Investigation of the Antibiotic-Resistant ESKAPE Pathogens in Ramadi Hospitals, Iraq

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Abstract

Bacterial species of the ESKAPE group (*Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Escherichia coli*) are often resistant to antibiotics. The purpose of this study was to find out the antibiotics resistance formed by ESKAPE pathogens in hospitals in the city of Ramadi, in Iraq. All bacteria within this clinically relevant ESKAPE group were isolated from September 2020 to April 2021. Identification of isolates was performed by Vitek 2 system and the Sensitivity Test was carried out using a disk diffusion method. The majority of pathogens isolated from patients at Ramadi Hospitals belong to the ESKAPE group. The percentage of bacterial isolates was *K. pneumoniae* (33.96%), *E. coli* (20.75%), *S. aureus* (20.13%), *P. aeruginosa* (11.32 %), *E. faecalis* (9.43%), *and A. baumannii* (4.41%). Some isolates of *E. coli* were Resistance to all antibiotics, while *P. aeruginosa* and *K. pneumoniae* were 100% resistant to Vancomycin and Tetracycline. The presence of the ESKAPE group of pathogens is a major problem in Ramadi city hospitals. The results of this study support the implementation of special antimicrobial strategies to specifically target these microorganisms.

Keywords: *ESKAPE, MDR, antibiotic, resistance, susceptibility*

Introduction

The term ESKAPE includes six genera of pathogens with multi-drug resistance (MDR) and high virulence. ESKAPE pathogens are responsible for the majority of nosocomial infections and are able to "escape" from the biocidal effect of antimicrobial agents [1]. The World Health Organization (WHO) has also recently included ESKAPE pathogens in the list of 12 bacteria that urgently need new methods and antibiotics [2]. Antimicrobial resistance between both Gram-positive and Gram-negative bacteria has been on the rise in the past few years [3]. The presence of multidrug resistance (MDR) pathogens has become a cause of serious concern with regard to nosocomial infections. In fact, the World Health Organization recently recognized antimicrobial resistance as one of the top three human health concerns [4]. The most common and threatening multidrug-resistant pathogens were grouped together under the acronym "ESKAPE", which stands for *E. faecalis, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, and E. coli*. [1]. Three decades ago, A. baumannii was sensitive to most antibiotics, but today it is exceptionally resistant to most antibiotics, with carbapenem resistance increasing more than 50% in some countries [5]. Recently, statistics have been recorded on meropenem resistance (MEM) of 69% [6]. A major factor contributing to antibiotic resistance is the production of extended-spectrum β-lactamases (ESBLs) by Enterobacteriaceae species, especially *K. pneumoniae* [7] ESBL producing Gram-negative pathogens in hospitals is an emerging global

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problem that deserves special attention [8]. Another important species of the ESKAPE group is *S. aureus*, especially methicillin-resistant *S. aureus* (MRSA), which has an incidence and prevalence that continues to increase rapidly in many regions of the world. Mortality associated with invasive MRSA infections is estimated to be around 20% [9] and bloodstream infections caused by these bacterial species are associated with high mortality and length of hospital stay [10]. Finally, *E. faecalis* isolates are the third to the fourth most prevalent pathogen in hospitals worldwide. Acquired resistance, in particular to glycopeptides, has been reported for a number of these isolates, which limits the number of therapeutic options [11]. Global and regional surveillance of ESKAPE pathogens is essential to control infections caused by these bacterial species [12]. The purpose of this study was to monitor the occurrence of ESKAPE pathogens and their resistance to diseases in hospitals in Ramadi, Iraq.

Materials and Methods

Sampling

This study was conducted at Al-Ramadi General Teaching Hospital and Women's and Children's Hospital, Iraq. This hospital provides medical care to its patients and residents. This study was conducted in the medical field (from September 2020 to April 2021), 53 clinical isolates were obtained from patients referred to these hospitals. The first private isolate species was recorded for each patient, regardless of a body location. Patient samples taken for diagnostic purposes only are included.

Identification of isolated bacteria

Two hundred samples from various sources such as urine, faeces, burns, wounds, and sputum were collected from patients in the Ramadi hospitals. Blood agar and MacConkey agar were used to culture the samples. Culture plates were incubated aerobically at 37 °C for 24 h. After incubation, bacterial isolates were identified by performing Gram staining and using the Vitek-2 Compact system (bioMerieux). A total of 159 bacterial isolates were collected from different clinical samples.

Antimicrobial susceptibility assays

The antibiotics susceptibility was determined using the disc diffusion according to Kirby-Bauer method by using Mueller's solid agar plates [13]. Antimicrobials were tested against both Gram-negative and Gram-positive bacteria including Piperacillin (PRL), Amikacin (AMK), Aztreonam (AZT), Ciprofloxacin (CIP), Imipenem (IPM), Vancomycin (VA), Azithromycin (AZM), Tetracycline (T), Ceftriaxone (CRO), and Tigecycline (TGC). Multidrug resistance **MDR** has been defined as resistance to three or more classes of antimicrobials. Class definitions used in this study were: Penicillins (PRL), Cephalosporins (CRO), Carbapenems (IPM), Beta-lactam (AZT), Fluoroquinolones (CIP), Aminoglycosides (AMK), Glycopeptide (VA), Tetracyclines (T), Macrolides (AZM), and Glycylcyclines (TGC). Extensive drug resistance **XDR** was defined as the lack of susceptibility to at least one agent in all but two or fewer classes of antibiotics (for example, bacterial isolates remain susceptible for one or two classes only) and Pandrug resistance **PDR** was defined as resistant for all agents in all classes of antibiotics [14]. The diameters of the inhibition zone were measured and compared with the susceptibility breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) [15].

Results

Two hundred different samples obtained from patients in Ramadi hospitals were processed. A total 159 (79.5%) of ESKAPE pathogens were included in this study. The ESKAPE pathogens were distributed into 112 (70.4%) Gram-negative isolates. The pathogens belonging to the family Enterobacteriaceae (*K. pneumoniae* & *E. coli*) were 78 (54.7%) and non-intestinal (*A. baumannii* & *P. aeruginosa*) 25 (15 .7%), and the Gram-positive isolates (*S. aureus* & *E. faecalis*) were 47 (29.6%). *K. pneumoniae* 54

(33.96%) was the most common isolate, followed by E. coli 22 (20.75%), *S. aureus* 32 (20.13%), *P. aeruginosa* 18 (11.32%), *E. faecalis* 15 (9.43%), and *A. baumannii* 7 (4.41%). The isolates were recovered mostly from feces (78 isolates), followed by urine samples 33 isolates, wounds 25 isolates, sputum 15 isolates, and finally burns 8 isolates. The distribution of ESKAPE pathogens isolated from different samples is shown in Table 1.

Table (1) The ESKAPE pathogens profile based on specimen type

Bacteria spices	Source of bacteria					No.	%
	urine	faeces	wounds	Burn	Sputum		
E. coli	15	18	-	-	-	33	20.75
S. aureus	18	-	8	-	6	32	20.13
K. pneumoniae	-	36	9	-	9	54	33.96
A. baumannii	-	-	5	2	-	7	4.41
P. aeruginosa	-	9	3	6	-	18	11.32
E. faecalis	-	15	-	-	-	15	9.43
Total	33	78	25	8	15	159	100.00

Antibiotic Resistance levels

The results of the current study showed the existence of a common resistance of ESKAPE groups toward classes of antibiotics such as penicillins, cephalosporins, fluoroquinolones, and aminoglycosides, which makes it have multi-resistance to the antibiotics, as the term multi-resistance to this group of bacteria is called (Multidrug resistance), which means the ability of bacteria to resist three or more antibiotics used, provided that the bacteria have the ability to resist at least one antibiotic within the class. The isolates were distributed according to Table (2), the types of resistance, and their proportion to the isolates of the (ESKAPE) group. Among all 159 isolates, 146 were found to be MDR (100% from *E. coli*, 94.4% from *K. pneumoniae*, 88.9% from *P. aeruginosa*, 87.5 from *S. aureus*, 86.6% from *E. faecalis*, and 71.4 from *A. baumannii*). There were only 13 isolates that were found to be sensitive to most antibiotics. *E. coli* isolates of the ESKAPE group that were understudy has an Extensive drug resistance (XDR) of 21.2%, followed by *K. pneumonea* of 18.5%, as the danger of Extensive resistance strains lies in their endemicity within hospitals, their rapid spread, the high rate of resistance and their transformation into strains resistant to all drugs. The term (Pandrug resistance) is applied to the recorded resistance of all classes of antibiotics studied. The isolates of the (ESKAPE) group were recorded with resistance to the ten classes of antibiotics, represented by three isolates of *E. coli* (9.1%), also three isolates of *K. pneumoniae* (5.5%), and one isolate of P. *aeruginosa* (5.5%) Table (2).

Table (2) Antibiotic resistance levels of ESKAPE isolates during the study period.

Bacteria spices	Resistance type		
	MDR %	XDR %	PDR %
E. coli	100.0	21.2	9.1
S. aureus	87.5	12.5	0.0
K. pneumoniae	94.4	18.5	5.5
A. baumannii	71.4	0.0	0.0
P. aeruginosa	88.9	11.1	5.5
E. faecalis	86.6	6.6	0.0

Antibiotic Susceptibility Pattern

Antibiotic sensitivity testing showed that the rate of resistance of *E. faecalis* to Amikacin and Tetracycline was 100%. It was noted that the lowest resistance was in the case of ciprofloxacin (6.67%), while *S. aureus* showed the highest resistance rate of Aztreonam which was (75%). The results also showed that Imipenem was the most successful antibiotic in the treatment of Gram-positive ESKAPE pathogens (Table 3).

Table (3). Antibiotic susceptibility pattern of Gram-positive ESKAPE isolates

Antibiotics	S. aureus (n=32)			E. faecalis (n=15)		
	S%	I%	R%	S%	I%	R%
Amikacin	21.88	25.00	53.13	0.00	0.00	100.00
Azithromycin	0.00	37.50	62.50	6.67	13.33	80.00
Aztreonam	0.00	25.00	75.00	40.00	0.00	60.00
Ceftriaxone	56.25	0.00	43.75	26.67	0.00	73.33
Ciprofloxacin	62.50	6.25	31.25	80.00	13.33	6.67
Imipenem	81.25	18.75	0.00	86.67	0.00	13.33
Piperacillin	68.75	31.25	0.00	0.00	20.00	80.00
Tetracycline	6.25	21.88	71.88	0.00	0.00	100.00
Tigecycline	43.75	25.00	31.25	20.00	0.00	80.00
Vancomycin	15.63	43.75	40.63	66.67	0.00	33.33

The frequency rate of resistance of *E. coli* to vancomycin, piperacillin, and tetracycline was 100%. As well, the resistance frequency rate of *K. pneumoniae* against vancomycin and piperacillin was 100%. Imipenem was again effective against (81.82%) of the tested *E. coli* isolates, while Ceftriaxone was effective against (81.48%) of *K. pneumoniae* (Table 4). None of the isolates were 100% sensitive to all antimicrobials tested. Furthermore, some *E. coli* and *K. pneumoniae* isolates were found to be highly drug-resistant as they showed resistance to all antimicrobial agents evaluated.

Table (4). Antibiotic susceptibility pattern of Enterobacteriaceae ESKAPE isolates

Antibiotics	E. coli (n=33)			K. pneumoniae (n=54)		
	S%	I%	R%	S%	I%	R%
Amikacin	69.70	0.00	30.30	9.26	27.78	62.96
Azithromycin	9.09	9.09	81.82	0.00	25.93	74.07
Aztreonam	0.00	15.15	84.85	16.67	20.37	62.96
Ceftriaxone	12.12	0.00	87.88	81.48	7.41	11.11
Ciprofloxacin	45.45	0.00	54.55	25.93	16.67	57.41
Imipenem	81.82	6.06	12.12	74.07	18.52	7.41
Piperacillin	0.00	0.00	100.00	0.00	0.00	100.00
Tetracycline	0.00	0.00	100.00	0.00	14.81	85.19
Tigecycline	63.64	27.27	9.09	3.70	14.81	81.48
Vancomycin	0.00	0.00	100.00	0.00	0.00	100.00

The susceptibility pattern of non-intestinal Gram-negative ESKAPE isolates displayed high resistance of *P. aeruginosa* to Vancomycin (100%), Tigecycline (100%), Tetracycline (83.33%), and Azithromycin (72.22%). Also, high resistance to Vancomycin (100), Tetracycline (100%), and Aztreonam (71.43%) was observed in *A. baumannii*. Otherwise, Imipenem and Ceftriaxone have the best antibacterial effect against *A. baumannii* and *P. aeruginosa* (Table 5).

Table (5). Antibiotic susceptibility pattern of non-intestinal Gram-negative ESKAPE isolates

Antibiotics	A. baumannii (n=7)			P. aeruginosa (n=18)		
	S%	I%	R%	S%	I%	R%
Amikacin	14.29	28.57	57.14	44.44	5.56	50.00
Azithromycin	42.86	14.29	42.86	16.67	11.11	72.22
Aztreonam	28.57	0.00	71.43	83.33	0.00	16.67
Ceftriaxone	71.43	0.00	28.57	88.89	0.00	11.11
Ciprofloxacin	57.14	42.86	0.00	77.78	16.67	5.56
Imipenem	100.00	0.00	0.00	94.44	0.00	5.56
Piperacillin	28.57	28.57	42.86	77.78	11.11	11.11
Tetracycline	0.00	0.00	100.00	11.11	5.56	83.33
Tigecycline	71.43	28.57	0.00	0.00	0.00	100.00
Vancomycin	0.00	0	100.00	0.00	0.00	100.00

Discussions

The emergence of isolates resistant to a wide range of antibiotics poses a threat to health and this is a result of the wide and indiscriminate use of these wide groups of antibiotics and the lack of care for the emergence of such resistant isolates, the increase of which poses a danger to patients, and this situation remains uncontrolled at the hospital and community levels, and this is the reason for the emergence of multidrug-resistant strains, especially the broad-spectrum Multidrug-resistant strains [16-17]. In a hospital environment, there may be different bacterial species that are the causative agents of infectious diseases. For them, with a high level of proliferation and association with antibiotic-resistant microorganisms, the ESKAPE group of pathogens deserves special attention. To control the incidence of infection caused by ESKAPE pathogens, site-by-site observational studies are necessary to establish hospital-specific guidelines for effective empirical therapy [18]. In this study, ESKAPE pathogens were followed up and the incidence of K. pneumoniae and the presence of resistance to Vancomycin in Ramadi hospitals were described. The organisms most often recovered from our bodies were intestinal infections from ESKAPE pathogens (54.61%), with a predominance of Gram-negative bacteria. The most common organisms in ESKAPE are K. pneumoniae, E. coli, and S. aureus. These common bacterial pathogens were found to be similar to other predominant pathogens reported in other countries [19]. In general, an elevated MDR was observed in E. coli deserves special attention because it showed a high MDR (100%). The reason is that these bacteria in the (ESKAPE) group possess many resistance mechanisms, such as the production of aminoglycoside-modifying enzymes and beta-lactamase enzymes, and their exogenous pumping mechanism and other mechanisms that work together causing the phenomenon of multiple resistance [20]. The noticeable rise in the percentage of isolates of the (ESKAPE) group with multiple resistance in Al-Ramadi Teaching Hospitals is an indication of the outbreak of multi-resistant strains of antibiotics, which leads to the possibility of failure of the treatments currently used to treat infections caused by these bacteria.

Conclusion

Rapid identification and susceptibility testing of ESKAPE pathogens with the compact VITEK-2 system helps treat these pathogens with appropriate antibiotics and leads to a reduction in total antibiotic consumption. It was found that the Enterobacteriaceae ESKAPE group (*K. pneumoniae* & *E. coli*) are the highest prevalence among other bacterial species, and all ESKAPE pathogens have shown multi-drug resistance (MDR). Also, most ESKAPE species have Extensive resistance to antibiotics, in addition to the occurrence of resistance to all classes of antibiotics in some isolates of *E. coli*. In this study. Most ESKAPE isolates were found to be sensitive against Imipenem and Ceftriaxone. These antibiotics can be good treatment options for infections due to the etiology of ESKAPE.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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