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Temporal trends and patterns in antimicrobial resistant Gram-negative bacteria implicated in intensive care unit-acquired infections: a cohort-based surveillance study in Istanbul, Turkey

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Highlights

- Surveillance of gram-negative bacterial infections in intensive-care patients in Istanbul.
- Overall infection rate substantially and progressively decreased over 4 years.
- Antimicrobial resistance patterns varied markedly by organism and time.
- A back-to-susceptibility trend was noted for *P. aeruginosa*.
- XDR proportions remained constant in *Klebsiella* spp and increased in *A. baumannii*.

ABSTRACT

Objectives: This study assessed trends and patterns in antimicrobial resistant intensive care unit (ICU)-acquired infections caused by Gram-negative bacteria (GNB) in Istanbul, Turkey.

Methods: Bacterial culture and antibiotic susceptibility data were collected for all GNB causing nosocomial infections in five adult ICUs of a large university hospital during 2012-2015. Multi-resistance patterns were categorised as multidrug (MDR), extensively-drug (XDR) and pandrug (PDR)-resistance. Patterns and trends were assessed using seasonal decomposition and regression analyses.

Results: Of 991 pathogenic GNB recorded, most frequent were *Acinetobacter baumannii* (35%), *Klebsiella* species (27%), *Pseudomonas aeruginosa* (18%), *Escherichia coli* (7%) and *Enterobacter* species (4%). The overall infection rate decreased by 41% from 18.4 to 10.9 cases per 1000 patient-days in 2012 compared to 2015 ($p<0.001$), mostly representing decreases in bloodstream infections and pneumonias by *A. baumannii* and *P. aeruginosa*. XDR proportion in *A.baumannii* increased from 52% in 2012 to 72% in 2015, but only one isolate was colistin-resistant. Multi-resistance patterns remained stable in *Klebsiella*, with overall XDR and possible PDR proportions of 14% and 2%, respectively. A back-to-susceptibility trend was noted for *P. aeruginosa* in which the non-MDR proportion increased

from 53% in 2012 to 71% in 2015. 88% of *E.coli* and 40% of *Enterobacter* isolates were MDR, but none was XDR.

Conclusions: Antimicrobial resistance patterns in pathogenic GNB continuously change over time and may not reflect single-agent resistance trends. The proportionate amount of antimicrobial-resistant GNB may persist despite overall decreasing infection rates. Timely regional surveillance data are thus imperative for optimal infection control.

Keywords: Gram-negative bacteria; Antibiotic resistance; Nosocomial infection; Surveillance; Time trends.

1. Introduction

The intensive care unit (ICU) is the epicentre of nosocomial infections [1]. The most recent estimate of the prevalence of infected patients in ICUs is 24% in Europe [2] and 51% worldwide [3]. Gram-negative bacteria (GNB) are largely responsible for ICU-acquired infections and resistance to multiple antibiotics in pathogenic GNB has been alarmingly increasing worldwide [1,4]. GNB infections have become more complicated to treat and associated treatment failure and deaths have risen [5].

Successful management of infections in ICU patients requires prompt initiation of effective empirical antibiotic therapy, which in turn relies upon knowledge of likely antimicrobial resistance (AMR) patterns and trends [6]. As both the spectrum of ICU pathogens and the rates of AMR may vary widely by geographic region and between countries, it is imperative that data from systematic monitoring of local and regional AMR patterns and trends of clinically important GNB isolates be available and accessible to optimize antibiotic usage.

AMR in GNB pathogens has been reported to increase over the last decade in Turkey, but data on the changing epidemiology of ICU-acquired pathogens remain scarce [7–9].

Therefore, this study aimed to document *in vitro* susceptibilities to several antibiotics and assess temporal trends and patterns in the development of AMR among GNB causing infections in ICU patients in Istanbul, Turkey.

2. Materials and Methods

2.1. Study design and setting

In this cohort-based surveillance study, we recorded and analysed data from bacterial cultures and antimicrobials susceptibility tests performed on all GNB causing nosocomial infections in adult ICU-patients at Bezmialem Vakif University Hospital from January 2012 to November 2015. The study centre is a 600-bed university-affiliated hospital and one of the largest hospitals in Istanbul offering secondary and tertiary-care services to approximately 70,000 inpatients annually. Data were collected from five ICUs, including three mixed medical-surgical units (anaesthesia and reanimation I and II, respiratory, and neurology units), one surgical unit (cardiovascular surgery) and one medical unit (coronary ICU).

2.2. Data collection

For each ICU-acquired GNB infection, we recorded specimen type and infecting organism, infection site and date of first diagnostic sample. Infections were detected through active daily surveillance according to the CDC criteria [10]. Infections occurring more than 48 hours following ICU admission were considered ICU-acquired. We also recorded patient age, gender and APACHE II score at infection onset. We derived monthly denominator data (ICU admissions and patient-days) from electronic administration records.

GNB species were identified by the Vitek2 automated system or MALDI-TOF Mass spectrometry. Vitek2 was also used for antibiotic susceptibility testing in accordance with

CLSI guidelines [11,12]. To ascertain complex multi-resistance patterns, each GNB isolate was classified in one of four non-overlapping categories as follows: (1) non multidrug-resistant (non-MDR) if non-susceptible to less than three antimicrobial groups; (2) multidrug-resistant (MDR) if non-susceptible to three or more but susceptible to at least two groups; (3) extensively drug resistant (XDR) if susceptible to only one or two groups; and (4) pandrug resistant (PDR) if non-susceptible to all agents in all antimicrobial groups. An isolate was reported as non-susceptible to an antimicrobial group if it was *in vitro* resistant or intermediate resistant to at least one agent within the group. Antimicrobial groups were defined according to Magiorakos et al. [13] for *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae, and according to German et al. [14] for *Stenotrophomonas maltophilia*. Because not all required antibiotics had been tested, XDR and PDR in this study should be interpreted as 'possible XDR' and 'possible PDR', respectively, for all organisms except *A. baumannii* and *S. maltophilia* for which XDR is definite.

2.3. Data analysis

Focusing on the likelihood that a specific patient will receive adequate therapy, we present AMR data for each GNB species as proportion of isolates that are non-susceptible to certain antimicrobials and proportion presenting complex multi-resistance phenotypes. To assess trends over time we used incidence density rates (number of new infections per 1000 patient-days in ICU) and compared these between different years using rate ratios with exact 95% confidence intervals (CIs) and mid-P tests. To estimate annual trends in infection rates we used the slope coefficient and associated CI in a linear regression model of time on quarterly data. Linear trends in annual antibiotic non-susceptibility proportions were assessed using logistic regression. We report study results in accordance with the STROBE guidelines.

3. Results

3.1. Study population

In total, 991 GNB causing 900 infections in 550 patients were recorded. Affected patients had a median age of 69 years (interquartile range, 57 – 79 years) and 59.1% were male. The median ICU stay before onset of first infection was 12 days (interquartile range, 7 – 21 days) and the median APACHE II score was 18 (interquartile range, 13 – 23). Most patients had been admitted to the anaesthesia and reanimation units I (44%) and II (15%), followed by the respiratory (20%), neurology (13%), coronary (5%) and cardiovascular surgery (3%) units.

3.2. Distribution of infections and GNB pathogens

Overall, 152 (17%) infections were polymicrobial (7% involving gram-positive bacteria) and varied significantly by type of infection: 18% of bloodstream infections were polymicrobial, 17% of pneumonias, 2% of urinary tract infections, and 33% of other types of infection (p=0.001). *A. baumannii* was most frequent (35% of isolates), followed by *Klebsiella* species (27%), *P. aeruginosa* (18%), *E. coli* (7%), *S. maltophilia* (5%), *Enterobacter* species (4%), *S. marcescens (2%)*, and other organisms (2%).

The rank-order distribution of infecting pathogens varied according to infection type (Table 1). *A. baumannii* was the leading pathogen in pneumonias and urinary tract infections (causing 50% and 25% of infections, respectively), while *Klebsiella* spp. was leading cause in bloodstream infections and "other" infections (38% and 41% of infections, respectively). *P. aeruginosa* was the third most prevalent pathogen irrespective of infection type.

3.3. Trends in GNB infection rates

Between 2012 and 2015, the overall incidence of GNB infections progressively decreased from 18.4 to 10.9 cases per 1000 patient days (IRR = 0.59, 95%CI 0.48 – 0.71, p<0.001) (Figure 1). This mainly represented statistically significant decreases in infections caused by *A. baumannii* and *P. aeruginosa* (overall decreases of 51% and 76%, respectively), especially pneumonias and bloodstream infections (Table 2). In contrast, we did not observe significant trends in the rate of *Klebsiella* infections, which were relatively constant throughout the study period.

3.4 Trends and patterns in AMR

For each of the most frequent GNB pathogens, temporal trends in single-agent AMR are shown in Table 3 and multi-resistance patterns in Table 4. *A. baumannii* was highly non-susceptible (>90%) to most of the antibiotics tested. XDR proportion in *A.baumannii* progressively increased from 52% in 2012 to 72% in 2015 (p<0.001). Non-susceptibility to carbapenems and tigecycline reached 98% and 39% in 2015, respectively, but only one *A. baumannii* isolate was resistant to colistin and classified as possible PDR.

P. aeruginosa showed fluctuating non-susceptibility to carbapenems, fluoroquinolones and cephalosporins (pooled proportions, 52%, 41% and 55%, respectively), and decreasing non-susceptibility to aminoglycosides and piperacillin-tazobactam. An overall trend back to susceptibility was observed in *P. aeruginosa*; MDR and XDR proportions decreased from 17% and 29% in 2012 to 12% and 18% in 2015, respectively, while non-MDR proportion increased from 53% in 2012 to 71% in 2015.

Non-susceptibility of *Klebsiella* to carbapenems and colistin was high (pooled proportions of 62% and 34%, respectively), but tigecycline resistance decreased significantly from 29% in 2012 to 9.5% in 2015 (p = 0.002). Complex multi-resistance patterns remained relatively stable throughout the study period. Overall, 73% of *Klebsiella* isolates were MDR

and an additional 14% were XDR. Five isolates (2%) were possible PDR.

E.coli had a high MDR proportion (88% of isolates) and non-susceptibility to carbapenems increased from 0% in 2012 to 17% in 2015, but no isolate was XDR. Similarly, 40% of *Enterobacter* spp isolates were MDR but none was XDR.

S. maltophilia and S. marcescens remained highly susceptible throughout the study period with 98% and 96% of the isolates being non-MDR, respectively, and only one isolate in each species classified as MDR.

4. Discussion

This study assessed temporal trends and patterns in AMR development among GNB recovered from patients with ICU-acquired infections in Istanbul during a 4-year period. A substantial decrease in the overall GNB infection rate was documented, mainly representing significant reductions in bloodstream infections and pneumonias caused by *A. baumannii* and *P. aeruginosa*. Phenotypic patterns of AMR varied markedly by organism and continuously changed over the course of this study. A back-to-susceptibility trend was noted for *P. aeruginosa*, but XDR proportions remained constant in *Klebsiella* spp and increased in *A. baumannii.*

The overall reduction in the rate of ICU-acquired infections by 41% in four years in the study ICUs is worthy of consideration. It parallels the progression of an overall effort to identify, recognise and implement effective infection control practices in the study ICUs that was intensified by radical changes in staff management and the design of the built environment. Indeed, following the establishment of hospital-wide surveillance of hand hygiene compliance and nosocomial infections in 2011, the practice of supplementing ICU nursing staff from non-ICU services was banned late 2013. In addition, the length of

infectious disease rotations increased from 2 weeks to 3 months in 2014, which reduced staff turnover. The decline in the GNB infection rate in this study was slow in the first 30 months, but following a change in slope mid 2014 it became much faster thereafter (figure 1). It is notable that the latter coincides with the rebuilt of the anaesthesia and reanimation unit, a bay-room unit with high patient turnover, which was converted to a single-room ICU design in summer 2014. Although we can only hypothesize a possible effect in this study, others have demonstrated that the single room ICU design contributes significantly to the reduction of cross transmission of MDR GNB [15].

The impact of infection control activities on antimicrobial resistant infections is not necessarily straightforward. Infections least likely to be preventable are those occurring in the most vulnerable patients who may also be most likely to require prophylactic or therapeutic antibiotics. Thus, infection control programmes may be less effective in decreasing infections in patients who are at greatest risk for antimicrobial-resistant organisms. Moreover, an assumption often made in clinical practice is that successful infection control programmes would decrease antimicrobial-resistant proportions in pathogenic organisms by an amount proportional to the overall decrease in nosocomial infection rates. This, however, may not always be the case. In 33,587 central line–associated bloodstream infections that were reported from 1,684 ICUs in the USA from 1997 through 2007, the overall infection rate decreased by 70% but the proportion of *S. aureus* that was methicillin-resistant increased by 26% [16]. Similarly, we recorded a significant decrease in ICU-acquired bloodstream infections and pneumonias caused by *A. baumannii* but the XDR proportion in *A. baumannii* isolates progressively increased from 52% to 72% over the study period. In effect, we were confronting increasingly fewer infections caused by *A. baumannii* but these were increasingly more complicated to treat. By contrast, both the overall rate and the proportion XDR remained relatively constant in *Klebsiella* infections whereas significantly decreased in

Pseudomonas infections in our ICUs.

There are currently no systematic or comparable data on complex multi-resistance patterns in GNB clinical isolates collected in major surveillance systems worldwide [13,17]. Using European expert consensus definitions [13], we documented high prevalence of XDR phenotypes in *A. baumannii*, *P. aeruginosa*, and *Klebsiella* spp. in the study ICUs (73%, 29% and 14%, respectively). Although not directly comparable, our results are in agreement with those of the CAESAR surveillance network report from more than 70 Turkish hospitals in 2015 [18]. According to CAESAR findings, combined resistance to fluoroquinolones, aminoglycosides and carbapenems occurred in 77% of *A. baumannii*; resistance to three or more antimicrobial agents among piperacillin-tazobactam, ceftazidime, fluoroquinolones, aminoglycosides and carbapenems occurred in 17% of *P. aeruginosa* isolates; and combined resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides was detected in 20% of *K. pneumoniae* isolated in blood or cerebrospinal fluid clinical cultures from Turkish patients in 2015 [18] .

AMR in *Acinetobacter* spp. is a widespread problem in Europe where combined resistance to fluoroquinolones, aminoglycosides and carbapenems was noted for almost half of invasive blood isolates reported to EARS-Net from 30 countries in 2015 [19]. Overall, 4% of *Acinetobacter* isolates reported to EARS-Net were resistant to polymyxins, with half of these originating from Greece and Italy [19]. In the USA, carbapenem-resistance was detected in 47% and 64% of *A. baumannii* isolates implicated in catheter-associated bloodstream and urinary tract infections in 2014, respectively, and a multi-resistance phenotype was recorded for 44% and 69% of the isolates, respectively [20]. In this study, the rate of infections caused by *A. baumannii* was reduced by half over the study period, but proportionate single-agent resistance remained high (>90%) for most antibiotics tested. Tigecycline resistance remained relatively lower and stable and only one isolate was resistant

to colistin, but the proportion of XDR in *A. baumannii* increased significantly from 52% to 72% over the course of this study. This indicates persistent accumulation of AMR in *A. baumannii* isolates rather than increases in single-agent resistance. Colistin remains the only efficient option against *A. baumannii* infections in ICUs in our region.

High and increasing percentages of AMR in *Klebsiella pneumoniae* are a public health concern worldwide. The emergence and increasing rates of colistin resistance in *K. pneumoniae* have been reported globally, including regions of Europe, North America, South America, Asia and South Africa [21]. A multicentre study in 13 European countries, plus Israel and Turkey, found colistin resistance in *K. pneumoniae* of between 5% and 10% in 2011–2012 [22]. According to most recent EARS-Net data, one third of carbapenem-resistant *K. pneumoniae* isolates were also resistant to polymixins in 2015; the vast majority (95%) of which were derived from Greece and Italy [19]. In the present study, *Klebsiella* spp. isolates implicated in ICU-acquired infections presented high non-susceptibility to carbapenems and colistin (62% and 34%, respectively), with 14% of the isolates classified as XDR and an additional 2% as possible PDR. Colistin resistance in settings with high proportions of multidrug and carbapenem resistance in *Klebsiella* infections is of special concern, as colistin remains the last-resort treatment option for these infections. It is therefore imperative that surveillance of colistin-resistant GNB, including faecal carriage screening, is established in ICUs and other high-risk hospital units to monitor carefully the evolution of resistance to this drug in our region. Reliable techniques for susceptibility testing such as the broth dilution and rapid diagnostic tests for polymyxin resistance should be promoted for this purpose [23]. In addition to their alarming AMR profile, infections caused by *Klebsiella* spp. remained constant over the period of this study despite the significant reduction in the overall GNB infection rate. This may be related to the capacity of *Klebsiella* to silently colonize patients and hospital workers [24]. Asymptomatic carriers may be colonized for long periods of time

and are at increased risk of subsequent symptomatic infections, thereby acting as reservoirs for continued transmission that makes the spread of *Klebsiella* spp. exceptionally difficult to control [24].

P. aeruginosa strains with high resistance rates to aminoglycosides, ceftazidime, quinolones, piperacillin-tazobactam and carbapenems are common in Southern and Eastern Europe [19]. However, a trend analysis for 2012 to 2015 indicated variable single-agent resistance trends and an overall stable combined resistance pattern for these antimicrobials across Europe [19]. Moreover, a 'back to susceptibility' trend has been observed in French hospitals where the proportion of *P. aeruginosa* clinical isolates that showed MDR and XDR resistance patterns decreased significantly between 2008 and 2011 [25], a decrease that appears to have been sustained until 2015 [19]. Similarly, surveillance data from China demonstrated decreasing resistance levels in *P. aeruginosa* for all fourteen antimicrobial agents tested over a decade [26]. In this study, the major decrease seen in *P. aeruginosa* infections was accompanied by an overall back-to-susceptibility trend that was characterized by decreasing proportions of MDR and XDR isolates and fluctuating single-agent resistance trends.

Carbapenem resistance in *E. coli* in Europe is rarely reported; the highest rates are found in Greece (1.2%) and Romania (1.9%) [19]. In Turkey, resistance of *E. coli* against carbapenems has been reported to substantially fluctuate over time in a large paediatric hospital in Ankara, reaching 12.5% in 2014 and with 4.5% of the isolates classified as XDR [7]. Although none of the isolates was classified as XDR in this study, the increase of carbapenem resistance from 0% in 2012 to 17% in 2015 observed for *E. coli* is alarming. Continually monitoring and analysing such trends in AMR and establishing timely feedback of data to stakeholders is critical for creating and refining approaches to controlling AMR and for guiding clinician decisions regarding optimal antimicrobial prescribing.

The strengths of this study include its longitudinal length and the large number of pathogenic GNB isolates analysed, the prospective identification of nosocomial infections using standardised criteria and active case finding, and the inclusion of isolates from any site of infection giving an overall view of the changing epidemiology of GNB infections in ICU patients over time. Particular limitations in this study should also be acknowledged when interpreting our findings. We used clinical breakpoints and semi-quantitative interpretations to describe and quantify resistance and not captured minimum inhibitory concentration of antimicrobial agents which may be important to ensure consistent comparisons in time and space [27]. We did not perform genetic or molecular testing of GNB isolates to examine for specific resistance genes or enzymes; hence, the origin, clonal relationship and nosocomial spread of GNB were not investigated in this study. We are can only provide probable explanations or hypotheses for the causes of observed trends in GNB infection incidence and AMR patterns; the surveillance data used in this study do not include process measures linked to prevention efforts and do not allow us to test specific causation hypotheses. Our data were derived from a single centre in one geographic area; but as AMR patterns may vary from one location to another, studies from multiple sites may provide a more comprehensive picture of AMR trends across our region.

In conclusion, this study provided a unique view of the magnitude and trends of AMR among GNB infections in ICU patients in Istanbul. It demonstrates that AMR patterns in pathogenic GNB continuously change over time, vary by pathogen species and may not reflect single-agent resistance trends. The proportionate amount of antimicrobial-resistant GNB may persist despite overall decreasing infection rates. Timely regional surveillance data are thus imperative in understanding the changing epidemiology of ICU-acquired infections and in guiding empirical therapy and rational antibiotic policies.

Declarations

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Competing interests: None declared.

Ethical approval: The study was approved by the hospital's General Directorate (approval no. 97706721-900).

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Figure 1

Scatterplot and loess smoothed curve of monthly incidence of ICU-acquired infections caused by Gram-negative bacteria, 2012 – 2015

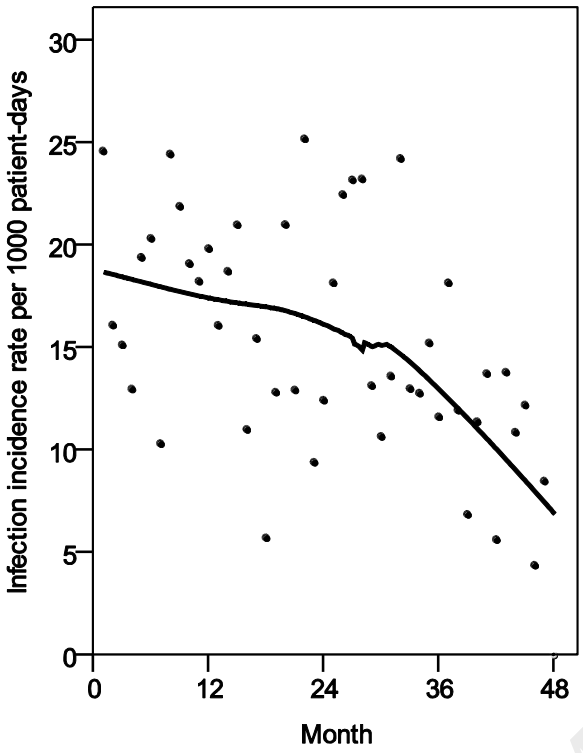

Table 1

Distribution and rank order of Gram-negative bacteria causing intensive care unit-acquired infections

	Pneumonia (n = 515)			**Bloodstream infection (n = 297)**			**Urinary tract infection (n = 61)**			**Other infection types[a] (n = 27)**		
Pathogen	No. of infections	Percent[b]	Rank	No. of infections	Percent[b]	Rank	No. of infections	Percent[b]	Rank	No. of infections	Percent[b]	Rank
Acinetobacter baumannii	257	49.9%	1	68	22.9%	2	15	24.6%	1	9	33.3%	2
Klebsiella spp.	126	24.5%	2	114	38.4%	1	14	23.0%	2	11	40.7%	1
Pseudomonas aeruginosa	112	21.7%	3	49	16.5%	3	12	19.7%	3	6	22.2%	3
Escherichia coli	32	6.2%	4	15	5.1%	6	14	23.0%	2	4	14.8%	4
Stenotrophomonas maltophilia	20	3.9%	5	30	10.1%	4	0	0.0%		0	0.0%	
Enterobacter *spp.*	10	1.9%	7	21	7.1%	5	6	9.8%	4	1	3.7%	5
Serratia marcescens	14	2.7%	6	10	3.4%	8	0	0.0%		1	3.7%	5
Other GNB species	4	0.8%	8	11	3.7%	7	0	0.0%		1	3.7%	5

[a] Other types of infection include surgical site infection (n = 14), skin and soft tissue infection (n = 10) and intra-abdominal infection (n = 3).

[b] Percentage of cases of infection caused by each pathogen. The sum of these percentages exceeds 100% as some infections were polymicrobial.

[c] Include *Achromobacter dentrificans* (n=1), *Achromobacter xylosidans* (n=1), *Acinetobacter junii* (n=1), *Burkholderia cepacia* (n=1), *Chryseobacterium gleum* (n=1), *Chryseobacterium indologenes* (n=2), *Morganella morgagni* (n=4), *Proteus mirabilis* (n=1), *Pseudomonas fluorescen*s (n=1), *Pseudomonas putida* (n=2), *Ralstonia pickettii* (n=1).

Table 2

Temporal trends in the rates of ICU-acquired infections caused by Gram-negative bacteria

Infection site and pathogen	Annual Incidence Rate[a]				Annual Trend Statistics[b]			
	2012	2015	IRR	95% CI for IRR	Trend[c]	95% CI for trend	P value	Adj. R^2
Pneumonia								
A. baumannii,	4.25	2.42	0.57	0.37 to 0.86	-0.69	-1.25 to -0.12	0.021	0.55
P. aeruginosa	2.59	.94	0.36	0.19 to 0.67	-0.49	-0.78 to -0.21	0.003	0.50
Klebsiella spp.	1.15	1.71	1.48	0.78 to 2.93	0.24	-0.26 to 0.73	0.316	0.00
Other GNB	1.08	1.36	1.26	0.63 to 2.59	0.15	-0.20 to 0.50	0.363	0.00
Bloodstream infection								
A. baumannii	1.95	.41	0.21	0.08 to 0.50	-0.53	-0.82 to -0.24	0.002	0.51
P. aeruginosa	1.59	.12	0.07	0.01 to 0.30	-0.46	-0.73 to -0.20	0.003	0.44
Klebsiella spp.	2.45	1.65	0.67	0.39 to 1.15	-0.29	-0.67 to 0.10	0.133	0.00
Other GNB	2.16	.83	0.38	0.19 to 0.74	-0.31	-0.72 to 0.09	0.119	0.31
Urinary tract infection								
A. baumannii	0.07	0.12	1.64	0.09 to 96.66	0.03	-0.11 to 0.17	0.641	0.00
P. aeruginosa	0.29	0.06	0.20	0.00 to 2.07	-0.07	-0.15 to 0.00	0.063	0.41
Klebsiella spp.	0.07	0.41	5.73	0.74 to 258.45	0.13	0.02 to 0.24	0.021	0.27
Other GNB	0.43	0.30	0.68	0.16 to 2.68	-0.06	-0.19 to 0.07	0.320	0.00
Other infection types								
A. baumannii	0.14	0.18	1.23	0.14 to 14.71	0.03	-0.07 to 0.12	0.559	0.00
P. aeruginosa	0.14	0.00	0.00	0.00 to 4.36	-0.03	-0.11 to 0.05	0.451	0.00
Klebsiella spp.	0.07	0.12	1.64	0.09 to 96.66	0.03	-0.08 to 0.14	0.568	0.00
Other GNB	0.00	0.24	NA	NA	0.09	0.01 to 0.16	0.024	0.39
Total (any infection)								
A. baumannii	6.41	3.13	0.49	0.34 to 0.69	-1.16	-1.76 to -0.56	0.001	0.69
P. aeruginosa	4.61	1.12	0.24	0.14 to 0.41	-1.06	-1.54 to -0.58	0.001	0.63
Klebsiella spp.	3.75	3.90	1.04	0.71 to 1.53	0.11	-0.60 to 0.83	0.737	0.00
Other GNB	3.68	2.72	0.74	0.49 to 1.12	-0.14	-0.76 to 0.48	0.638	0.00

GNB, Gram-negative bacteria; IRR, incidence rate ratio; CI, confidence interval.

[a] Number of infections caused by each specified pathogen per 1000 patient-days in 2012 and 2015.

[b] Estimated using ordinary least-squares linear regression of time on quarterly infection incidence rates.

[c] The trend is the estimated annual average change in the incidence rate of infection.

Table 3

Annual proportions of antibiotic resistance in most common Gram-negative bacteria causing ICU-acquired infections

Pathogen	Antibiotic agent or group[a]	Proportion non-susceptible (%)					2015-2012 % change[b]	p-value[c]
		Pooled	2012	2013	2014	2015		
A. baumannii (n = 350)	GEN, AMK	92.0	88.3	93.9	96.5	87.0	-1.4	0.559
	IPM, MEM	98.0	97.1	99.1	97.7	97.8	0.7	0.850
	CIP, LVX	97.7	97.1	99.1	96.5	97.8	0.7	0.920
	TZP	98.6	97.1	99.1	98.8	100.0	2.9	0.171
	CAZ, CFP/SUL, FEP	98.3	97.1	99.1	97.7	100.0	2.9	0.352
	SXT	80.6	65.0	87.8	88.4	82.6	17.6	0.001
	SAM	98.3	97.1	99.1	97.7	100.0	2.9	0.352
	CST	0.3	0.0	0.0	0.0	2.2	2.2	0.077
	TET	93.7	88.3	98.3	95.3	91.3	3.0	0.305
	TGC	26.6	35.0	14.8	25.6	39.1	4.2	0.804
P. aeruginosa (n = 179)	GEN, AMK	38.0	40.0	53.3	26.2	17.6	-22.4	0.043
	IPM, MEM	52.0	40.0	71.1	57.1	41.2	1.2	0.272
	CIP, LVX	41.3	42.7	51.1	33.3	29.4	-13.3	0.210
	TZP	62.6	65.3	75.6	50.0	47.1	-18.3	0.056
	CAZ, CFP/SUL, FEP	54.7	52.0	66.7	38.1	76.5	24.5	0.718
	CST	0.6	0.0	0.0	0.0	5.9	5.9	0.050
Klebsiella spp. (n = 265)	GEN, AMK	75.1	62.1	78.4	82.9	74.6	12.5	0.104
	IPM, MEM	61.5	43.1	73.0	68.6	57.1	14.0	0.237
	CIP, LVX	81.1	77.6	91.9	81.4	71.4	-6.2	0.148
	TZP	83.4	84.5	85.1	85.7	77.8	-6.7	0.352
	CAZ, CFP/SUL, FEP	93.6	87.9	97.3	91.4	96.8	8.9	0.174
	SXT	40.0	34.5	44.6	31.4	49.2	14.7	0.307
	SAM	95.1	98.3	94.6	92.9	95.2	-3.0	0.395
	CST	34.3	19.0	36.5	57.1	20.6	1.7	0.362
	TET	80.4	79.3	82.4	85.7	73.0	-6.3	0.481
	TGC	22.6	29.3	32.4	18.6	9.5	-19.8	0.002
E. coli (n = 66)	GEN, AMK	48.5	35.3	76.5	50.0	33.3	-2.0	0.522
	IPM, MEM	7.6	0.0	5.9	7.1	16.7	16.7	0.070
	CIP, LVX	69.7	76.5	76.5	85.7	44.4	-32.0	0.063
	TZP	54.5	41.2	52.9	42.9	77.8	36.6	0.054
	CAZ, CFP/SUL, FEP	87.9	70.6	100.0	92.9	88.9	18.3	0.191
	SXT	56.1	52.9	64.7	50.0	55.6	2.6	0.914
	CST	0.0	0.0	0.0	0.0	0.0	0.0	-
	TET	78.8	82.4	82.4	85.7	66.7	-15.7	0.296
	TGC	15.2	29.4	11.8	21.4	0.0	-29.4	0.037
Enterobacter spp. (n = 38)	GEN, AMK	10.5	15.4	16.7	10.0	0.0	-15.4	0.255
	IPM, MEM	23.7	23.1	50.0	30.0	0.0	-23.1	0.258
	CIP, LVX	15.8	7.7	50.0	20.0	0.0	-7.7	0.611
	TZP	39.5	46.2	50.0	30.0	33.3	-12.8	0.418
	CAZ, CFP/SUL, FEP	42.1	46.2	50.0	20.0	55.6	9.4	0.931
	SXT	10.5	15.4	16.7	10.0	0.0	-15.4	0.255
	CST	0.0	0.0	0.0	0.0	0.0	0.0	-
	TET	31.6	46.2	16.7	30.0	22.2	-23.9	0.276
	TGC	2.6	0.0	16.7	0.0	0.0	0.0	0.738

GEN, gentamicin; AMK, amikacin; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; LVX, levofloxacin; TZP, piperacillin-tazobactam; CAZ, ceftazidime; CFP/SUL, cefoperazone-sulbactam; FEP, cefepime; SXT, trimethoprim-sulfamethoxazole; SAM, ampicillin-sulbactam; CST, colistin; TET, tetracycline; TGC, tigecycline.

[a] An isolate is reported as non-susceptible to a group of antibiotics if it was non-susceptible to at least one antibiotic agent within the group.

[b] Difference of non-susceptibility proportions between 2015 and 2012.

[c] P-value calculated using logistic regression to assess the statistical significance of an increasing or decreasing linear trend in annual non-susceptibility proportions.

Table 4

Annual proportions of multidrug-resistant, extensively drug-resistant and pandrug-resistant Gram-negative bacteria causing intensive care unit-acquired infections

Pathogen	Resistance level[a]	Proportion non-susceptible (%)					2015-2012 % change[b]	p-value[c]
		Pooled	2012	2013	2014	2015		
A. baumannii (n = 350)	Non-MDR	1.4	2.9	0.0	2.3	0.0	-2.9	0.356
	MDR	25.7	44.7	18.3	12.8	26.1	-18.6	<0.001
	XDR[d]	72.6	52.4	81.7	84.9	71.7	19.3	<0.001
	Possible PDR	0.3	0.0	0.0	0.0	2.2	2.2	0.077
P. aeruginosa (n = 179)	Non-MDR	54.7	53.3	40.0	66.7	70.6	17.3	0.092
	MDR	16.2	17.3	20.0	11.9	11.8	-5.6	0.408
	XDR[d]	29.1	29.3	40.0	21.4	17.6	-11.7	0.239
	Possible PDR	0.0	0.0	0.0	0.0	0.0	0.0	-
S. maltophilia (n = 50)	Non-MDR	98.0	100.0	100.0	95.7	100.0	0.0	0.591
	MDR	2.0	0.0	0.0	4.3	0.0	0.0	0.591
	XDR[d]	0.0	0.0	0.0	0.0	0.0	0.0	-
	Possible PDR	0.0	0.0	0.0	0.0	0.0	0.0	-
Klebsiella spp. (n = 265)	Non-MDR	10.6	12.1	6.8	11.4	12.7	0.6	0.655
	MDR	73.2	77.6	70.3	70.0	76.2	-1.4	0.895
	XDR[d]	14.3	10.3	20.3	14.3	11.1	0.8	0.772
	Possible PDR	1.9	0.0	2.7	4.3	0.0	0.0	0.868
E. coli (n = 66)	Non-MDR	12.1	23.5	5.9	0.0	16.7	-6.9	0.513
	MDR	87.9	76.5	94.1	100.0	83.3	6.9	0.513
	XDR[d]	0.0	0.0	0.0	0.0	0.0	0.0	-
	Possible PDR	0.0	0.0	0.0	0.0	0.0	0.0	-
Enterobacter spp. (n = 38)	Non-MDR	60.5	53.8	50.0	70.0	66.7	12.8	0.418
	MDR	39.5	46.2	50.0	30.0	33.3	-12.8	0.418
	XDR[d]	0.0	0.0	0.0	0.0	0.0	0.0	-
	Possible PDR	0.0	0.0	0.0	0.0	0.0	0.0	-
S. marcescens (n = 25)	Non-MDR	96.0	100.0	100.0	91.7	100.0	0.0	0.611
	MDR	4.0	0.0	0.0	8.3	0.0	0.0	0.611
	XDR[d]	0.0	0.0	0.0	0.0	0.0	0.0	-
	Possible PDR	0.0	0.0	0.0	0.0	0.0	0.0	-

MDR, multidrug-resistant; XDR, extensively drug-resistant; PDR pandrug-resistant.

[a] Resistance level was categorised in non-overlapping groups of increasing multi-resistance level in accordance with the definitions of Magiorakos et al. [13] for *A. baumannii*, *P. aeruginosa* and Enterobacteriaceae, and German et al. [14] for *S. maltophilia* .

[b] Difference of non-susceptibility proportions between 2015 and 2012.

[c] P-value calculated using logistic regression to assess the statistical significance of an increasing or decreasing linear trend in annual non-susceptibility proportions.

[d] XDR should be interpreted as 'possible XDR' for all organisms, except *A. baumannii* and *S. maltophilia* for which XDR is definitive [13,14].