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ORIGINAL ARTICLE

Multidrug-Resistant Gram-Negative ESKAPE Pathogens from a Tertiary-Care Hospital: Prevalence and Risk Factors

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ABSTRACT

Background: *Antibiotic-resistant ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) are commonly implicated in health-care associated infections (HAIs).* ***Objectives:*** *The purpose of this study is to assess the antimicrobial susceptibility profile of Gram-negative ESKAPE pathogens, with prime emphasis on the prevalence and risk factors for infections by multidrug-resistant (MDR) strains.* ***Methodology:*** *In this retrospective cohort study, we reviewed the electronic medical and laboratory records of our tertiary health-care facility throughout the period from January 2019 to December 2020. Adult patients identified with infections by any of the Gram-negative ESKAPE bacteria were eligible for our study. The risk factors associated with acquisition of MDR organisms were analyzed using univariate and multivariate models.* ***Results:*** *During the period of interest, a total of 614 Gram-negative ESKAPE isolates were identified, of which 121 were found to be MDR (19.7%). A. baumannii was the leading MDR organism (43.1%), whereas MDR P. aeruginosa was the least common (10.7%). The independent risk factors associated with acquisition of MDR infections included long hospital stays (P < 0.0001), undergoing surgical procedures (P= 0.001), ischemic heart disease (P= 0.005), mechanical ventilation (P= 0.005), and presence of indwelling urinary catheter (P= 0.03).* ***Conclusions:*** *Infections with MDR Gram-negative ESKAPE organisms have an alarming magnitude in our institution. Continued vigilance by the involved health-care workers, stringent compliance to the infection control guidelines, and effective implementation of the antimicrobial stewardship programs are critical measures to decrease the burden of this health problem.*

INTRODUCTION

In the past few years, bacterial species from the ESKAPE set of pathogens have shown a rising public health concern owing to their multifaceted antimicrobial resistance profiles [1]. The acronym "ESKAPE" stands for a group of six Gram-positive and Gram-negative bacteria, namely; E: *Enterococcus faecium*, S: *Staphylococcus aureus*, K: *Klebsiella pneumoniae*, A: *Acinetobacter baumannii*, P: *Pseudomonas aeruginosa*, and E: *Enterobacter* species [2]. This term was initially coined by Rice in 2008 to reflect the ability of these germs to "escape" the devastating action of different antibiotics [3]. Members of the ESKAPE group of organisms are major drivers of healthcare-associated infections (HAIs) worldwide. They culminate into serious and usually fatal human infections, particularly amongst critically-ill and immunosuppressed individuals [4].

The Gram-negative ESKAPE organisms, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* species, constitute an even more worrisome clinical challenge because of their trends in acquiring rapid resistance to the currently available antibiotics [5]. Antibiotic alteration, bacterial target site modification, decreased intracellular antibiotic accumulation, and biofilm formation are among the underlying resistance mechanisms [6]. In addition, production of enzymes such as β-lactamases and extended-spectrum β-lactamases (ESBLs) is one of the key mechanisms by which Gram-negative ESKAPE bacteria can attack the β-lactam ring of β-lactam antibiotics (i.e., penicillins, cephalosporins, and monobactams) [7].

Infections by antimicrobial-resistant (AMR) Gram-negative bacteria, including ESKAPE pathogens, are among the looming clinical challenges to healthcare providers [8]. According to the European Centre for Disease Control (ECDC) and Centre for Disease

Control & Prevention (CDC) terminology, multidrug resistance (MDR) is defined as lack of susceptibility to at least one agent in three or more antimicrobial categories. On the other hand, extensively drug resistance (XDR) denotes bacterial susceptibility to only one or two antimicrobial categories, and pandrug resistance (PDR) involves nonsusceptibility to all agents in all antimicrobial categories [9].

Despite the rampant emergence of MDR Gram-negative ESKAPE infections worldwide, there is a dearth of data from the Kingdom of Saudi Arabia (KSA) describing the collective magnitude of this problem from hospitalized patients. So, we sought to assess the antimicrobial susceptibility profile of Gram-negative ESKAPE pathogens, as well as the prevalence and risk factors for infections by MDR Gram-negative ESKAPE isolates from adult patients admitted to Dr. Soliman Fakeeh Hospital (DSFH), Jeddah, KSA.

METHODOLOGY

Ethical considerations:

The current research was conducted in keeping with the ethical principles of the Declaration of Helsinki. The research protocol was approved by the institutional review board (IRB) of DSFH (Approval no. 192/IRB/2021), and the need for informed consent was waived since all data were anonymized before analysis. Patients' data privacy and confidentiality were respected in all steps of our research.

Study eligibility, design and setting:

From January 2019 to December 2020, adult patients (> 18 years) who were hospitalized in DSFH were enrolled into the present retrospective cohort study if they had positive cultures for any of the Gram-negative ESKAPE pathogens after 48 hours of their admission. DSFH is a 500-bedded tertiary-care center in the Western region (Jeddah) of the KSA. It provides tertiary medical and surgical care for the dwellers of the Kingdom.

Laboratory procedures and bacterial identification:

Different clinical samples collected from the study cohort were processed in the Microbiology Laboratory of DSFH with reference to the standard protocols of the hospital laboratory. The VITEK TWO (BioMérieux, Brazil) automated system was used for the identification and antibiotic susceptibility testing of the recovered isolates using the identification and susceptibility cards for the Gram-negative bacilli (ID-GN and AST 291) following the manufacturer's instructions (BioMérieux, Brazil). The antimicrobial test panel in each run differed according to the bacterial identity. ESBL production in *K. pneumoniae* isolates, also, was determined using the VITEK TWO system.

Interpretations of antimicrobial susceptibility results were established in agreement with the published guidelines of the Clinical and Laboratory Standards Institute (CLSI) [10, 11]. The breakpoints set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used to interpret tigecycline susceptibility results [12]. *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853 were included for quality control purposes in each antimicrobial susceptibility testing batch. Isolates with MDR phenotypes were identified according to the previously mentioned terminology [9].

Study participants' data collection:

The hospital electronic medical records of the enrolled patients were reviewed using the medical record numbers (MRN) to capture the relevant data to the study group. In addition, we analyzed the electronic database of the Microbiology Laboratory of DSFH to extract the data entailing the causative microorganisms and their antimicrobial susceptibility profiles.

Exclusion criteria:

Patients with missing clinical data, incomplete susceptibility test profiles, polymicrobial infections, multiple infections from different body sites, and patients < 18 years old were excluded from our cohort.

Statistical analysis:

The data were entered and analyzed using IBM®SPSS® Statistics program version 26.0 for Windows (SPSS Inc., Chicago, IL, USA). *P*- values < 0.05 (2-tailed) were considered statistically-significant.

RESULTS

Demographic and clinical features of the study population:

During the study period, a total of 614 adult patients (> 18 years) who met our inclusion criteria were enrolled into the current study. The average age of the involved patients was 62.95 ± 15.24 years (range; 26–98 years), with a gender distribution of 402 (65.5%) males and 212 (34.5%) females. Diabetes mellitus (DM) was the most frequent underlying morbidity (38.6%). Almost 15% of our cohort underwent surgical interventions, while 18.4% were admitted to the ICU. The average length of hospital stays was 13.58 ± 12.71 days (range; 4–133 days). About 37% of the study participants received prior antibiotics in the last month before acquiring infection.

Distribution of the study isolates:

Overall, 614 non-duplicate (one isolate/patient) consecutive isolates belonging to the Gram-negative ESKAPE group were identified during the study period. Of these, *K. pneumoniae* was the leading isolate accounting for 47.4% (n= 291), followed by *P. aeruginosa* (29.2%; n= 179). *Enterobacter* species constituted 14% of the test strains (60 *E. cloacae* and 26 *E. aerogenes*), whereas *A. baumannii* was the least frequent isolate (9.4%; n= 58). Sample-wise distribution of the recovered isolates is shown in **table 1**.

Table 1: Sample-wise distribution of the isolated Gram-negative ESKAPE pathogens

Samples	*K. pneumoniae*		*P. aeruginosa*		*Enterobacter* spp.		*A. baumannii*		Total	
	n	%	n	%	n	%	n	%	n	%
Urine	142	69.0	20	9.7	21	10.2	23	11.1	206	100
Wound swab	58	30.2	87	45.3	28	14.6	19	9.9	192	100
Sputum	54	39.4	51	37.3	25	18.2	7	5.1	137	100
Blood	21	55.3	6	15.8	9	23.7	2	5.2	38	100
ETA	7	33.3	11	52.4	0	0.0	3	14.3	21	100
Body fluid	9	45.0	4	20.0	3	15.0	4	20.0	20	100

Note: Data are expressed as numbers and percentages.
Abbreviations: *K. pneumoniae*, *Klebsiella pneumoniae*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *Enterobacter* spp., *Enterobacter* species; *A. baumannii*, *Acinetobacter baumannii*; ETA, endotracheal aspirate.

Results of antibiotic susceptibility testing of the isolates:

In the present study, resistance to ≥ three antimicrobial agents (MDR) was found in 19.7% of our Gram-negative ESKAPE isolates (n= 121). MDR was most predominant in *A. baumannii* (43.1%), followed by *K. pneumoniae* (21.3%), and *Enterobacter* species (17.4%), while *P. aeruginosa* showed the least MDR rate (10.7%). **Table 2** shows the number and percentage of Gram-negative ESKAPE isolates resistant to different classes of antibiotics and their MDR pattern.

Table 2: Antimicrobial resistance profile of the isolated Gram-negative ESKAPE pathogens

Antibiotics	*K. pneumoniae*		*P. aeruginosa*		*Enterobacter spp.*		*A. baumannii*	
	n= 291	%	n= 179	%	n= 86	%	n= 58	%
AMC	95	32.6	NT	–	NT	–	NT	–
SAM	NT	–	NT	–	NT	–	31	53.4
TZP	63	21.6	35	19.6	21	24.4	35	60.3
CXM	123	42.3	NT	–	NT	–	NT	–
CAZ	111	38.1	78	43.6	25	29.1	37	63.8
CRO	108	37.1	80	44.7	25	29.1	NT	–
FEP	103	35.4	42	23.5	11	12.8	29	50
AK	26	8.9	7	3.9	0	0.0	17	29.3
GN	48	16.5	13	7.3	4	4.7	22	37.9
TOB	NT	–	11	6.2	4	4.7	13	22.4
CIP	71	24.4	55	30.7	10	11.6	33	56.9
LEV	NT	–	57	31.8	NT	–	33	56.9
F*	114/142	80.3	NT	–	17/21	81.0	NT	–
SXT	114	39.2	NT	–	12	14	24	41.4
IPM	26	8.9	47	26.3	15	17.4	24	41.4
MEM	26	8.9	46	25.7	10	11.6	25	43.1
TGC	10	3.4	NT	–	2	2.3	6	10.3
COL	NT	–	0	0.0	NT	–	0	0.0
MDR isolates	62	21.3	19	10.7	15	17.4	25	43.1

Notes: Data are expressed as numbers and percentages. Nitrofurantoin (F) was tested only against the urinary isolates.
Abbreviations: *K. pneumoniae*, *Klebsiella pneumoniae*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *Enterobacter spp*., *Enterobacter species*; *A. baumannii*, *Acinetobacter baumannii*; NT, not tested; AMC, amoxicillin-clavulanate; SAM, ampicillin-sulbactam; TPZ, piperacillin/tazobactam; CXM, cefuroxime; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; AK, amikacin; GN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LEV, levofloxacin; F, nitrofurantoin; SXT, trimethoprim/ sulfamethoxazole; IPM, imipenem; MEM, meropenem; TGC, tigecycline; COL, colistin; MDR, multidrug-resistant.

Sources and risk factors for acquisition of MDR Gram-negative ESKAPE pathogens:

Out of 121 identified MDR isolates, 43.8% were recovered from urine samples, whereas 24.8% and 22.3% from wound swabs and sputum samples, respectively. Endotracheal aspirates and blood samples showed lower frequency of MDR isolates (5% and 3.3%, respectively). From body fluid samples, only one MDR isolate was retrieved from pleural fluid (0.8%). Risk factors and independent predictors for acquisition of MDR Gram-negative ESKAPE infections are depicted in **tables 3 and 4**, respectively.

Table 3: Risk factors associated with acquisition of MDR Gram-negative ESKAPE infections

Risk factors	MDR Gram-negative ESKAPE n= 121 (%)	Non-MDR Gram-negative ESKAPE n= 493 (%)	χ^2	*P*-value
Age, years (± SD)[a]	68.55 ± 15.23	61.57 ± 14.94	– 4.58	< 0.0001*
Age groups < 60 years ≥ 60 years	 35 (28.9) 86 (71.1)	 207 (42.0) 286 (58.0)	6.94	0.009*
Gender				
Male Female	86 (71.1) 35 (28.9)	316 (64.1) 177 (35.9)	2.09	0.17
Underlying disease				
DM	68 (56.2)	169 (34.3)	19.69	< 0.0001*
IHD	29 (24)	24 (4.9)	44.93	< 0.0001*
CKD	27 (22.3)	38 (7.7)	21.89	< 0.0001*
Malignancy	16 (13.2)	24 (4.9)	11.14	0.002*
Pulmonary disease	15 (12.4)	37 (7.5)	2.99	0.10
Chronic liver disease	14 (11.6)	26 (5.3)	6.32	0.01*
Invasive procedures				
Surgery	48 (39.7)	46 (9.3)	68.97	< 0.0001*
MV	39 (32.2)	33 (6.7)	61.21	< 0.0001*
PVC	44 (36.4)	114 (23.1)	8.91	0.004*
CVC	18 (14.9)	41 (8.3)	4.81	0.03*
IUC	20 (16.5)	45 (9.1)	5.62	0.02*
Prior antibiotics	75 (62)	150 (30.4)	41.68	< 0.0001*
ICU admission	33 (27.3)	80 (16.2)	7.89	0.006*
LOS, days (± SD)[a]	22.74 ± 20.54	11.33± 8.52	– 9.47	< 0.0001*
LOS categories < 14 days ≥ 14 days	 49 (40.5) 72 (59.5)	 376 (76.3) 117 (23.7)	58.35	< 0.0001*
30-day mortality	35 (28.9)	25 (5.1)	62.70	< 0.0001*

Notes: Data are expressed as numbers and percentages unless otherwise indicated; [a]Significance was tested using the independent samples T-tests; **P* < 0.05 (statistically significant).

Abbreviations: MDR, multidrug-resistant; χ^2, Pearson's Chi-Square; SD, standard deviation; DM, diabetes mellitus; IHD, ischemic heart disease; CKD, chronic kidney disease; MV, mechanical ventilation; PVC, peripheral venous catheter; CVC, central venous catheter; IUC, indwelling urinary catheter; ICU, intensive care unit; LOS, length of hospital stay.

Table 4: Independent predictors for acquisition of MDR Gram-negative ESKAPE infections

Risk factors	Univariate			Multivariate		
	OR	(95% CI)	*P* value	OR	(95% CI)	*P* value
Age	1.78	1.16–2.74	0.009	0.81	0.49–1.34	0.41
DM	2.46	1.64–3.69	< 0.0001	0.63	0.39–1.02	0.06
IHD	6.16	3.43–11.06	< 0.0001	0.35	0.17–0.73	0.005*
CKD	3.43	2.00–5.91	< 0.0001	0.79	0.39–1.58	0.49
Malignancy	2.98	1.53–5.80	0.002	0.66	0.28–1.50	0.32
Chronic liver disease	2.35	1.18–4.65	0.01	0.58	0.24–1.37	0.23
Surgery	6.39	3.97–10.27	< 0.0001	0.37	0.20–0.66	0.001*
MV	6.63	3.94–11.15	< 0.0001	0.39	0.19–0.75	0.005*
PVC	1.91	1.24–2.91	0.004	0.80	0.48–1.35	0.41
CVC	1.93	1.06–3.49	0.03	0.98	0.45–2.11	0.95
IUC	1.97	1.12–3.48	0.02	2.37	1.05–5.23	0.03*
Prior antibiotic	3.73	2.46–5.64	< 0.0001	0.65	0.39–1.23	0.11
ICU admission	1.94	1.22–3.09	0.006	1.11	0.59–2.05	0.75
LOS	4.72	3.11–7.17	< 0.0001	0.37	0.23–0.59	< 0.0001*

Notes: Data are expressed as numbers; *$P < 0.05$ (statistically significant).
Abbreviations: MDR, multidrug-resistant; DM, diabetes mellitus; IHD, ischemic heart disease; CKD, chronic kidney disease; MV, mechanical ventilation; PVC, peripheral venous catheter; CVC, central venous catheter; IUC, indwelling urinary catheter; ICU, intensive care unit; LOS, length of hospital stay; OR, odds ratio; CI, confidence interval.

DISCUSSION

During the period of interest, *K. pneumoniae* was the most frequent amongst our Gram-negative ESKAPE isolates (47.4%), whereas *A. baumannii* was the least prevalent organism (9.4%). The high prevalence of *K. pneumoniae* concurs with that reported from a recent Brazilian study [13]. Quite the reverse, *A. baumannii* was the leading pathogen in an earlier study from Mexico [14]. Different study design, setting, and selection criteria of the study participants could have contributed to such discrepancy. In our study, about one-fifth (19.7%) of the isolates were found to be MDR, in keeping with the results from a previous work [15]. This considerably high rate is awful, since these hospital-acquired strains are likely to be pooled into the community leading to farther aggravation of this serious health problem.

Amongst the recovered MDR isolates, *A. baumannii* displayed the most troublesome profile, where 43.1% of these isolates were MDR. This observation is supported by recent literature from the Ministry of National Guard Health Affairs of Saudi Arabia [16]. On the flip side, data from Eastern Saudi Arabia highlighted that MDR *Acinetobacter* species were the least common isolates; however, recruitment of patients from all age groups, as well as attendees of the outpatient clinics are likely explanations to this incongruence [17].

Consistent with our results, Arbune et al [18] reported 24.4% MDR amongst *K. pneumoniae* isolates. On the contrary, higher rates were reported from Iran (58%) [19]. Outstandingly, 95.5% of *K. pneumoniae* isolates were found to be MDR from a recent multicenter study in Ethiopia [20], which is far higher than that observed in our study. The rate of ESBL-producing *K. pneumoniae* isolates in this study (38.1%) is concomitant with previous studies from Saudi Arabia (42.1%) [21], but lower than that reported from Ethiopia (58.1%) [22], and higher than that described from Uganda (28.5%) [23]. Despite the disparity among different studies, a surge in the incidence of ESBL-producing *K. pneumoniae* is globally noticed, possibly due to pervasive administration of cephalosporins, associated with poor adherence to the antimicrobial stewardship guidelines.

At the present time, MDR *Enterobacter* species are increasingly become preponderant worldwide [24]. In our study, 17.4% of the test isolates were found to be MDR, in line with data from Romania (21.3%) [18]. Interestingly, no resistance to amikacin was observed amongst our isolates, whilst, low resistance profiles to tigecycline (2.3%) and gentamicin (4.7%) were recorded, compatible to a previous study by Cabral and his colleagues [25]. Susceptibility testing revealed that 10.7% of our *P. aeruginosa* isolates were MDR, comparable to a prior study from Iraq (12.4%) [26]. On the other side, current data from Egypt demonstrated a relatively higher rate up to 42.4% [27].

In our univariate analysis, increased age beyond 60 years was significantly associated with acquiring MDR Gram-negative ESKAPE infections (P= 0.009), similar to another study from Saudi Arabia [17]. In elderly patients, jeopardized immune system sets them at a higher risk for hospitalization and having infections with virulent strains, including antibiotic-resistant bugs.

With the exception of pulmonary diseases, patients with chronic underlying morbidities were more likely to acquire MDR Gram-negative ESKAPE infections amongst our cohort. A current study by Shi et al[28]. identified DM as a risk factor for infection by Gram-negative bacilli (P= 0.023), which endorses our

findings. A systematic review and meta-analysis concluded that patients with type 2 DM are more likely to experience infections with antibiotic-resistant bacteria, owing to the adverse impact of DM on the immune system [29].

In this study, patients having indwelling urinary catheters were 2.37 times more likely to develop infections with MDR strains (CI, 1.05–5.23; *P*= 0.03). In addition, patients submitted to mechanical ventilation (*P*= 0.005) were at a greater risk **(Table 4)**. These findings align with the results described by Al Hamdan et al [17]. Undergoing surgical intervention was another independent predictor for MDR Gram-negative ESKAPE infections, which corroborates other researchers [30]. This could be traced to routine preoperative prescription of antibiotics for patients scheduled for surgery, and the role of these antibiotics in modifying the patients' microbiota.

Recently, Lin et al[31]. examined the predisposing factors for infection by MDR Gram-negative bacilli amongst patients who endured abdominal surgery. Comparable to our findings, longer length of hospitalization, and receipt of carbapenems and fluoroquinolones, were independent predictors. In the past decade, over use of carbapenems constituted one of the most important reasons for the emergence of carbapenem-resistant *Enterobacteriaceae*. It was estimated that prior exposure to carbapenems can increase the risk of infection by resistant organisms three to four folds [32]. Accordingly, the judicious use of the currently-available antibiotics is the mainstay to combat resistant bacteria.

Our study has limitations that worth-mentioning. First, molecular mechanisms underlying the emergence of MDR Gram-negative ESKAPE pathogens were not defined, because they are not routinely done in our hospital laboratory. Second, data about XDR and PDR isolates could not be formulated, because a number of antimicrobial classes were not included in the test panel. Third, the burden of ESBL-producing organisms, other than *K. pneumoniae*, could not be explored, because of missing hospital laboratory database in this regard.

CONCLUSION

Periodic tracing and surveillance of antibiotic-resistant bacteria in the hospital settings is of paramount importance, to provide meaningful data necessary to mitigate the problem, and to renew the current antibiotic prescription protocols. Future molecular studies are recommended to uncover the genetic mechanisms underlying the resistant phenotypes.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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