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Surveillance of multidrug resistance of gram negative bacteria in blood stream infection at a tertiary care Indian teaching hospital

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Abstract

Background: Bloodstream infections (BSI) are associated with high morbidity and mortality. This scenario worsens with the emergence of drug-resistant pathogens, resulting in infections which are difficult to treat or even untreatable with conventional antimicrobials. The aim of this study is to describe the epidemiological aspects of BSI caused by multiresistant gram-negative bacilli (MDR-GNB).

Methods: We conducted a laboratory-based surveillance for gram-negative bacteremia over a 1-year period. The bacterial isolates were identified by conventional method and the automated BacT/ALERT 3D system. the antimicrobial susceptibility testing was performed and documented.

Results: From 300 septicemia cases, cellulitis (62), pneumonia (25), UTI (96), lungs abscess (64) and unknown (51) cases revealed. From blood culture, *E. coli*, *K. pneumonia* and *P. aeruginosa* common gram negative bacteria were found. Among Gram negative bacilli, ampicillin was found to 80.9% resistant (MIC \geq 32), followed by cefipime, which was 76.2% resistant (MIC \geq 16).

Conclusions: Here we demonstrate that clinically relevant antibiotic resistance genes are prevalent in this setting. We hope our findings support the development of intervention measures by policy makers and healthcare professionals to face antibiotic resistance.

Keywords: Bacteremia, antibiotic resistance, gram-negative bacteria, MDR, blood culture

Introduction

Bloodstream infection (BSI) is one of the leading causes of morbidity and mortality in healthcare associated infections in India ^[1]. Approximately, 200,000 cases of bacteraemia and fungemia occur annually with mortality rate ranges from 20-50% worldwide ^[2]. Pneumonia is the most common cause accounting for about half of all cases, followed by intraabdominal and urinary tract infections ^[3, 4]. Intra-abdominal sepsis usually occurs after trauma or surgical resection, or intrinsic disease of the intestine, which includes appendicitis, peritonitis, diverticulitis or biliary tract infection, cholecystitis, and cholangitis ^[5].

A wide spectrum of organisms has been described that cause BSIs and this spectrum is subject to geographical variation ^[6-8]. Gram negative bacteria is the major pathogen in septic patient, but since 1987 until 2000, Gram positive bacteria has been major cause of sepsis with an increasing rate of 26.3% ^[9]. In many studies a wide range of bacteria has been described in sepsis patients including Gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella species*, *Neisseria meningitidis*, *Haemophilus influenzae*, and Gram positive such as *Coagulase negative staphylococci (CONS)*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Enterococcus faecium* ^[10-14]. *Escherichia coli* is a major cause of urinary tract infection, *Staphylococcus aureus* in wound infection, and *Pseudomonas aeruginosa* plays a key role in nosocomial infections. The common organisms isolated from patients with respiratory tract infections are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas Spp.*, and *Haemophilus Spp* ^[15-16]. The common organisms isolated from patients with intraabdominal infections are *Staphylococcus aureus*, *Streptococcus group D*, *Escherichia coli*, and *Candida Spp* ^[16].

The conventional blood culture methods currently being used are time consuming, labour intensive and suffer from poor sensitivity due to lack of use of nutritionally enriching

substances, inability to neutralize the inherent antimicrobial components in blood and patients receiving antimicrobial treatment [17, 18]. This method includes two-week culture in order to enable slow growth of some microorganisms which are cultured on specific media [19]. This method is simple, affordable and precise [19]. The limitations of conventional blood culture system are high risk of contamination due to repeated blind sub culturing, slow isolation rate especially slow-growing microorganisms, more turnaround time, unable to detect minimum inhibitory concentration (MIC) of antimicrobials.

The BacT/Alert 3D/60 (bioMérieux, Inc., Durham, N.C.) blood culture system is an automated, continuous monitoring system that is widely used in clinical laboratories. This system allows rapid and accurate detection of microorganisms in blood. The BacT/ALERT system detects microbial growth through non-invasive colorimetric detection of CO₂ produced during bacterial metabolism. The bottles, which are agitated throughout the recommended 7 days incubation period, are monitored every 10 min by the instrument, & growth is determined by an algorithm based on an initial high CO₂ production, or an acceleration in the rate of CO₂ production [20]. Isolates from obtained growth will be identified by VITEK-2 (bioMérieux, Durham, USA) by using identification cards. Antimicrobial profile for positive isolates will be determined by using AST cards. Minimum inhibitory concentration (MIC) of antimicrobials will also be detected by VITEK-2 system. Automated Blood culture manufacturers have devised methods for detection of bacterial pathogens in patients on antimicrobial therapy [20]. These include antibiotic-inactivating resins and charcoal containing media [20]. There are many clinical studies which demonstrates enhanced recovery of common bacterial pathogens and yeasts in charcoal containing media [21].

Automated blood culture system give better rapid result, reduced error and minimum turnaround time. They help in the reduction of disease mortality, morbidity and minimizes the risks of contamination. The most important advantage of this system is the time it gains for treatment, due to rapid isolation, especially slow-growing microorganisms [22]. And reduction of drug resistance bacterial strain development.

Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance of blood borne isolates is increasing and it also varies in accordance with geographical and regional location. The infection caused by MDR organisms is more likely to prolong the hospital stay, increase the risk of death, and require treatment with more expensive antibiotics. Keeping in view of the above facts, this prospective study was carried out to isolate & identify of pathogens causing BSI. Also evaluated the antimicrobial susceptibility pattern (AST) of isolated organism by using both conventional (Kirby-Bauer's disc diffusion) method and automated culture system (VITEK-2, bioMérieux).

Material and Methods

The study "Bacteriological profile of blood stream infection (BSI) by using both automated & conventional blood culture system in a tertiary care hospital" was a prospective study conducted in the department of Microbiology in collaboration with Department of Medicine, Paediatrics, Obstetrics & Gynaecology & Intensive care units of S.C.B. medical college and Hospital, Cuttack, Odisha.

A total of 600 blood culture specimens were collected aseptically from 300 patients. Samples were collected from both left & right cubital fossa, with 1 hour interval, before the start of antimicrobial therapy.

Blood culture

BHI culture bottles were incubated at 37° C for a maximum up to 7 days. Blind subculture was made on blood agar & MacConkey agar on day 1st, day 3rd and finally on 5th day. The culture bottles were discarded after 7 day.

Identification

Identification of isolates from sub cultured plates were done depending upon colony morphology, gram staining, rapid tests like catalase, coagulase, oxidase, and other requisite biochemical tests [23]. One presumptive identification of the organism & sensitivity was put on the same day to provide immediate report to the patient, so that proper treatment should be started as soon as possible. The final identification was done on the next day onwards after doing subcultures as mentioned above in blood culture and other biochemical tests.

AST was done on Mueller-Hinton agar following Kirby-Bauer's disc diffusion method which has been used for all positive isolates according to the guidelines of clinical & Laboratory standard institute (CLSI 2012) and zones of inhibition were interpreted accordingly.

Automated blood culture system

Inoculated BacT/ALERT culture bottles were loaded in to the automated BacT/ALERT 3D system as per manufacturer's guidelines (bioMérieux, USA). All the culture bottles were incubated under continuous agitation & monitoring up to 7 days. The increase amount of CO₂ produced by bacterial growth diffuses through a semi-permeable membrane in the base of culture bottle.

Flagged culture bottle were sub cultured on blood agar & MacConkey's agar and incubated at 37° C for 24-48 hours. The time required for bacterial growth in BacT/ALERT bottles had been detected & displayed on the 3D monitor of BacT/ALERT which had noted.

Identification

The isolated colony from sub cultured plate had been added to sterile saline solution to make a suspension equivalent to 0.5 McFarland standard, adjusted by using a DensiCHEK Plus (bioMérieux, Inc.) based on colorimetric principle.

Identification of positive isolates had been by VITEK 2 system (bioMérieux, USA). For identification of gram positive isolates GP ID card & for gram negative isolates GN ID card had been used. Samples yielding yeasts had been identified by using YST card. The reagent cards have 64 wells which contain 41 biochemical tests.

With a vacuum device, the card were inoculated with 0.5 McFarland suspension of positive organisms & then automatically sealed and manually inserted inside the VITEK 2 reader inoculator module. Fluorescence were measured every 15 minutes and results were determined after 3 hours [25].

Antimicrobial susceptibility testing (AST)

AST of all positive isolates had been performed with the VITEK 2 system, as recommended by the manufacture's

guidelines. For AST of gram positive isolates P628 card & for gram negative isolates N281 card. For yeasts AST-YS07 card had been used for antifungal susceptibility testing. Results had been given as sensitive(S), intermediate (I) and resistant(R) as per database in instrument, which is regularly updated by the manufacturer.

Minimum inhibitory concentration (MIC) of all positive isolates by using micro broth dilution method had been

detected. Resistant pattern of isolates by advanced expert study (AES) finding had also been detected.

Results

From 300 septicemic cases, 198 (66.6%) were male & 102 (33.3%) were female. Majority of patients were belong to the age group 31-40 year (32%).

Table 1: Age and sex distribution of septicemic cases (n=300)

Age group (Yrs.)	Male (n=198)	Female (n=102)	Total
0-10	11 (5.55%)	4 (3.9)	15 (5%)
11-20	07 (3.5%)	02 (1.9)	9 (3%)
21-30	52 (26.2%)	30 (29.4)	82 (27.3%)
31-40	63 (31.3%)	33 (32.3%)	96 (32%)
41-50	49 (24.7%)	27 (26.5)	76 (25.3%)
51-60	09 (4.54%)	03 (2.9)	12 (4%)
>60	07 (3.5%)	03 (2.9)	10 (3.3%)
total	198 (66.6%)	102 (33.3)	300 (100%)

Table 2: Study on various risk factors and number of isolates obtained (n=300)

Risk factors	No. of patients	No. of isolates obtained
Cellulitis	62 (20.7%)	10 (20.8%)
Pneumonia	25 (8.3%)	03 (6.2%)
UTIs	98 (32.7%)	17 (35.4%)
Lung abscess	64 (21.3%)	10 (20.8%)
Unknown	51 (17%)	8 (16.7%)
Total	300 (100%)	48 (100%)

Maximum no. of positive isolates was found to have risk factor of UTIs (35.41%) followed by lung abscess (20.83%) (Table 1).

From 300 patients, the risk factors were identified in 249 (83%) of cases, among of them, most common risk factor observed was Urinary tract infections (32.7%), followed by lung abscesses (21.33%) and cellulitis (20.7%) ((Table 2).

Table 3: Blood culture isolates identified in conventional (n=39) and automated culture system (n=48)

Organisms isolated	Conventional method (n=39)	Automated system (n=48)
<i>Escherichia coli</i>	13 (33.3%)	15 (31.2%)
<i>Klebsiella pneumoniae</i>	5 (12.8%)	6 (12.5%)
<i>Acinetobacter baumannii</i>	3 (7.7%)	4 (8.3%)
<i>Pseudomonas aeruginosa</i>	3 (7.7%)	3 (6.2%)
<i>Burkholderia cepacia</i>	0	1 (2.1%)

The most common Gram positive organisms in automated (25%) & in conventional (23.1%) were *CoNS*, followed by *S.aureus* in automated (7.7%) & in conventional (6.2%)

cases. Among Gram negative isolates, most common in automated (31.2%) & in conventional (33.3%) were *E.coli*, followed by *Klebsiella Spp.*(12.5%) (Table 3).

Table 4: Time of recovery of isolates by both automated and conventional blood culture systems

Organisms isolated	1 st day		2 nd day		3 rd day		4 th day		5 th day		6 th day		7 th day	
	Conv	Auto	Conv	Auto	Conv	Auto	Conv	Auto	Conv	Auto	Conv	Auto	Conv	Auto
<i>E.coli</i>	2	12	-	3	9	-	-	-	2	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	2	6	-	-	1	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i>	2	3	-	1	1	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	2	2	1	1	-	-	-	-	-	-	-	-	-	-
<i>Burkholderia cepacia</i>	-	-	-	-	-	1	-	-	-	-	-	-	-	-

Auto = Automated culture system, Conv = Conventional culture system

Out of 48 culture positive cases, 37 isolates were obtained within 24 hours in automated culture system and, 10 in 48 hours & 1 isolate after 72 hour of incubation, with mean time of detection was 14 hours (± 1.09) (Table 4).

In conventional culture method, 18 isolates obtained within 24 hours, 12 isolates were in 72 hours & 4 were after 96 hours of incubation, with mean time of detection was 60 hours (± 12.08).

Table 5: Antimicrobial resistant patterns of gram negative isolates

Antibiotics	<i>E.coli</i>		<i>Klebsiella pneumoniae</i>		<i>Pseudomonas aeruginosa</i>		<i>Acinetobacter baumannii</i>		<i>Burkholderia cepacia</i> (n=1)
	Automated system (n=15)	Conventional system (n=12)	Automated system (n=6)	Conventional system (n=5)	Automated system (n=3)	Conventional system (n=3)	Automated system (n=4)	Conventional system (n=3)	Automated system (n=1)
Ampicillin	13 (86.6%)	10 (83.3%)	5 (83%)	4 (80%)	3 (100%)	3 (100%)	4 (100%)	3 (100%)	1 (100%)
Ceftazidime	NA	NA	4 (67%)	3 (60%)	1 (33%)	1 (33%)	2 (50%)	1 (33%)	1 (100%)
Cefipime	8 (53.3%)	7 (60%)	4 (67%)	3 (60%)	3 (100%)	3 (100%)	3 (75%)	2 (66.6%)	1 (100%)
Ceftriaxone	6 (40%)	5 (40%)	3 (50%)	2 (40%)	2 (66.6%)	2 (66.6%)	2 (50%)	2 (66.6%)	0 (0%)
Imipenem	1 (6.66%)	1 (5%)	2 (33%)	2 (40%)	1 (33%)	1 (33%)	1 (25%)	1 (33%)	0 (0%)
Meropenem	1 (6.66%)	1 (5%)	2 (33%)	2 (40%)	1 (33%)	1 (33%)	1 (25%)	1 (33%)	0 (0%)
Co-trimoxazole	5 (33.3%)	4 (30%)	4 (67%)	3 (60%)	2 (66.6%)	2 (66.6%)	2 (50%)	2 (66.6%)	0 (0%)
Ciprofloxacin	6 (40%)	5 (40%)	4 (67%)	3 (60%)	2 (66.6%)	2 (66.6%)	2 (50%)	2 (66.6%)	1 (100%)
Amikacin	2 (13.3%)	2 (10%)	2 (33%)	2 (40%)	2 (66.6%)	2 (66.6%)	1 (25%)	2 (66.6%)	0 (0%)
Tigecycline	5 (33.3%)	4 (30%)	3 (50%)	2 (40%)	2 (66.6%)	2 (66.6%)	2 (50%)	2 (66.6%)	0 (0%)
Colistin	5 (33.3%)	4 (30%)	3 (50%)	2 (40%)	1 (33%)	1 (33%)	2 (50%)	1 (33%)	1 (100%)

From Gram negative isoates, *E.coli* found highly resistant to ampicillin (86.6%), followed by cefipime (53.3%), whereas *Klebsiella spp.* resistant to ampicillin (83%), followed by

ciprofloxacin (67%). The non-fermenters were, found 100% resistant to ampicillin, followed by ciprofloxacin (70%) and cefipime (66%) (Table 5).

Table 6: Minimum inhibitory concentration (MIC) of gram negative isolates in automated blood culture system (n=29)

<i>Enterobacteriaceae</i> (n=21)						<i>Pseudomonas spp.</i> (n=3)						<i>Acinetobacter spp.</i> (n=4)					
Normal MIC ranges			No. of isolates			Normal MIC ranges			No. of isolates			Normal MIC ranges			No. of isolates		
S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin						Ampicillin						Ampicillin					
≤8	16	≥32	4 (19.1%)	-	17 (80.9%)	-	-	-	-	-	-	≤8	16	≥32	-	1 (25%)	3
Ceftazidime						Ceftazidime						Ceftazidime					
≤4	8	≥16	-	-	-	≤8	16	≥32	2 (66.7%)	1 (33.3%)	-	≤8	16	≥32	-	-	-
Cefepime						Cefepime						Cefepime					
≤2	-	≥16	5 (23.8%)	-	16 (76.2%)	≤8	16	≥32	1	-	2	≤8	16	≥32	1	1	3 (75%)
Ciprofloxacin						Ciprofloxacin						Ciprofloxacin					
≤1	2	≥4	3 (14.2%)	5 (23.8%)	13 (62%)	≤1	2	≥4	1	-	2	≤1	2	≥4	-	1	3
Gentamicin						Gentamicin						Gentamicin					
≤4	8	≥16	15 (76.4%)	3 (14.3%)	3 (14.2%)	≤4	8	≥10	1	-	2	≤4	8	≥16	3 (75%)	2	-
Imipenem						Imipenem						Imipenem					
≤1	2	≥4	17 (80.9%)	3 (14.2%)	1 (4.8%)	≤2	4	≥8	1	2	-	≤2	4	≥8	3	1	-
Meropenem						Meropenem						Meropenem					
≤1	2	≥4	17 (80.9%)	3 (14.2%)	1 (4.8%)	≤2	4	≥8	1	2	-	≤2	4	≥8	3	1	-
Colistin						Colistin						Colistin					
-	-	-	-	-	-	≤2	4	≥8	2	1	-	≤2	-	≥4	3	1	-
Piperacillin-tazobac						Piperacillin-tazobac						Piperacillin-tazobac					
≤16/4	32/4-64/4	≥128/4	5 (23.8%)	2 (9.5%)	14 (66.6%)	≤16/4	32/2	≥128/2	1	-	2	≤16/4	32/4	≥128/4	1	3	-

Among Gram negative bacilli, ampicillin was found to 80.9% resistant (MIC≥32), followed by cefipime, which was 76.2% resistant (MIC≥16) among *Enterobacteriaceae*. Piperacillin-tazobactam was found to be 66.7% resistant (MIC≥128/2) and 75% intermediate resistant (MIC=32/4) among non-fermenters, followed by 70.5% resistant (MIC≥32) and 25% intermediate resistant (MIC=16) to cefipime. Least resistance was noted in imipenem and meropenem (4.8%) with MIC≥4 among *Enterobacteriaceae* and 25% resistant (MIC≥8) in non-fermenters (Table 6).

Discussion

Blood stream infection (BSI) is one of the most important factor affecting morbidity & mortality in patients. Immune-compromised patients especially suffering from cancer and neutropenia, have even higher risk of developing BSI [26]. Clinical suspicion, early diagnostic measures, prompt initiation of rational antimicrobial therapy and

comprehensive supportive measures are the cornerstone of successful management of BSI [27]. Blood culture is a critical diagnostic test to guide management of BSI and sepsis. The significance of providing rapid and reliable information to clinicians when a blood culture first becomes positive and reduction in turnaround time to final results has been well documented [28].

The present study "Bacteriological profile of blood stream infection (BSI) by using both automated and conventional blood culture system in a tertiary care hospital" was conducted in S.C.B. Medical college & hospital from October 2014 to September 2016 in 300 suspected cases of BSIs admitted to various departments.

The study was conducted to identify the isolates and its speciation, mean time of detection and antimicrobial resistance pattern in both conventional and automated blood culture system. Minimum inhibitory concentration (MIC) was determined by automated blood culture system.

In present study, the gender distribution of 300 blood samples, was found to be 66.6% of cases in males & 33.3% in females, which is similar to Vanitha *et al.* and Kante *et al.*, where they found 60.2% and 61.7% cases in males and 39.7%, 38.2% of cases in females respectively [29]. In most of the studies of septic shock report a male preponderance [29]. This male preponderance could be due to a higher prevalence of co-morbidities in men and women are protected due to hormonal factors (more estrogen production) and non-hormonal factors (higher secretion of cytokines, interleukin-6) through the immune system that occur in BSIs [30].

Our study showed, majority of patients (31%) were within 31-40 years of age group, which is similar to the findings of Meenakshi *et al.* and Vanitha *et al.*, where they have found 28% of cases were in the age group of 20-40 years and 83.7% of cases were in adult age group (>18years) respectively [29]. Most sepsis episodes are observed in patients older than 60 years [29]. Advanced age is a risk factor for acquiring nosocomial blood stream infection in the development of severe sepsis. Neonates are also vulnerable to infections because of their weak immunological barrier [29].

From 300 patients, the risk factors were identified in 249 (83%) of cases, among of them, most common risk factor observed was Urinary tract infections (32.7%), followed by lung abscesses (21.33%) and cellulitis (20.7%),. Our result is in contrast to the findings of Ivan *et al.*, where they found lung was the primary source of infection in both severe sepsis and septic shock, followed by the abdominal infections, the urinary tract infections and soft tissue infections [30].

In present study, blood culture was positive in 48 (16%) of cases in automated blood culture system, which is in accordance with findings done by Surase *et al.* (32%), Lunagaria *et al.* (16.9%) Goel *et al.* (9.2%) and Parihar *et al.* (28.9%) [31].

Blood culture positivity in our study by conventional blood culture system was 39 (13%) cases, which is in accordance with findings done by in Surase *et al.* (19.9%), Arora *et al.* (20%), Sharma *et al.* (33%) and Roy *et al.* (16.4%) [32].

In present study, the automated blood culture system detected nine additional organisms as compared to the conventional system, which is in accordance to the findings done by Surase *et al.* and Kareen *et al.* [32]. The reason behind this higher recovery rate of isolates by the automated system could be due to the continuous agitation, use of SPS (sodium polyanethol sulfonate) as an anticoagulant and the presence of activated charcoal as a neutralizing agent in BacT/ALERT culture bottle.

Various studies from different parts of India and around the world, showed varying blood culture positive reports by Nasa *et al.* (10.6%), Mathur *et al.* (10.6%), Arora *et al.* (20.02%), Sharma *et al.* (33.9%) and Ramirez Barba *et al.* (39%) [33]. In our study showed, the percentage of isolation rate in both automated and conventional method is low. This could be due to of patients reported to our hospital were referred from secondary care hospitals and these patients were already prescribed with antibiotics. Other reason could be due to self-medication, as antibiotics are available easily over the counter.

According to an another study done by Surase *et al.*, where they have found blood culture positivity in 32% of cases in

automated and 19.9% in conventional system, which is higher findings from our study.

In our study, Gram negative bacilli were predominant isolate in 60.4% of cases, followed by Gram positive cocci in 33.3% and yeasts in 6.2% in automated blood culture system, which is similar to the findings of Lunagaria *et al.*, where they have found Gram negative isolates in 55.3% of cases, Among Gram negative bacilli, *E.coli* 31% was the predominant isolate, followed by *Klebsiella spp.* 12%, which is in accordance with other studies of Mehta *et al.*, Karlowsky *et al.*, Kamga *et al.* and China & Gupta [35]. One *Burkholderia cepacia* 2.1% was isolated by automated blood culture system in present study, which is in contrast to the finding of Lunagaria *et al.*, where they have found 41 (4.8%) from 825 isolates [35].

Our study showed, among Gram positive cocci isolates, *Coagulase negative Staphylococci* (25%) of cases predominant, followed by *S.aureus* (6.2%) and *Enterococcus spp.* (2.2%), which is similar to the findings of Mulat *et al.*, where they have found CoNS (42.3%), followed by *S.aureus* (23.4%) [35]. According to an another study done by Kalpesh *et al.*, where they have found *S.aureus* (38.6%) was most common isolate, followed by *CoNS* (4.5%) & *Enterococcus spp.* (3.8%) [33]. In present study, from twelve CoNS isolates, *S.haemolyticus* (50%) was most common isolate, followed by *S.hominis* (41.6%). This is in accordance with the findings of Lunagaria *et al.*, where they have found *S.hominis* (28.6%) as predominant, followed by *S. haemolyticus* (22%) and *S.epidermidis* (21%) [32]. Several other studies have reported increasing incidence of infections due to *CoNS* [35]. *Coagulase negative staphylococci* is a well described pathogen in immune-compromised patients causing nosocomial BSI, UTIs, surgical wound infections, infections of prosthetic valves and ophthalmic infections [36].

In our study, automated blood culture system recovered 96.8% of isolates within 48hour, which is similar to the study of Surase *et al.*, where 86.8% of isolates obtained within 48 hours [30]. In present study, Using automated blood culture system, the mean detection time of isolation was 14 hours (± 1.09) for all isolates, which is similar to the findings of Ramana *et al.*, where they found MTTD to be 14.5 hours (± 5.7) [37]. In comparison to automated system, conventional blood culture system in this present study, MTTD was found to be 60 hours (± 12.08), which is similar to the findings of Surase *et al.*, where they have found MTTD to be 67 hours (± 80). Other studies have also reported a mean detection time of 19-24 hours by automated system and 5-7 days by conventional system [38].

In present study, antibiotic resistant pattern among Gram negative isolates showed that, most of the isolates found resistant to ampicillin (80%), followed by cefipime (75%), which is similar to the findings of Vanitha *et al.* [16]. Sensitivity pattern in our study showed, imipenem (80%), colistin (75%) and amikacin (70%) were found to be most effective antibiotics for all Gram negative bacterial isolates including non-fermenters, which is in accordance with the findings done by Lunagaria *et al.*, where they have found colistin (80.9%) the most sensitive antibiotic, followed by tigecycline (66%), amikacin (65%) and imipenem (62%) [32].

Among Gram negative bacilli, our MIC study showed, ampicillin was found to 80.9% resistant ($MIC \geq 32$), followed by cefipime, which was 76.2% resistant ($MIC \geq 16$) among

Enterobacteriaceae. Piperacillin-tazobactam was found to be 66.7% resistant ($\text{MIC} \geq 128/2$) and 75% intermediate resistant ($\text{MIC} = 32/4$) among non-fermenters, followed by 70.5% resistant ($\text{MIC} \geq 32$) and 25% intermediate resistant ($\text{MIC} = 16$) to cefipime. Least resistance was noted in imipenem and meropenem (4.8%) with $\text{MIC} \geq 4$ among *Enterobacteriaceae* and 25% resistant ($\text{MIC} \geq 8$) in non-fermenters.

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