Original Article

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Microbiology of Non-Tunnelled Catheter-Related Infections

Microbiology Section

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ABSTRACT

Introduction: Aerobic bacterial infections often complicate vascular access in patients receiving haemodialysis, leading to Catheter-Related Blood Stream Infections (CRBSI). Various studies report Gram - positive bacteria, *Staphylococcus aureus* (*S. aureus*) in particular, as the most common aetiologic agent. Studies on microbiological analysis in this subset of population from India are very few.

Aim: To examine clinical and bacteriological profiles of haemodialysis patients developing CRBSI, the antibiotic susceptibility of the bacteria isolated from these patients and determine nasal carriage of *S. aureus* in the study subjects.

Materials and Methods: Using a prospective observational design 127 patients receiving haemodialysis (84 males; 43 females) from October 2011 to March 2013 were enrolled in this study. At each dialysis session, catheters were examined for any evidence of infection. In case of suspicion for infection, pus swab, blood culture and the catheter tips were sent to microbiology laboratory for site specific investigations. Vancomycin injection was empirically administered to these patients pending culture results. Data obtained was examined for relationship of CRBSI with clinical and socio-demographic risk factors.

Results: Out of 127 patients, 19 developed CRBSI, 10 developed exit-site infections and 33 patients were noted to have colonization of their catheters. The most common organisms included *S. aureus* in 24 (45.2%) catheter tips, followed by *Pseudomonas aeruginosa* in 9 (17%), *Acinetobacter* spp. in 5 (9%), *Enterobacter* spp. in 4 (7.5%) and *Klebsiella pneumoniae* in 3 (5.6%) catheter tips. Bacteraemia was found in 19 (20.7%) patients and *P. aeruginosa* was the most commonly isolated organism amongst them (38.8%). Staphylococcal nasal carriage was seen in 60 (69%) patients and 36 (41.4%) of these isolates were methicillin-resistant. Significant factors associated with CRBSI included history of bacteraemia, presence of diabetes mellitus, long duration (>15 days) of catheterization and antibiotic use within three months ($p<0.05$ for all).

Conclusion: Although *S. aureus* was the most common colonizer of non-tunnelled central access catheters among haemodialysis patients, CRBSI was most frequently caused by *P. aeruginosa,* which may have a bearing on our current antibiotic policy.

Keywords: Central venous catheters, Haemodialysis, Renal dialysis

INTRODUCTION

Patients undergoing maintenance haemodialysis are prone to a plethora of infections with greater frequency than the general population [1]. Approximately 55,000 patients have been estimated to receive haemodialysis every year and this population is reported to be growing at a rate of 10% to 15% per year. This is probably an underestimate due to lack of a national End Stage Kidney Disease (ESRD) registry. Chronic Kidney Disease (CKD) registry recently developed in India, states that nearly 48% of patients are in Stage 5 at the time of presentation. Such patients undergo dialysis usually through Central Venous Catheter (CVC) for vascular access [2].

CVCs are associated with a spectrum of infections that include exit site and tunnel infections, and bacteraemia. However, bacteraemia is clinically the most important because of its potential to progress to sepsis [3]. The incidence of catheter-related bloodstream infection (CRBSI) should be less than 10% at 3 months according to K/DOQI clinical practice guidelines for haemodialysis [4].

Non-tunnelled catheters are frequently used to initiate dialysis by nephrologists in India taking into account the high cost of the tunnelled ones. The latter are permanent catheters and are preferred only in a subset of patients [5]. Mostly skin derived organisms especially *Staphylococcus epidermidis* and *Staphylococcus aureus* have been implicated to be the aetiological agents of CRBSI [3]. However, few studies report Gram negatives, especially *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as the culprits [6-8].

Recent guidelines recommend empiric treatment of catheter-related bloodstream infection (CRBSI) with vancomycin in institutions with high prevalence of these infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) [9]. Also, the empiric coverage for Gram-negative bacilli should be based on local antimicrobial susceptibility data and the severity of disease. It is crucial for institutions to identify local patterns of microorganisms and their susceptibilities in order to appropriately inform choice of empiric antibiotics for these infections and to promote antibiotic stewardship.

To the best of our knowledge, bacteriological profile of CRBSI in haemodialysis patients and the antibiograms of the isolates are not well studied in South India. Most of the reported data are from Intensive Care Unit (ICU) studies that do not emphasize on haemodialysis patients [10]. Accordingly, we sought to examine the culture and sensitivity patterns observed in CRBSI, catheter colonization and staphylococcal nasal carriage in patients receiving haemodialysis using a non-tunnelled catheter in a tertiary care hospital in South India during 2011 to 2013.

MATERIALS AND METHODS

Study Design and Cohort

Using a prospective observational design,127 patients were enrolled, receiving haemodialysis, with CVC as their vascular access treated at a tertiary care hospital in South India over a period of 18 months from October 2011 to March 2013. The study

approval was obtained from the Ethics Committee of Manipal University. For inclusion criteria, patients had to be more than 18 years of age with end-stage renal disease receiving haemodialysis without definite vascular access Arteriovenous Fistula (AVF) or Arteriovenous Graft (AVG) not present or mature at the time of the study. Initially,130 patients were enrolled based on the inclusion criteria out of which three patients were excluded because of accidental breach in aseptic precautions during catheter insertion. So, a final sample of 127 patients was included in this study. For catheter insertion, non-tunnelled, double lumen catheters were used and adhered strictly to aseptic technique. The femoral route was used in 57% of the patients, while 38% of them had the catheter inserted into the internal jugular vein using the central approach. Subclavian catheters were utilized in the remaining 5% of patients. These temporary non-tunnelled catheters were used for haemodialysis till the AVF or AVG became functional (usually 2-3 months).

Data Collection

Demographic data and clinical variables including age, sex, duration of dialysis, immunosuppression and other treatment history for each patient were collected at the initial visit using a detailed questionnaire. An active enquiry was made into the suggestive signs and symptoms of CRBSI including the presence of fever, local discomfort or discharge from the exit-site at each patient visit for haemodialysis. We followed up each patient till the removal of the catheter for reasons including infection, catheter blockage due to intra luminal clotting or a kink and maturation of AVF. In case of any purulent discharge at the exit-site, pus swabs were taken and sent to the laboratory for further processing. In all cases of catheter removal the catheter was flushed with 10 ml of 0.9% saline, followed by skin preparation with tincture of iodine for antisepsis. Catheters were sectioned at the distal 5 centimeters with sterile forceps. These catheter tips were then sent to the laboratory for semi-quantitative analysis in a sterile container [11].

Laboratory Processing

Nasal swabs: Two sterile dry cotton wool swabs were used for each patient. These were inoculated onto sheep blood agar and phenol red mannitol salt agar followed by incubation at 35°C for upto 48 hours. Organisms with a yellow colour (mannitol fermenters) were identified as *Staphylococcus aureus* by standard methods, including Gram stain and tube coagulase test [11].

Pus swabs: These were placed onto blood agar and incubated aerobically for 48-72 hours. This was done in 14 patients who had signs suggestive of exit-site infection.

Catheter tips: The external surface of the catheter tip was rolled back and forth on the surface of a Columbia blood agar plate supplemented with 5% sheep blood at least 4 times and then the plate was incubated for 72 hours at 5% CO_2 and 35°C, after which the colony forming units were quantitated [11].

Blood cultures: These were done in 23 suspicious cases of CRBSI in whom the antibiotic therapy had not yet been initiated. Samples were incubated aerobically at 37°C for 7 days. They were subcultured on day 2, 4 and 7 onto blood and chocolate agar followed by incubation at 37°C. The grown organisms from culture of catheter tips, pus swabs and blood culture were speciated and typed using standard laboratory techniques [11]. All the isolates were subjected to antibiotic sensitivity by using laboratory protocols (by Kirby-Bauer disk diffusion assay) as recommended by the Clinical Laboratory Standards Institute [12].

The antibiotic disks were obtained from Himedia, Mumbai, India. For Gram- positives, antibiotics that were used for sensitivity testing included cefoxitin (30µg), cephalothin (30µg), clindamycin (2µg), rifampicin (5µg), teicoplanin (30µg), netilmycin (30µg), erythromycin (15µg), high level streptomycin (300µg) and high level gentamycin (120µg). The latter two were for *Enterococcus* spp.

The Gram–negatives were tested for sensitivity to ampicillin (10µg), cefotaxime (30µg), ceftazidime (30µg), gentamycin (10µg), amikacin (30µg), ofloxacin (5µg), imipenem (10µg), meropenem (10µg), cefoperazone-sulbactam (75/10µg), netilmycin (30µg), piperacillin (100µg), and piperacillin-tazobactam (100/10µg).

Screening for Methicillin-Resistant *Staphylococcus Aureus* (MRSA) was performed using a cefoxitin (30µg) disk on Mueller Hinton agar. Screening for extended spectrum beta-lactamases (ESBL) was done by double disk approximation or double disk synergy using cefotaxime (30µg), cefotaxime-clavulanic acid (30/10µg) and ceftazidime (30µg), ceftazidime-clavulanic acid (30/10µg) at a distance of 30 mm between the center of the two disks. American type culture collections were used as control strains [12].

Laboratory protocol for catheter cultures: Semiquantitative culture method described by Dennis G Maki et al., 1977, was followed [13].

Definitions and Interpretations

The agar plates were examined at 24 hours, 48 hours and 72 hours (in case of blood culture). Significant growth on catheter tip culture was defined as ≥ 15 colony forming units (CFU) by Maki's roll plate method in case of CRBSI and ≤ 15 colonies per plate reflected catheter colonization [13].

Multidrug resistance was defined as resistance to at least three of the four following groups: (1) imipenem or meropenem; (2) ceftazidime; (3) piperacillin or piperacillin–tazobactam and (4) ofloxacin [14].

The CDC definitions for infections with or without bacteraemia were used. Catheter exit-site infection was defined as a positive semi-quantitative culture of the purulent drainage material obtained from the exit site accompanied with redness and crusting. In the absence of clinical signs of infection at the catheter exit site, <15 CFU (colony forming units) on semi-quantitative cultures (roll-plate technique), catheter colonization was considered.

Confirmed catheter-related bacteraemia was defined as the isolation of the same organism from a quantitative culture of the distal segment of the catheter and from the blood of a patient with clinical symptoms of sepsis in the absence of any other noticeable source of infection [15].

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 16.0 for Windows (IBM Corporation, New York, USA). Categorical variables were reported as number (percentages) and continuous variables were reported as mean (SD). Chi-square test was used for univariate analysis for factors in relation to CRBSI. Statistical significance was determined at a 5% level of significance.

RESULTS

Our study included a total of 127 patients receiving haemodialysis using CVC as their vascular access. Among these, 19 patients developed CRBSI, 10 developed exit-site infections and 33 patients were noted to have colonization of their catheters. In the latter subset, the patients had signs and symptoms suggestive of CRBSI.

The study population comprised of 84 males and 43 females. The mean age (range: 18-77 years) of the study population was 53 ± 14 years. Hypertension was the most common co-morbid condition (72.4%). Diabetes mellitus came second (52%). Factors associated with CRBSI have been shown in detail in [Table/Fig-1].

CRBSI

Out of a total of 127 patients, laboratory confirmed CRBSI was documented in 19 patients (15%). That is in all these cases, catheter tip and blood cultures revealed the same bacterium

along with their similar antibiograms. Gram-negative organisms constituted 79% of the pathogens whereas 21% of them were Gram- positive. The most common organism causing CRBSI was *P. aeruginosa* (36.8%). *S. aureus* accounted for 21.1% of the isolates. The organisms implicated in CRBSI are shown in [Table/Fig-2].

Variable	Total Patients (n =)	CRBSI	CRBSI (%)	p value
Age				
< 60 years >60 years	91 36	10 9	10.98 25.00	0.701
Gender				
Men Women	84 43	13 6	15.47 13.95	0.901
Duration of catheterization				
< 15 days >15 days	63 64	4 15	6.34 23.44	<0.001
History of diabetes mellitus	127	19	63.15	0.001
Nasal carriage of *Staphylococcus aureus*				
Yes No	85 42	11 8	0.13 0.19	0.812

[Table/Fig-1]: Univariate analysis of factors related to bacteraemia in CRBSI group.

Organism name	No. of isolates (Count and %)
Staphylococcus aureus	4 (21%)
Pseudomonas aeruginosa	9 (47.4%)
Acinetobacter spp.	2 (10.5%)
Stenotrophomonas maltophilia	1 (5.3%)
Escherichia coli	2 (10.5%)
Burkholderia spp	1 (5.3%)
Total	19 (100.0%)

[Table/Fig 2]: Number and percentage of organisms isolated from blood culture at peripheral vein in cases of CRBSI (organisms implicated in case of CRBSI).

In patients with CRBSIs, all the *S. aureus* isolates were 100% sensitive to cephalothin, netilmycin, rifampicin and teicoplanin, while the *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. Only 1 (25%) out of four of the total *S. aureus* isolates was methicillin-resistant and 26.3% of the Gram-negative bacterial isolates was Extended Spectrum Beta Lactamase (ESBL) producing organisms. Detailed antibiograms of these isolates are shown in [Table/Fig-3].

Exit Site Infections

Out of a total of 10 culture positive cases of exit site infections, *S. aureus* was isolated in 4 (40%) cases, *E. faecalis* and *Enterobacter* spp in one each (10%), *Citrobacter* spp and *P. aeruginosa* in two (20%) each.

All of the four *S. aureus* isolates from the wound swab were sensitive to methicillin, cephalothin, netilmycin, clindamycin, rifampicin, and teicoplanin. There was one isolate of *E. faecalis* which was pandrug resistant. The one *Enterobacter* spp isolate was sensitive only to cefaperazone-sulbactam and ofloxacin. One of the two isolates of *P. aeruginosa* was sensitive to imipenem, meropenem, and piperacillin-tazobactam. No ESBL isolate was obtained from pus swabs.

Catheter Colonization

Out of the total 127 patients, catheter colonization was seen in 33 (23.6%) cases. *S. aureus* was the most common organism to colonize catheters at 66.6% (22 isolates) followed by *Acinetobacter* spp at nine percent (3 isolates). *S. aureus* was found along with Gram-negative bacilli in five catheters. Out of these, three catheters were colonized by *S. aureus* and *Enterobacter* spp; one (0.9%) each catheter grew *S. aureus* and *Citrobacter* spp and *S. aureus* and *Klebsiella pneumoniae*. The sensitivity pattern of these isolates is not being reported as it is clinically insignificant. The bacteria implicated in catheter colonization are shown in [Table/Fig-4].

Bacteria	Name of organism	Number of isolates	Percentage (%)
Gram - positive	*Staphylococcus aureus*	22	66.6
	Staphylococcus epidermidis	1	3
Gram - negative	*Enterobacter* spp	2	6
	Acinetobacter spp	3	9
	Citrobacter spp	1	3
	Klebsiella pneumoniae	2	6
	Pseudomonas aeruginosa	2	6
Total		33	100

[Table/Fig-4]: Bacteria isolated from infected catheter tips (in catheter colonization cases)

Nasal Carriage of *S. aureus*

S. aureus from the anterior nares was detected in 85 (67%) patients of 127 study subjects; 52 (61.2%) isolates were methicillin resistant.

DISCUSSION

Our study is one of the first describing clinical and microbiological profiles of CRBSI in patients receiving haemodialysis at a South-Indian tertiary care hospital. We noted several findings relevant to

Gram positives	Meth	Ch	E	Cd	Rif	Tei	Net					
Staphylococcus aureus (4)	1 (25%)	4 (100%)	2 (50%)	2 (50%)	4(100%)	4(100%)	4 (80%)					

Gram negatives	A	CT	CA	G	Ak	OF	IMP	MRP	CFS	Net	Pc	PIT
Pseudomonas aeruginosa (9)	R	R	6(66.7%)	4(44.4%)	8(88.9%)	3 (33.3%)	8 (88.9%)	8 (88.9%)	1 (11.1%)	1 (11.1%)	R	8 (88.9%)
Acinetobacter spp.(2)	1(50%)	R	R	R	1(50%)	NA	1(100%)	2(100%)	1(50%)	R	NA	NA
Stenotrophomonas maltophilia (1)	1(100%)	R	1(100%)	1(100%)	1(100%)	1 (100%)	1(100%)	1(50%)	R	1(100%)	R	R
Burkholderia spp. (1)	1(100%)	1(100%)	1(100%)	R	R	NA	1(100%)	1(100%)	1(100%)	NA	1(100%)	1(100%)
Escherichia coli (2)	1(100%)	R	R	R	1(50%)	R	2(100%)	2(100%)	2(100%)	2(100%)	NA	2(100%)

[Table/Fig-3]: Sensitivity profiles of CRBSI isolates
R = Resistant; NA = Not tested; Meth = Methicillin, Ch = cephalothin, E=erythromycin, Cd = clindamycin, Rif = rifampicin, Tei = teicoplanin, Net = netilmicin, A = ampicillin, CT = cefotaxime, CA = ceftazidime, G = gentamicin, Ak = amikacin, OF = ofloxacin, IMP = imipenem, MRP = meropenem, CFS = cefoperazone-sulbactam, Pc = piperacillin, PIT = piperacillin-tazobactam

management of CRBSI in these patients. Firstly, longer duration (>15 days) of haemodialysis and presence of diabetes mellitus were significantly associated with the risk of CRBSI while age, sex and nasal carriage of *S. aureus* were not found to be significant predictors. Interestingly, Gram-negative bacilli (*P. aeruginosa*) outnumbered Gram-positive cocci (*S. aureus*) in causation of CRBSI. Additionally, *S. aureus* was found to be the most common catheter colonizer in our study.

The duration of catheterization is an important factor that determines the risk of catheter-related infections [16]. Our study confirmed the fact that catheterization for more than 15 days was significantly associated with the risk of infection ($p< 0.05$). In general, internal jugular and subclavian vein catheters are suitable for two to three weeks of use although longer periods have been reported [17]. Femoral catheters are generally limited to a single dialysis session in ambulatory patients, and three to seven days in bed-bound patients [18]. After two weeks, the rate of infection rises in both the femoral and internal jugular positions [19]. The mean duration of haemodialysis with the non-cuffed, double-lumen catheter in our study was 16 days, which is in accordance with the National Kidney Foundation- Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) vascular access guidelines [4]. Moreover, we also noted the presence of diabetes to increase the risk of developing CRBSI, in accordance with prior studies [20]. However, in contrast with previous investigators, we did not identify nasal carriage of *S. aureus* to be significantly associated with the risk of developing CRBSI ($p = 0.81$) [21-23]. We cultured the nasal swab only at initial visit and did not repeat it serially at every visit, which may account for this discordance. Regardless, the nasal carriage rate of 67% of *S. aureus* is quite alarming.

Regarding the spectrum of bacteria implicated in CRBSI infections, our study shows that most (79%) of the episodes were caused by Gram-negative organisms as compared with 21% of those caused by Gram-positive organisms. Although a recent study from Singapore describing CRBSI associated with tunnelled catheters reported *P. aeruginosa* to be the most common organism [6]. Our findings differ from a vast body of literature where in Gram-positive organisms are implicated for majority of the dialysis catheter-related infections [24-26]. Some other recent studies also noted a significant proportion of Gram-negative bacterial cultures, but *Enterobacter spp, E. coli*, and *Klebsiella spp* were more frequently isolated [27,28]. More Indian studies are needed to determine whether the bacteriological profile of non-tunnelled catheters, that are still widely used here different from that of tunnelled and cuffed catheters that are used in the Western world and also to determine the source of the Gram-negative bacteria.

The rate of exit site infections in our study was 7.8%, similar to one reported from Australia (7.6%) that also documented a significant relationship between diabetes mellitus and exit site infections [29]. The colonization incidence of CVC's varies widely in published studies.

The usual incidence of colonization is stated as being between 15 and 40%, similar to findings from our centre (23.6%) [30]. Haemodialysis patients have been reported to have a high rate of colonization (50-60%) with *S. aureus,* and this is reflected in the disproportionate numbers of *S. aureus* associated catheter colonization (22 out of 30) and exit site infections (40%) in our study [26,29,31].

Potential sources of bacterial colonization of non-cuffed, non-tunnelled catheters include the skin around the catheter insertion site, the catheter hub and contaminated infusate [29].

LIMITATION

Firstly, not every vascular catheter inserted during the study period was sampled; however every effort was made to maximally recruit patients attending our haemodialysis unit into the study. Second, blood cultures were performed only in cases of suspected CRBSI, so we could have missed subclinical bacteraemic episodes. However, it is highly unlikely that a patient with bacteraemia does not develop symptoms. Third, we did not determine vancomycin resistance in case of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates as only one isolate from CRBSI was methicillin-resistant.

CONCLUSION

This is one of the first few reports clearly illustrating an increased role of Gram-negative bacilli, *Pseudomonas aeruginosa* in particular, in causation of CRBSI. We speculate that a possible cause of the increased incidence of Gram-negative infections may be the use of empiric gram-positive antibiotic coverage with vancomycin in our institute without appropriate gram-negative therapy. Because 37% of all isolates were *P. aeruginosa* in our study, it would be prudent to include an anti-pseudomonal antibiotic for empiric management of patients in our institute. Any of the commonly prescribed antibiotics (ceftazidime, piperacillin/tazobactam, or a carbapenem) may be prescribed. Future studies for identifying more risk factors for gram-negative CRBSI in our patients would be important to improve the selection of empiric antibiotics. Also, for quality improvement and antibiotic stewardship, all institutions should determine their microbiological profiles of CRBSI and antibiograms of the isolates to positively affect patient outcomes.

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