A Ten-year study of the antibiotic resistance rates and trends of the ESKAPE bacteria isolated from sterile body sites (2010-2019) at a single hospital

Jamal Wadi Al Ramahi¹,
Walid Jamal²

¹ The University of Jordan, and Al Khalidi Hospital and Medical Center, Department of Infection Prevention and Control.

² Department of the Laboratory, Al Khalidi Hospital and Medical Center, Ibn Khaldoun St. 39, Amman, Jordan 11183.

Abstract

Background: To assess the rates and trends of resistance among ESKAPE pathogens during 2010-2019.

Methods: A retrospective, single-center study between 2010-2019, non-duplicate isolates from six sterile sources were studied. Pathogens were processed through the automated VITEK-2. The Clinical Laboratory Standards Institute (CLSI) breakpoints were referenced. The aim was to detect the rates and resistance trends of the ESKAPE pathogens, the rates of ESBL-producing *K. pneumoniae*, and the carbapenem-resistant (CR)-*K. pneumoniae*, CRAb, CRPa, VRE, and MRSA for the inpatients. Trends for the prevalence and resistance rates were analyzed by linear regression. Missing values were averaged based on the neighboring values. Data analysis was done using SPSS version 25, and statistical significance is considered for one-tailed $P < 0.05$.

Results: The ESKAPE bacteria (4286 isolate) comprised (45.57%) of the inpatients' isolates, the sterile sources consisted of 1421 (33.15%): *K. pneumoniae* 272 isolates, the ESBL-producing *K. pneumoniae* significantly declined (Pearson $R=0.877$, $P = 0.001$), CR-*K. pneumoniae* showed no significant trends ($P = 0.475$). *P. aeruginosa* 202 isolates; resistance to carbapenem (CRPa) averaged 42%. *S. aureus* 198 isolates; MRSA rates averaged 45%. *A. baumannii* 165 isolates; carbapenem-resistance (CRAb) average 93%. Vancomycin-resistant (VR)*E. faecium* = 33%, and VRE. *faecalis* = 15% with a weighted average 17%. Enterobacter spp. resistance rates were: Amikacin 3.6%, Third

Contact information:

Jamal Wadi Al Ramahi M.D, FIDSA.

Address: The University of Jordan, and Al Khalidi Hospital and Medical Center Department of Infection Prevention and Control.

Tel: +962 777 536 503.

✉ jamalwadimd@yahoo.com

and fourth generation cephalosporines 28% and 20% respectively, Quinolones 27% $\pm$ 3%, Piperacillin/tazobactam resistance 29%, Imipenem 15%, and Meropenem 27.

Conclusion: ESBL-*K. pneumonia* showed a declining trend but not other ESKAPE pathogens, though all were highly resistant, with a stable rate making treatment more complicated, and compromise the initial empiric treatment.

Keywords

ESKAPE; Carbapenem-Resistant Enterobacteriaceae; Carbapenem-Resistant *P. Aeruginosa*; Carbapenem-Resistant *A. Baumannii*; Resistance Trends; Extended-Spectrum β -Lactamases Producers; Jordan.

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Introduction

The acronym ESKAPE includes six common pathogenic bacteria (*Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp.). They exhibit multidrug resistance (MDR) pattern, virulence, and represent a substantial burden of the nosocomial infections [1]. Mortality associated with their infections is high and their treatment is challenging, costly, and carries poorer prognosis [2]. The rates of the ESKAPE pathogens have been on the rise in our region in the last decades as well as their resistance rates [3]. Furthermore, their resistance rates may increase with the length of hospital stay of the afflicted patients, especially when several surgical interventions were needed [4]. This study aims to identify the rates of the resistant ESKAPE pathogens over the ten years 2010-2019 and if there are trends in the rates and resistance. *E. coli* and *E. faecalis* were evaluated for the same sources, duration, and resistance to commonly used antimicrobial agents in their treatment during the same period for comparisons.

Materials and Methods

Settings

The study was conducted at Al Khalidi Hospital and Medical Center, a one-hundred-ten bed hospital including a 25-bed ICU, a 2-bed bone marrow transplant unit, and active surgical operating rooms; general surgery, brain and spine, orthopedics and cardiovascular surgery. Attached to the hospital is a medical center with out patient clinics, outpatient rooms for parenteral chemotherapy, antimicrobials therapy, radiology, Al Khalidi Women's Health & Breast Center, and diabetes centers. It is accredited by the Joint Commission International (JCI) since 2013 and the Jordanian Health Care Accreditation Council (HCAC). It serves as a referral hospital for patients from many Arab countries, and numerous multi-clinic complexes in its vicinity other than its outpatient department.

Methods

Non-duplicate isolates from six sterile sources (Blood, CSF, Pleura, Bones and joints, BAL, and Abdomen) were collected and processed through

an automated identification and susceptibility method: VITEK 2 (bioMérieux Middle East FZ-LLC. DHCC Al Baker Building 26. Office 107. P.O.Box 505 201. Dubai). Aerobic and anaerobic blood cultures were collected by experienced staff, 5 ml of blood per each bottle was extracted from the patients. Ertapenem was used to detect the carbapenem-resistant Enterobacteriaceae (CRE). Imipenem/meropenem for detecting *P. aeruginosa* (CRPa) and carbapenem-resistant-*A. baumannii* (CRAb). Extended-spectrum β -lactamases producers (ESBL) were identified by the automated VITECK 2. Methicillin-resistant *S. aureus* (MRSA) was identified based on Oxacillin susceptibility by the VITECK 2. Vancomycin-resistant enterococci (VRE) were detected based on vancomycin resistance in *E. faecium* and *E. faecalis*. Breakpoints used are those published by the Clinical Laboratory Standards Institute (CLSI) 2015.

Study outcome

A ten years' study between January 2010-December 2019, the aim was to detect the rates and resistance trends of the ESKAPE pathogens, and to detect the rates of ESBL-producing *K. pneumoniae*, the carbapenem-resistant (CR)-*K. pneumoniae*, CRAb, CRPa, VRE, and MRSA isolated from the six infected sterile body sources for the hospital inpatients.

Statistical analysis

The Rates and susceptibility of the ESKAPE pathogens *E. coli*, and *E. faecalis* were described (descriptive statistics) and the yearly trends over the ten years were analyzed by linear regression. The rates of ESBL-*K. pneumoniae* and ESBL-Enterobacteriaceae were described, and their trends were evaluated by linear regression analysis. The MDR patterns and bacteria (CRE, MRSA, CRPa, CRAb, and VRE) were analyzed by linear regression analysis for trends. Few missing values were calculated based on the average of the neighboring values. Data were analy-

zed using SPSS version 25 (IBM corporation), and statistical significance is considered for one-tailed $P < 0.05$.

Results

Over the ten years (2010-2019) there were 9405 inpatient isolates. The ESKAPE bacteria comprised 4286 (45.57%) bacteria isolates. The sterile sources consisted of 1421 (33.15%): *Enterobacter spp.* 35 isolates; 13 from blood, 8 from the abdomen, 8 from bones and joints, 3 from the pleura, and 3 from BAL. *S. aureus* 198 isolates; 112 from blood, 32 from BAL, and 28 isolates from bone and joints. *K. pneumoniae* 272 isolates; 151 isolates from blood, 55 from BAL, and 50 from the abdomen. *A. baumannii* 165 isolates; 61 from blood, and 76 from BAL. *P. aeruginosa* 202 isolates; 65 from blood, 69 from BAL, and 51 from the abdomen. *E. faecium* 45 isolates; blood 23 and 19 from the abdomen. *E. coli* 436 isolates; 259 were isolated from blood, followed by 118 isolates from the abdomen. *E. faecalis* 68 isolates; 51 from blood, 14 from the abdomen, and 3 from bones and joints (figure 1A).

Figure 1A: Frequency of the ESKAPE bacteria, *E. coli*, and *E. faecalis* from the sterile sources.

CSF: Cerebrospinal Fluid. BAL: Broncho-Alveolar Lavage.

Figure 1B: The rates of the ESKAPE pathogens for the years 2010-2019.

Figure 2: The percent of the ESBL-K.pneumonia and CRE-K. pneumoniae.

Among the ESKAPE pathogens, adequate numbers for analysis was for *K. pneumoniae* and *P. aeruginosa*, the other pathogens were discussed without charts as their counts were low. The yearly distribution of bacteria did not show a significant change in the burden over the ten years ($P > 0.05$), with the lowest rates for *Enterobacter* spp. and *E. faecium*. In the year 2016, the rate sighted for *A. baumannii* was the mean of the neighboring values

due to the absence of recorded cases in the sterile sources (**Figure 1B**). The multidrug resistance (MDR) pattern for the ESKAPE pathogens; the ESBL-producing *K. pneumoniae* showed a declining rate over the ten years (Pearson $R=0.877$, $P = 0.001$). CR-*K. pneumoniae* showed an increase in the years 2012 to 2016 then a decline in 2019 with no significant overall trends ($P = 0.475$), and all ESBL-producing *Enterobacteriaceae* drifted down from almost 60% in 2010 to 47% in 2019 with an average of 47.6% (Pearson $R = -0.582$, $P = 0.039$). MRSA showed variable rates between 31% to 62% (average 45%) and no trends. CRPa resistance rates were ranged between 29% in 2018 to 62% in the years 2010 (average 42%) with no significant trends. CRAB resistance rates were between 82% to 100% (average 93%) and no trends. VRE rates were 15% and 33% for *E. faecalis* and *E. faecium*, respectively, and the weighted average was 17%, with no significant trends (**Figure 2**).

The antimicrobial resistance patterns of the ESKAPE pathogen for the ten years (2010-2019) were variable (**Table 1**), the total average showed that *Enterobacter* spp. resistance rates were as follow: Amikacin 3.6%, ceftazidime 28%, cefepime 20%, ciprofloxacin was 30% and levofloxacin 24%, piperacillin/tazobactam resistance 29%, imipenem 15%, ertapenem 23%, and meropenem 27, tigecycline 24% and colistin "0%". *S. aureus* resistance i.e. MRSA averaged 45% with no trends ($P > 0.05$), imipenem 14%. *K. pneumoniae* resistance rates for amikacin 13%, ceftazidime 54%, piperacillin/tazobactam 52%, levofloxacin 42%, imipenem 20%, ertapenem 31% and meropenem 12%, colistin and tigecycline 13% and 12 %, respectively (**Figure 3A**). *A. baumannii* resistance for amikacin was 48%, for the third and fourth generations and quinolones (98-100%), 93% for imipenem and meropenem, and 97% and 94% for levofloxacin and ciprofloxacin, respectively with no trends ($P > 0.05$). *P. aeruginosa* resistance for amikacin was 24%, ceftazidime, cefepime 35%, ciprofloxacin and levofloxacin 39%

Table 1. The total average of the ESKAPE resistance rates to several antimicrobial agents for the years 2010-2019. In addition to rates, all cells were color-coded (heat table) to easily demonstrate the extent of resistance rates. The lower bounded two-rows of the table include *E. coli* and *E. faecalis*.

	Amikacin	Cefazoline	Ceftriaxone	Ceftazidime	Cefepime
Enterobacter spp.	3.6	96	38	28	20
<i>S. aureus</i>	NT	46	46	NT	44
<i>K. pneumoniae</i>	13	70	60	54	37
<i>A. baumannii</i>	48	100	100	98	99
<i>P. aeruginosa</i>	24	100	100	35	35
<i>E. faecium</i>	NT	100	100	NT	100
<i>E. faecalis</i>	NT	100	100	NT	100
<i>E. coli</i>	1.5	69	60	44	22
	Ciprofloxacin	Levofloxacin	Ertapenem	Imipenem	Meropenem
Enterobacter spp.	30	24	23	15	27
<i>S. aureus</i>	26	27	43	14	46
<i>K. pneumoniae</i>	52	42	31	20	12
<i>A. baumannii</i>	97	94	94	93	93
<i>P. aeruginosa</i>	39	41	100	42	41
<i>E. faecium</i>	100	75	100	68	100
<i>E. faecalis</i>	100	56	NT	0	NT
<i>E. coli</i>	63	60	1.8	1	25
	PIP/TAZ	Colistin	Linezolid	Tigecycline	PIP/TAZ: Piperacillin/ tazobactam. NT: Not tested.
Enterobacter spp.	29	0	NT	24	
<i>S. aureus</i>	46	NT	NT	0	
<i>K. pneumoniae</i>	52	13	NT	12	
<i>A. baumannii</i>	97	2	NT	40	
<i>P. aeruginosa</i>	29	15	NT	89	
<i>E. faecium</i>	67	NT	11	0	
<i>E. faecalis</i>	0	NT	13	0	
<i>E. coli</i>	25	2	NT	0	

and 40%, respectively. Further resistance to piperacillin/tazobactam 29%, imipenem and meropenem 41% and 15% for colistin (**Figure 3B**). *E. faecium* was 100% resistant for ertapenem and meropenem, imipenem 68%, and piperacillin/tazobactam 67%.

The resistance of *E. coli* and MSSA were assessed at the same period for comparison: The average ESBL-*E. coli* rate was 54%, with no trends (R 0.49, P = 0.15); resistance to amikacin was 2%, ertapenem

2%, and imipenem 1%, meropenem 0%, piperacillin/tazobactam 25%, ciprofloxacin and levofloxacin 63% and 60%, respectively, Also, ceftazidime and ceftriaxone 39% and 60%, respectively, whereas tigecycline "0%" and Colistin 2% (**Figure 3C**). The MSSA demonstrated "0%" resistance for imipenem and tigecycline, and 1%-2% for cefazoline, ceftriaxone, cefepime, piperacillin/tazobactam and ertapenem, and 9% for quinolones (**Figure 3D**).

Figure 3A: Susceptibility of the ESKAPE pathogens for *K. pneumoniae*.

Figure 3B: Susceptibility of the ESKAPE pathogens for *P. aeruginosa*.

Figure 3C: Susceptibility of the ESKAPE pathogens for *E. coli*.

Figure 3D: Resistance rates of *S. aureus* (MRSA not included) for the ten-years 2010-2019.

Discussion

Blood isolates dominated the sources of the isolated ESKAPE pathogens over the ten years, and each species was fluctuating within a narrow range. The total numbers of the bacteria started with adequate counts but dropped dramatically down for the *Enterobacter* spp. and *A. baumannii* as we sought the sterile sources. However, a total average was calculated for the ten years to move closer to real rates. Despite there was a surge in the CRE rates between 2012-2016, the years outside this period may have had represented the local rates more accurately as many war trauma patients arrived in Jordan from the Arab countries for treatment, most of them had multiple surgical interventions with antimicrobial exposures, extended length of

hospital stay, and highly resistant isolates on arrival [5]. Plans were needed to cope with the increasing MDR-bacteria in their home country and Jordan [6]. The ESBL-producing *K. pneumoniae* showed a dramatic decline in the rates (**Figure 2**) during 2010-2019 (P trends = 0.001), to re-examine this phenomenon at the time of the increased resistance due to many factors, all ESBL-*Enterobacteriaceae* including *E. coli* were evaluated and showed a similar significant phenomenon though was less impressive (P trends = 0.039), mostly attenuated by the lack of trends by the ESBL-*E. coli* (P = 0.15). This was similar to what was reported by a Japanese group where they demonstrated stable rates at 24.6% for *E. coli*, but the resistance pattern declines significantly over five years (P < 0.01) [7]. Carbapenems were reliable agents in the

treatment of *A. baumannii* but the development of resistance (CRAB) compromise their activity, our resistance rates escalated to 93-100%, and were much similar to a Jordanian study and higher than the recorded rates from South and Southeast Asia, where rates were 50-60 % in some countries and 85% in others [8, 9]. A worldwide study revealed that the global resistance of *A. baumannii* for tigecycline was as high as 25.6% before 2018, lower than what we had at 40% [10]. CRPa was highly resistant to the carbapenems at an average of 41% with no trends ($P > 0.05$), our rates were higher than some hospitals in our region. A recent published work from Jordan demonstrated that *P. aeruginosa* isolated from clinical respiratory specimens expressed multiple resistant genes with 52.5% MDR phenotype [11, 12], but lower than other countries where CRPa rate was as high as 90% [13]. The resistance for the traditional anti-pseudomonal β -lactams in this study was 35%, meanwhile, it ranged 15-23% elsewhere [14].

Our MRSA rates were 45%, where as a study from two large tertiary hospitals in Jordan showed that 73.2% of *S. aureus* contained mec gene and were designated as MRSA, other studies from several countries demonstrated high MRSA similar to what we found in our hospital [15, 16]. MSSA were tested for susceptibility to commonly used agents, their resistance rates for the cephalosporines (ceftriaxone, cefazoline and cefepime) were 2%, and zero for imipenem. Unfortunately, many practitioners still use the broad-spectrum antimicrobial agents in the treatment of MSSA though the first generation cephalosporines agents are appropriate, and they have a low resistance rate. Canonically, penicillin was justified and recommended for use in the treatment of MSSA bacteremia if it tested penicillin-sensitive [17]. Enterococci were dominantly isolated from blood and abdomen, VRE rates were 17%, for *E. faecium* was 33% but low counts over years, although the low count may have blown up the ratio. It is well perceived that

E. faecium assumes a high vancomycin resistance rate, an example from the region showed that VRE rates from Turkey has reached up to 22% [18]

Conclusion

The ESKAPE pathogens isolated in this study from sterile sources were resistant to the traditional antimicrobials used in their treatment; particularly *A. baumannii* isolates for carbapenems, and *P. aeruginosa* isolates for ceftazidime, cefepime and meropenem. The first-generation cephalosporins and anti-staphylococcal penicillins for *S. aureus* and ampicillin and vancomycin for Enterococci. The MDR pattern is increased and became stable, except for the ESBL-*K. pneumoniae* that showed a declining trend. Therefore, this would hamper the utilization of the commonly used antimicrobials, and resorting to more complicated regimens.

Conflict of interest

None for this manuscript.

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