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Measurement and prediction of antimicrobial resistance in bloodstream infections by ESKAPE pathogens and *Escherichia coli*

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ABSTRACT

Objectives: This study aimed to evaluate a cumulative antimicrobial resistance index (ARI) as a possible key outcome measure of antimicrobial stewardship programmes (ASPs) and a tool to predict the antimicrobial resistance (AMR) trend.

Methods: Antimicrobial susceptibility for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli* (ESKAPEEc) pathogens recovered from blood cultures during a 5-year period (2014–2018) was analysed to obtain a cumulative ARI. For each antibiotic tested, a score of 0, 0.5 or 1 was assigned for susceptibility, intermediate resistance or resistance, respectively, and the ARI was calculated by dividing the sum of these scores by the number of antibiotics tested. Cumulative ARIs of ESKAPEEc micro-organisms were compared and a mathematical prediction model for AMR trend was obtained.

Results: In total, 1858 ESKAPEEc isolates were included in the study. The cumulative ESKAPEEc mean ARI increased significantly from 0.200 ± 0.01 in 2014 to 0.276 ± 0.02 in 2018 ($P < 0.001$). In multivariable regression analysis, factors significantly associated with ARI ≥ 0.5 were *E. faecium*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* infection ($P < 0.001$) and infection occurring after 2014 ($P < 0.05$). Based on the prediction model obtained, in the absence of any interventional measure, a tendency to pandrug resistance of the ESKAPEEc group could be expected in the next 8–15 years.

Conclusion: The ARI could be a useful tool to measure the impact of ASPs on AMR. The increasing incidence of AMR among ESKAPEEc organisms underscores the needing for ASPs.

1. Introduction

The World Health Organization (WHO) has identified antimicrobial resistance (AMR) as among the top public-health concerns of the 21 st century [1]. In 2017, the European Centre for Disease Prevention and Control (ECDC) declared Italy as one of the member states with the highest levels of AMR in Europe [2]. Indeed, a major driver of AMR has been considered the misuse of antimicrobials in humans and animals [3], with antimicrobial use accelerating the development of AMR [4]. The challenge with antimicrobial prescribing lies in the need to balance two conflicting goals, namely the provision of adequate therapy to treat documented or presumed infection, and the minimisation of antimicrobial use to avoid the emergence of AMR.

Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp. and Escherichia coli pathogens, defined as ESKAPEEc by De Angelis et al. [5], are responsible for a substantial percentage of severe infections and of multidrug-resistant organisms in hospitals [6]. During the last 5 years, in our hospital ESKAPEEc pathogens ranged from 75.7–78.9% of total bacterial isolates causing bloodstream infections (BSIs).

In an era of increasing AMR with limited availability of new and effective antibiotic agents, antimicrobial stewardship programmes (ASPs) have emerged as a fundamental component of healthcare systems [7], being theoretically able to avert trends in AMR and thus preserve future antimicrobial susceptibility. Indeed, the primary reason why antimicrobial stewardship (AMS) is necessary is specifically the growth of AMR [8]. Accordingly, AMR should be the main metric of the impact of an ASP.

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Reporting of antimicrobial susceptibility testing results has been shown to be crucial for targeting antimicrobial therapy in single clinical cases. However, information on a single pathogen or a single antibiotic cannot be sufficient to measure the efficacy of institutional programmes of antibiotic use. Unfortunately, there are no proposed aggregate measures of ASP success in the literature, considering resistance to multiple antimicrobials in multiple organisms [9]. The low evidence for the impact of AMS on AMR is likely related also to difficulties in measuring its outcome.

The primary aim of this study was to identify a simple way to decode and predict the AMR trend in the next years based on surveillance of ESKAPEEc organisms recovered from BSIs. A cumulative antimicrobial resistance index (ARI), obtained in a real-life setting, was generated to quantify the hospital AMR trend and for use by healthcare stakeholders to address specific ASPs. A secondary objective was to evaluate factors associated with a high ARI.

2. Methods

This was a monocentric observational retrospective study.

2.1. Data collection and description

Antimicrobial susceptibility testing results related to ESKAPEEc organisms isolated from blood cultures of patients admitted to Perugia General Hospital (Perugia, Italy) from 1 January 2014 to 15 October 2018 were collected from the Laboratory Information System (LIS) archive (TD-Synergy®; Siemens, Grenoble, France). Identification of ESKAPEEc organisms in blood samples was considered a proxy of true BSIs given its high positive predictive value. Pathogens other than ESKAPEEc organisms were excluded from the analysis. For each pathogen/patient combination, only the first BSI episode was considered.

For the years 2016, 2017 and 2018, BSI was classified as hospital-onset or community-onset if the first positive blood culture was collected >48 h or ≤48 h after admission, respectively. The above differentiation for the years 2014 and 2015 was not done, as the day of hospital admission for these years was not recorded in the LIS archive.

The microbiology laboratory provides diagnostic services to the 800-bed Perugia General Hospital, serving a population of approximately 200 000 people.

In our hospital, an infectious diseases specialist (IDS) is available on an on-call basis in all hospital wards at bedside 24 h/7 days. During the period 2014–2018, there was no local restriction policy on antibiotic use, including carbapenems or other broad-spectrum antibiotics, nor was there a specific ASP with pre-authorisation requirements for antimicrobial agents [10]. Only an 'educational/persuasive' approach was used by the IDS consultant, only upon request by ward clinicians.

To date, no change in the AMS policy has been made in the hospital. However, in the last observational year (2018), laboratory management of blood cultures has improved. First, thanks to a satellite incubator, all blood cultures are rapidly incubated within 1 h from collection, also outside of the operating hours of the microbiology laboratory. Second, following the introduction of a laboratory automation system, a new method to process positive blood cultures has been implemented with 8-h digital reading of subculture plates, followed by immediate identification and antimicrobial susceptibility testing. This method has been shown to significantly reduce the time to report and to shorten the duration of broad-spectrum empirical therapy to approximately 32 h [11]. Moreover, from 2018 a more stringent collaboration between the IDS and clinical microbiologist has been established, with an everyday briefing to discuss the most serious cases and blood culture results from the surgical ward and intensive care unit.

2.2. Blood culture and antimicrobial susceptibility testing

Blood cultures were received within 1 h from collection from different hospital wards inoculated into BD BACTEC™ Plus Aerobic/F and BD BACTEC™ Lytic/10 Anaerobic/F bottles and were incubated immediately in a BD BACTEC™ FX instrument (Becton Dickinson, Sparks, MD, USA). Positive blood cultures were processed for Gram staining and subculture on solid media, performed manually or, in the last 2 years of the study, automatically on a Work Cell Automation System (Becton Dickinson) as previously described [11]. Colonies were identified using a MALDI Biotyper® instrument (Bruker Daltonik GmbH, Bremen, Germany) as described elsewhere [12]. Antimicrobial susceptibility testing was performed with a BD Phoenix™ Automated Microbiology System (Becton Dickinson). For screening of methicillin resistance, the cefoxitin disk diffusion test was performed on *S. aureus*, whilst the combination disk test was used for extended-spectrum β-lactamase (ESBL) detection in Enterobacteriaceae. *Klebsiella pneumoniae*, *A. baumannii* and *P. aeruginosa* organisms suspected to be multidrug-resistant (MDR) (e.g. patient surveillance swabs or other specimens positive for MDR *K. pneumoniae*, *A. baumannii* or *P. aeruginosa* and/or colonies grown on blood CNA agar plates) were tested by the Sensititre™ microdilution method (Thermo Fisher Scientific, Cleveland, OH, USA). *Klebsiella* isolates were tested for carbapenemases by the Xpert® Carba-R assay (Cepheid, Sunnyvale, CA, USA) according to the manufacturer's instructions. Minimum inhibitory concentrations (MICs) were translated in clinical categories (susceptible, intermediate resistant or resistant) according to contemporary European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [13–17].

Acinetobacter baumannii and *P. aeruginosa* organisms were considered MDR if they exhibited resistance to at least one antibiotic in at least three relevant antimicrobial categories [18].

2.3. Antimicrobial resistance index (ARI) and prediction model for antimicrobial resistance trend

To calculate the ARI, the model for measuring antibiotic resistance in *P. aeruginosa* in patients with cystic fibrosis used by Ewing et al. [19] was followed. Briefly, for each antibiotic tested in each ESKAPEEc micro-organism, a score of 0 for susceptibility, 0.5 for intermediate resistance or 1 for resistance were assigned, and the ARI was calculated by dividing the sum of these scores by the number of antibiotics tested, giving a maximum score of 1. Thus, an ARI of 0 corresponded to a pandrug-susceptible organism and an ARI of 1 to a pandrug-resistant organism.

Then, the ARI of each ESKAPEEc species and the cumulative ARI for the entire ESKAPEEc group were obtained for each year included in the study and were compared.

Low/intermediate AMR was defined when the cumulative ARI was <0.5, and high/very high AMR was defined for ARI ≥ 0.5. Factors associated with a cumulative ARI ≥ 0.5 were evaluated.

Prediction of the ARI in the coming years was obtained from the data of the cumulative ARI for the ESKAPEEc organisms in each observational year, as follows: defining X_n as the ARI in the year 'n', the annual ARI increase (i.e. the increase of ARI between the two consecutive years 'n–1' and 'n') was:

$$I\_n = X\_n - X\_(n-1)$$

Hence, the rate T_n of the year-by-year increase/decrease was given by the formula:

$$T\_n = I\_n / X\_(n-1)$$

Now, in order to use a *T_n* rate that did not depend on the year '*n*', we considered the average *T* of the year-by-year *T_n* rates:

$T = (T\_1 + T\_2 + \ldots + T\_n)/n$

This value *T*, considering the current conditions stable, led us to predict the ARI in next years, according to the following formula:

$X\_n = (1 + T)X\_(n-1)$, for > 1

2.4. Ethics statement

Samples were collected and results were delivered to wards as part of standard care. Data included in the database were extracted from the archive of the microbiology laboratory and were de-identified before access. No personal information was stored in the study database. All collected data were analysed anonymously.

2.5. Statistical analysis

Standard descriptive statistics were used to summarise data, e.g. mean and standard deviation or standard error, and percentage, as appropriate. Differences between categorical variables were evaluated by Pearson's χ^2 test. Bacterial isolates were each compared versus any others. Unadjusted absolute changes in the ARI was calculated from 2015–2018 at each time point by analysis of variance (ANOVA) test. Mantel–Haenszel test was used for trend analysis.

Univariable and multivariable logistic regression analyses were used to evaluate factors associated with ARI $\geq$ 0.5. The dependent variable was ARI $\geq$ 0.5 as a dichotomic variable in the logistic models. Variables with a significance level of <0.1 were included in the multivariable analysis. Results of the logistic analysis are presented as odds ratio (OR) and 95% confidence interval (CI). Multivariable analysis included the following variables: age; hospital ward; bacterial isolate; and observational year. The analysis was not adjusted for hospital-onset BSIs as data for the years 2014 and 2015 were not available.

All statistical tests were performed using IBM SPSS Statistics v.22.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Antimicrobial resistance index in Perugia General Hospital from 2014–2018

A total of 8348 consecutive positive blood culture were analysed in the 5-year study period (2014–2018). After exclusion of 6490 samples according to the exclusion criteria (4640 not ESKAPEEc micro-organisms and 1850 redundant samples), 1858 ESKAPEEc isolates were included in the current study, comprising 385 in 2014, 415 in 2015, 391 in 2016, 408 in 2017, and 259 in 2018 (until 15 October 2018).

Patients' demographic characteristics and bacterial isolates included in the study are shown in Table 1. No statistical difference was observed for patient age, sex or hospital ward. More than the one-half of the BSIs were caused by two bacterial species, namely *E. coli*, which was the most frequent pathogen (34.0%), and *S. aureus* (25.5%), with no significant differences among the 5 years. Selected bacterial species were not evenly distributed in the five

Table 1
Patients' demographic characteristics and bacterial isolates from positive blood cultures in the 5-year study period[a].

Variable	All years	2014	2015	2016	2017	2018	*P*-value[b]
Patients (*n*)	1858	385	415	391	408	259	
Age (years) (mean ± S.D.)	68.0 ± 19.1	67.7 ± 18.8	67.5 ± 19.3	67.7 ± 20.6	69.2 ± 18.4	66.2 ± 20.7	0.346
Sex male	1077 (58.0)	223 (57.9)	230 (55.4)	234 (59.8)	239 (58.6)	151 (58.3)	0.783
Hospital ward							0.144[*]
Medical	1282 (69.0)	287 (74.5)	271 (65.3)	276 (70.6)	289 (70.8)	159 (61.4)	
Surgical	302 (16.3)	48 (12.5)	67 (16.1)	63 (16.1)	71 (17.4)	53 (20.5)	
ICU	274 (14.7)	50 (13.0)	77 (18.6)	52 (13.3)	48 (11.8)	47 (18.1)	
Source of bloodstream infection[c]							0.002
Hospital-onset	426 (42.5)	N/A	N/A	130 (37.4)	168 (41.9)	128 (50.4)	
Community-onset	577 (57.5)	N/A	N/A	218 (62.6)	233 (58.1)	126 (49.6)	
Bacterial isolates							
Bacterial isolates							
Gram-positive bacteria	603 (32.5)	132 (34.3)	137 (33.0)	127 (32.5)	121 (29.7)	86 (33.2)	0.704
Staphylococcus aureus	473 (25.5)	103 (26.8)	113 (27.2)	100 (25.6)	87 (21.3)	70 (27.0)	0.283
Enterococcus faecium	130 (7.0)	29 (7.5)	24 (5.8)	27 (6.9)	34 (8.3)	16 (6.2)	0.644
Gram-negative bacteria	1255 (67.5)	253 (65.7)	278 (67.0)	264 (67.5)	287 (70.3)	173 (66.8)	0.704
Escherichia coli	631 (34.0)	147 (38.2)	147 (35.4)	129 (33.0)	132 (32.4)	76 (29.3)	0.161
Pseudomonas aeruginosa	131 (7.1)	28 (7.3)	27 (6.5)	33 (8.4)	28 (6.9)	15 (5.8)	0.733
Klebsiella pneumoniae	315 (17.0)	52 (13.5)	58 (14.0)	70 (17.9)	88 (21.6)	47 (18.1)	0.014
Acinetobacter baumannii	61 (3.3)	12 (3.1)	12 (2.9)	5 (1.3)	22 (5.4)	10 (3.9)	0.025
Enterobacter spp.	117 (6.3)	14 (3.6)	34 (8.2)	27 (6.9)	17 (4.2)	25 (9.7)	0.004
Resistant isolates[d]							
MRSA	132 (27.9)	35 (34.0)	32 (28.3)	26 (26.0)	21 (24.1)	18 (25.7)	0.581
VREfm	33 (25.4)	4 (13.8)	6 (25.0)	8 (29.6)	9 (26.5)	6 (37.5)	0.466
3GC-resistant *E. coli*	152 (24.1)	21 (14.3)	42 (28.6)	35 (27.1)	31 (23.5)	23 (30.3)	0.529
MDR *P. aeruginosa*	11 (8.4)	4 (14.3)	3 (11.1)	2 (6.1)	2 (7.1)	0 (0.0)	0.527
CR *K. pneumoniae*	123 (39.0)	19 (36.5)	28 (48.3)	30 (42.9)	21 (23.9)	25 (53.2)	0.004
MDR *A. baumannii*	54 (88.5)	10 (83.3)	8 (66.7)	5 (100)	21 (95.5)	10 (100)	0.062
3GC-resistant *Enterobacter* spp.	33 (28.2)	3 (21.4)	14 (41.2)	0 (0.0)	7 (41.2)	9 (36.0)	0.003

S.D., standard deviation; ICU, intensive care unit; N/A, not available; MRSA, methicillin-resistant *S. aureus*; VREfm, vancomycin-resistant *E. faecium*; 3GC, third-generation cephem; MDR, multidrug-resistant; CR, carbapenem-resistant.

[a] Data are number (%) unless otherwise stated.

[b] Analysis of variance (ANOVA) test for multiple comparison of continuous variables and χ^2 test for categorical variables.

[*] Mantel–Haenszel test was used for trend analysis. All *P*-values refer to the comparison between individuals with or without the selected bacteria, e.g. those with versus those without the isolate.

[c] Missing data for hospital- or community-acquired bloodstream infection definition: 385 in 2014, 415 in 2015, 43 in 2016, 7 in 2017, and 5 in 2018.

[d] Percentages are out of the number of isolates of that bacterial species per year.

Table 2
Antimicrobial resistance index (ARI) for ESKAPEEc bacteria in each year of the 5-year study period[a]

Species	2014	2015	2016	2017	2018	*P*-value[b]	Trend
Enterococcus faecium	0.339 ± 0.03	0.354 ± 0.04	0.420 ± 0.03	0.416 ± 0.03	0.453 ± 0.05	0.184	
Staphylococcus aureus	0.168 ± 0.01	0.150 ± 0.01	0.169 ± 0.01	0.162 ± 0.01	0.160 ± 0.01	0.776	
Klebsiella pneumoniae	0.335 ± 0.05	0.544 ± 0.05	0.516 ± 0.04	0.487 ± 0.04	0.557 ± 0.04	0.011	
Acinetobacter baumannii	0.606 ± 0.07	0.634 ± 0.07	0.866 ± 0.01	0.874 ± 0.03	0.850 ± 0.02	<0.001	
Pseudomonas aeruginosa	0.210 ± 0.05	0.126 ± 0.04	0.169 ± 0.04	0.138 ± 0.04	0.096 ± 0.04	0.568	
Enterobacter spp.	0.129 ± 0.03	0.188 ± 0.02	0.123 ± 0.01	0.241 ± 0.04	0.171 ± 0.03	0.056	
Escherichia coli	0.121 ± 0.01	0.119 ± 0.01	0.137 ± 0.01	0.134 ± 0.01	0.167 ± 0.02	0.203	
Cumulative ESKAPEEc	0.200 ± 0.01	0.221 ± 0.01	0.244 ± 0.01	0.284 ± 0.01	0.276 ± 0.02	<0.001	

[a] Values are the mean ± standard error.
[b] Analysis of variance (ANOVA).

observational years. Hospital-onset BSIs increased significantly from 37.4% in 2016 to 50.4% in 2018 ($P = 0.002$).

A significant increase in ARI was observed for some, but not all, bacterial species (Table 2). The ESKAPEEc group cumulative ARI progressively and significantly increased during the 5-year study period from 0.200 ± 0.01 in 2014 to 0.276 ± 0.02 in 2018 ($P < 0.001$) (Fig. 1A) owing to the increase in isolates with high ARI ≥ 0.5. (Fig. 1B).

The ARI in hospital-onset BSI was significantly ($P < 0.001$) higher than in that in community-onset infection (Fig. 2).

In the multivariable regression analysis (Table 3) including age, hospital ward, ESKAPEEc organism and observational year, factors significantly associated with ARI ≥ 0.5 were: *E. faecium* (OR = 15.052, 95% CI 8.075–28.059), *K. pneumoniae* (OR = 52.891, 95% CI 30.531–91.626), *P. aeruginosa* (OR = 4.889, 95% CI 2.369–10.088) and *A. baumannii* infection (OR = 296.811, 95% CI 115.696–761.451) (all $P < 0.001$), and infection occurring after 2014 ($P < 0.05$). An increased OR was observed both in the univariable and multivariable analysis in any subsequent observational year following 2014.

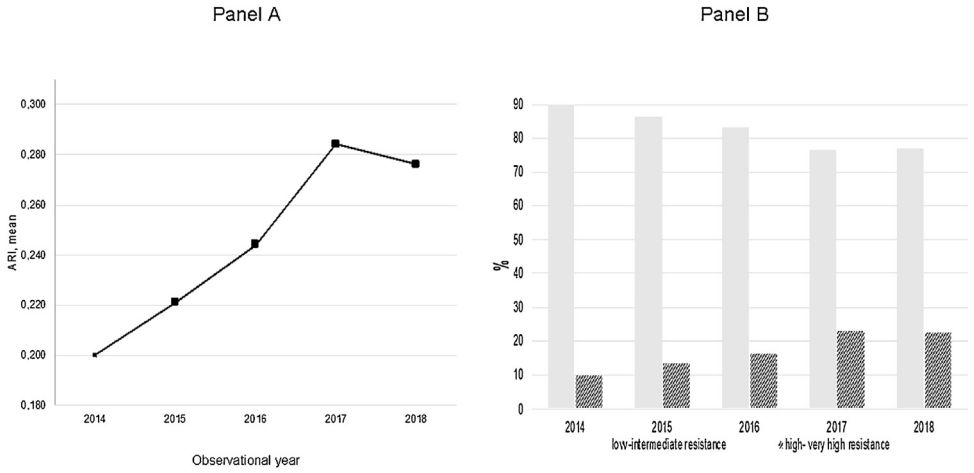

Fig. 1. (A) Mean antimicrobial resistance index (ARI) of all ESKAPEEc group organisms in the 5-year study period (2014–2018). (B) Percentage of isolates with low/intermediate resistance (ARI < 0.5) (whole grey bar) and with high/very high resistance (ARI ≥ 0.5) (striped bar). ESKAPEEc, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli*.

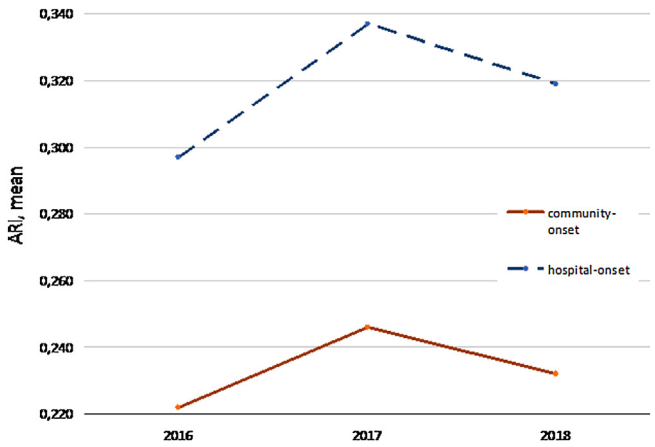

Fig. 2. Antimicrobial resistance index (ARI) from 2016–2018 in hospital-onset and community-onset bloodstream infections.

3.2. Prediction model of antimicrobial resistance

Since from 2014 (year 0) to 2018 (year 4), the ARI data were, respectively:

$X\_0 = 0.200$; $X\_1 = 0.221$; $X\_2 = 0.244$; $X\_3 = 0.284$; $X\_4 = 0.276$

then, according to the formula:

$I\_n = X\_n - X\_(n-1)$

the annual increase/decrease of ARI for each observational year was:

$I\_1 = 0.021$; $I\_2 = 0.023$; $I\_3 = 0.040$; $I\_4 = -0.008$

Next, according to the formula:

$T\_n = I\_n / X\_(n-1)$

the rate T_n of each year-by-year increase/decrease was:

$T\_1 = 0.105$; $T\_2 = 0.104$; $T\_3 = 0.164$; $T\_4 = -0.028$

The mean T from 2014–2018 was $T = (T\_1 + T\_2 + T\_3 + T\_4)/4 = 0.086$, hence the corresponding annual prediction given by the formula $X\_n = (1 + T)X\_(n-1)$ provides:

$X\_n = 1.086 \cdot X\_(n-1)$

(Fig. 3, line A). Because the I_4 for the year 2018 showed an opposite trend with respect to the previous years, the average T from 2014–2017 was also calculated, resulting in $T = (T\_1 + T\_2 + T\_3)/3 = 0.124$, leading to the alternative annual prediction:

$X\_n = 1.124 \cdot X\_(n-1)$

(Fig. 3, line B).

4. Discussion

The advent of multidrug resistance among pathogenic bacteria is jeopardising the value of antibiotics, which have previously transformed medical sciences. The global spread of AMR, predominantly due to the misuse of these agents and the unavailability of new drugs [20], points to the importance of ASPs aimed at optimising antibiotic use and minimising resistance [21].

There is a growing interest in the use of laboratory or clinical records to provide information supporting ASPs and to measure their impact in clinical practice. Although it is widely accepted that AMS is effective in increasing compliance with antibiotic policy and reducing the duration of antibiotic treatment, nevertheless

Table 3
Univariable and multivariable analysis for factors associated with high/very high antimicrobial resistance index (ARI ≥ 0.5)[a].

Variable	All	ARI ≥ 0.5	Univariable analysis		Multivariable analysis[b]	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Patients (*n*)	1858	315				
Age (years) (mean ± S.D.)	68.0 ± 19.1	66.2 ± 16.9	0.994 (0.988–1.00)	0.047		NS
Sex						
Female	781 (42.0)	121 (38.4)	1 (Ref.)			
Male	1077 (58.0)	194 (61.6)	1.198 (0.935–1.536)	0.153		
Hospital ward						
Medical	1282 (69.0)	202 (64.1)	1 (Ref.)			
Surgical	302 (16.3)	39 (12.4)	0.793 (0.549–1.146)	0.041		NS
ICU	274 (14.7)	74 (23.5)	1.978 (1.457–2.687)	<0.001		NS
Source of bloodstream infection						
Community-onset	577 (57.5)	92 (43.8)	1 (Ref.)			
Hospital-onset	426 (42.5)	118 (56.2)	2.023 (1.488–2.752)			
Bacterial isolates						
Escherichia coli	631 (34.0)	17 (5.4)	1 (Ref.)			
Staphylococcus aureus	473 (25.5)	1 (0.3)	0.077 (0.01–0.577)	0.013	0.072 (0.010–0.544)	0.011
Enterococcus faecium	130 (7.0)	37 (11.7)	14.369 (7.774–26.560)	<0.001	15.052 (8.075–28.059)	<0.001
Pseudomonas aeruginosa	131 (7.1)	16 (5.1)	5.025 (2.468–10.233)	<0.001	4.889 (2.369–10.088)	<0.001
Klebsiella pneumoniae	315 (17.0)	186 (59.0)	52.077 (30.602–88.621)	<0.001	52.891 (30.531–91.626)	<0.001
Acinetobacter baumannii	61 (3.3)	54 (17.1)	278.622 (110.691–701.325)	<0.001	296.811 (115.696–761.451)	<0.001
Enterobacter spp.	117 (6.3)	4 (1.3)	1.29 (0.422–3.870)	0.664	1.060 (0.342–3.283)	0.919
Observational year				<0.001*		
2014	385	39 (10.1)	1 (Ref.)			
2015	415	57 (13.7)	1.413 (0.916–2.179)		1.819 (1.030–3.208)	0.039
2016	391	65 (16.6)	1.769 (1.157–2.705)		2.357 (1.357–4.095)	0.002
2017	408	95 (23.3)	2.693 (1.800–4.029)		2.459 (1.451–4.167)	0.001
2018	259	59 (22.8)	2.617 (1.685–4.065)		4.167 (2.270–7.651)	<0.001

OR, odds ratio; CI, confidence interval; S.D., standard deviation; NS, not significant; ICU, intensive care unit.

[a] Values are number (%) unless otherwise stated.

[b] Multivariable analysis included the following variables: age; hospital ward; bacterial isolate; and observational year. The analysis was not adjusted for hospital-onset bloodstream infection as data were not available for 2014 and 2015.

* Mantel–Haenszel test for trend among observational years.

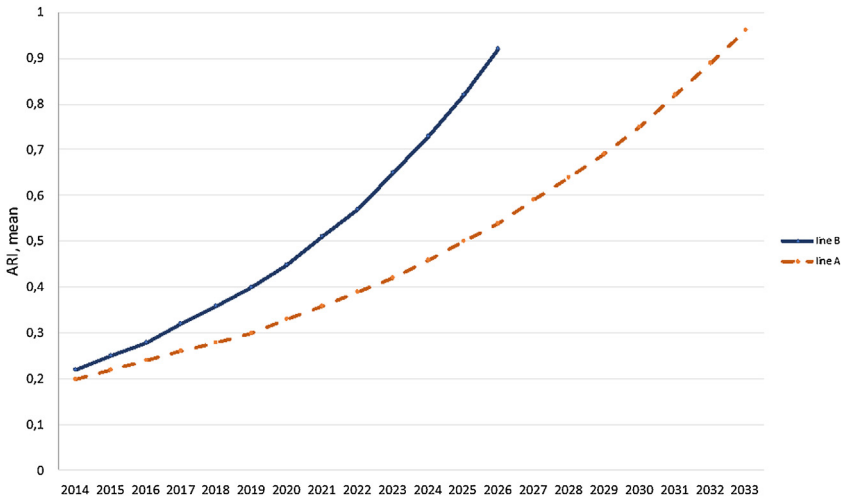

Fig. 3. Prediction model of antimicrobial resistance index (ARI) for ESKAPEEc group pathogens, including data from 2018 (line A) and excluding data from 2018 (line B). ESKAPEEc, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli*.

there is a surprising paucity of literature demonstrating the real impact of AMS on AMR [22], probably due to the difficulty in measuring this outcome [23]. It has been reported that decreased use of antibiotics probably does not increase mortality and possibly reduces the length of hospital stay [24], and a reduction in *Clostridioides* (*Clostridium*) *difficile* infections can be considered a measure of the outcome of AMS interventions [10].

This study proposes a simple method measuring AMR in a hospital and predicting AMR trend based on a cumulative ARI of all ESKAPEEc organisms. According to this model, it was found that AMR patterns in blood sample isolates progressively increased from 2014 to 2018 in Perugia General Hospital and, in the absence of a specific ASP, antimicrobial pandrug resistance could be expected within the next 8–15 years.

The ARI of some bacterial species did not significantly vary in different years (Table 2), whilst the cumulative ARI of the entire ESKAPEEc group significantly increased from 2014 to 2018 (Fig. 1A). This finding can be explained by the fact that cumulative ARI depends not only on the resistance of single bacterial species but also on the relative percentage of highly resistant organisms isolated each year. This result highlights the usefulness of the ARI in depicting the real weight of AMR in a particular setting. The bacterial species mainly contributing to high/very high ARI in the multivariable analysis were *E. faecium, K. pneumoniae*, *A. baumannii* and *P. aeruginosa* and, as expected, the ARI was higher in hospital-onset compared with community-onset infections, in line with the notion that infections by MDR organisms are more common in hospitals than in the community. Similarly, the progressive increase of AMR in our hospital, with pandrug resistance predicted within 8–15 years, is dramatically in line with the global AMR crisis, rendering infections virtually untreatable by the year 2050 [25].

Actually, in our setting, a minimal reversal of the AMR trend was observed in 2018. Although we cannot exclude that this finding is incidental, it could nevertheless be a consequence of two changes in the management of sepsis in our hospital in the last year: a significant reduction in time to report for blood culture results following the introduction of molecular technologies and automation in the laboratory workflow on positive blood cultures [11]; and a fruitful, close collaboration between the IDS and clinical microbiologist. Thus, assuming that the reversal trend in AMR in 2018 was due to the improved management of sepsis in our hospital, pandrug resistance can be predicted by 15 years. On the other hand, according to a less optimistic vision, considering the decreased 2018 ARI as a coincidence thus excluding it from the formula, pandrug resistance could be expected within 8 years. Nevertheless, according to the prediction model proposed in this study, an increasing trend in AMR is expected in the next years in our hospital.

The ARI can be intelligible to non-experts and useful to experts and could be applied as a measurement tool of different ASPs over time. It is not clear what kind of ASP could be more effective in our context to contain AMR trends. Many different methods to improve the prescription of antibiotics in hospitals have been studied. These programmes can be distinguished by persuasive or restrictive methods to reduce unnecessary antibiotic use. Persuasive methods advise physicians about how to prescribe or give them feedback about how they prescribed. Restrictive methods put a limit on how they prescribe and are considered able to achieve a faster effect than persuasive methods [10]. A recent meta-analysis supported the use of restrictive interventions when the need is urgent but suggested that persuasive and restrictive interventions are equally effective after 6 months from their application [10]. However, the timeframe of 6 months may be too short to produce significant results in terms of a reduction both in the incidence of infections by MDR organisms and of the ARI. Moreover, the ARI may not be appropriate to evaluate the efficacy of an ASP in a time period shorter than 1 year. In contrast, in clinical practice a short-term tool would be useful.

We found that the 2018 ARI, although lower than 2017, was higher than in previous years. It is also possible that the lack of specific implementation of AMS policy in our hospital could explain the increase of ARI observed in 2018. Furthermore, our 'soft persuasive' strategy on antimicrobial prescription may not have succeeded in reducing unnecessary use of antibiotics and the widespread prescription of broad-spectrum antimicrobials. On the contrary, this study underlines the needing for urgent strong specific actions in antimicrobial and infection control according to recent recommendations from the ECDC [2] and the Italian Group for Antimicrobial Stewardship (GISA) [26].

The present study has some strengths and limitations that deserve some comments. The ARI, a tool to get a view of the AMR trend that is simple to calculate, was set up based on the most appropriate and up-to date methods for antimicrobial susceptibility testing that included all first isolates of ESKAPEEc group organisms per single patient over 5 years.

As this was a single-centre study performed in a teaching hospital with a medically complex patient population, replication

of the results in other institutions and in other patient populations is necessary to enhance the generalisability of the findings. In addition, we are not able to evaluate in the multivariate analysis the role of hospital-onset BSIs as data were not availability for the years 2014 and 2015 in the database.

To minimise bias due to the fact that, for a few antibiotic molecules, data on the susceptibility of single bacterial species were not always available throughout the 5-year study period, due to the availability of new drugs or laboratory diagnostic tests, the ARI was normalised by the number of molecules tested. Other potential limitations include the absence of any consideration in the prediction model of infection control interventions as well as the difference among antibiotics and single species as the development of resistance to different antibiotics is considered equally, independent of the class and the bacterial species.

In conclusion, the increasing ARI rates among ESKAPEEc organisms in our hospital underscores the needing for a systematic approach and ASPs to effectively manage AMR in BSIs. The method proposed, in combination with others measurement tools of AMS, could be useful to measure the effects of such programmes in our hospital but also to compare them with those obtained with different approaches in other settings.

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Competing interests

None declared.

Ethical approval

This study followed the principles of the Declaration of Helsinki. Data included in the database were extracted from the archive of the microbiology laboratory and were de-identified before access. No personal information was stored in the study database. All collected data were analysed anonymously.

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