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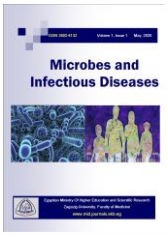

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Original article

Multidrug resistant pathogens and their antibiotic susceptibility profile isolated from blood stream infections in a tertiary care hospital

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ABSTRACT

Background: Increasing burden of hospital-acquired BSI caused by multidrug-resistant (MDR) pathogens are associated with poor patient outcome compared to susceptible bacteria. **Objectives:** The aim is to isolate and identify multidrug resistant (MDR) pathogens from positive blood cultures and to study their antibiotic resistant pattern. **Methods:** A cross sectional study was done on blood samples sent for culture and sensitivity. Identification and antibiotic susceptibility of isolated pathogens were done as per guidelines and Gram-negative bacteria resistance to at least one antibiotic in three or more antimicrobial classes was taken as MDR pathogen and further studied. **Results:** Out of 4770 blood samples were cultured, 78.3% were sterile and 7.2% were proven culture positive for pathogen. Among 342 positive blood culture, 88(25.7%) were MDR, 146 (42.7%) were ESBL producers and 76(22.2%) were carbapenem resistant isolates. Gram negative bacteria was predominantly isolated (83%) of which 67% of *Klebsiella pneumoniae (K. pneumoniae)*, 57% of *Acinetobacter baumanni* (*A. baumannii)* were MDR pathogens. 75% of *Klebsiella pneumoniae*, 69% of *Escherichia coli (E. coli),* 50% of *A. baumanni* were screened positive for ESBL producers. Resistance to carbapenems were 64%, 50%, 31% and 6% for *K. pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *E. coli* respectively. **Conclusion**: In our study, among 342 positive blood culture, 25.7% were MDR, 42.7% were ESBL producers and 22.2% were carbapenem resistant isolates. Measures to curtail the spread of MDR pathogen in the community settings and policies to adopt appropriate use of antibiotics in the hospital as well in the community need to be emphasized.

Introduction

Bloodstream infections (BSI) are considered to be one of the most serious and life-threatening conditions which are associated with high rate of mortality and morbidity. The increasing burden of hospital-acquired BSI caused by Multidrug-resistant (MDR) pathogens including extended-spectrum beta-lactamases (ESBL) and carbapenemase are associated with poor patient outcome compared to susceptible bacteria.

The definition of MDR pathogen includes organism resistance to at least one antibiotic in three or more antimicrobial classes. *Enterobacterales*,

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Pseudomonas aeruginosa and *Acinetobacter baumanni* are the most common gram-negative pathogens, that are resistant to carbapenem groups and the leading cause of nosocomial infections. MDR bacteria cause around 700, 000 deaths worldwide every year and it is estimated they will cause 10 million deaths by 2050, with a severe loss of economic resources [1].

The major contributing factor MDR in carbapenem resistance in Gram-negative bacteria is the emergence of carbapenem resistance which ultimately leads to pan drug resistance (PDR). When these organisms cause invasive infections, they are often associated with high morbidity and mortality. Therefore, detection of these resistant pathogen has got a significant role in prompt and appropriate antibiotic therapy as well as hospital infection control.

The aim is to isolate and identify multidrug resistant (MDR) pathogens from positive blood cultures and to study their antibiotic resistant pattern.

Methods

This cross sectional study done for a period of ten months from October 2022 to May 2023 in a tertiary care hospital, Puducherry. Blood samples sent for culture and sensitivity to microbiology laboratory were studied. Isolates from positive blood culture, which were considered as possible/ probable pathogens were included in this study. Skin commensals/ contaminants from positive blood cultures were excluded.

Blood samples were processed in BacT / Alert automated blood culture system. Blood culture bottles that flag positive signals were subcultured in blood Agar, MacConkey Agar and chocolate agar and further incubated at 37^{o}C. Identification and antibiotic susceptibility were done using VITEK-2 compact system and the results were interpreted as per CLSI guidelines [2].

Gram negative bacilli resistance to at least one antibiotic in three or more antimicrobial classes were taken as MDR pathogen. All data was be entered into MS Excel 2010 and analyzed. Qualitative variables represented by frequency and percentage. The study was approved by the Institutional Human Ethics Committee as per Indian Council of Medical Research guidelines 2017.

Results

During the entire study period, 4770 blood samples were cultured and 342 (7.2%) positive blood stream infections were reported and 3733(78.3%) were sterile. Of 342 positive blood culture, 88(25.7%) were MDR, 146 (42.7%) were ESBL producers and 76(22.2%) were carbapenem resistant isolates **(Table 1).**

In **figure (1),** out of the 342 culture-positive samples, 218 (64%) were males and 124 (36%) were females. The maximum positive BSIs were reported from patients>61-80 years of age (37%). Among 342 positive blood samples 176 (51%),79 (23%), 56 (16%), 16 (5%),9(3%) and 6(2%), were isolated respectively from intensive care units (ICUs), Emergency departments, Medical wards, Surgical wards, Obstetrics and Gynecology ward and Pediatric ward respectively. The majority of isolates were from by intensive care unit (ICU) (51%), followed by emergency department (ED) (23%) and medical ward patients (16%). Among 176 (51%) isolates from intensive care units, 65(74%), 57(75%) and 81(55%) were multidrug resistant (MDR), carbapenem resistant and ESBL producers **(Figure 2).**

Out of 342 pathogens reported, Gram negative bacteria is predominantly isolated (83%) followed by Gram-positive bacteria (14%) and yeast (3%). Among Gram negative bacteria, *Enterobacterales* (73%) was frequently isolated followed by non-fermenting Gram-negative bacilli (NFGNB) (25%) **(Table 4).** *Escherichia coli* (29%) is the common bacteria isolated in blood stream infections followed by *Klebsiella pneumonia* (19%), *Staphylococcus aureus* (8%), *Acinetobacter baumannii* (7%) and *Pseudomonas aeruginosa* (5%). Yeast was isolated in 11(3%) patients, and all were non albicans *Candida*, of which 2 *Candida* auris were isolated **(Figure 3)**

The antimicrobial susceptibility pattern of Gram-negative bacterial isolates is shown in **table (3 & 4).** Resistance pattern of *Enterobacteriaceae* in our study showed ciprofloxacin (70% in *E. coli* and 77% in *K. pneumoniae*), gentamicin (36% in *E. coli* and 63% in *K. pneumoniae*) and ceftriaxone (69% in *E. coli* and 75% in *K. pneumoniae*). The resistance rate to different agents of carbapenem was much higher in *K. pneumoniae*, which showed resistance to imipenem (63%) and meropenem (66%), than in *E. coli*, which had 6% resistance to both imipenem and meropenem. A similar pattern was shown for

tigecycline (28% of *K. pneumoniae* vs 2% of *E. coli*) and colistin resistance (3% of *K. pneumoniae* vs no resistance observed in *E. coli*).

Among the non-fermentative Gram-negative bacteria, resistance pattern showed ciprofloxacin (57% of *A. baumannii* and 44% of P. aeruginosa), gentamicin (57% of *A. baumannii* and 31% of *P. aeruginosa*), ceftazidime (50% of *A. baumannii* and 25% of *P. aeruginosa*). The proportion of resistance to carbapenem agents was 61% (imipenem and meropenem) for *A. baumannii*, while for *P. aeruginosa*, it showed 31% (imipenem and meropenem). The proportion of colistin resistance was 4% for both *A. baumannii* and 6% for *P. aeruginosa.*

The antimicrobial susceptibility pattern of Gram-positive bacterial species is shown in **figure (4).** Among 29 *Staphylococcus aureus* isolates, 100% resistance shown for penicillin, more than 70% were resistant to ciprofloxacin. *Staphylococcus aureus* strain resistance to cefoxitin was 31%, screened positive for MRSA. All the 29 isolates of *S. aureus* were 100% sensitive to vancomycin, linezolid and teicoplanin.

In our study, we have analyzed the percentage of multidrug-resistant (MDR) strains among the blood stream isolates, by taking into consideration resistance to at least three different antibiotic groups: aminoglycosides, cephalosporins, carbapenems, tetracyclines and fluoroquinolones. Overall, among the Gram-negative bacteria, 67% of *Klebsiella pneumoniae*, 57% of *A. baumannii* were found to be MDR pathogen, resistant to more than three class of antimicrobial groups. 75% of *K. pneumoniae*, 69% of *E. coli*, 50% of *A. baumannii* were screened positive for ESBL producers. Resistance to carbapenems were 64%, 50%, 31% and 6% for *K. pneumoniae, A. baumannii, Pseudomonas aeruginosa* and *E. coli* respectively. **(Figure 5).**

Table 1. Analysis of sample for blood culture and sensitivity.

Sample analysis	**Number**	**Percentage (%)**
Total No of samples	4770	
Sterile	3733	78.3
Pathogen	342	7.2
MDR	88	25.7
ESBL	146	42.7
Carbapenem Resistant	76	22.2

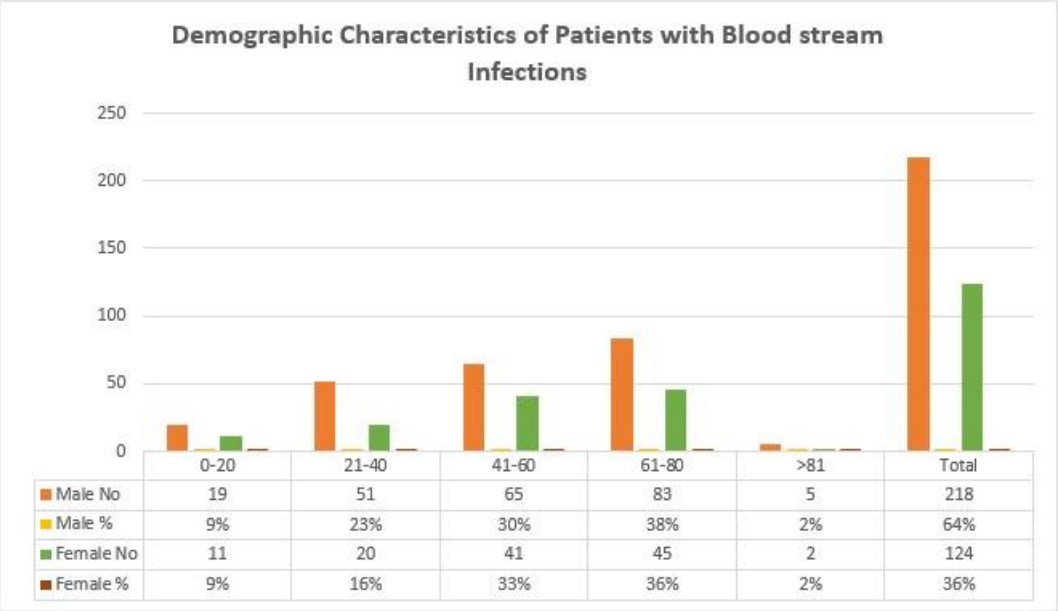
Table 2. Antimicrobial susceptibility pattern of *Escherichia coli* (n=100) and *Klebsiella pneumoniae* (n= 64) isolated from bloodstream infections.

Antibiotics	*Escherichia coli* (100)			*Klebsiella pneumoniae* (64)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ceftriaxone	27%	4%	69%	25%	0%	75%
Cotrimoxaole	47%	1%	52%	36%	6%	58%
Meroenem	93%	1%	6%	33%	2%	66%
Imipenem	94%	0%	6%	28%	9%	63%
Amikacin	98%	0%	2%	39%	38%	23%
Gentamicin	63%	1%	36%	38%	0%	63%
Tigecycline	97%	1%	2%	63%	9%	28%
Ciprofloxacin	16%	14%	70%	14%	9%	77%
Cefperazone Sulbactum	93%	0%	7%	33%	2%	66%
Piperacillin Tazobactam	86%	2%	12%	33%	2%	66%
Colistin	0%	100%	0%	0%	97%	3%

Table 3. Antimicrobial susceptibility pattern of *Acinetobacter* spp (n=28) and *Pseudomonas aeruginosa* (n= 16) isolated from bloodstream infections

Antibiotics	*Acinetobacter* spp (28)			*Pseudomonas aeruginosa* (16)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ceftazidime	46%	4%	50%	75%	0%	25%
Cotrimoxazole	39%	4%	57%	69%	0%	31%
Meropenem	39%	0%	61%	56%	13%	31%
Imipenem	39%	0%	61%	63%	6%	31%
Amikacin	46%	14%	39%	81%	6%	13%
Gentamicin	39%	4%	57%	69%	0%	31%
Tigecycline	61%	0%	39%	81%	0%	19%
Ciprofloxacin	43%	0%	57%	56%	0%	44%
Levofloxacin	50%	0%	50%	63%	0%	38%
Cefperazone Sulbactum	50%	4%	46%	56%	6%	38%
Piperacillin Tazobactam	43%	0%	57%	63%	6%	31%
Colistin	0%	96%	4%	0%	94%	6%

Figure 1. Demographic characteristics of patients with blood stream infections.



	0-20	21-40	41-60	61-80	>81	Total
Male No	19	51	65	83	5	218
Male %	9%	23%	30%	38%	2%	64%
Female No	11	20	41	45	2	124
Female %	9%	16%	33%	36%	2%	36%

Figure 2. Distribution trends of group of patients associated with BSIs.

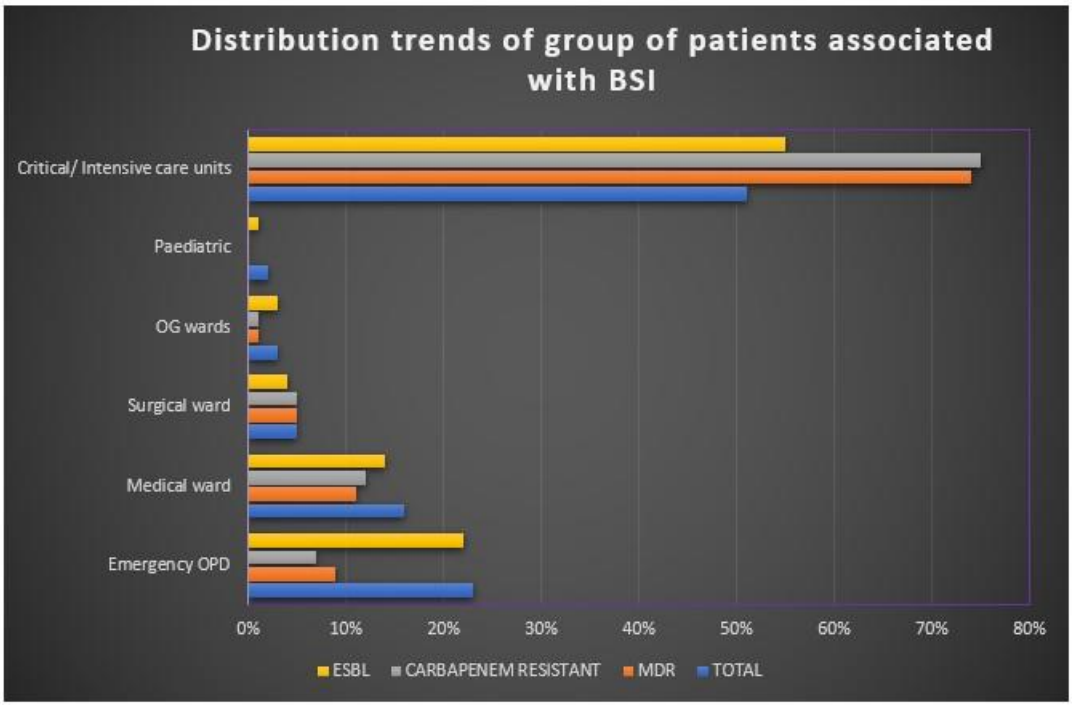


Figure 3. Perecentage of causative pathogens isolated from BSIs (n=342).

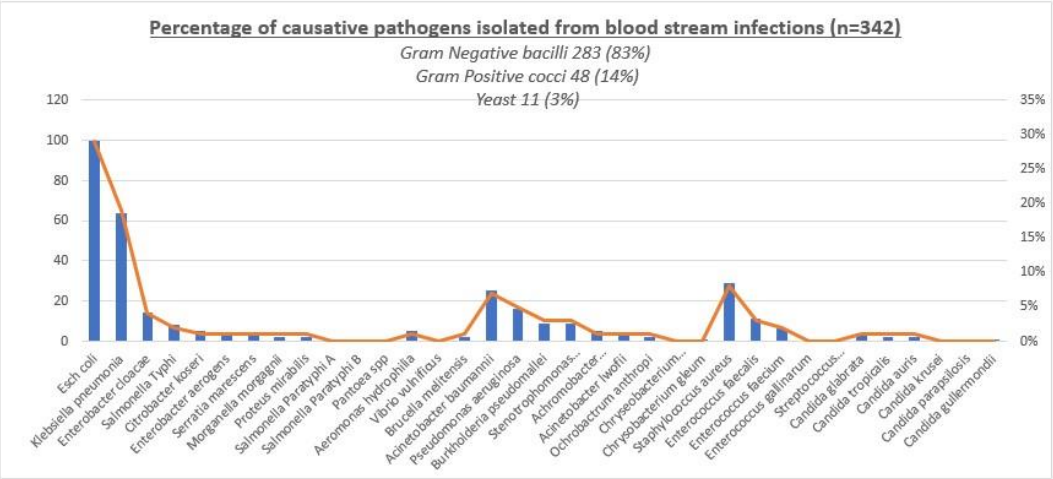


Figure 4. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from BSIs (n=29).

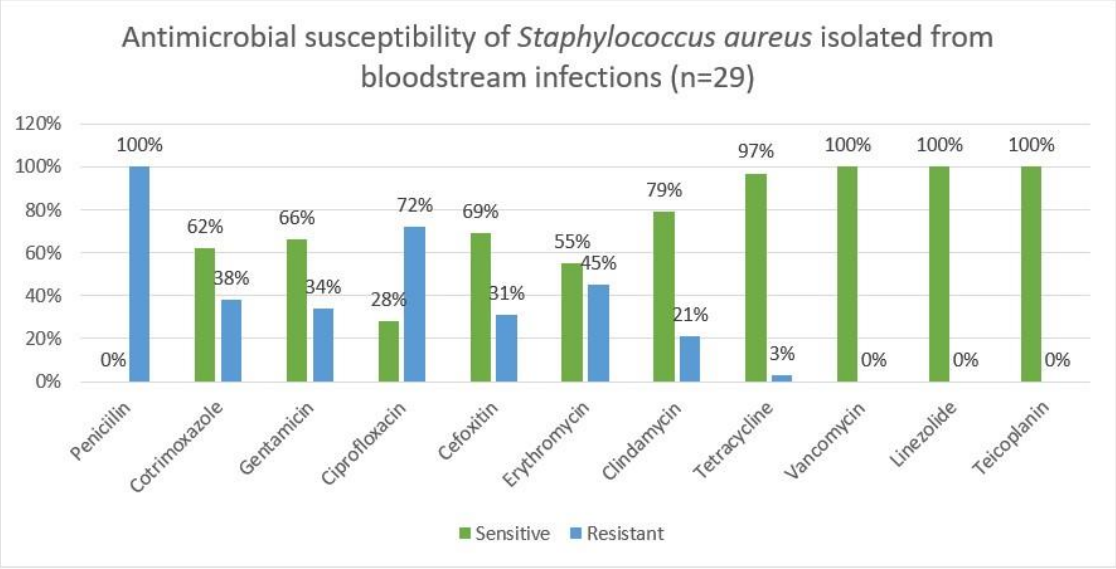
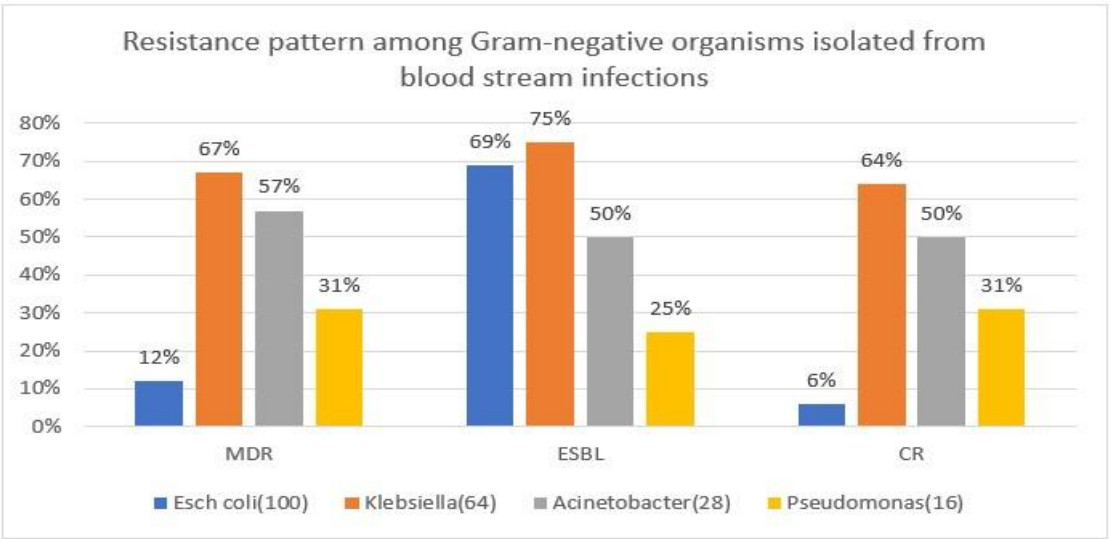


Figure 5. Resistance pattern among Gram-negative organisms isolated from BSIs.



Discussion

Multidrug resistance (MDR) has grown to be a major burden in management of patient with bacterial infections and turning to be a greatest threat to public health worldwide. As per the fact sheet of World Health Organization (WHO) fact sheet, infections by antimicrobial resistant organisms can result in treatment failure, elevated cost, frequent hospitalization and increased socioeconomic burden [3].

Among available β-lactam antibiotics, carbapenems are capable to resist many β-lactamase enzymes [4]. This feature renders them the last resort drugs to treat serious infections of many ESBL producing Gram-negative bacilli [5,6]. However, overuse of carbapenems has led to the emergence of carbapenem resistant *Enterobacteriaceae* (CRE), carbapenem resistant *A. baumannii* (CRAB) and carbapenem resistant *P. aeruginosa* (CRP).

In our study, the percentage of blood culture positive was 7.2%, Studies had shown positivity of 7.66% [7], similar to our study. Few studies had shown positive rate of.16.04% and 14.24% higher than our study[8,9].

Demographic profile of the current study showed 64% of males and 36% of females were culture positive. Majority were in the age group 61-80years (37%), followed by 41-60 years (31%). Study by **Maharath et al.** showed male preponderance of 59% with 41% similar to our data [10].

In the present study, among 342 positive blood culture, the majority of isolation was from intensive care units (51%) followed by Emergency departments (23%). **Bhardwaj et al.** in their study had similar isolation rate of 51% from intensive care units and 1% from emergency OPD, which was in a higher side of 23% in our study [11].

We have also found that among 176 (51%) isolates from intensive care units, 65(74%) were multidrug resistant (MDR) pathogens, of which 75% and 55% were carbapenem resistant and ESBL producers respectively.

In our study, of total 342 bacterial isolates, 83% were Gram-negative bacteria predominantly isolated followed by gram-positive bacteria (14%). Similar findings of bacterial isolation were found in other studies concordant with our study [7,11,12]. Among Gram negative bacteria, *E. coli* was commonly isolated followed by *K. pneumoniae* in *Enterobacterales* and among non-fermenters *Acinetobacter* spp was predominantly isolated and the results were consistent with previous study [13].

Antibiotic susceptibility pattern of *E.coli* showed maximum resistant to cephalosporins, trimethoprim/sulfamethoxazole and fluoroquinolones, whereas *K. pneumoniae* was resistant to cephalosporins, aminoglycosides, carbapenems, fluoroquinolones, and β-lactam combination agents. Our findings were congruent with other studies [13-16], where they have discovered similar pattern of resistance among *E.coli* and *K. pneumoniae.*

More than 50% *Acinetobacter* were found to be resistant to ceftazidime, cotrimoxazole, meropenem, imipenem, gentamicin and fluroquinolones and 96% intermediate sensitive to colistin. In contrary, *Pseudomonas* showed 60-80% sensitivity to ceftazidime, cotrimoxazole, aminoglycosides carbapenems, fluroquinolones, piperacillin tazobactam and cefperazone sulbactum, with 81% sensitivity to tigecycline and 96% intermediate sensitive to colistin. *Staphylococcus aureus*, in our study showed 100% resistant to penicillin and 31% were methicillin resistant and all were sensitive to vancomycin, linezolid and teicoplanin.

In our study, we have analyzed the percentage of multidrug-resistant (MDR) strains among the blood stream isolates, by taking into consideration resistance to at least three different antibiotic groups. The high percentage of MDR strains, was isolated from *K. pneumoniae* second most common Gram-negative microorganisms in our study of which 64% were carbapenemase producers and 75% were ESBL producers. Second most common MDR strain in our study was *Acinetobacter* spp (57%), of which 50% were both ESBL producers and carbapenem resistant. **Nagvekar et al.** [17], study on total number of positive blood cultures for a period of three years showed MDR-GNB positivity was around 22-37%, and we have recorded 25.7% in our study.

In study conducted at academic medical centers in the United States between 2015 and 2019 showed most CRE were *K. pneumoniae* (63.2%), followed by *E. coli* (14.5%) concurrent to our study [18]. Similarly, *Porwall et al.* identified the most common carbapenem resistant Gram negative isolate was CRKP (44%) followed by CREC (26%) [19]. In an another similar type of study, they have isolated common multi-drug resistant bacteria in the

ICU were *A. baumanni* and ESBL positivity was determined in 62.4% isolates of *E. coli* and 56.8% isolates of *Klebsiella* spp comparable to our data[20]. In our study we have isolated *A. baumanni* being the second most common MDR pathogen.

Of all the isolates studied, more than 90% of the blood isolates were found to be resistant to cephalosporins. On the other hand, 42.7% of blood isolates were found to be ESBL producer comparable to similar study [13].

In our study we have reported the total number pathogens from blood stream infections with reference to multidrug resistant pattern and their distribution in hospital settings, however we have few limitations of the study in not differentiating them as community and hospital-acquired BSIs which would add better analysis pattern. However, this study will give a comprehensive idea of sensitive pattern among multidrug resistant pathogen isolated from blood stream infection and their occurrence in ward setting, which would help in formulating infection control and antibiotic policies for better patient management and infection control.

Conclusion

In our study, among 342 positive blood culture, 25.7% were MDR, 42.7% were ESBL producers and 22.2% were carbapenem resistant isolates. The alarming increasing burden of hospital-acquired blood stream infections caused by multidrug-resistant (MDR) pathogens including are commonly associated with poor patient outcome compared to susceptible bacteria. Hence measures to curtail the spread of MDR pathogen in the community settings and policies to adopt appropriate use of antibiotics in the hospital as well in the community need to be emphasized.

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Competing interest

The authors declares that they have no competing interest.

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