Original Research Article

Bacterial Profile and Antibiogram of Pathogens Causing Surgical Site Infections at Mnazi Mmoja Hospital in Zanzibar, Tanzania

ABSTRACT

Aims: To determine bacterial profile and antibiogram of pathogens causing surgical site infections at Mnazi Mmoja Hospital
Study design: This was a hospital based cross sectional study
Place and Duration of Study: Mnazi Mmoja Hospital in Zanzibar, Tanzania, from May to July 2017.
Methodology: We enrolled a total of 121 patients who underwent different surgical procedures and met study inclusion criteria. Pus swabs were collected and cultured as per standard operative procedures. Bacterial identification was then performed using biochemical tests and Analytical Profile Index test for Enterobacteriaceae (API 20E). Antimicrobial drug susceptibility testing was performed using Kirby Bauer disc diffusion technique. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21.0.
Results: Of 121 patients that were enrolled in the study, 75 (62%) developed SSI. A total of 106 isolates were recovered from patients with positive culture results. Majority of the isolates, 92/106 (87%) were Gram-negative bacteria. The most predominant bacteria was *Pseudomonas aeruginosa,* 29/106 (27%) in all surgical Departments. Some of the isolated pathogens were multi-drug resistant (29/106; 27%), including Extended Spectrum Beta Lactamase (ESBL) producers (24/92; 26%), Methicilin Resistant *Stahylococcus aureus* (MRSA) (3/9; 33%) and Induced Clindamycin Resistance (ICR) strains (2/14; 14%). *K. pneumoniae* isolates showed the highest resistance towards most tested antibiotics (24.1-100%), and 24% of *P. aureginosa* isolates were resistant to meropenem.
Conclusion: *P.aureginosa* was the most common isolated pathogen from patients with SSI in Zanzibar. The majority of pathogens showed high resistance to commonly prescribed antimicrobial agents used in the hospital settings.

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*Keywords: Surgical site infection***,** *Bacterial isolates, Antibiogram, multi-drug resistance, Zanzibar*

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1. INTRODUCTION

Surgical site infections (SSIs) are among common healthcare associated infections (HAIs) that have far reaching implications on patient morbidity and mortality as well as significant financial implications [1]. SSI can give rise to local signs and symptoms at wound site including heat, redness, pain and swelling and in more serious cases with systemic signs of fever or raised white blood cell count (2 ,3). SSIs are the second leading cause of all nosocomial infections worldwide accounting for 20% to 25% (4,5). Globally, SSI rate is estimated to range from 2.3% to 41.9% (6,7). Published data from Tanzania Mainland indicated that there is an increased trend of SSI from 26% in 2011 to 35.6% in 2012 [8–10].

The aetiology of SSI is known to be diverse; with *P. aeruginosa* reported to be the most common causative agent [11,12]. Previous studies that were conducted in Tanzania Mainland have reported prevalence and causative agents of SSI with varying antimicrobial susceptibility patterns [9,13]. In the recent years, the increased trend of multi drug resistance (MDR) strains is becoming worrisome, which might be attributed by the misuse and mismanagement of antibiotics, limiting the treatment options [13–15]. A previous study done at Muhimbili National Hospital, Tanzania reported the prevalence of 63% of MDR pathogens causing SSI [16]. To date, there is scarcity of data regarding the magnitude and causative agents of SSI in Zanzibar, which might have a contribution in challenges facing the development of evidence-based interventions for treatment, control and prevention of SSIs in the area. The study was therefore conducted to provide data on magnitude, causative agents and their antibiogram among patients with SSI at Mnazi Mmoja Hospital in Zanzibar, Tanzania.

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2. MATERIAL AND METHODS

2.1 Study design and settings

This was a descriptive hospital-based cross-sectional study that was conducted at Mnazi Mmoja Hospital (MMH), in Zanzibar, Tanzania. MMH is a tertiary hospital responsible for teaching and management of referrals in Zanzibar Island with a bed capacity of 630. The study was conducted from April to July 2017.

2.2 Study population, sample size and sampling procedure

The study included randomly selected patients from obstetrics and gynecology (maternity), general surgery and orthopedic wards. All patients who underwent both elective and emergency surgical procedures were recruited in the study, while surgical patients with pyogenic infections such as abscess, furuncle and carbuncles and those who refused to give consent for participating were excluded to participate in the study.

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2.3 Data collection

Social demographic and clinical characteristics from all patients who underwent surgical procedures were collected using a structured questionnaire. Information gathered included, age, sex, medical history, presence of other chronic illness, history of previous antibiotic use and history of previous hospitalization.

2.4 Laboratory investigations

2.4.1 Culture and identification

Two pus swabs were collected aseptically from all participants who underwent surgery. The swabs were inserted into Amies' transport media immediately after collection and they were transported to the laboratory for processing**.** Gram stain technique was performed from the first swab and the second swab was used for inoculation onto MacConkey agar (MCA) and blood agar (BA). MCA plates were incubated aerobically and BA was incubated under microaerophilic environment at 37°C for 18-24hrs. Biochemical tests were used for identification of bacteria to a species level [17].

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2.4.2 Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed using Kirby Bauer disc diffusion method on Mueller Hinton agar (MHA) and the results were interpreted as per Clinical and

Laboratory Standards Institute (CLSI) guidelines [18]. Briefly, homogenous colonial suspensions were prepared using 3-5 colonies from a pure culture comparable to 0.5 McFarland turbidity standard. Standardized suspensions were inoculated on MHA, and then incubated at 37 ºC aerobically for 24 hours. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used as control organisms for Gram positive and Gram negative bacteria, respectively. *P.aureginosa* (ATCC 27853) was used as a control organism for *Pseudomonas spp.*

2.4.3 ESBL screening

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ESBL production was screened by double disc diffusion test. Briefly, ceftazidime (30µg) and cefotaxime (30µg) disks were first tested alone and they were then tested in combination with clavulanate i.e. ceftazidime/clavulanate (30/10µg) and Cefotaxime/clavulanate (30/10µg). ESBL production was confirmed when there was ≥5mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate versus when tested alone after 18-24hours incubation at 37ºC [18]. *Klebsiella pneumoniae* ATCC 700603 was used as a control organism.

2.4.4 MRSA screening and ICR detection

Methicillin resistant *Staphylococcus aureus* (MRSA) was determined by disc diffusion technique using cefoxitin (30µg) disc on MHA, while Induced Clindamycin resistance (ICR) among *Staphylococcus aureus* was detected by pacing erythromycin (15 µg) and clindamycin (2 µg) discs 15 mm apart and incubating the plates at 35± 2ºC for 16-18 hours. The flattening of the zone of inhibition adjacent to Erythromycin (D-zone) were interpreted as inducible Clindamycin resistance [18].

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2.5 Data analysis

Data were entered and analyzed using SPSS version 21.0. Frequencies and percentages were used to summarize the data.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Characteristics of study participants

A total of 121 participants with clinical evidence of SSIs consented to participate in the study. Of these, 86 (71.1%) were females and 35 (28.9%) were males. The overall mean age of the patients was 36 years (SD=13.0). Half of the specimen (61/121; 50.4%), came from general surgery ward and 49% of specimens were from patients who underwent caesarian section procedure. More than half (69/121; 57%) of the study participants had a history of previous antibiotic use and almost half of them (58/121; 47.9%) had a history of previous hospitalization (Table 1).

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3.1.2 Aetiology of SSI

Of 121 non-repeat wound swabs collected from patients who underwent surgery, 75 showed bacteria growth within 24hours of incubation giving a total of 106 bacteria isolates. Majority of the isolates, 92/106 (86.8%), were Gram-negative bacteria. *Pseudomonas aeruginosa* was the most predominant pathogen (29/106; 27.4%) isolated followed by *Proteus spp* (18/106; 17%). *Acinetobacter baumanii* was the least isolated Gram negative bacteria (8/92; 8.7%). Among the Gram positive bacteria, *Staphylococcus aureus* was the most frequent (9/14) isolated organism (Figure 1).

With regard to pathogen distribution, the majority of isolates were from maternity ward accounting for 55.7% of all isolates. Table 2 summarizes the distribution of bacteria isolated from different surgical departments. Most of the isolated Gram positive pathogens were from general surgery ward (*S.aureus* 7/9; 14.9% vs. 2/9; 3.4% and CoNS 5/5; 100% vs. 0/5;0%).

3.1.3 Antimicrobial susceptibility testing patterns of isolated pathogens

Most Gram negative isolates were very resistant to ampicillin (88.9-100%) and among them *K.pneumoniae* isolates showed high resistance towards all tested antibiotics (46.2-100%).

All *Proteus* species were resistant to ceftriaxone. More than half (57.1%) of the *E.coli* isolates were resistant to ciprofloxacin. Nearly quarter (24.1%) of the *P.aureginosa* isolates were resistance to meropenem and 10.3% were resistant piperacillin. Among the Gram positive isolates, majority of *S.aureus* (76%) were resistant to penicillin, while most of CoNS (80%) were resistant to gentamycin. Thirty three percent of *S.aureus* isolates were Methicilin Resistant *S. aureus* (MRSA). (Table 3). We also isolated two induced clindamycin resistant isolates which were *S. aureus* (data not shown).

Of the 92 Gram negative isolates, 24 (26%) were ESBL producers, and *E.coli* contributed the most number (10/24). Majority of ESBL producers exhibited greatest resistance towards ampicillin (80-100%) and amoxicillin/Clavunate (83-90%). None of the ESBL producers were resistant to meropenem. Less than half (37.5%) of ESBL producing *K.pnemoniae* isolates displayed resistance towards chloramphenicol. Table 4 shows these results

DISCUSSION

To the best of our knowledge, this is the first study to report on aetiology and antibiogram of pathogens causing surgical site infections among patients undergoing different surgical procedures in Zanzibar. We report high prevalence of SSI due to Gram negative bacteria and existence of multidrug resistant bacteria among the study population.

In this study we report an overall prevalence of SSI to be 62% among the study population, which is higher compared to many studies in developing countries. Reports from the Northern part of Tanzania documents the prevalence of SSI to be 26% in Bugando Hospital Mwanza and 19.4% in Kilimanjaro Christian Medical Centre, Moshi [8,9]. The possible explanation for this variation may be due to difference in geographical locations, different type of surgical procedures between the studies and lack of specific national guideline for treatment and management of surgical wound infection.

In the present study we demonstrate the predominance of Gram-negative bacteria as a cause of SSIs (86.8%). This finding is in agreement with reports from many sub Saharan African countries including Tanzania [16][19]. This observation may be explained by the fact that more antibiotic resistance is observed in Gram negative bacteria compared to Gram positives, making them more persistent in infected wounds [13]. In line with reports from other studies, *P. aeruginosa* was the most predominant isolated pathogen in this study. The predominance of *K.pneumoniae* recovery from infected wounds after a Caesarian section observed in this study is in line with reports from Ethiopia [20]. However, contrary to our finding, *S.aureus* is documented as a leading cause of SSI in both general and obstetric surgeries in Tanzania mainland [21,22]. Different types of prophylactic antibiotic given may have contributed to this difference, as there was no specific policy followed in giving the antibiotics.

In line with reports from other resource-constrained countries, we document the presence of multi drug resistance bacteria among the causatives agents of SSI [8,23]. Here we report the prevalence of MDR to be 27.4%. Although the reported prevalence is lower compared to that reported in Dar es Salaam Tanzania by Manyahi et al [16], there is a reason to be concerned since most commonly prescribed antibiotics may be ineffective against a significant proportion of isolates causing SSI in Zanzibar. We have also demonstrated a high resistant pattern of *S.aureus* isolates to penicillin group of antibiotics which is comparable to what others have reported [23]. This may be attributed by the long use of these antibiotics and their oral route of administration that affects their rate of absorption into blood stream. The detection of meropenem resistant *P. aureginosa* (24.1%) isolates demonstrated in the present study is a major threat to patient care, since this antibiotic is usually among the last treatment options for infections caused by the pathogen.

4. CONCLUSION

Prevalence of SSI was found to be high among patients admitted at Mnazi Mmoja Hospital. *Pseudomonas aeruginosa* was found to be the most predominant pathogen isolated, followed by *Proteus spp* and *E.coli*. Prevalence of multidrug resistance was also found to be high at the Hospital.

CONSENT

All authors declare that written informed consent was obtained from the patients.

ETHICAL APPROVAL

Ethical clearance was obtained from Muhimbili University of Health and Allied Sciences Senate Research and Publications committee.

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APPENDIX

Table 1: Social Demographic and Clinical Characteristics of Patients with Surgical Site Infections Attending Mnazi Mmoja Hospital

Variable	Frequency	Percentage%
Age (years)		
<20	3	2.5
20-34	71	58.7
35-49	27	22.3
50-64	13	10.7
65-79	7	5.8
Gender		
Female	86	71.07
Male	35	28.93
Source of sample		
Obstetrics/Gynecology	60	49.6
General surgery	61	50.4
Surgical procedure		
Appendectomy	16	13.2
Amputation	7	5.8
Laparotomy	15	12.4
Caesarian section	59	49

Others	24	19.8
Nature of surgery		
Elective	50	41.3
Emergency	71	58.7
History of antibiotic use		
Yes	69	57.0
No	52	43.0
History of previous hospitalization		
Yes	58	47.9
No	63	52.1

Others: Myomectomy, Sequestrectomy, Open reduction & internal fixation, Laminectomy, Hernioraphy, Prostectomy, Cholecystectomy, CSF fistula, Fasciotomy, Amputation, Splenectomy

Table 2: Distribution pattern of Isolated Bacteria in Surgical Departments

Isolated Bacteria	Surgical Department		Total
	General surgery n (%)	**OBGY (Maternity) n (%)**	
A. baumannii	4(8.5)	4(6.8)	8(7.6)
CoNS	5(10.6)	0	5(4.7)
E. Coli	6(12.8)	8(13.6)	14(13.2)
K. pneumoniae	3(6.4)	10(16.9)	13(12.3)
Proteus spp	9(19.1)	9(15.3)	18(16.9)
P. aeruginosa	10(21.3)	19(32.2)	29(27.4)
S. aureus	7(14.9)	2(3.4)	9(8.5)
Other GNR	3(6.4)	7(11.8)	10(9.4)
Total	**47(44.3)**	**59(55.7)**	**106(100)**

Other GNR: *Raoultella planticola, Serratia marcescens,Providencia qrettegari* and *Morganella Morganii*

Table 3: Antimicrobial resistance pattern of isolated pathogens

Bacterial isolate	**Antimicrobial agents resisted (%)**															
	P	AP	C	CD	TE	SXT	E	CIP	CN	Fox	AMC	CAZ	CRO	CTX	MEM	PIP
S.aureus	76	-	11	33	56	33	56	33	44	33	-	-	-	-	-	-
CoNS	60	-	60	60	40	40	60	40	80	-	-	-	-	-	-	-
P.aureginosa	-	-	-	-	-	-	-	13.8	27.6	-	-	51.7	-	-	24.1	10.3
Proteus spp	-	88.9	11.1	-	66.7	66.7	-	38.9	66.7	-	83.3	77.8	100	77.8	0	-
E.coli	-	92.9	17.1	-	50	78.6	-	57.1	64.3	-	71.4	78.6	92.9	78.6	-	-
K.pneumoniae	-	100	46.2	-	61.5	61.5	-	38.5	69.2	-	76.9	69.2	92.3	76.9	-	-
A.baumanii	-	100	-	-	37.5	37.5	-	50	50	-	-	75	87.5	75	0	25
Other GNR	-	100	40	-	70	80	-	20	50	-	100	100	100	60	0	-

P=Penicilin, AP = Ampicillin, C=Chloramphenicol,TE=Tetracycline, SXT=Sulphamexazole/trimethoprim, E=Erythromycin,

CIP = Ciprofloxacin, CN = Gentamicin, Fox=Cefoxitin, AMC = Amoxicillin/clavulanicacid, CAZ = Ceftazidime, CRO = Ceftriaxone CTX = Cefotaxime,

MEM = Meropenem, PIP=Piperacillin

Table 4: Antimicrobial Resistance Pattern of ESBL Producing Enterobacteriaceae

Antimicrobial agent	***E.coli* % n=10**	***K. pneumoniae* % n=8**	***Proteus* spp% n=6**	**Total n=24(%)**
AP	80	100	100	22
AMC	90	88	83	21
C	60	37.5	83	14
CRO	90	88	100	22
CIP	70	50	67	15
CN	60	88	100	19
MEM	0	0	0	0
SXT	80	75	67	18
TE	50	63	83	16

AP = Ampicillin, AMC = Amoxicillin/clavulanic acid, C=Chloramphenicol,CRO = Ceftriaxone, CIP = Ciprofloxacin, CN = Gentamicin, MEM = Meropenem, SXT=Sulphamexazole/ trimethoprim, TE=Tetracyclin

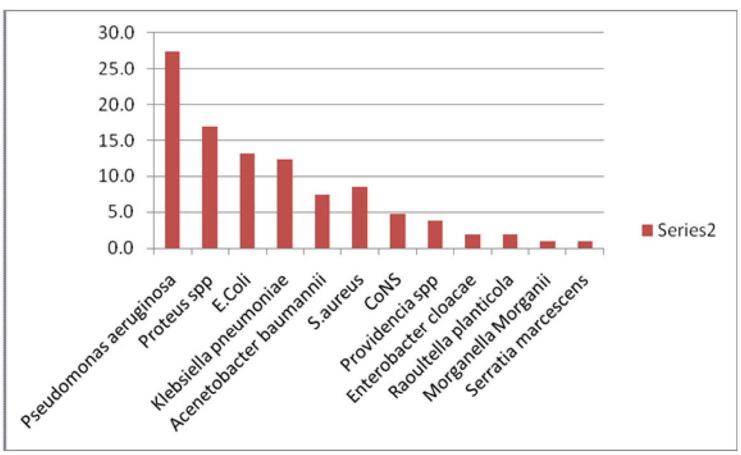

Figure 1: **Frequency of pathogenic bacterial isolates from surgical site infection**

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