

ANTIMICROBIAL RESISTANCE OF ESKAPE-PATHOGENS IN CULTURE-POSITIVE PNEUMONIA

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Abstract

The aim of our study was to characterize the antimicrobial resistance in ESKAPE pathogens (*E. faecium*, *S. aureus*, *Klebsiella* spp., *A. baumannii*, *P. aeruginosa* and *Enterobacter* spp.) isolated from 606 culture-positive pneumonia: community-acquired (CAP), healthcare-associated (HCAP), hospital-acquired and ventilator-associated (HAP/VAP). A retrospective analysis was performed in tertiary care settings from Cluj-Napoca (2007-2013). Antibiotic resistance was determined according to Clinical and Laboratory Standards Institute (CLSI) and the trend by multiple antibiotic resistance index (MAR). ESKAPE pathogens (52%) were more likely found in HCAP and HAP/VAP. Co-resistance to ceftazidime, ciprofloxacin and gentamicin was dominant (48%). Highly resistant pathogens, Extended-spectrum beta-lactamase (ESBL)-*Klebsiella* spp., carbapenem-resistant *A. baumannii* and *P. aeruginosa* were identified mainly in HAP/VAP versus HCAP (39.3% vs. 2.3%, 92% vs. 7.5% and 40% vs. 1.4%, respectively). Except for *S. aureus*, multiple antibiotics resistance (MAR) index of ESKAPE pathogens revealed an increasing trend. In conclusion, ESKAPE pathogens are commonly identified in HCAP and HAP/VAP. An alarming frequency of highly resistant pathogens in hospital-acquired and ventilator-associated pneumonia was noticed.

Rezumat

Scopul studiului a fost caracterizarea rezistenței la antibiotice a patogenilor ESKAPE (*E. faecium*, *S. aureus*, *Klebsiella* spp., *A. baumannii*, *P. aeruginosa* și *Enterobacter* spp.) izolați din 606 pneumonii confirmate bacteriologic: comunitare (CAP), asociate îngrijirilor medicale (HCAP), spitalizării sau ventilației mecanice (HAP/VAP). Studiul retrospectiv (2007-2013) s-a derulat în cadrul serviciilor medicale terțiare din Cluj-Napoca. Rezistența la antibiotice a fost evaluată conform CLSI și tendința prin indicele de multirezistență la antibiotice (MAR). Patogenii ESKAPE (52%) au predominat în HCAP și HAP/VAP. Co-rezistența la ceftazidim, ciprofloxacin și gentamicină a fost de 48%. Patogenii cu rezistență extinsă, *Klebsiella* cu ESBL, *A. baumannii* și *P. aeruginosa* carbapenem rezistenți au dominat în HAP/VAP față de HCAP (39,3% vs. 2,3%, 92% vs. 7,5% și 40% vs. 1,4%). Indicele MAR al patogenilor ESKAPE a demonstrat o tendință de creștere cu excepția *S. aureus*. În concluzie, patogenii ESKAPE sunt frecvent identificați în pneumoniile non comunitare. S-a constatat o frecvență îngrijorătoare a patogenilor cu rezistență extinsă în pneumoniile contactate în spital și asociate ventilației mecanice.

Keywords: ESKAPE pathogens, pneumonia, highly resistant pathogens

Introduction

Antimicrobial resistance is one of the most important health concerns. The presence of multidrug-resistant pathogens in community and hospitals has been on rise in the last decade along with the problem of nosocomial infections. Health care-associated and nosocomial pneumonia are related to a high frequency of multidrug-resistant pathogens that have been grouped under the acronym ESKAPE comprising: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. [3, 15]. Rice et al. reported the

ESKAPE pathogens as responsible for the majority of nosocomial infections and capable of 'escaping' the biocidal action of antibiotics [3, 14-16].

The purpose of this study was to characterize the bacterial aetiology and antimicrobial resistance profiles of culture-positive acute pneumonia, focused on ESKAPE pathogens, in patients admitted in tertiary care hospitals including intensive care units (ICU's) in Cluj-Napoca, Romania.

Materials and Methods

We conducted a retrospective observational study of all culture-positive pneumonia between January

2007 and December 2013. The recorded data were obtained from medical wards, medical ICU, surgical and trauma ICU (Hospital of Infectious Diseases and University Emergency County Hospital, Cluj-Napoca). The inclusion criteria were: adult patients with radiographic, clinical signs of pneumonia and a concomitant positive respiratory bacterial culture or blood culture. Exclusion criteria were: patients younger than 18 years, pregnancy and HIV infection. According to the definitions for different types of pneumonia we identified: community-acquired pneumonia (CAP), health care-associated pneumonia (HCAP), nosocomial pneumonia including hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) [1, 11, 18]. All bacterial strains were referred and identified in a single microbiological laboratory. The strains identification was done by conventional methods, Vitek2 Compact (bioMérieux, Marcy

l'Etoile, France), API 10S and API Staph (bioMérieux, France). Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method (Bio-Rad discs, Marnes-la-Coquette, France) and minimal inhibitory concentrations (MICs) determinations with Vitek2 Compact system. The results were interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI). Extended-spectrum beta-lactamases (ESBLs) were detected in *E. coli* and *Klebsiella* spp. by using the CLSI criteria for screening of ESBLs and disk confirmation tests (Bio-Rad). For statistical purposes intermediate susceptibility was considered as resistant. Based on a set of definitions introduced by Kluytmans-VandenBergh et al., we identified the highly resistant microorganisms within the ESKAPE group, as seen in Table I [6, 21].

Table I

Definitions of highly resistant (HR) organisms

Microorganism	FLOX	GLY	PEN	ESBL	CAR	QUI	AMG	CFT	PIP	TMP-SMZ
<i>S. aureus</i>	A									
<i>E. faecium</i>		B	B							
<i>Klebsiella</i> spp.				A	A	B	B			
Other <i>Enterobacteriaceae</i>				A	A	B	B			B
<i>Acinetobacter</i> spp.					A	B	B	B		
<i>P. aeruginosa</i>					C	C	C	C	C	

HR to a drug listed as A = the organism must show resistance against an antibacterial agent from 1 of the indicated groups of this category; HR to a drug listed as B = the organism must show resistance against an antibacterial agent from at least 2 groups; HR to a drug listed as C = the organism must show resistance against an antibacterial agent from at least 3 groups. AMG, aminoglycosides; CAR, carbapenems; CFT, ceftazidime; ESBL, extended-spectrum betalactamase production; FLOX, flucloxacillin; GLY, glycopeptides; PEN, penicillins; PIP, piperacillin; QUI, fluoroquinolones; TMP-SMZ, trimethoprim-sulfamethoxazole.

The multiple antibiotics resistance (MAR) index is defined as a/b, where “a” represents the number of antibiotics to which the isolate was resistant and “b” represents the number of antibiotics tested [9, 17]. Descriptive statistics included mean and standard deviations or medians and interquartile range for continuous variables and percentages for categorical variables. Fisher’s exact test and chi-square test were used for categorical variables and the Mann-Whitney U test for nonparametric quantitative variables. Any value of $P < 0.05$ was considered statistically significant. GraphPad Prism version 5.03 Software for Windows (GraphPad Software, La Jolla, California, USA) was used. The study protocol was approved by the local ethical committee.

Results and Discussion

A total of 606 cultures-positive pneumonia were included: 330 CAP, 55 HCAP, and 221 HAP/VAP (Table II). Out of 221 nosocomial pneumonia, 197 were VAP (89%), the majority being recorded from surgical patients in ICU (132/197, 67%). The aetiology in HCAP was dominated by *P.*

aeruginosa, *E. coli* and *S. aureus*, similar to other studies performed in USA [7, 12]. Consistent to several international and European studies performed in ICUs, we found that the most prevalent pathogens responsible for HAP and VAP were: *A. baumannii*, *S. aureus*, *P. aeruginosa* and *Klebsiella* spp., [5, 8, 19, 20]. HAP and VAP are the second most common nosocomial infections in ICU, 3 to 20 times higher in surgical and trauma patients that underwent mechanical ventilation [2, 4, 13]. In the current study, most of the nosocomial pneumonia occurred in ventilated patients admitted in the above mentioned ICUs (89%). The ESKAPE pathogens were more frequently identified in HCAP compared to CAP, (RR 4.618, CI 95% 2.77-7.69) and in HAP/VAP versus HCAP (RR 1.8, CI 95% 1.26 to 2.55).

Among Gram-positive pathogens of ESKAPE group, the majority were *S. aureus* strains (12.2%). In HAP/VAP versus HCAP, the following resistance rates of *S. aureus* were identified to: oxacillin (69% vs. 9.5%, $p = 0.002$), gentamicin (62% vs. 1.3%, $p = 0.0001$), levofloxacin (63.5% vs. 6.7%, $p = 0.002$), clindamycin (67.6% vs. 6.7%, $p = 0.0002$). *S. aureus* isolates exhibited complete

susceptibility to linezolid, vancomycin and teicoplanin. Compared to HCAP, in HAP/VAP we found a high frequency of Gram-negative ESKAPE pathogens resistant to beta-lactam antibiotics, fluoroquinolones and aminoglycosides, $p = 0.003$ (Figure 1). Causative microorganisms of HCAP resemble those found in HAP/VAP but the resistance profiles were significantly different compared to the same pathogens identified in

HCAP/VAP. *Klebsiella* spp. (84 isolates) had the highest ceftazidime resistance rates with ESBL phenotypes in 41.4% (2.3% in HCAP, 39.3% in HAP/VAP). Among non-fermentative Gram-negative bacilli, 74% *A. baumannii* strains had a carbapenem-resistant profile (7.5% in HCAP, 92% in HAP/VAP) and 41.4% *P. aeruginosa* strains were resistant to carbapenem and ciprofloxacin (1.4% in HCAP, 40% in HAP/VAP).

Table II

Distribution of pathogens in culture-positive pneumonia

Pathogens	Culture positive pneumonia	CAP No. (%)	HCAP No. (%)	HAP/VAP No. (%)	p value HCAP vs. CAP	p value HAP/VAP vs. HCAP
All pathogens	606	330	55	221		
ESKAPE pathogens	316 (52)	76 (23)	36 (65.5)	204 (92.3)	0.0001	0.0001
<i>E. faecium</i>	4 (0.6)	0	1 (1.8)	3 (1.4)		
<i>S. aureus</i>	74 (12.2)	11 (3.3)	11 (20)	52 (23.5)	0.0001	NS
<i>Klebsiella</i> spp.	84 (13.8)	42 (12.7)	2 (3.6)	40 (18)	0.049	0.007
<i>A. baumannii</i>	66 (11)	0	5 (9)	61 (27.6)	-	0.003
<i>P. aeruginosa</i>	70 (11.5)	10 (3)	17 (31)	43 (19.5)	0.0001	NS
<i>Enterobacter</i> spp.	18 (3)	13 (4)	0	5 (2.3)	-	-
Non-ESKAPE pathogens	290 (48)	254 (77)	19 (34.5)	17 (7.7)	0.0001	0.0001
<i>S. pneumoniae</i>	108 (17.8)	101 (30.6)	4 (7.3)	3 (1.4)	0.0003	0.043
<i>S. pyogenes</i>	1 (0.2)	1 (0.3)	0	0	-	-
<i>H. influenzae</i>	112 (18.5)	110 (33.3)	0	2 (0.9)	-	-
<i>E. coli</i>	49 (8)	33 (10)	12 (21.8)	4 (1.8)	0.011	0.0001
Other Gram-negative bacilli	20 (3.3)	9 (2.7)	3 (5.5)	8 (3.6)	NS	NS

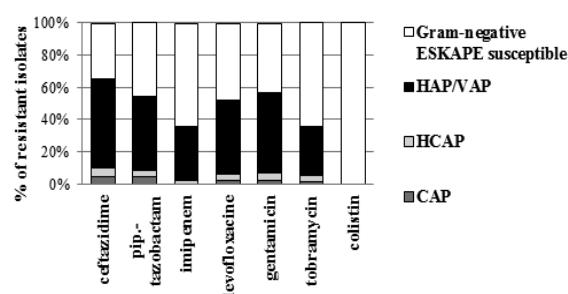


Figure 1.

Resistance profiles of Gram-negative ESKAPE pathogens

Overall, the most common resistance pattern in Gram-negative pathogens of ESKAPE group was the co-resistance to ceftazidime, gentamicin and ciprofloxacin (115/238 isolates - 48%). Out of 316 ESKAPE strains, 210 (66.5%) were highly resistant (HR) pathogens with a distribution that varied between the types of pneumonia: 3.6% in CAP, 30.9% in HCAP, 82% in HAP/VAP, $p < 0.0001$ (Figure 2). A surveillance study of ESKAPE pathogens in an ICU from Mexico reported a high prevalence of ESKAPE pathogens in respiratory infections and a prominent multidrug resistance for *A. baumannii*, *Enterobacter* spp., *Klebsiella* spp. and *P. aeruginosa* [10]. VAP, as one of the most

frequent complications in critical care settings, is more likely caused by highly resistant ESKAPE organisms related to antibiotic selective pressure and frequent genetic exchanges [14, 15].

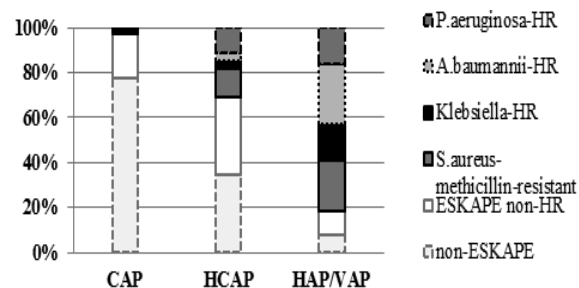


Figure 2.

Distribution of highly resistant ESKAPE pathogens

For each isolated species MAR index ranged from 0.0 to 0.76 (Table III). Compared with CAP, the median value of MAR index was significantly higher in HCAP and HAP/VAP [0.0, (IQR 0.0-0.13); 0.36, (IQR 0.2-0.58); 0.66, (IQR 0.57-0.80); $p < 0.0001$]. A high value of the median MAR index was found in ESKAPE versus non-ESKAPE pathogens, $p < 0.0001$ (Figure 3).

Table III

MAR index of relevant pathogens in pneumonia

	Non-ESKAPE pathogens			ESKAPE pathogens				
	<i>S. pneumoniae</i>	<i>H. influenzae</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>Klebsiella</i> spp.	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>Enterobacter</i> spp.
No. of isolates	108	112	49	74	84	66	70	18
MAR index, Median (IQR)	0.07 (0-0.2)	0.0 (0-0.09)	0.07 (0-0.22)	0.61 (0.5-0.66)	0.39 (0-0.63)	0.76 (0.6-0.83)	0.56 (0.18-0.9)	0.08 (0-0.23)

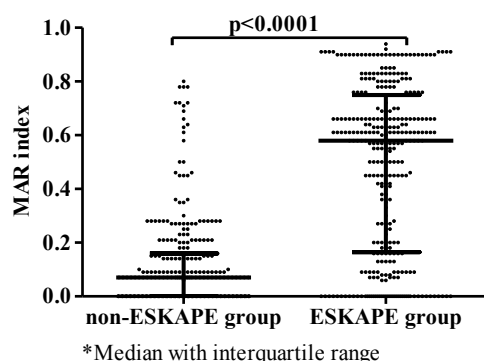


Figure 3.

MAR index of ESKAPE and non-ESKAPE pathogens

In receiver operating characteristic (ROC) analysis, a cut-off point of 0.29, the MAR index was a good predictor for highly resistant ESKAPE pathogens (AUC 0.99, 95% CI 0.991 to 0.999; $p < 0.0001$) and at cut-off point above 0.405, MAR index was 98% sensitive for highly resistant ESKAPE pathogens with a likelihood ratio of 20.8.

Analysing the temporal trend of antibiotic resistance in ESKAPE group, we found a slight increase of the MAR index for Gram-negative bacilli, but a significant decrease for *S. aureus* ($p = 0.001$) that might be explained by a better control of the spread of MRSA in ICU (Figure 4).

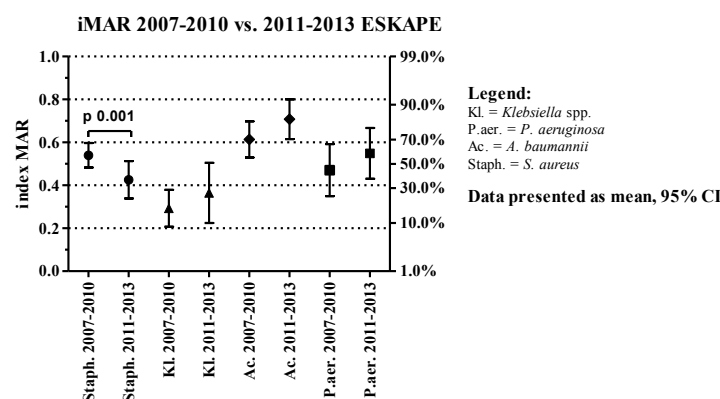


Figure 4.

MAR index trend of ESKAPE pathogens (2007-2010 vs. 2011-2013)

To our knowledge, this is the first assessment of the ESKAPE pathogens in pneumonia in tertiary referral centres, Cluj-Napoca, Romania. The data collection enabled us to present the ESKAPE pathogens, the resistance patterns and trends in culture-positive pneumonia. The main limitation of our study was the subject selection bias since only hospitalized patients with CAP and HCAP were included, while many of them are not referred to tertiary care settings. Since we focused on resistant respiratory pathogens we excluded culture-negative pneumonia, therefore the relative proportion of pathogens in CAP and HCAP might be different.

Despite the limitations, the current study supports the distinction between the three types of pneumonia, suggesting that ESKAPE pathogen distribution in HCAP shares more similarities with those found in HAP/VAP rather than CAP. Surveillance cultures are needed since the population at risk of HCAP is expanding and HCAP patients are more at risk for infections with multidrug-resistant pathogens. However, our study emphasizes a significantly increased proportion of resistant strains in nosocomial pneumonia compared with HCAP, correlated to host and environment characteristics, previous

hospitalization and immunosuppression. The most worrisome finding was the outstanding burden of highly resistant ESKAPE pathogens in nosocomial pneumonia consistent with MAR index appraisal. The ESKAPE pathogens remain by far the most common cause of healthcare-associated and nosocomial pneumonia.

Conclusions

Community-acquired, healthcare-associated and nosocomial pneumonia should be distinctly considered. The ESKAPE pathogens distribution in HCAP shares more similarities with HAP/VAP than with CAP. Our results highlight the alarming frequency and increasing trend of highly resistant ESKAPE pathogens with a common pattern of co-resistance to ceftazidime, gentamicin and ciprofloxacin in nosocomial pneumonia. The knowledge of local antimicrobial resistance can help in selecting an appropriate empirical therapeutic regimen in pneumonia.

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