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Antibiotic susceptibility profile of multidrug resistant ESKAPE pathogens isolated from clinical specimens in a tertiary care teaching hospital of central India:- an emerging challenge to clinician to efficiently use reserve drugs.

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Abstract

Background

A global threat to human health is posed by antimicrobial-resistant ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Stenotrophomonas maltophilia, Klebsiella pneumoniae, Acinetobacter baumannii complex, Pseudomonas aeruginosa, and Enterobacter species*) infections. This study aimed to determine the prevalence and antibiotic susceptibility profile of multidrug resistant (MDR) ESKAPE pathogens.

Materials and methods

This prospective observational study conducted in the department of microbiology from January 2023 to December 2023 at a tertiary care teaching institute of central India identified MDR ESKAPE pathogens from clinical specimens using standard protocol and antibiotic susceptibility testing done as per as Clinical Laboratory Standard Institute (CLSI) guidelines.

Results

A total 6883 ESKAPE pathogens were isolated from 8540 culture positive clinical samples, of which 3294 (47.8%) were MDR. *Escherichia coli* and *Klebsiella pneumoniae* are leading MDR Gram negative organism (21.6%)and (19.5%) respectively. 3.7% of *Pseudomonas aeruginosa* were resistant to reserve drug colistin. 3.4% of *Enterococcus faecium* were vancomycin resistant.

Conclusion

Our study has a concerning number of MDR ESKAPE organism infections. Crucial steps to lessen the impact of this health issue include strict adherence to infection control recommendations, efficient implementation of antimicrobial stewardship programs to slow the emergence of antimicrobial drug resistance.

Keywords- antimicrobial drug resistance, multidrug- resistant, ESKAPE, colistin resistance.

INTRODUCTION

A major threat to public health in India is antimicrobial resistance due to the high rate of infectious illness and massive, uncontrolled antibiotic use (1). AMR is becoming a greater

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concern for as hospital-acquired infections as well as community-acquired illnesses because of the effective introduction and dissemination of MDR clones from nosocomial settings (2). It is predicted that by 2050, the number of fatalities caused by antibiotic resistance may reach 10 million annually (3).

The "ESKAPE" group of pathogens merits the highest attention from both a clinical research and development perspective due to their overall mortality and economic effect (4). Bloodstream infections, pneumonia, and urinary tract infections are among the serious and sometimes fatal illnesses that are frequently caused by ESKAPE bacteria. They have the potential to significantly harm critically ill and immunocompromised patients with life-threatening infections. Their clinical significance is based on their virulence, their capacity to create defense mechanisms against antimicrobial resistance, their increased use of inappropriate therapy, and their detrimental effects on the outcomes of ICU patients (5).

ESKAPE's multidrug resistance mechanisms can be broadly classified as drug inactivation, drug modification, drug accumulation, and biofilms. Drug inactivation is usually irreversible cleavage by an enzyme, drug modification is modification of the antibiotic's target site where it may bind, drug accumulation is reduced by reduced permeability or increased efflux and biofilms are able to physically prevent the host's immune response cells from responding to both antibiotics and the pathogen (6). Biofilms protect specialized dormant cells known as persister cells, which are sensitive to antibiotics that cause difficult to treat infections (7).

While multidrug-resistant ESKAPE infections are on the rise around the world, there is an acute lack of data from central India on the prevalence of MDRs among hospitalized patients. Therefore, we set out to evaluate the antimicrobial susceptibility profile of ESKAPE pathogens and the prevalence and risk factors associated with MDR organisms.

MATERIALS & METHOD

This prospective study was conducted at the department of microbiology of a tertiary care teaching centre in central India for a duration of 12 months.

ESKAPE pathogens were isolated from a varied specimens obtained from individuals suspected of having pyogenic illnesses. All specimens received in the aerobic section of bacteriology laboratory were inoculated on Sheep blood agar, MacConkey, and chocolate agar, and the plates were then incubated at 37°C in ambient air before being reported as sterile. Further identification based upon Gram staining characteristics, observing colony morphology, motility, catalase test, oxidase test, and a panel of standard biochemical tests (8).

Antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates as per Clinical and Laboratory Standard Institute guidelines (M100; 33rd edition) (9). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as the controls for disc diffusion testing. "MDR phenotype" refers to isolates that are resistant to three or more antibiotic classes (10).

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MRSA detection: The Cefoxitin disk diffusion test was used to identify methicillin resistance. Mueller-Hinton agar plates were used for lawn cultivation. For 24 hours, a disc containing 30 µg cefoxitin was incubated at 3°C. Methicillin-resistant *S. aureus* were defined as having a zone of inhibition of < 21 mm.

VRE screening was performed on *Enterococcus faecium* isolates that were shown to be resistant to the vancomycin screen agar.

Pseudomonas aeruginosa ATCC 27853 (MIC: 0.5–4 µg/mL) served as the negative control for the colistin broth microdilution, while an *Proteus mirabilis*, colistin intrinsically resistant (MIC >16 µg/mL) served as the positive control. MDR phenotype was assigned to isolates that were resistant to three or more antibiotic classes (11).

RESULTS

A total 6883 ESKAPE pathogens were isolated from 8540 culture positive clinical samples, of which 3294 (47.8%) were multidrug resistant. Most of the MDR ESKAPE pathogens isolated were from various medical and surgical wards (54.2%), followed by ICU(intensive care unit) patients (28.9%) and OPD (outpatient department) patients (16.8%).
Most of the MDR ESKAPE pathogens isolated were from urine 1025 (31.1%) , followed by blood 809 (24.5%), pus 742(22.5%), sterile fluids 482 (14.6%) and respiratory samples 236 (7.1%). Majority of isolates were from the age group of 46-65 years (28.7%), and the least were from the age group of 0-1 years (13%).

Table 1. Distribution of ESKAPE pathogens during the study period.

Gram Stain	**Bacterial Family/Genus/Species**	**Frequency (%)**
Gram Positive n=1011(30.6%)	*Enterococcus faecium*	404(12.2%)
	Staphylococcus aureus	607(18.4%)
Gram Negative n=2283(69.3%)	*Stenotrophomonas maltophilia*	10(0.3%)
	Klebsiella pneumoniae	643(19.5%)
	Acinetobacter baumannii	406(12.3%)
	Pseudomonas aeruginosa	219(6.64%)
	Enterobacterales: Escherichia spp.	712(21.6%)
	Enterobacterales: Proteus spp.	150(4.5%)
	Enterobacterales: Providencia spp	23(0.69%)
	Enterobacterales:Morganella spp.	25(0.75%)
	Enterobacterales: Enterobacter spp..	31(0.94%)
	Enterobacterales: Citrobacter spp.	30(0.9%)

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	Enterobacterales: Salmonella spp.	22(0.6%)
	Enterobacterales: Serratia spp	11(0.33%)
Total		3294(100%)

Among the Gram positive cocci *Staphylococcus aureus* was the most predominant species and *Escherichia coli* in Gram negative MDR ESKAPE pathogens.

Table 2:- Various MDR ESKAPE pathogens isolated from clinical specimens

S.no	MDR isolates	Sample type					Total
		Pus	Blood	Urine	Respiratory sample	Sterile body fluids	
1	*Enterococcus faecium*	56	84	191	15	58	404
2	*Staphylococcus aureus*	119	337	51	25	75	607
3	*Stenotrophomonas maltophilia*	3	0	0	1	6	10
4	*Klebsiella pneumoniae*	125	116	273	62	67	643
5	*Acinetobacter baumannii*	140	24	46	54	142	406
6	*Pseudomonas aeruginosa*	54	48	38	36	43	219
7	*Enterobacterales: Escherichia spp.*	148	145	369	16	34	712
	Enterobacterales: Proteus spp.	62	12	24	14	38	150
	Enterobacterales: Providencia spp	7	3	8	2	3	23
	Enterobacterales:Morganella spp.	8	2	8	3	4	25
	Enterobacterales: Enterobacter spp.	4	14	6	5	2	31
	Enterobacterales: Citrobacter spp.	12	4	4	2	8	30
	Enterobacterales: Salmonella spp.	0	18	4	0	0	22
	Enterobacterales: Serratia spp	4	2	3	1	2	12
	Total	742	809	1025	236	482	3294

Most of the MDR ESKAPE pathogens isolated were from urine 1025 (31.1%) , followed by blood 809 (24.5%), pus 742(22.5%), sterile fluids 482 (14.6%) and respiratory samples 236 (7.1%).

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Table 3:- **Antibiotic susceptibility profile of multidrug-resistant Gram positive ESKAPE pathogens isolated from clinical specimens as per as CLSI**

Antimicrobials (mcg)	***Enterococcus faecium***	***Staphylococcus aureus***
Erythromycin (15)	48.2%	35.4%
Clindamycin(2)	-	44.4%
Cefoxitin(30)	-	64%
Ciprofloxacin(5)	55.4%	68.3%
Gentamicin(10)	-	74.1%
Tetracycline(30)	52.4%	67.8%
Doxycycline(30)	47%	58.8%
Linezolid(30)	100%	100%
Penicillin(10)	86.6%	61.2%
Cotrimoxazole (25)	-	52.7%
Vancomycin MIC≤ 2 µg/ml(susceptible)	97.7%	100%

Enterococcus faecium and Staphylococcus aureus showed 100% susceptibility to Linezolid. *3.4% of Enterococcus faecium* were resistant to reserve drug Vancomycin where as 100% susceptibility of Vancomycin seen in *Staphylococcus aureus.*

Table 4:- **Antibiotic susceptibility profile of multidrug-resistant Gram negative ESKAPE pathogens isolated from clinical specimens as per as CLSI**

Antimicrobials (mcg)	*Klebsiella pneumoniae*	*Escherichia spp.*	*Proteus spp*	*Morganella spp*	*Providencia spp*	*Enterobacter spp..*	*Citrobacter spp.*	*Salmonella spp.*	*Serratia spp*
Amikacin (30)	55.2%	70.2%	86.6 %	80%	86.9%	93.5%	87.5%	-	81.8%
Ciprofloxacin (5)	46.6%	37.2%	54.6 %	48%	52.1%	61.2%	45%	77.2 %	63.6%
Gentamicin (10)	65.6%	63. %	60.6 %	56%	60.8%	77.4%	70%	-	72.7%
Ceftazidime (30)	13.2%	38.3%	43.3 %	36%	30.4%	41.9%	30%	-	27.2%
Cefotaxime (30)	14.7%	40.7%	46.6 %	36%	26%	41.9%	30%	68.1 %	27.2%
Ceftriaxone (30)	30.3%	47.8%	52%	40%	34.7%	45.1%	32.5%	72.7 %	45.4%
Cefepime (30)	31.1%	49.1%	53.3 %	56%	47.8%	48.3%	35%	-	54.5%

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Doxycycline (30)	33.1%	48.4%	34%	48%	43.4%	51.6%	40%	-	45.4%
Aztreonam(30)	48.3%	44.9%	40%	44%	39.1%	58%	37.5%	-	54.5%
Piperacillin-tazobactam (100/10)	50.5%	54.7%	66.6 %	64%	73.9%	67.7%	52.5%	-	63.6%
Cotrimoxazole (25)	34.5%	19.6%	20%	32%	21.7%	54.8%	42.5%	68.1 %	54.5%
Meropenem (10)	77.7%	84.2%	76.6 %	72%	78.2%	80.6%	72.5%	90.9 %	90.9%
Colistin MIC ≤ 2 µg/mL (intermediate)	97.97 %	98.3%	IR	IR	IR	100%	100%	-	IR

2.1% of *Klebsiella pneumoniae* and 1.7% of *Escherichia coli* were resistant to reserve drug colistin.

Table 5:- **Antibiotic susceptibility profile of multidrug-resistant Non fermenting Gram negative ESKAPE pathogens isolated from clinical specimens as per as CLSI**

Antimicrobials (mcg)	*Pseudomonas aeruginosa*	*Acinetobacter baumannii complex*	*Stenotrophomonas maltophilia*
Amikacin (30)	73%	70.1%	-
Ciprofloxacin (5)	44.7%	42.3%	-
Gentamicin (10)	66%	61.5%	-
Ceftazidime (30)	22.8%	27.5%	80%
Cefotaxime (30)	-	31.2%	-
Ceftriaxone (30)	-	54.6%	-
Cefepime (30)	51.1%	57.3%	-
Doxycycline (30)	34.2%	35.2%	-
Aztreonam(30)	52.5%	44.3%	-
Piperacillin-tazobactam (100/10)	59.3%	59.8%	-
Cotrimoxazole (25)	31.9%	30.5%	70%
Minocycline (30)		78.8%	80%

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Meropenem (10)	79.9%	86.2%	-
Colistin MIC ≤ 2 µg/mL (intermediate)	96.3%	96.5%	100%

Stenotrophomonas maltophilia showed 100% susceptibility to colistin where as 3.7% and 3.5% of *Pseudomonas aeruginosa and Acinetobacter baumannii complex are resistant to colistin respectively.*

DISCUSSION

According to the CDC, the ESKAPE bacteria pose a serious threat to world health as infection can raise morbidity and death. These microorganisms have the potential to lengthen hospital stays and raise expenses for people and the healthcare system (12). The WHO has listed all ESKAPE infections as critical and high priority pathogens for antimicrobial research and development, underscoring the organisms' clinical significance (13).

A total 6883 ESKAPE pathogens were isolated from 8540 culture positive clinical samples, of which 3294 (47.8%) were multidrug resistant. Most of the MDR ESKAPE pathogens isolated were from various medical and surgical wards (54.2%), followed by ICU(intensive care unit) patients (28.9%) and OPD (outpatient department) patients (16.8%).

Most of the MDR ESKAPE pathogens isolated were from urine 1025 (31.1%) , followed by blood 809 (24.5%), pus 742(22.5%), sterile fluids 482 (14.6%) and respiratory samples 236 (7.1%), which is similar to a study conducted by Pandey et al in which most of the ESKAPE pathogen isolated was from Urine (13). Majority of isolates were from the age group of 46-65 years (28.7%), and the least were from the age group of 0-1 years (13%).

Klebsiella pneumoniae and *Staphylococccus aureus* were the most common MDR ESKAPE pathogen found in our study, which is similar to study conducted by Pandey et al(13) and Arbune et al (14). In this study, 36% of *S. aureus* were methicillin- resistant which is similar than studies conducted in Nepal which showed MRSA to be 39.6% (15). *Enterococcus faecium and Staphylococcus aureus* showed 100% susceptibility to Linezolid. *3.4% of Enterococcus faecium* were resistant to reserve drug Vancomycin where as 100% susceptibility of Vancomycin seen in *Staphylococcus aureus* which is similar to study conducted by Jagadevi et al where 100% of Vancomycin and Linezolid susceptibility was seen in *Staphylococcus aureus* (16).

MDR *Escherichia coli* showed good susceptibility to meropenem*(88.2%)* where as least susceptibility to Cotrimoxazole (19.6%). *Klebsiella pneumoniae showed 88.2% susceptibility to meropenem but* least susceptibility to Ceftazidime(13.2%), similar to a study conducted by *Armin S et al.*, where least susceptibility was seen in 3rd generation cephalosporins (12).

2.1% of *Klebsiella pneumoniae* and 1.7% of *Escherichia coli* were resistant to reserve drug colistin. *Stenotrophomonas maltophilia* showed 100% susceptibility to colistin where as 3.7% and 3.5% of *Pseudomonas aeruginosa and Acinetobacter baumannii complex are resistant to colistin respectively.* Resistance to this antibiotic class has emerged as a result of the usage of polymyxins, one of the only effective treatments for infections brought on by

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MDR *Enterobacteriaceae, P. aeruginosa, and A. baumannii.* This has raised serious concerns for public health. Bacterial resistance to polymyxins can be encoded on transposable genetic elements, chromosomal and linked to LPS modification (17).

CONCLUSION

Given the dramatic increase of MDR Gram-negative bacteria, particularly ESKAPE pathogens, it is critical that practitioners in every area be informed about the most recent prevalence and pattern of antibiotic susceptibility of the circulating pathogens. Furthermore, because these bacteria may thrive in a hospital setting, better infection-control practices and antibiotic stewardship are necessary to stop or restrict the progression and spread.

The usage of polymyxins as the final effective treatment option is growing. Colistin and other antibiotics can be used in synergy when there is multidrug resistance. In order to make sure that colistin is still a viable therapeutic choice, it is critical to assess and create recommendations for its usage.

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