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Bloodstream infections and antimicrobial sensitivity patterns in a tertiary care hospital of India

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Abstract

Background: Invasion of the bloodstream by microorganisms constitutes one of the most serious situations in infectious disease. Microorganisms present in circulating blood whether continuously, intermittently, or transiently are a threat to every organ in the body. Prevalence and antimicrobial susceptibility of microorganisms vary depending upon the geography and the use of antibiotics.

Methods: A cross-sectional study to determine the prevalent organisms causing bloodstream infection was conducted. BACTEC BD 9050 system was used to identify the causative organism, and sub-cultures were done on MacConkey Agar and Blood Agar. Antibiotic susceptibility test (AST) was done using Kirby B Disk diffusion method.

Results: A total of 170 patients were enrolled, and blood samples of 53 patients showed growth of organisms. *Staphylococcus aureus* was the most commonly isolated organism. Most of the Gram-positive cocci (GPC) were susceptible to vancomycin and linezolid. Most of the Gram-negative bacilli (GNB) showed sensitivity to cefoperazone/sulbactam followed by imipenem.

Keywords: Blood Stream infections, Antibiotic Sensitivity, Antimicrobial resistance BACTEC

Introduction

Invasion of the bloodstream by microorganisms constitutes one of the most serious situations in infectious disease. Microorganisms present in circulating blood whether continuously, intermittently, or transiently are a threat to every organ in the body. Prevalence and antimicrobial susceptibility of microorganism vary depending upon the geography and the use of antibiotics. The excessive and irrational use of antibiotics has led to an increase in the multidrug-resistant bugs and thus worsened the condition. Bloodstream infections have serious consequences like shock, disseminated intravascular coagulation, multiple organ failure, and even death. Increased hospital stay and associated costs are the most troublesome consequences.

Treatment of bloodstream infections is based on the knowledge of prevalent microorganisms and their antimicrobial sensitivity patterns. This information also forms the basis for making recommendations for initial empirical therapy to be started when a bloodstream infection is suspected.

Specific therapy can only be started once the organisms are isolated and their antimicrobial sensitivity patterns are studied. The procedure is time consuming and depends upon the growth of the organisms in culture media. Many faster and automated culture techniques are available. BACTEC 9050 is one of the automated blood culture methods and has a detection rate from 15% to 50%. In this study, we aimed to detect the most prevalent microorganisms in the region and their antimicrobial sensitivity patterns.

Methods

A cross-sectional observational study was carried out from March 2014 to June 2015; blood samples were collected from patients suspected of

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having bloodstream infection attending and admitted in National Institute of Medical Sciences and Research, NIMS University, Jaipur. Details like hospital identity, number, age, gender of the patients, and type and place of collection of specimen were recorded on a formatted proforma. A total of 170 suspected patients attending the various intensive care units (ICUs), out patient departments (OPDs), and indoor wards of Internal Medicine, Pediatrics, Obstetrics and Gynecology, Orthopedics, and General Surgery were included in the study. Patients of all age groups with fever (both high and low grade) due to infective causes were included. Written and informed consent was taken from all who fulfilled the criteria. Patients having both leukocytosis and leucopenia were included in the study. Those who refused consent were excluded along with patients having autoimmune or chronic diseases like tuberculosis or sarcoidosis. Patients on steroids, having heat stroke, or having a suspected viral or parasitic infection were also excluded.

Blood was collected with all aseptic precautions from the bedside of the patients suspected of having bloodstream infection using a sterile syringe. Approximately 5–10 mL of blood was collected from adult patients, while 1–5 mL blood was collected from pediatric patients, and 1–2 mL from neonates for blood culture. The sample taken was immediately processed for blood culture by BACTEC BD 9050 blood culture system (Figure 1). In the BACTEC BD 9050 Blood Culture System, when growth of bacteria occurred in sufficient amount, the system automatically generated a signal on the front panel. If no growth of bacteria occurred within 5 days of blood culture, then the blood culture was reported as sterile on culture for pyogenic aerobic organisms.

Identification of the bacterial isolates

Subculture was made on MacConkey Agar and Blood Agar from the BACTEC blood culture bottles, which generated a beep in the Automated BACTEC BD 9050 blood culture system indicating growth of the organism. The organisms were identified as per standard protocol [Ananthanarayan and Paniker, 2013; Collee *et al.* 1996; Forbes *et al.* 2014; Winm *et al.* 2006].

Gram-negative bacilli. The colony character on culture media was observed, and Gram staining, motility, and biochemical tests – indole, methyl red, Voges–Proskauer, citrate utilization, urease test, phenyl pyruvic acid test, triple sugar iron agar, oxidase, amino acids decarboxylase test, and sugar fermentation reaction – were conducted (Figure 2a and b).

Figure 1. BACTEC BD 9050 system used for automated cultures.

Gram-positive cocci. On the basis of colony character, Gram stain, catalase test, and coagulase test.

Antimicrobial susceptibility testing

This was performed by Kirby Bauer disk diffusion method as per the Clinical and Laboratory Standards Institute (CLSI, [2013]) guidelines. The antibiotics disks (Hi-media, Mumbai) were used as mentioned in Tables 1–3.

The following are quality control strains for antimicrobial sensitivity testing:

(1) *Pseudomonas aeruginosa* (ATCC27853),
(2) *Escherichia coli (ATCC25922)*,
(3) *Staphylococcus aureus (ATCC25923)*.

Data were entered in excel sheet to prepare a master chart. Quantitative data were shown as mean ± standard deviation (SD), and qualitative data as numbers and percentages.

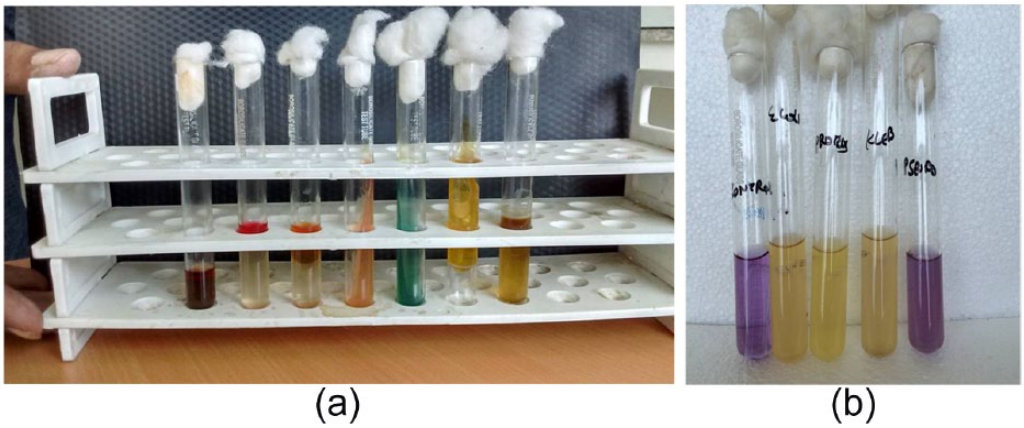

Figure 2. (a) IMVIC tests for *Escherichia. coli* and (b) fermentation tests for GNB.
GNB, Gram-negative bacilli.

Table 1. Antibiotics for GPC.

Antibiotic	Potency (μg)	Symbol
Amikacin	30	AK
Cefoxitin	30	CX
Ciprofloxacin	5	CIP
Clindamycin	2	CD
Erythromycin	15	E
Linezolid	30	LZ
Penicillin G	10 units	P
Vancomycin	30	VA

GPC, Gram-positive cocci.

Table 2. Antibiotics for GNB.

Antibiotic	Potency (μg)	Symbol
Ampicillin	10	AMP
Ceftriaxone	30	CTR
Piperacillin/tazobactam	100/10	PIT
Gentamycin	10	GEN
Cefoperazone/sulbactam	75/30	CFS
Chloramphenicol	30	C
Ofloxacin	5	OF
Cefepime	30	CPM
Amikacin	30	AK
Imipenem	10	IPM

GNB, Gram-negative bacilli.

Table 3. Antibiotics for *Pseudomonas* spp.

Antibiotic	Potency (μg)	Symbol
Amikacin	30	AK
Aztreonam	30	AT
Ceftazidime	30	CAZ
Ciprofloxacin	5	CIP
Colistin	10	CL
Piperacillin/tazobactam	100/10	PIT
Tobramycin	10	TOB
Imipenem	10	IPM

Results

This study was carried out from March 2014 to June 2015 with 170 non-repetitive blood samples collected from patients suspected of having bloodstream infections attending and admitted in National Institute of Medical Sciences and Research, NIMS University, Jaipur. Details like hospital identity, registration number, laboratory number, age and sex of the patients, and type and place of collection of specimen were recorded in a formatted proforma.

Culture positivity was seen in 53 (31.2%) samples, and 117 (68.8%) samples were sterile as detected with the BACTEC BD 9050 blood culture system. The maximum number of positive blood culture was from age group of 31 to 40 years, and the minimum from age group 11 to 20 years (Figure 3). Of the 53 culture-positive patients, 30 were adults admitted to medical ICUs. The mean time taken to culture positivity was 33.58 ± 21.64 h. Gram-positive cocci (GPC) were the most common organism isolated, with *S. aureus* being the most common of them (Figure 4), and *E. coli* was the most commonly isolated Gram-negative bacilli (GNB) (Figure 5).

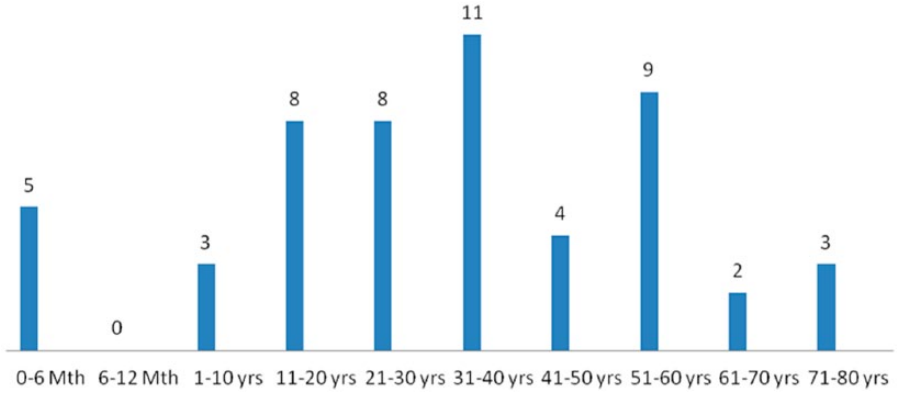

Figure 3. Age-wise distribution of culture-positive patients.

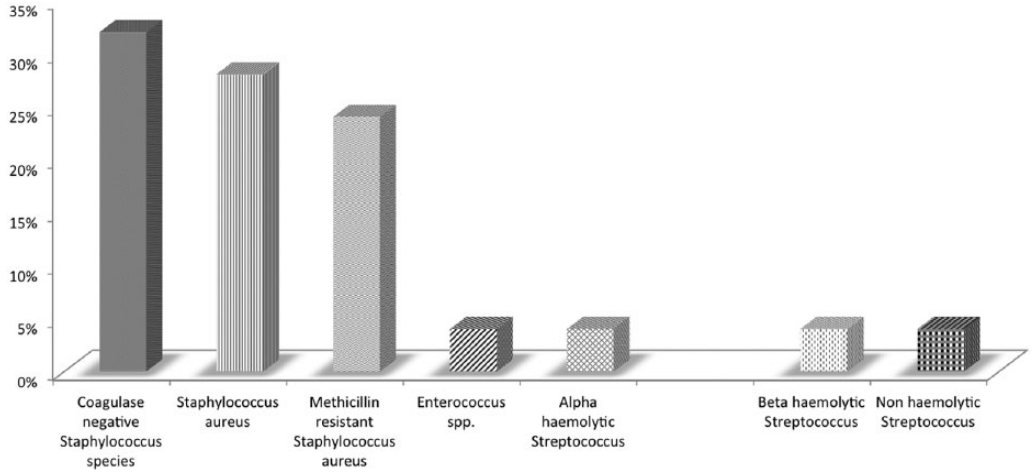

Figure 4. Distribution of GPC isolated from the cultures.
GPC, Gram-positive cocci.

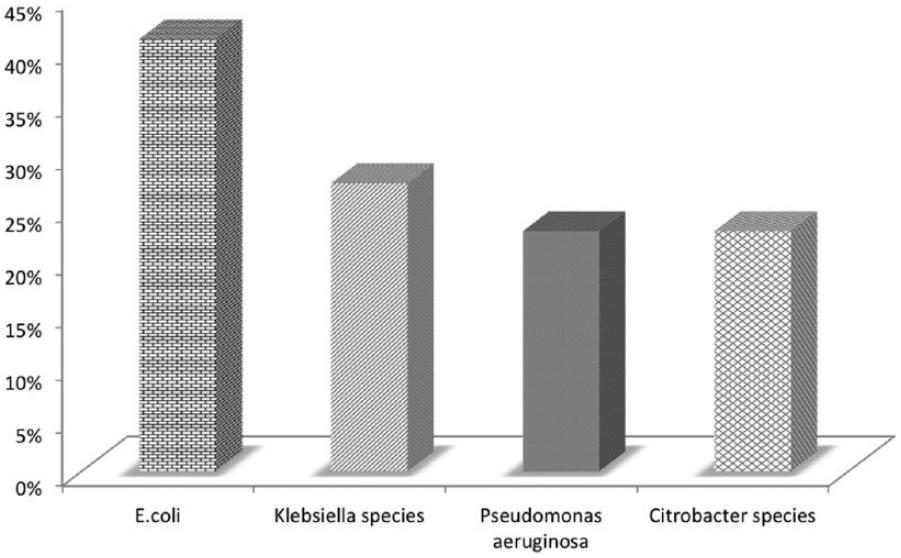

Figure 5. Distribution of GNB isolated from the cultures.
GNB, Gram-negative bacilli.

Table 4. Antibiotic sensitivity pattern of GPC.

Antibiotics	*Staphylococci*			*Enterococcus* (*n* = 2)	*Streptococcus. pneumoniae* (*n* = 2)
	Coagulase-positive *Staphylococcus. aureus* (*n* = 13)		Coagulase-negative *Staphylococcus* species (*n* = 8)		
	MSSA (*n* = 7)	MRSA (*n* = 6)			
Vancomycin	100%	100%	100%	100%	100%
Linezolid	100%	100%	100%	100%	100%
Amikacin	85.71%	33.33%	100%	0%	100%
Penicillin G	0%	0%	0%	0%	100%
Clindamycin	85.71%	50%	87.5%	0%	100%
Ciprofloxacin	85.71%	16.66%	87.5%	0%	100%
Cefoxitin	100%	0%	100%	100%	100%
Erythromycin	–	–	–	50%	–

GPC, Gram-positive cocci; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.

Table 5. Antibiotic sensitivity pattern of GNB (Figure 6a).

Antibiotic	*Escherichia coli* (*n* = 9)	*Klebsiella* (*n* = 6)	*Citrobacter* (*n* = 2)
Cefoperazone/sulbactam	100%	83.33%	100%
Imipenem	88.88%	100%	50%
Piperacillin/tazobactam	88.88%	83.33%	100%
Amikacin	66.66%	83.33%	100%
Cefepime	66.66%	16.66%	100%
Ciprofloxacin	33.33%	33.33%	50%
Ceftriaxone	22.22%	33.33%	100%
Chloramphenicol	0%	0%	50%
Ampicillin	0%	0%	0%
Gentamycin	22.22%	33.33%	0%

GPC, Gram-negative bacilli.

There were six *Candida* fungus isolated, of which four were non albicans species.

Antibiotic sensitivity pattern

The antibiotic sensitivity patterns of GPC are shown in Table 4 and those of GNB are shown in Table 5.

Some other antibiotic sensitivity patterns were also noted for *P. aeruginosa*, which are listed in Table 6.

Discussion

Bloodstream infection is a challenging problem, and sometimes, it may be life threatening; therefore, timely detection, identification, and antimicrobial susceptibility testing of blood-borne pathogens are one of the most important functions of diagnostic microbiology laboratory. In this study, the culture positivity of the BACTEC BD 9050 blood culture system was 31.2%. This positivity rate was consistent with the study done by Kohli-Kochhar *et al.* [2011]. This positivity rate was consistent with the Kenyan study by Kohli-Kochhar *et al.* [2011] in neonates who reported blood culture positivity of 23% but not consistent with Karunakaran *et al.* [2007] and Viswanathan *et al.* [2012]. Kolkata whose study yielded positivity rate of 16.7% and 46.3% respectively.

In this study, maximum number of positive cases was found in the middle age group less than 60 years (31–40 years of age). A study done by Prashanth *et al.* [2011] also reported maximum number of positive cases in age group of 31–45 years.

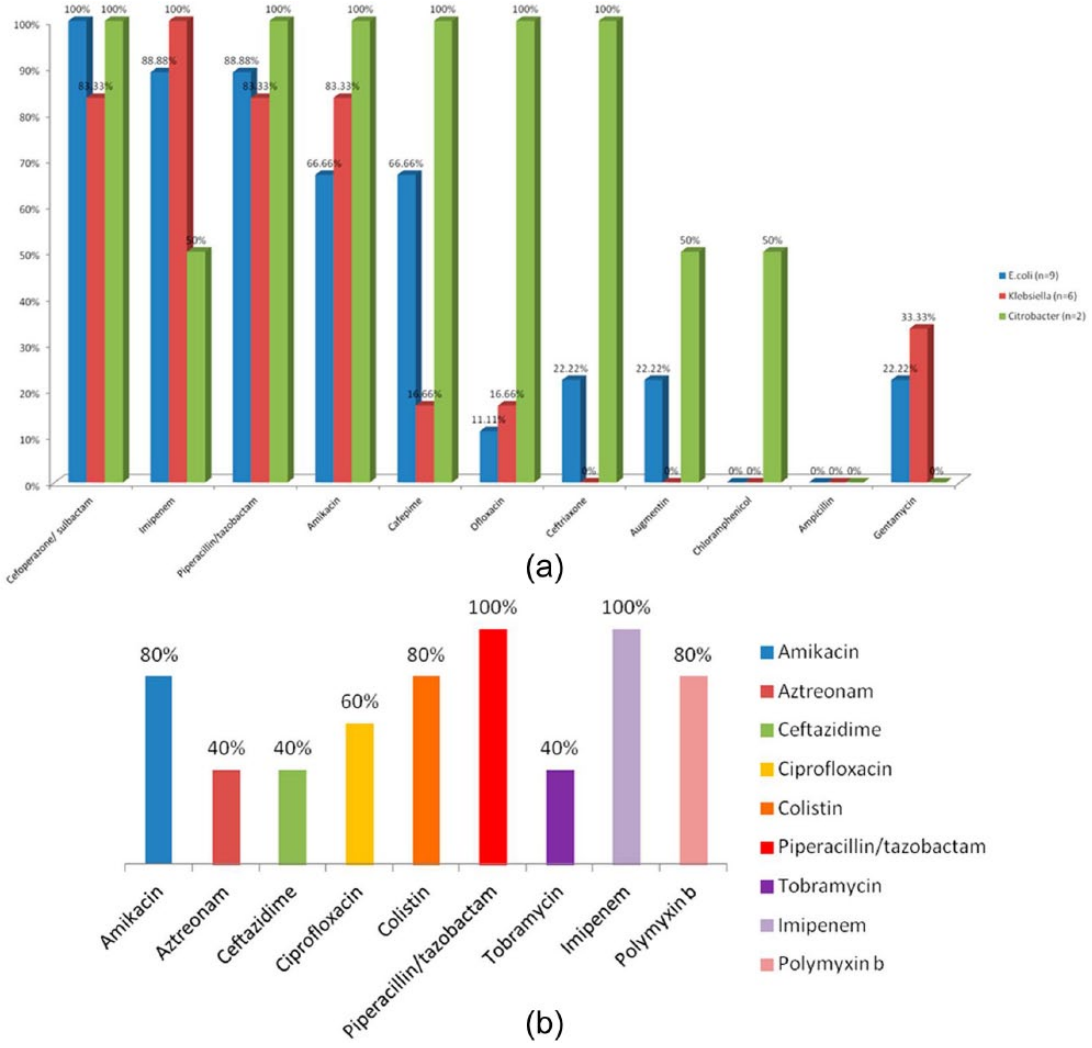

Figure 6. (a) Antibiotic susceptibility pattern of gram negative bacilli and (b) antibiotic susceptibility pattern of *Pseudomonas.*

Table 6. Antibiotic sensitivity patterns for *Pseudomonas aeruginosa* (Figure 6b).

Antibiotics	*P. aeruginosa* (*n* = 5)
Amikacin	80%
Aztreonam	40%
Ceftazidime	60%
Ciprofloxacin	60%
Colistin	80%
Piperacillin/tazobactam	80%
Tobramycin	40%
Imipenem	100%

In this study, men had high culture positivity as compared with women. The result was consistent with the study done by Kaur and Singh [2014] who reported high culture positivity in 65.22% men. The finding was also similar to study by Hussein *et al.* [2005] that reported 66.66% positivity in men and 33.33% in women, and similar observation of male dominance – 86.92% over 13.08% in female – was reported in study by Salari [2002]. Men are the active and are the main earning members of most families, so they are more privileged to visit physician chamber for treatment. However, Zenebe *et al.* [2011] reported more high culture positivity in women, 59.2%, than men, 40.8%, in their study.

In the present study, maximum number of blood culture positivity was found in the ICUs, especially medical intensive care unit (MICU) followed by wards and OPD as determined by both blood

cultures. This is likely to be due to the admission of the patients more in ICU and wards as blood-stream infections are a serious illnesses and have to be monitored round the clock in the hospital.

In this study, maximum number of GPC which were isolated by the BACTEC BD 9050 blood culture system was of *S. aureus* 13 (52%) followed by coagulase-negative *Staphylococcus* spp. 8 (32%), *Enterococcus* spp. 2 (8%), and *Streptococcus pneumoniae* 2 (8%), whereas the maximum number of GNB which were isolated were *E. coli* (40.91%) followed by *Klebsiella* spp. (27.27%), *P. aeruginosa* (22.73%), and *Citrobacter* spp. (9.09%). These above findings were consistent with the studies done by Fayyaz *et al.* [2013] and Karlowsky *et al.* [2004] who reported maximum number of *E. coli* in GNB in their studies. Similar findings were also found in the studies done by Karunakaran *et al.* [2007] and Aiken *et al.* [2011] for GNB. However, Karlowsky *et al.* [2004] and Karunakaran *et al.* [2007] reported more of coagulase-negative Staphylococci in their study, and Kaur and Singh [2014] reported higher prevalence of *Salmonella typhi* among GNB in their study.

In this study, *Candida* spp. 6 (11.32%) were isolated by the BACTEC BD 9050 blood culture system out of which non albicans *Candida* spp. were 4 (7.55%) and *Candida albicans* were 2 (3.77%). The results were consistent with study by Karunakaran *et al.* [2007] who reported positivity rate of 3.8% for *Candida* spp. with predominance of non albicans *Candida* spp. and with Kohli-Kochhar *et al.* [2011] who reported a rate of 3% for yeast isolates.

In this study, it was seen that GPC were 100% sensitive to vancomycin and linezolid. The results were consistent with the studies done by Fayyaz *et al.* [2013] and Marshall *et al.* [1998]. However, the results were inconsistent with the study done by Kaur and Singh [2014] who reported sensitivity of 57.14% to vancomycin. The sensitivity rate of amikacin was 85.71% for methicillin-sensitive *S. aureus*, 33.33% for methicillin-resistant *S. aureus*, 100% for coagulase-negative *Staphylococcus* spp. and *S. pneumoniae*, and 0% for *Enterococcus* spp. This result was consistent for methicillin-sensitive *S. aureus and* coagulase-negative *Staphylococcus* spp. with the study by Fayyaz *et al.* [2013] who reported sensitivity of amikacin to be 93.33%. The sensitivity rate of clindamycin is 85.71% for methicillin-sensitive *S. aureus*, 50% for methicillin-resistant *S. aureus*, and 87.5% for coagulase-negative *Staphylococcus* spp., which were consistent with the study by Marshall *et al.* [1998].

In this study, the sensitivity rates of ciprofloxacin was 87.5% for coagulase-negative *Staphylococcus* spp., 85.71% for methicillin-sensitive *S. aureus*, 16.66% for methicillin-resistant *S. aureus*, and 0% for *Enterococcus* spp., which were consistent with the study by Marshall *et al.* [1998].

In addition to the above, cefoxitin was 100% sensitive for coagulase-negative *Staphylococcus* spp., methicillin-sensitive *S. aureus*, *Enterococcus* spp., and *S. pneumoniae*, and 100% resistant for methicillin-resistant *S. aureus*.

All the GPC showed 100% resistance to penicillin G except *S. pneumoniae*, which were 100% sensitive. In the present study, among GNB, imipenem showed 88.88% sensitivity to *E. coli*, 100% to *Klebsiella* spp., and 50% to *Citrobacter* spp. The results for *Klebsiella* spp. were consistent with the study done by Saghir *et al.* [2009] who reported 96% sensitivity of imipenem for Enterobacteriacae family but was not compatible with *E. coli* and *Citrobacter* spp. The results of *E. coli* and *Klebsiella* spp. were also consistent with the study done by Jyothi *et al.* [2013] who reported sensitivity of 93% for *E. coli* and *Klebsiella* spp. For *Citrobacter* spp., the results were not consistent. This might be due to the fact that only two isolates of *Citrobacter* spp. were isolated. However, the results were not consistent with the findings of Fayyaz *et al.* [2013] who reported sensitivity of imipenem to be 59.2% for GNB.

In the present study, ceftriaxone showed 22.22% sensitivity to *E. coli*, 33.33% to *Klebsiella* spp., and 100% to *Citrobacter* spp. These findings for *E. coli* and *Klebsiella* spp. were consistent with the studies done by Saghir *et al.* [2009] and Fayyaz *et al.* [2013] who reported 28% and 22.44%, respectively, sensitivity to Enterobacteriacae family. However, the findings of *Citrobacter* spp. for ceftriaxone are consistent with the study done by Zenebe *et al.* [2011] who showed 100% sensitivity to ceftriaxone. The observation of ceftriaxone resistance pattern is suggestive of the fact that 77% *E. coli* and 66% of *Klebsiella* spp. isolates were extended spectrum beta-lactamase (ESBL) producers.

In this study, sensitivity to amikacin for *E. coli*, *Klebsiella* spp., and *Citrobacter* spp. was 66.66%, 83.33%, and 100%, respectively. The results for

Klebsiella spp. were consistent with the study by Fayyaz *et al.* [2013] but not consistent for *E. coli* and *Klebsiella* spp. showing higher sensitivity of 85.71%, and were consistent with the studies by Kaur and Singh [2014] for *Citrobacter* spp.

In this study, *E. coli, Klebsiella* spp., and *Citrobacter* spp. showed 88.88%, 83.33%, and 100% sensitivity to piperacillin/tazobactam, respectively. This finding was consistent with the study by Karlowsky *et al.* [2002] who reported a sensitivity of 89.9% for *Klebsiella* spp., but not compatible for *E. coli* and *Citrobacter* spp.

In this study, *E. coli* and *Klebsiella* spp. showed 22.22% and 33.33% sensitivity, respectively, to gentamycin, and *Citrobacter* spp. were 100% resistance to gentamycin; sensitivity to ciprofloxacin was 33.33% for *E. coli* and *Klebsiella* spp. and was 50% for *Citrobacter* spp. The results were consistent for *E. coli* and *Klebsiella* spp. with the study done by Fayyaz *et al.* [2013] for gentamycin and ciprofloxacin, which showed sensitivity of 32.7% and 26.53%, respectively.

In this study, *E. coli, Klebsiella* spp., and *Citrobacter* spp. showed 66.66%, 16.66%, and 100% sensitivity, respectively, to cefepime. This was consistent with the study by Saghir *et al.* [2009] for *Klebsiella* spp. who reported 20% sensitivity of for Enterobacteriacae family to cefepime. However, the findings were not consistent for *E. coli* and *Citrobacter* spp.

In this study, *E. coli, Klebsiella* spp., and *Citrobacter* spp showed 100% resistance to ampicillin. The results were consistent with the study done by Jyothi *et al.* [2013] showing 97% resistance to ampicillin. Also the results were consistent with the study done by Fayyaz *et al.* [2013] whose study showed that *E. coli, Klebsiella* spp. and *Citrobacter* spp. were 93.88% resistant to ampicillin.

In this study, *P. aeruginosa* showed 40% sensitivity to aztreonam, 60% sensitivity to ciprofloxacin, and 80% sensitivity to amikacin. This finding was consistent with the study by Hafsa *et al.* [2011] for ciprofloxacin and amikacin, that is, 50% sensitivity to ciprofloxacin and 75% sensitivity to amikacin, and was also consistent with the study by Fayyaz *et al.* [2013], that is, 37.5% sensitivity to aztreonam, 60% sensitivity to ciprofloxacin, and 72.5% sensitivity to amikacin.

In this study, *P. aeruginosa* showed 100% sensitivity to imipenem, which was consistent with the study by Hafsa *et al.* [2011] that reported a sensitivity of 100%.

In this study, *P. aeruginosa* showed 60% sensitivity to ceftazidime, which was consistent with the study done by Fayyaz *et al.* [2013], reporting a sensitivity of 60%, and by Rabirad *et al.* [2014], reporting a sensitivity of 65.8% to ceftazidime. In this study, *P. aeruginosa* showed 80% sensitivity to piperacillin/tazobactam, which is consistent with the study done by Fayyaz *et al.* [2013], reporting a sensitivity of 70%.

There was variation in the antibiotic sensitivity rate of various organisms isolated in the present study when compared to different past studies. This may be due to the fact that sensitivity of organisms to antibiotics is variable and depends upon prevalence of strains, antibiotics use, and its resistance patterns in a particular area.

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Conflict of interest statement

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