Original Research Article

PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BACTERIAL ISOLATES AND RISK FACTORS OF ACCESS RELATED INFECTION AMONG HEMODIALYSIS PATIENTS AT BENJAMIN MKAPA HOSPITAL

ABSTRACT

Background: Patients suffering from chronic kidney diseases (CKD) on dialysis are at risk of dying mainly due to cardiovascular complication or infections. Infections are the second leading cause of death and hospitalization among hemodialysis (HD) patients. Blood stream infection is the main source of infection through the vascular access. Factors attributed to this are mainly patient characteristics and principle of Infection Prevention and Control (IPC) of the Hospital or unit. The risk of bacteremia in hemodialysis patients is 26-fold higher than in the general population, and ~~gram positive~~gram-positive bacteria are the causative organisms. The most common site of infection causing bacteremia is internal prostheses. Infection control principle is recommended by the Center for Disease Control and Prevention (CDC) in reducing bacteremia in hemodialysis patients with either a Central Venous catheter (~~CVC) or~~CVC) or Arterio venous Fistula (AVF). ~~Objectives:To~~**Objectives:** To determine prevalence, Antimicrobial Susceptibility Testing Pattern sand risk factors of Access related infection among hemodialysis patients at Benjamin Mkapa Hospital (BMH). Methodology: Across-sectional prospective study conducted for the period of six (6) months. Informed consent was sought from all participants who met the criteria. A swab from the site of vascular access site and venous blood sample was collected. The data was entered in the SPSS for analysis. **Results:** We studied 35 individuals who were on maintenance ~~haemodialysis~~hemodialysis services at our ~~haemodilaysis~~hemodialysis unit of which 57% were male. The majority of participants (40%) were aged above 60 years. The prevalence of vascular access ~~bacterial infection~~bacterial infection was 28.6%. Most patients with swab and blood culture infections were those on CVC by 87.5% and 90% on swab and

blood cultures respectively. Staphylococcus aureus was 87% from the swab culture and 80% from the blood culture. The sensitivity tests showed that staphylococcus aureus was sensitive to all antibiotics but more sensitive to Ceftriaxone and vancomycin by 85% in the swab culture and 87.5% by 75% in the blood culture for ceftriaxone and vancomycin respectively. Metronidazole and azithromycin sensitivity was 57% and 71% in the swab culture while in the blood culture was 50% and 71% respectively. Staphylococcus aureus was less sensitive both in the swab (28%) and blood culture (37.5%). Escherichia coli (*E.coliE. coli*) was very sensitive to ceftriaxone meropenum (100%) and less sensitive (100%) to azithromycin and metronidazole. **Conclusion:** Gram positive cocci (*Staphylococcus aureus*) were the most identified bacteria in patients on haemodialysishemodialysis from both swab and blood culture and indeed the source of infection in the blood is from the site infection due to contamination or improper care of the site especially those with CVC. So having CVC as the vascular access for haemodialysishemodialysis bearbears a high risk of acquiring infection. *Staphylococcus aureus* was found to be highly sensitive to ceftriaxone and vancomycin and less sensitive to meropenum. **Recommendation:** Improve IPC practice in haemodialysis unit and health education about access care will reduce access the risk of infection in haemodialysis patients.

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INTRODUCTION

The prevalence of CKD is increasing globally both in developed and developing countries. In Tanzania, there is still paused of the magnitude of CKD but of the few studies conducted in the community showed the prevalence of 7-12.9% in Northern Tanzania and Kisarawe in the cost region respectively. [1, 2] Subsequently CKD will progress to end stage renal disease (ESRD) and ultimately require renal replacement therapy in form of ~~haemodialysis~~hemodialysis or peritoneal therapy. It has been reported that Hemodialysis patients have increased risk of infections [3, 4] and CKD itself is a risk for bacterial infection. Patients with CKD experience a varying level of ureamia complications and for immunity it impairs the function   through interferes of T-cell and B-cell function, macrophage phagocytosis, and antigen presentation as well as chronic activation of the immune system. [4, 5]

 In CKD patients on ~~haemodialysis~~hemodialysis therapy, infection is the second leading cause of death after cardiovascular complications [6, 7, 8] contributing to 15% of all deaths in CKD patients. (9) There is high prevalence of access related bacterial infections [10] that varies from 29.8% to 60.3%. (6, 11) Both gram positive and gram negative bacterial have been isolated but ~~gram positive~~gram-positive bacteria are the commonest identified. [8,12, 4] Commonly the infections ~~is~~are blood stream bacterial infections originating from vascular access. [3,9] Other sources are blood borne pathogens, respiratory infection, urinary tract infection and less likely to originate from the oral cavity. [3] For vascular access related bacterial infection, CVC is the most common site of infection compared to arteriovenous fistula (AVF). [3,6,7,13,14] Of these infections the most isolated microorganisms are staphylococcus aureus, staphylococcus epidermidis, ~~serratia~~Serratia marcescens, pseudomonas aeruginosa and Enterococcus faecalis. [3,6, 8,10] Other studies isolated coagulase negative staphylococcus more (54.6%) over staphylococcus aureus (18.2%) and the other microorganisms were Klebsiella pneumoniae and Enterobacteriaceae (15.2%, each). [13] Staphylococcus bacteria is reported to be sensitive to vancomycin ~~by 77~~by 77%. [15]

As it has been shown that bacterial infection in ~~haemodialysis~~hemodialysis patients is ~~common~~common, but no study has been conducted at our setting on the prevalence, predisposing ~~factors~~factors, and microbial pattern for ~~haemodialysis~~hemodialysis patients. The aim of this study was to determine prevalence, Antimicrobial Susceptibility Testing Patterns and risk factors of Access related infection among hemodialysis patients at BMH. The findings ~~of study~~of study has shade light on the prevalence, predisposing factors and aetiology of haemodialysis infection as well as antimicrobial susceptibility

pattern. These results will assist to establish best IPC ~~practices in~~practices in hemodialysis unit and ensure availability of antimicrobial drugs based on sensitivity pattern at the Hospital.

METHODOLOGY

Study area

The study was conducted at Benjamin Mkapa Hospital in the ~~Haemodialysis~~hemodialysis unit. The study involved swab and blood samples that were collected from dialysis patients. The hospital large, ~~compelex~~complex composed of several departments including Laboratory, Pharmacy, Radiology and imaging, wards (medical, surgical, urology, ~~paedriatrics~~pediatrics, Obstetrics & gynecology, VIP/ private), theaters, and internal medicine, General Surgery, Urology, Pediatric and child healthcare, Obstetrics & gynecology, Ophthalmology, ENT (Ear, Nose & Throat), Physiotherapy, Nephrology (Haemodialysis and Kidney transplant), Cardiology (including Cath lab), Orthopaedics, Gastroenterology, Oral health, Oncology, Haematology, CTC clinic, ICU and Emergency medicine (including trauma unit).

Study design and population

A cross-sectional prospective study was conducted in a period of 6 months for 35 patients who were on ~~Haemodialysis~~hemodialysis therapy for more than three months who voluntary agreed to participate and signed a consent form.

Sampling techniques

The patients who were undergoing ~~haemodialysis~~hemodialysis therapy ~~were~~was consecutively entered in the study after consenting. Patients demographic and clinical biodata were entered into the structured questionnaire. Both pus swab and blood samples were collected from each participant for microbiological testing at Benjamin Mkapa Hospital Laboratory.

Sample collection

The pus swab samples from the surface of the entrance point of CVC accesses and the injection site for AVF were collected using sterile swab stick then put into amies transport media. A 5ml~~s~~ of patient blood was collected using sterile syringe into medium broth (Brain Heart Infusion Broth Medium). All samples were transported to the laboratory for further processing in Microbiology section. Each sample were assigned serial number and patient hospital identity.

Isolation and identification bacteria

Blood samples in the broth medium were incubated for 24 hours at 37°C following daily check if turns positive by appearence of turbid in the broth. After three days the blood sample with or without turbidity were inoculated using sterile wire loop onto Blood agar, Chocolate agar and MacConkey agar at 37 ^{0}C anaerobic incubation for 24 hours. As for blood sample, a swab samples were inoculatedusing sterile wire

loop onto Blood agar, Chocolate agar and MacConkey agar at 37 ^{0}C anaerobic incubation for 24 hours. Among the plates which grew a single to three similar colonies were picked up to prepare smear for gram stain ~~in order to~~to identify two major groups of bacteria namely gram positive and gram negative. Thereafter, gram positive bacteria were identified using biochemical tests called catalase test, coagulase test, novobiocin, optochin and bacitracin disks and ~~gram negative~~gram-negative bacteria were identified using Kligler Iron Agar (KIA), Sulfur Indole Motility (SIM), citrate, urea, Lysine Iron Agar (LIA)and oxidase test. We performed catalase and coagulase tests to identify staphylococcus aureus and KIA, SIM, citrate, urea, LIA and oxidase tests for ~~E.coli~~E. coli bacteria. After bacterial identification, the antimicrobial susceptibility testing was performed.

Antimicrobial Susceptibility Testing (AST)

A two to three colonies of confirmed isolates were picked a sterile and immersed into a bottle of sterile normal saline. The turbidity of these bottles was compared with 0.1 McFarland Equivalent standards to get the desired number microorganism prior to spreading onto Muller Hinton agar (MH). Using a sterile swab, diluted isolates were spread onto MH agar and disks of Azithromycin (30µg), Vancomycin (20µg), Ceftriaxone (30µg), Meropenum (10µg) and Metronidazole (30ug) were placed on top of inoculums by Kirby Bauer disc diffusion method to determine the drug susceptibility patterns. Zone diameter of inhibition was measured using a millimeter scale around each antimicrobial disk on the under surface of the plate in a period of 18-24 hours of incubation at 37 °C. The zone size around each antimicrobial disk was interpreted as sensitive, ~~intermediate~~intermediate, or resistant. [16,17]

Quality Control

A reference strain of gram-negative bacteria *~~E.coli~~E. coli* ATCC 25922 and gram-positive bacteria *Staphylococcus aureus* ATCC 25923 were used for quality control of Microbiological procedures using existing Standard Operating Procedures of BMH and Clinical Laboratory Standard Institute guideline. [16, 18]

Data analysis

A statistical package for social science (SPSS) version 17: software was used to analysis the results into mean and proportions.

RESULTS

Demographic characteristics and bacterial growth

In the study of 35 ~~haemodialysis~~hemodialysis patients 20/35 (57%) were male and 15/35 (43%) were female. The majority of participants 14/35 (40%) were patients of age above 60 years old and few 2/35 (6%) of age group below 18 years old. Out of 35 patients,24 (69%) were not in the formal employment, 17 (49%) had secondary level of education. Bacteria were isolated in 28.6% (10/35) of patients, and 70% of

participants with bacterial isolates were male patients. The age group which isolates was high 5/10 (50%) was in the age above 60 years. No bacteria isolated in patients who have no education (Table 1).

Table 1, Demographic Characteristic and bacterial growth among patients

Description	Bacterial Growth	No Bacterial Growth	Total [%]
Sex/Gender			
Male	7	13	20 (57)
Female	3	12	15 (43)
Age			
Below 18	1	1	2 (6)
18-45	1	8	9 (26)
46-60	3	7	10 (29)
Above 60	5	9	14 (40)
Education Level			
No Education	0	2	2 (6)
Primary	2	2	4 (11)
Secondary	3	14	17 (49)
College	5	7	12 (34)
Occupation			
Employed	5	6	11 (31)
Not in the formal Employment	5	19	24 (69)

Demographic characteristics, bacterial isolates and type of access

About 85.7% (30/35) of patients were using CVC access at the time of study. Of those on CVC 60% (18/35) were male patients and 70% were unemployed. Patients on AVF 60% were in the age of 18-45 years. Microorganism was isolated more 70% in male patients, 50% age above 60 years, and 50% patients with college education. (Table 2).

Table 2. Demographic characteristics against Bacterial growth and type of vascular access

Demographic features	Bacterial growth		Type of vascular access	
	BG	NBG	AVF	CVC
Sex				
Male	7 (70%)	13(52%)	2 (40%)	18 (60%)
Female	3(30%)	12(48%)	3(60%)	12(40%)
Age				
Below 18	1(10%)	1(4%)	0(0%)	2(7%)
18-45	1(10%)	8(32%)	3(60%)	6(20%)
46-60	3(30%)	7(28%)	1(20%)	9(30%)
Above 60	5(50%)	9(36%)	1(20%)	13(43%)
Education Level				
No Education	0(0%)	2(8%)	0(0%)	2(7%)
Primary	2(20%)	2(8%)	1(20%)	3(10%)
Secondary	3(30%)	14(56%)	2(40%)	15(50%)
College	5(50%)	7(28%)	2(40%)	10(33%)
Occupation				
Employed	5(50%)	6(24%)	2(40%)	9(30%)
Not in the formal employment	5(50%)	19(76%)	3(60%)	21(70%)

BG-Bacterial growth, **NBG**- No bacterial growth

Vascular access and bacterial infection

Of the 35 individuals in the study, bacterial isolates in swab culture was 22.9% while that blood culture was 28.6%. Isolates from CVC were 23.3% and 30% on the swab and blood swab cultures respectively while on AVF 20% for both swab and blood cultures. Staphylococcus aureus was the predominant 87.5%

and 80% isolate in swab and blood cultures respectively. *Staphylococcus aureus* was most isolated 85.7% in swab and 87.5% in blood culture for CVC as compared for those with AVF 14.3% and 12.5% for swab and blood respectively. There were no isolates of E. coli both in swab and blood culture for AVF patients. Table No 3

Table 3. Vascular access and bacterial infection

ACCESS TYPE	Microbes isolated from swab specimen			Microbes isolated from Blood specimen		
	NBG (%)	*Staphylococcus aureus*	~~E.coli~~*E. coli* (%)	NBG (%)	*Staphylococcus aureus (%)*	~~E.coli~~*E. coli* (%)
AVF	4 (14.8)	1 (14.3)	0 (0.0)	4 (16.0)	1 (12.5)	0 (0.0)
CVC	23 (85.2)	6 (85.7)	1 (100.0)	21 (84.0)	7 (87.5)	2 (100.0)
TOTAL	27(77)	7 (20.0)	1 (2.8)	25 (71.0)	8 (22.9)	2 (5.7)

AVF-Arteriovenous fistula, **CVC**-Central venous catheter, **NBG**-No bacterial growth

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

Staphylococcus aureus was found to be sensitive to all antibiotics but was highly sensitive to Ceftriaxone and vancomycin by 85% in the swab culture while in the blood culture only ceftriaxone by 87.5%. but highly resistant to Meropenum by 72% and 62.5% in swab and blood culture respectively. ~~E.coli~~E. coli was highly sensitive to ceftriaxone and meropenum in both swab and blood culture by 100% and highly resistant to vancomycin, azithromycin and metronidazole by 100% in both swab and blood culture. Table No 4

Table 4. ~~Antimicrobial Susceptibility~~Antimicrobial Susceptibility patterns of bacterial isolates

Antimicrobials	AST results for isolate from swab specimens				AST results for isolates from Blood specimens			
	Staphylococcus aureus		*E.coli*		*Staphylococcus aureus*		*E.coli*	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ceftriaxone	6(85)	1(15)	1(100)	0	7(87.5)	1(12.5)	2(100)	0 (0)
Vancomycin	6(85)	1(15)	0 (0)	1(100)	6(75)	2(25)	0 (0)	2(100)

Meropenum	2(28)	5(72)	1(100)	0 (0)	3(37.5)	5(62.5)	2(100)	0 (0)
Azithromycin	5(71)	2(29)	0 (0)	1(100)	6(71)	2(29)	0 (0)	2(100)
Metronidazole	4(57)	3(43)	0 (0)	1(100)	4(50)	4(50)	0 (0)	2(100)

AST- Antimicrobial Susceptibility Testing, **S**-Sensitive, **R**-Resistant

DISCUSSION

In many Sub--Saharan African countries, haemodialysis therapy is the main dialysis option compared to peritoneal dialysis therapy. At BMH temporary central venous catheter is the most commonly used access and rarely permanent central venous catheter. This is because haemodialysis therapy is initiated as an emergency treatment since majority of patients reach to the Hospital in critical situation. Very few individuals have had AVF constructed before initiation of haemodialysis therapy. Haemodialysis access related bacterial infection is very common in patients on haemodialysis. [3, 4, 5] In our study the prevalence of vascular access related bacterial infection was 28.6%. The existence of bacteria infection in haemodialysis patients inpatients in our setting is similar to findings of 29.8% and 60.3% reported in other studies. [7, 10]

We assessed access related bacterial infection in 35 patients with chronic kidney disease on haemodialysis therapy for more than 3 months and found that 85.7% of patients were using CVC vascular access. This type of access (CVC) is commonly used in our setting because the patients present with uraemia in need of urgent renal replacement therapy at the time of diagnosis therefore CVC become the only option for initiation of haemodialysis therapy. Although patients with CKD have impaired immune defense mechanism due to uraemia, vascular access increases the risk of vascular access related bacterial infection. In our study we found that the vascular access central venous catheter had a high risk of vascular access related bacterial infection of 90%compared to 10% of AVF. This finding is in line with other studies which reported high blood stream infection for patients on CVC compared to AVF. [3, 8, 14]

In our study the predominant bacteria identified in both swab and blood culture was *Staphylococcus aureus* (gram positive cocci) by 80% and 87% respectively. and *E.coli* (gram negative anaerobic microorganism) was found in 20% of the patients both in swab and blood culture. The gram positive cocci (*Staphylococcus*) being the most microorganism isolated in this study is in line with other studies of seebastiano et al, 2010 and Palumbo et at, 2013 which reported staphylococcus aureus and enterococcus species were among the most microorganisms identified in their studies [4, 6] where as in the study by Abdulrahman et al 2018 reported staphylococcus is the most common cause of bacterial infection in haemodialysis by 77% and staphylococcus epidermidis was the most common among the staphylococcus. [19]

Staphylococcus aureus was found to be highly sensitive to ceftriaxone (85%) and vancomycin (85%) in swab culture and 87.5% by 75% respectively in blood culture for our study. This study is also revealed results of previous studies of which vancomycin was highly sensitive more than 77than 77% in gram

positive bacteria. [16] Ceftriaxone is still effective more than 85% in our study and 90 % in a study by Bushra et al, 2016 [20] but more surveillance is needed because the drug is widely used. The in vitro study reported that, overall clinical response ~~of Meropenum~~of Meropenum against ~~nonfastidious~~non-fastidious pathothogens was 93% including staphylococcus aureus (92%) [21] but in our study the resistant rate of meropenum was 72% in swab culture and 62.5% in blood culture may be due to small sample size used so more to be done to establish the microbial susceptibility pattern of meropenum at our setting .

CONCLUSION

At BMH and globally, staphylococcus aureus and other gram negative bacteria are the common source of access related bacterial infection in haemodilaysis patients especially those using CVC. Currently, *Staphylococcus aureus* is high sensitive to vancomycin and less sensitive to meropenum at Benjamin Mkapa Hospital.

LIMITATION OF THE STUDY

This study was performed on small sample size of 35 ~~patients on~~patients on haemodialysis therapy due to availability of individuals who were on haemodialysis at that time. The study did not perform molecular characterization of bacterial isolates and gene sequencing on the drug resistance.

RECOMMENDATION

Improve IPC practice in haemodialysis unit and health education about access care will reduce access infection in haemodialysis patients. Perform regular bacterial surveillance to identify type of bacterial over the type of access the patient is using and treatment of infection as per antibiogram. Patients with CKD and those on chronic haemodialysis services should have early AVF construction for better haemodialysis therapy and lesser infection.

Dissemination and Publication

The findings of this study has been presented at BMH continues medical education, local and International scientific conferences.

NK;HNKLNKLK Ethical clearance

Ethical clearance was granted by the Central Zone Health Research Ethic Review ~~Committee (~~Committee (CZHREC) and Benjamin Mkapa Hospital authority allowed to conduct the study in Dialysis of BMH. The study was complying with the principals of Helsinki for Good laboratory practices that Confidentiality to be kept for all information gathered from study

The Central Zone Health Research Ethics Review Committee (CZHREC) approved and granted certificate with Ref No.002/2020 (Appendix I). BMH authority granted permission to conduct this study at Benjamin Mkapa Hospital. The study was complying with the principals of Helsinki for Good laboratory practices that Confidentiality to be kept for all information gathered from study

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