ORIGINAL ARTICLE

Antibacterial Susceptibility Pattern of Gram-Negative *ESKAPE* Pathogens Isolated From Hospitalized Patients

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ABSTRACT

Objective: To determine in-vitro antimicrobial susceptibility pattern of multidrug resistant (MDR) strains of Gram-negative *ESKAPE* pathogens using VITEK 2 Compact system.

Study Design: Descriptive Cross sectional study.

Place and Duration of Study: The study was conducted at Pakistan Railway hospital (PRH) Rawalpindi in collaboration with Armed Forces Institute of Pathology (AFIP) Rawalpindi from 1st September 2018 to 1st September 2019.

Material and Methods: A total of 320 clinical specimens were collected. The samples included urine, blood, pus, wound, effusions, C.S.F and sputum. After processing the isolates by standard microbiological methods, the antibiotic susceptibility pattern was carried out using VITEK 2 Compact system.

Results: A total of 190 clinical isolates of Gram-negative *ESKAPE* pathogens isolated from 320 clinical specimens. Among them 150 MDR Gram negative *ESKAPE* pathogens were detected. Out of 190 Gram-negative *ESKAPE* pathogens, (30.5%) were members of the family *Enterobacteriaceae* and (69.4%) were from *non Enterobacteriaceae.* The commonest isolated organism was *Acinetobacter baumannii*, 36% followed by *Pseudomonas aeruginosa*, 33.7%, *Klebsiella pneumoniae*, 26.3% and *Enterobacter spp.* 4.2%. From all isolates 88% of *Klebsiella pneumoniae*, 86.9% of *Acinetobacter baumannii* and 73.4% of *Pseudomonas aeruginosa* were found MDR.

Conclusion: Rapid identification and susceptibility testing of Gram-negative *ESKAPE* pathogens by VITEK-2 compact system helps in reducing total consumption of antibiotics. MDR was observed in majority of Gram-negative *ESKAPE* pathogens except for *Enterobacter spp*. These pathogens revealed comparatively better susceptibility against Minocycline, Tigecycline, and Colistin.

Key Words: *Colistin, Enterobacteriaceae, ESKAPE Pathogens, Multidrug-Resistance, Vitek 2 Compact System.*

Introduction

In the last decade along with the problem of Healthcare associated Infections (HCAIs), the prevalence of multidrug-resistant (MDR) organisms in hospitals has been greatly increased. The most frequently isolated six MDR bacterial species around the world have been grouped under the acronym *ESKAPE* in 2008 by Infectious Diseases society of America. It includes two Gram-positive cocci (*Enterococcus faecium* and *Staphylococcus aureus)* and four Gram-negative bacilli (*Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp*).[1] These superbugs are widely distributed and are frequently resistant to antibiotics.[2] They have the capability of 'ESCAPING' the biocidal action of antibiotic and communally representing new paradigms in transmission, pathogenesis, and resistance.[1] Today they are considered to be major threats and are responsible for two-thirds of all Healthcare-associated Infections (HCAIs) in both developed and developing world.[3] They lead to increase morbidity & mortality, due to severe and life-threatening infections, especially if the host is debilitated or immunosuppressed (e.g. AIDS, cancer and transplant patients, patients with autoimmune diseases, old age, neonates).[4] The World Health Organization selected anti-microbial resistance (AMR) as the theme for World Health Day 2011. Their slogan was "Combat Drug Resistance – No action

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today, no cure tomorrow." [4]

ESKAPE pathogens with built in abilities have a variety of mechanisms to find new ways to be resistant to multiple classes of current antibiotics. Gram-negative *ESKAPE* pathogens are particularly concerning because their multidrug-resistant phenotypes frequently present clinicians with few therapeutic choices.[5]The important mechanisms of antibiotic resistance are: Chromosomally-encoded enzymes such as Extended-spectrum beta-lactamase (ESBLs), Cephalosporinases (AmpC) and Carbapenemase. In addition decreased permeability through Porin channels loss due to mutations and activation of multi-drug efflux pumps.[6] Acquisition of plasmids and mobile genetic elements carrying multiple resistance genes also contributes to the development of multidrug-resistant phenotypes.

A recent study found 2,609,911 new patients with HCAIs annually in the European Economic Area and the European Union. *Acinetobacter* species and the *Klebsiella pneumoniae* were extremely resistant to multiple anti-microbials. The lack of new anti-biotics has mounted huge burden in European Union.[7]

In Southeast Asian countries a systematic review and meta-analysis regarding HCAIs found an overall prevalence rate of 9.1%. The most common micro-organisms being *A. baumannii, P. aeruginosa,* and the *Klebsiella species*.[8]

In order to keep regular monitoring of MDR at species level the changing trends in the susceptibility patterns of Gram-negative *ESKAPE* pathogens should be known. It is essential for detection or at least controlling the outbreaks, identifying the population most at risk, designing and evaluating intervention strategies. This study was therefore, planned to know the susceptibility pattern of all the Gram negative *ESKAPE* pathogens isolated at our set up.

Material and Methods

It was a descriptive cross-sectional study, conducted at PRH Rawalpindi in collaboration with AFIP Rawalpindi, after getting formal approval from Institutional Ethical Review Committee. All the samples coming to Microbiology labs for culture and sensitivity report were processed. Gram negative *ESKAPE* pathogens (*Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* & *Enterobacter species*) recovered from these clinical specimens were included in our study. Duplicate samples were excluded from the study. A demographic proforma specially designed for this purpose was filled to avoid duplication of samples.

Three hundred and twenty samples from different sources like urine, blood, pus, wound, throat infection, nose infection, effusions, C.S.F and sputum were collected from hospitalized patients. The samples were inoculated on suitable culture media depending upon the type of specimen. CLED agar was used for inoculation of urine specimens. Blood agar and MacConkey agar were taken for all other specimens. The culture plates were incubated aerobically at 37°C for 24 hours & re-incubated for next 24 hours if growth was not sufficient. After incubation, bacterial isolates were identified by performing Gram staining and standard biochemical tests which included catalase test, oxidase test, and Analytical profile index (API).

Sub-culture of mixed colonies for GNR was done on MacConkey agar to obtain a pure culture. *Escherichia coli* ATCC 25922 was used for quality control. A total of 320 samples were collected from different clinical samples. Culture positive samples (n=190) were included in the study. These isolates were stored in glycerol broth, as MICs was runs in batches by using Vitek-2 Compact system (bioMérieux). Preserved specimens were thawed at room temperature then subculture on MacConkey agar and incubated for 24-48 hours at 37°C. Antimicrobial Susceptibility tests were performed with AST-N222 cards which contained the dehydrated form of the following antimicrobial agents.

Amikacin, Ticarcillin, Ticarcillin- Clavalanic acid, Piperacillin, Tazobactam-Piperacillin , Ceftazidime, Ciprofloxacin, Imipenem, Levofloxacin, Minocycline, Tobramycin, Trimethoprim-Sulfamethoxazole, Gentamicin, Meropenem, & Colistin. The susceptibility breakpoints were those recommended by CLSI.

Data analysis was done by using SPSS 21. For qualitative variables (gender of patient, type of samples and organisms isolated), percentages and frequencies were calculated. Descriptive numerical (continuous) variables of age (years) was calculated in terms of Mean ± SD (standard deviation).

Results

Three hundred and twenty different samples obtained from hospitalized patient were processed.

One hundred and ninety (59.3%) Gram-negative *ESKAPE* isolates were included in the study. The pathogens belonging to family *Enterobacteriaceae* (*Klebsiella pneumoniae* & *Enterobacter species*) were 58(30.5%) and *non Enterobacteriaceae* (*Acinetobacter baumannii* & *Pseudomonas aeruginosa*) were 132(69.4%). *Acinetobacter baumannii* 68(36%) was the commonest isolate, followed by *Pseudomonas aeruginosa* 64(33.7%), *Klebsiella pneumoniae* 50(26.3%) and *Enterobacter spp*. 8(4.2%).

They were mostly recovered from Medical Intensive Care Unit patients' specimens, followed by Surgical Intensive Care Unit, and Pulmonology ward. Isolates were mostly yielded from pus, followed by blood, and urine specimens. The distribution of Gram-negative *ESKAPE* pathogens recovered from different specimens is presented in table I.

Out of the total isolates, 54.7% were recovered from male patients and 45.3% from female patients. Mean age of the patients was 47.19 years ± 19.91 SD. Majority 44 (23%) of the subjects had ages between 40-49 years.

Among all 190 isolates, 150 were found to be MDR (88% of *Klebsiella pneumoniae*, 86.9% of *Acinetobacter baumannii* and 73.4% of *Pseudomonas aeruginosa*). There were only 8 isolates of *Enterobacter spp*. Which were found sensitive to most of antibiotics, we excluded them from final analysis.

Antibiotic Sensitivity Testing by Vitek-2 Compact system showed that the resistance frequency of *Acinetobacter baumannii* against Ticarcillin, Ticarcillin-Clavulanate and Tazobactam-Piperacillin was 96.6%. Highest resistance was observed in case of Ciprofloxacin (100%). Colistin was again the most successful antibiotic.

The resistance frequency of *Klebsiella pneumoniae* against Ticarcillin and Piperacillin was 100% with a maximum MIC of ≥128 µg/ml and Cefipime and Ceftazidime with an MIC of ≥ 64 µg/ml. The resistance frequency of *Pseudomonas aeruginosa* against Ticarcillin and Tazobactam-Piperacillin was 95.7% with a maximum range of ≥ 128 µg/ml. Colistin was effective against (62%) of the total isolates of *Pseudomonas aeruginosa* tested.

None of the isolates was 100% susceptible to all of the tested anti-microbials. Furthermore, 25.4% (15/59) of *A. baumannii* and 27.2% (12/44) of *K. pneumoniae* isolates were found to be Extensively Drug Resistant because they showed resistance to all antimicrobial agents evaluated.

The antibacterial susceptibility pattern of MDR strains of *non-Enterobacteriaceae* is displayed in table II and antibacterial susceptibility pattern of MDR strains of *K. pneumoniae* is shown in Table III.

Table I: Distribution of Gram-Negative *ESKAPE* Pathogens Recovered From Different Specimens

Specimens	*Klebsiella pneumoniae*	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*	*Enterobacter Spp.*
	n (%)			
Pus	11(22)	21(30.9)	22(34.4)	3(37.5)
Urine	10(20)	10(14.7)	17(26.6)	5(62.5)
Blood	6(12)	17(25)	8(12.5)	-
Catheter Tip	3(6)	2(2.9)	4(6.3)	-
Tissue	6(12)	3(4.4)	2(3.1)	-
CSF	-	9(13)	1(1.6)	-
Sputum	3(6)	-	5(7.8)	-
EB washing	11(22)	6(8.8)	5(7.8)	-

Table II: Antibacterial Susceptibility Pattern of MDR Strains of *Non-Enterobacteriaceae* by Vitek- 2 Compact System

Antibiotics	*A. baumannii* (n=59)			*P. aeruginosa*(n=47)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ticarcillin	2(3.4)	-	57(96.6)	2(4.2)	-	45(95.7)
Ticarcillin/ Clavulanic acid	1(1.7)	2(3.4)	56(94.9)	3(6.3)	1(2.1)	43(91.5)
Pipracillin	1(1.7)	1(1.7)	57(96.6)	3(6.3)	1(2.1)	43(91.5)
Tazobactam/ Piperacillin	2(3.4)	-	57(96.6)	2(4.2)	-	45(95.7)
Ceftazidime	1(1.7)	1(1.7)	57(96.6)	4(8.5)	-	43(91.4)
Cefipime	3(5)	3(5)	53(89.8)	3(6.3)	1(2.1)	43(91.4)
Imipenem	23(38.9)	-	36(61)	7(14)	-	40(85.1)
Meropenem	5(8.4)	5(8.4)	49(83)	9(19.1)	-	38(80.9)
Gentamycin	6(10.1)	6(10.1)	47(79.6)	15(31.9)	-	32(68.1)
Tobramycin	14(23.7)	3(5.1)	42(71.1)	6(12.8)	4(8.5)	37(78.7)
Ciprofloxacin	2(3.3)	-	57(96.6)	5(10.6)	-	42(89.4)
Minocycline	27(45.7)	-	32(54.2)	29(59.1)	-	20(40.8)
Trimethoprim/ Sulfamethoxazole	19(32.2%)	-	40(67.9)	35(74.4)	-	12(25.5)
Colistin	37(62.7)	-	22(37.2)			

R: Resistance I: Intermediate S: Sensitive

Table III: Antibacterial Susceptibility Pattern of MDR Strains of *K. Pneumoniae* by Vitek- 2 Compact System

Antibiotics	*Klebsiella pneumoniae* (n=44)		
	S (%)	I (%)	R (%)
Ticarcillin	-	-	44(100)
Ticarcillin/Clavulanic acid	-	2(4.5)	39(95.1)
Pipracillin	-	-	41(93.1)
Tazobactam/Piperacillin	2(4.5)	-	42(95.5)
Ceftazidime	-	4(9.1)	40(90.9)
Cefipime	1(2.3)	1(2.3)	42(95.5)
Imipenem	7(15.9)	2(4.5)	35(79.5)
Meropenem	6(13.6)	-	38(86.4)
Gentamycin	4(9.1)	3(6.8)	37(84.1)
Tobramycin	4(9.1)	2(4.5)	38(86.4)
Ciprofloxacin	-	-	44(100)
Minocycline	24(54.5)		28(45.5)
Trimethoprim/Sulfamethoxazole	10(25)	-	30(75)
Colistin	28(68.2)	-	13(31.7)

R: Resistance I: Intermediate S: Sensitive

Discussion

It is worrisome to note the high rates of resistance of *non Enterobacteriaceae* members of gram negative *ESKAPE* pathogens i.e. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* to the commonly used flouroquinolone (ciprofloxacin) in our study i.e. 77.9 % and 81.3% respectively, while *Klebsiella pneumoniae* showed 58% resistance and *Enterobacter spp*. 100% sensitivity to ciprofloxacin.

In present study MDR *Acinetobacter baumannii*, was the most common pathogen recovered from clinical specimens (36 %). Similar findings have been reported in another study conducted in an Intensive Care Unit of Monterrey, Mexico, in which *Acinetobacter baumannii*, was also found to be the most common isolate.[2]

Multidrug resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* are now emerged as one of the very important healthcare-associated infections to control and treat. Patients admitted in intensive care unit (ICU) and those with central intravenous catheters and respiratory devices are the main targets of these organism.[9]

MDR *Acinetobacter baumannii* demonstrated high rate of resistance to Ciprofloxacin 38% Piperacillin,78% & Ceftazidime 45% in the previous studies by Bruno et al., and Simgamsetty et al.,[10] which were contradictory to the results found in our study, that is resistance to Ciprofloxacin was 96.4%, to Ceftazidime 96.6% and to Piperacillin 96.6%. Viaggi et al., reported 96% *Klebsiella pneumoniae* isolates resistant to Meropenem (MIC>16 mg/L) while (92%) were resistant to Tigecycline (MIC >1 mg/L), Colistin (MIC >2 mg/L), and Gentamicin (MIC >2 mg/L).[11] Our result showed only 31.7% resistance of *Klebsiella pneumoniae* to Colistin (MIC >16), 84.1% to Gentamicin and 86.4% to Meropenem having MIC of >16.

Colistin is considered as one of the therapeutic option against isolates of *Acinetobacter baumannii* and *Klebsiella pneumoniae*.[12] *Pseudomonas aeruginosa* MDR isolates in our study showed > 90% resistance to Ticarcillin, Meropenem, Cefipime, Ceftazidime and Piperacillin, while 74.5% sensitivity to Cotrimoxazole, but contradictory findings were observed by Shashwati et al.,[13] and Ibrahim of Thi-Qar university.[14] They reported that majority of *Pseudomonas aeruginosa* isolates were susceptible to Cefepime, Cefoperazone-Sulbactum, Meropenem, Levofloxacin, & Amikacin. A study conducted by Ghazal et al., demonstrated 93.7% of *Pseudomonas aeruginosa* resistant against Ceftazidime, 72% against Ciprofloxacin and 52% against Amikacin.[15] It is comparable with our study which revealed almost similar susceptibility pattern.

The study being laboratory based is the limitation of this research. No clinical outcome of antibiotic therapy was determined. A multicenter study should be carried out involving all main hospitals of the city to establish MDR pattern in Gram-negative *ESKAPE* pathogens. However, this study will help doctors in our locale while deciding antimicrobial options for treating infectious diseases.

Conclusion

It is concluded that rapid identification and susceptibility testing of Gram-negative *ESKAPE* pathogens by VITEK-2 compact system helps in earlier switches in antibiotic therapy, and reducing total consumption of antibiotics. Moreover**,** MDR was observed in majority of Gram-negative *ESKAPE* pathogens except for *Enterobacter spp*. in our setup. Most of Gram-negative *ESKAPE* isolates were found to be susceptible against Colistin, Tigecycline and Minocycline. These antibiotics could be the good therapeutic options for infections due to *ESKAPE* pathogens. However, it is recommended that Colistin has to be used cautiously due to more significant adverse effects like neurotoxicity and nephrotoxicity and should be kept for use for more resistant Gram negative *ESKAPE* pathogens.

Future Prospects

The anticipated regional variations of MDR, requires consistent checking of disease control processes and ordinary surveillance of antimicrobials susceptibility profile in our hospitals. It should be the joint effort of microbiologists and clinical practitioners in introducing current and suitable antimicrobials according to AMR trend and locally designed antibiogram.

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