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Isolation and identification of aerobic bacteria and their antibiogram profile in catheter related bloodstream infection among the hemodialysis patient in a tertiary care hospital

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ABSTRACT

Background: Catheter-related bloodstream infection (CRBSI) is quite evident in hemodialysis (HD) patients. The pattern of the isolates from the catheter tip as well as blood culture and their sensitivity against antibiotics varies and set a huge challenge for the physician for its management. **Aims and Objectives:** The present study was undertaken to isolate and identify, causative organism in CRBSI from the HD patient and to determine the antibiotic susceptibility profile of each isolate and determines the current trend. **Materials and Methods:** A total (n = 101) patients admitted for HD in the nephrology unit of a tertiary care hospital with preexisting comorbidities such as diabetes and hypertension (HT) were recruited according to inclusion/exclusion criteria after obtaining written consent. Blood samples from peripheral blood vessel of suspected CRBSI patients were collected as well as the catheter tip was cut and collected in sterile test tube and transported to microbiology laboratory for isolation, identification, and antibiotic susceptibility test. **Results:** The HD catheter tip culture was found positive in 54 (53.46%) samples out of the total 101 samples. The distribution of catheter tip culture positive patients and their association with diabetes mellitus was highest at age group > 60 years with a $p < 0.0001$. The association between catheter tip culture positive and HT was also found significant with $P<0.0001$. After analysis of culture reports of HD catheter tip and peripheral blood, culture-positive reports were found in 15 (14.85%) patients as CRBSI. The distribution of CRBSI predominantly found in the age group of 41–50 years (33%) which was significant with $P=0.004$. The causative organisms for CRBSI were Gram negative under this study and multidrug resistant too. The only drug polymyxin B was 100% sensitive to Gram-negative organism whereas vancomycin and linezolid were 100% sensitive to Gram-positive organisms. **Conclusion:** Patients with kidney disease receiving HD with a central venous catheter experience high rates of bloodstream and catheter tip infection commonly in those suffering from diabetes, HT, and dyslipidemia. The causative organisms for CRBSI were mainly Gram negative with a concern over existence of multidrug resistance strain.

Key words: Bacteria; Hemodialysis; Infection; Intravenous catheter

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INTRODUCTION

Hemodialysis (HD) is very essential intervention required for the end-stage renal disease (ESRD). Infections are the major cause of morbidity and the second cause of death following cardiovascular events in HD patients. Catheters are used in approximately 80% of patients initiating HD and in 25% of all prevalent patients as a bridge to

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a permanent vascular access or because the patient has exhausted all options for a permanent access.[1] Catheter-dependent patients are at increased risk for all-cause infection.[2-5] Septicemia accounts near about three-fourths of deaths caused by infections.[6,7] Several clinical, treatment, and sociodemographic characteristics make ESRD patients, particularly susceptible to catheter-related bloodstream infection (CRBSI).[7]

Episodes of CRBSI account for the major cause of infections in this population, followed by pneumonia. The annual mortality due to bacteremia is 100–300 times higher[8,9] in HD patients compared to the general population. Most episodes of bacteremia are associated with vascular access and especially with central venous catheters (CVCs). Central catheter infection may manifest as infection at the skin insertion site, as cellulites along the soft tissues overlying the tunneled portion, or as bacteremia without evidence of external infection at either of these superficial sites. Bacteremia occurs secondary to infection of the central catheter or as a manifestation of more serious complications, including septic thrombophlebitis or endocarditis.[10] The risk of infection from CVC is estimated to be 10 times higher compared to arteriovenous fistula infection. For the diagnosis of CVC-associated bacteremia and identification of the responsible microorganism, typically CVC removal as well as blood and catheter tip cultures are required.

From the existing literature, it is evident that *Staphylococcus* continues to predominate as the most frequently encountered pathogens in device-related infections. Although *Staphylococcus aureus* is a frequent cause of device-associated infection, the coagulase-negative staphylococci (CoNS) have become the most common causes of these infections in the past two decades, especially in immune compromised patients and those in whom long-term central venous access is required. Nonetheless, the incidence of *S. aureus* bacteremia is increasing, driven largely by the blossoming epidemic of community-associated methicillin-resistant strains.[6] Other different organisms found in CRBSI are *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae,* and *Candida* spp.[1] The increasing trend in the incidence of antibiotic resistance among these patients has also accentuated the overall morbidity and mortality.

Aims and objectives

This study aims to isolate bacteria from HD catheter tip and peripheral blood sample by conventional aerobic culture and correlate clinically in the current set up of the HD unit of the institute and also to determine the antibiotic susceptibility profile of each isolates.

MATERIALS AND METHODS

After obtaining a prior approval from the Institutional Ethics Committee (RKC/455 Dated January 17, 2019), a cross-sectional observational study was conducted in patients (n=101) admitted in the Department of Nephrology indoor ward undergoing HD having clinically suspected CRBSI in collaboration with the Department of Microbiology of R.G. Kar Medical College and Hospital, Kolkata, India, for a period of 1 year (March 2019–February 2020). Patients although on HD otherwise asymptomatic and those not willing to give consent to participate in the study were excluded from the study. The sample size was calculated based on the proportion of CRBSI obtained from a study by Gupta[11] as the protocol, procedure and setting of the study were similar to the present study. No sampling technique was applied as all episodes of infection (as per inclusion and exclusion criteria) were taken into account during the study period.

After diagnosis of HD patient with CRBSI, that is, presence of fever, chills, and/or hypotension, pain, redness, swelling or warmth, pus, or bad smell around the catheter with no other apparent cause of bloodstream infection (BSI), then the catheter tip was collected in sterile test tube and transported to Microbiology Laboratory, R G Kar Medical College and Hospital promptly. Blood sample from the same patient was collected from peripheral vein at the time of catheter tip collection in blood culture bottle containing proper culture media as per standard protocol, was transported to microbiology laboratory as early as possible for isolation, identification, and antibiotic susceptibility test.

Any growth was characterized by colony morphology and Gram staining from the plates. Confirmation was done by routine biochemical tests. Antibiotic sensitivity testing was done by modified Kirby–Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute guidelines using proper antibiotic disks.

Statistical analysis

The statistical analysis of the data was done with the help of Statistical software Microsoft Excel 2010. Intergroup comparison was analyzed using Chi-square test.

RESULTS

A total of n=101 samples were collected during this 12 months study with suspected CRBSI among patients age ranging from 19 to 78 years with mean and median age of 46.25 years and 47 years, respectively. Total patient was divided into six groups according to different age group and the distribution is depicted in Figure 1.

The distribution of male and female patients was 55.44% and 44.56%, respectively. The patients on HD presented with different comorbidities such as diabetes mellitus (DM), hypertension (HT), and dyslipidemia and their distribution is depicted in Figure 2.

The HD catheter tip culture was found positive in 54 (53.46%) samples out of the total 101 samples. The catheter tip culture positive predominantly found in the age group of 51–60 years followed by other groups as shown in Figure 3.

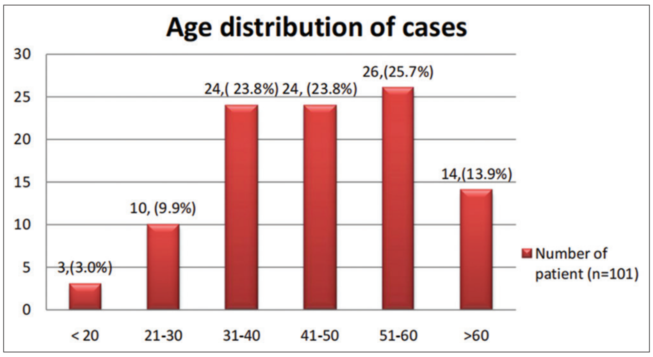

Figure 1: Distribution of study participants according to different age groups

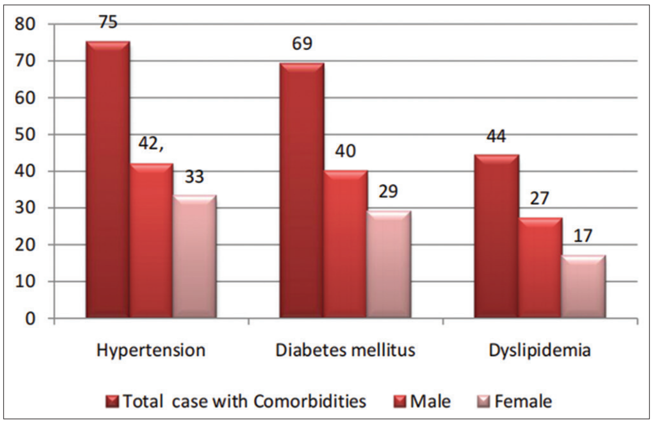

Figure 2: The distribution of the hypertensive patient according to sex ratio

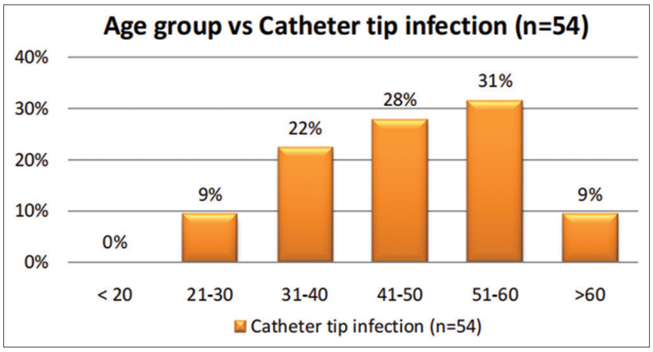

Figure 3: Distribution of hemodialysis catheter tip infection in the different age groups

The sex ratio among the catheter tip culture-positive male: female was 34 (62.96 %):20 (37.04%). The distribution of catheter tip culture positive and DM with respect to age is given in Table 1. It was found that with increasing age, the incidence of DM had also risen. At the same time, the percentage of catheter tip infection is also highest at age group >60 years. The association between catheter tip culture and DM found to be significant with Chi-square value 23.529 with P<0.0001.

The age distribution of HT in catheter tip culture-positive patient is given in Table 2.

Although there was no significant correlation between age of patient with hypertension as well as culture positivity, the association between patients with catheter tip culture positive and hypertension was found significant with Chi-square value 23.5294 with $p < 0.0001$. Out of total 54 positive catheter tip culture patients, 30 (55.56%) had dyslipidemia. The association between catheter tip culture positive and dyslipidemia was insignificant with Chi-square value 0.39; P=0.531. Out of total 101 peripheral blood culture samples, 19 (18.81%) were found culture positive. The blood culture positivity was found significant in the age group of 41–50 years. Out of these 19 samples, 12 (63.16%) and 7 (36.84%) catheter tip belong to male and female patient, respectively. Any association between sex of the patients and blood culture positivity cannot be established as Chi-square=0.5635 and P=0.452.

There was no statistical significance found between peripheral blood culture positive patient and HT as well as peripheral blood culture positive and DM since the Chi-square value=0.26928; P=0.603 and Chi-square value=1.2218; P=0.269 were found, respectively.

After the analysis of culture reports of HD catheter tip and peripheral blood, culture-positive reports were found in 15 (14.85%) patients as CRBSI. The distribution of CRBSI predominantly found in the age group of 41–50 years (33%) which was significant with P=0.004.

In this study, it was observed that out of total organism responsible for causing CRBSI, 40% was Gram-positive cocci and 60% was Gram-negative bacilli. The pattern of microorganism observed in the culture media is depicted in Figure 4 with predominance of *P. aeruginosa* and CoNS.

The pattern of microorganisms found in the HD catheter tip is shown in Figure 5.

The distribution of organisms found according to Gram's stain in catheter tip culture and peripheral blood culture samples are given in Table 3.

The antibiotic sensitivity pattern against *P. aeruginosa*, *Acinetobacter* spp., CoNS, Enterobacteriaceae, and methicillin0resistant staphylococci aureus (MRSA) was observed after testing in this study is depicted in Figure 6i-v. Isolates of *Acinetobacter* spp. which were responsible for CRBSI were resistant to amoxycillin-clavulanate, ciprofloxacin, chloramphenicol, and 3rd generation cephalosporins with resistance rate being 100% each. Even aminoglycosides, cotrimoxazole, and piperacillin-tazobactam also showed resistance with the sensitivity rates being 50%, 50%, and 100%, respectively. The doxycycline and meropenem showed also 50% sensitivity against the isolates each, whereas the only drug having high sensitivity was polymyxin B with 100% sensitivity against these isolates.

Table 1: Distribution of diabetes mellitus in hemodialysis catheter tip positive patients

Age (years)	All catheter tip culture positive	Catheter tip culture positive with diabetes mellitus (DM)	% of DM with culture positivity
<20	0	0	0
21–30	5	2	40
31–40	12	6	50
41–50	15	13	87
51–60	17	15	88
>60	5	5	100

Table 2: Distribution of hypertension in hemodialysis catheter tip positive patients

Age (years)	All catheter tip culture positive	Catheter tip culture positive with hypertension (HT)	% of HT with culture positivity
<20	0	0	0
21–30	5	4	80
31–40	12	8	67
41–50	15	11	73
51–60	17	12	71
>60	5	2	40

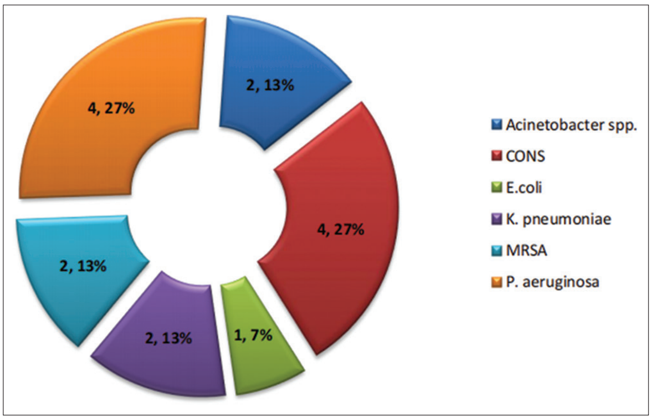

Figure 4: Distribution of CRBSI isolates (CONS: Coagulase-negative staphylococci, MRSA: Methicillin-resistant staphylococci aureus)

Bacteria causing BSIs and belonging to the family *Enterobacteriaceae* showed sensitivity to amoxycillin-clavulanate, piperacillin-tazobactam, aminoglycosides, 3rd generation cephalosporins, doxycycline, and to meropenem with resistance rate were 0%, 67%, 67%, 33%, 67%, and 67%, respectively. Similarly, quinolone group and phenicols were showed 67% resistance rate too. Polymyxin B and monobactam were found with 100% sensitivity against these isolates.

DISCUSSION

CVCs are the primary vascular access nearly for 20% of prevalent HD patients.[10] CRBSI is one of the most life-threatening consequences of HD catheter use due to its associated increased risk of morbidity and mortality.[12] In one large observational study of nearly 500 HD patients, the risk of CRBSI exceeded 50%.[6] Hence, there might be marked diversity in the presentation, clinical course, and outcomes of HD CRBSI. Such patients are successfully treated with intravenous antibiotics administered at their outpatient dialysis units, while others develop potentially life-threatening complications necessitating a high level of inpatient care.

However, there are few published studies investigating whether the clinical presentation and outcomes of CRBSI differ by infecting organism.[13]

The present study was undertaken to determine the microbiological profile and the susceptibility pattern of the microorganisms isolated from various samples of patients admitted in dialysis unit and suspected of having CRBSI at R. G. Kar Medical College and Hospital, a tertiary care center in Kolkata, India.

In this study, a total of 101 samples were collected based on the inclusion and exclusion criteria. Out of the

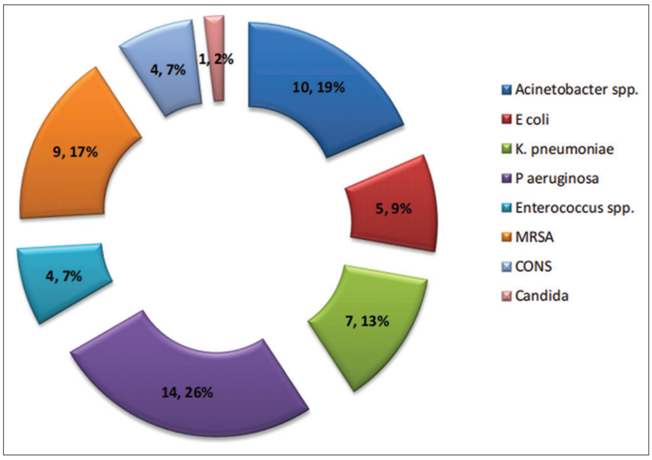

Figure 5: Distribution of isolates found in hemodialysis catheter tip (CONS: Coagulase-negative staphylococci, MRSA: Methicillin-resistant staphylococci aureus)

total 101 patients in this study, catheter tip samples of 54 (53.46%) and peripheral blood samples of 19 (18.81%) patients developed microbial growth. All samples were processed in the laboratory of the Department of Microbiology of the same institute.

All the reports acquired and their results were analyzed at the end of the study. Finally, there were 15 (14.85%) subjects diagnosed as CRBSI in the present set-up which was little higher with respect to a study conducted by Powe et al.,[14] where it was found to be 11.7%. However, Gupta[11] conducted a study in South India in 2016 which showed that the incidence of CRBSI was 15% which is near to our study.

Age of the study subjects was evaluated as a factor for association with infection. The lowest age of patient was 19 years and the highest age was 78 years in this study. The distribution of CRBSI predominantly found in the age group of 41–50 years (53%) which was statistically P=0.004. According to the study of Gupta,[11] the incidence of CRBSI is high at the age group of >60 years. However, the study conducted by Nabi et al.,[9] showed highest incidence of CRBSI in 41–60 years age group.

Table 3: Distribution of organisms according to Gram's stain

Culture positive parameter	Gram negative organism	Gram positive organism
CRBSI positive (n=15)	9 (60%)	6 (40%)
Catheter tip culture positive (n=54)	36 (67%)	18 (33%)
Blood culture positive (n=19)	13 (68%)	6 (32%)

In the present study, CRBSI subjects were distributed in 55.44% and 44.56% (male-female), respectively, according to their gender which was near similar distribution of sex in the study conducted by Gupta[11] (68:32). In the present study, DM, HT, and dyslipidemia were observed as comorbid condition. Among them, diabetes was detected in 100% CRBSI patients between 21–30 years and 51–60 years age groups. In the present study, investigators found that 69.31% had history of diabetes among total participants and 86.67% were diabetic among CRBSI cases. The study of Dagher et al.,[8] showed the prevalence of diabetes in 63.3%, Gupta[11] showed 52%, and Nabi et al.,[9] reported 54.5% of cases which were similar with the present study.

In this study, 75 (74.25%) were found to have HT almost similar to the studies conducted by Gupta,[11] Nabi et al., and Dagher et al.,[8] that is, 72.4%, 81.8%, and 83.3%,

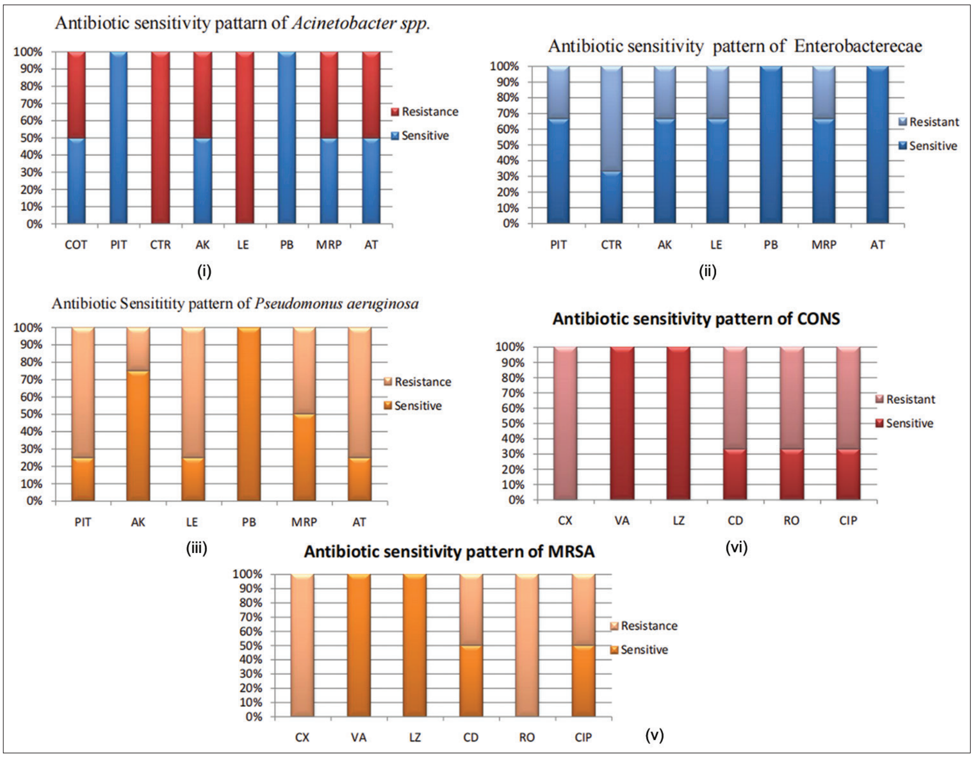

Figure 6: (i) Antibiotic sensitivity pattern of *Acinetobacter* spp., (ii) antibiotic sensitivity pattern of *Enterobacteriaceae*, (iii) antibiotic sensitivity pattern of *Pseudomonas aeruginosa*, (iv) antibiotic sensitivity pattern of CONS, antibiotic sensitivity pattern of MRSA. COT: Cotrimoxazole, PIT: Piperacillin-tazobactam, CTR: Ceftriaxone, AK: Amikacin, LE: Levofloxacin, PB: Polymyxin B, MRP: Meropenem, AT: Aztreonam, CX: Cefoxitin, VA: Vancomycin, LZ: Linezolid, CD: Clindamycin, RO: Roxithromycin, CIP: Ciprofloxacin, CONS: Coagulase-negative staphylococci, and MRSA: Methicillin-resistant staphylococci aureus

respectively. Although among the CRBSIs patients, 80% (n=15) were hypertensive, it was just statistically significant (P=0.05).

Out of total 15 CRBSI, 5 (33%) were found to have dyslipidemia and it was significant with P=0.032. A significant association was also found between catheter tip culture positivity and peripheral blood culture status with P=0.01 with degree of freedom 1.

There was vast distribution of causative organisms for CRBSI in different studies. Gupta[11] concluded in a study that Gram-negative organisms constituted 79% of the pathogens whereas 21% of them were Gram positive. In that study it was also revealed that predominant organism responsible was *S. aureus* among the Gram-positive organism and *P. aeruginosa* (9, 47%) among the Gram-negative organism. Other organisms were *detected Acinetobacter* spp. (2, 10.5%) and *E. coli* (2, 10.5%). In a study conducted by Sahli et al.,[15] microorganisms isolated were *K. pneumonia*e 26.5%, CoNS 23.5%, and *S. aureus* 23.5%.

According to the study conducted by Dagher et al.,[8] it was found that CRBSI from the HD catheters were *E. coli* in 22 (24.4 %), *Staphylococcus coagulas*e negative in 20 (22.2 %), *K. pneumoniae* in 7 (7.8 %), *P. aeruginosa* in 7 (7.8 %), *Enterococcus* species in 6 (6.7 %), *S. aureus* 5 (5.6 %), *Candida* species in 5 (5.5 %), *Proteus mirabilis* in 4 (4.4 %), *Serratia* species in 3 (3.3 %) and *Acinetobacter baumannii* in 2 (2.2%). Parameswaran et al., [16] found responsible organisms were *S. aureus* (40%) and that among patients with local catheter infections were CoNS. Candida caused 16% of CRBSI and 10% of local catheter infections.[16]

In the present study, it was found that out of total organism responsible for CRBSI, 40% was Gram-positive cocci and 60% was Gram-negative bacilli. *P. aeruginosa* 4 (27%) were found the most common bacteria causing BSIs in our set-up as found in a study of Gupta.[11] Coagulase-negative *Staphylococcus* was found as causative agent for CRBSI in 27% (n=15) which was also a predominant causative agent found by Parameswaran et al.,[16] *E. coli* was only 1 (7%) of total CRBSI causative agent in this study. However, this organism was predominantly found in a study conducted by Dagher et al.[8] Apart from all these organisms, the current investigators had found MRSA 2 (13%), *K. pneumoniae* 2 (13%), and *Acinetobacter* spp. 2 (13%) as causative agent of CRBSI in our study.

A nationwide surveillance study conducted in 49 hospitals in the USA showed a large prevalence of Gram-positive bacteria causing BSI's compared with Gram-negative organisms. However, a trend toward an increasing incidence of Gram-negative organisms causing BSI's has been observed more recently.[1] Findings of a study by Marra et al.,[17] from a Brazilian hospital were slightly different with Gram-negative organisms causing 58.5% of BSIs, Gram-positive organisms causing 35.4%, and fungi 6.1%. The most common pathogens were *S. aureus* (14.0%), CoNS (12.6%), *Klebsiella* spp. (12.0%), and *Acinetobacter spp*. (11.4%). The findings of the present study were also different from the study by Orsini et al.,[18] in the USA in 2010, where they found 59% Gram-positive bacteria, 31.1% Gram-negative organisms, and 9.8% fungi caused BSI. Among the Gram-positive isolates, the most common organism identified was CoNS (38.8%) followed by *S. aureus* (20.8). The most common Gram-negative bacteria isolated were *K. pneumoniae* (26.3%).

In this study, it was evolved that many isolates were multidrug resistant. *P. aeruginosa* showed high-level resistance to β-lactam (piperacillin-tazobactam) and to fluoroquinolone with resistance rate being 75% each.

Aminoglyoside was practically very useful with 75% sensitivity rate, whereas the only drug having high sensitivity was polymyxin B with 100% sensitivity against these isolates. All isolates of *P. aeruginosa* showed 75% resistance to carbapenem. The study conducted by Gupta[11] also showed antibiogram pattern of *P. aeruginosa*, sensitive to β-lactam (piperacillin-tazobactam) by 88.9% but sensitive to carbapenem by 88.9%. Isolates of *Acinetobacter* spp. which were responsible for CRBSI were resistant to amoxycillin-clavulanate, ciprofloxacin, chloramphenicol, and 3rd generation cephalosporins with resistance rate being 100% each. Even aminoglycosides, cotrimoxazole, and piperacillin-tazobactam also showed resistance with the sensitivity rates being 50%, 50%, and 0%, respectively.[11] The doxycycline and meropenem showed also 50% sensitivity against the isolates each, whereas the only drug having high sensitivity was polymyxin B with 100% sensitivity against these isolates. Gupta[11] study showed antibiogram pattern of *Acinetobacter* spp., 100% sensitive to carbapenem (meropenem). Bacteria causing BSIs and belonging to the family *Enterobacteriaceae* showed sensitivity to amoxycillin-clavulanate, piperacillin-tazobactam, aminoglycosides, 3rd generation cephalosporins, doxycycline, and to meropenem with resistance rate were 0%, 67%, 67%, 33%, 67%, and 67%, respectively. Similarly, quinolone group and phenicols were showed 67% resistance rate too.

Polymyxin B and monobactam were found with 100% sensitivity against these isolates. In the study done by Gupta[11] they found the sensitivity pattern to *Enterobacteriaceae* to Carbapenem (MRP) and to β lactam (piperacillin - tazobactam) were 100%. But their resistant to aminoglycoside was 50%.

All Gram-positive isolates were showed 100% sensitive to vancomycin and linezolid. Among the Gram-positive isolates, that is, MRSA and coagulase-negative *Staphylococcus* both were 100% resistant to cefoxitin.

In a study done by Marra et al.,[17] on 16 Brazilian hospitals showed that cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems were not active against >50% of the isolates of *Acinetobacte*r spp. tested. Relatively high proportions of *Klebsiella* spp. displayed resistance to ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, and cefepime (54.5%, 33.5%, 54.4%, and 50.2%, respectively). However, resistance to meropenem was seen in only 1.3% of the isolates which is much less than our finding. Methicillin resistance was detected in 43.7% of *S. aureus* isolates.

Gupta[11] showed in their study that patients with CRBSIs, *P. aeruginosa* isolates were sensitive to ceftazidime, amikacin, imipenem, meropenem, and piperacillin-tazobactam (88.9% for each antibiotic). A total of 4 (21%) isolates were multidrug resistant including one each of *Acinetobacter* spp. and *P. aeruginosa* and two of *E. coli*. One strain of *P. aeruginosa* was resistant to all the drugs used routinely. The antibiogram of organisms, responsible for CRBSI in our study, was near tallied with study of Gupta[11] and by Marra et al.[17]

Limitations of the study

The present study was only a single centered on a small cohort of patients. Moreover, only aerobic bacteriological profile and antibiotic susceptibility testing were performed, and their molecular identification and anaerobic bacteriological profile estimation could not be performed due to logistic constrain.

CONCLUSION

Patients with kidney disease receiving HD with a CVC experience high rates of BSI. A significant association found with increased age, dyslipidemia, diabetes, and increased chances of infection in this set-up. Most common organism isolated were Gram negative with few multidrug resistance strains too. It is important to know the possible pathogenic microorganisms and their sensitivity patterns for the treatment success of such cases. The most important strategy in the prevention of infection could be avoiding unnecessary placement of indwelling catheter, ensuring staff hygiene, and avoiding repeat catheterizations.

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