burden can induce selection bias by limiting the participation of certain laboratories. This is particularly problematic when estimating resistance rates within relatively low-frequent pathogens (i.e. *Acinetobacter spp*). Therefore, it should be noted that EARS-BE data collection proceeds without any funding, in contrast with other national surveillances of HAI including antimicrobial resistance reporting. This is in contradiction with the widespread use of its results[1] combined with the substantial efforts needed to obtain these (both by laboratories and Sciensano).

In the next few years, strategies will be developed to overcome or reduce these limitations. The priority is to reduce the workload of laboratories and increase their participation. To this aim, general measures can be suggested. A first strategy is the harmonisation of protocols from the various surveillances organized within Sciensano. Of note, an harmonised data collection protocol for the EARS-BE and the AMR national surveillances will be proposed as a pilot phase for the 2019 data collection. Another strategic plan could be focusing on validated extraction procedures for specific lab systems. A last approach is to recognize the resources needed when producing timely, valid local and national indicators on AMR using a standardized AST-based laboratory data mapping.

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