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Bacteria Isolated from Hospital Surface Contamination and their Antimicrobial Susceptibility Patterns at Muhimbili National Hospital, Dar es Salaam, Tanzania

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Abstract

Background

An estimated 20%-40% of hospital acquired infections (HAI) are attributed to cross infection through health care workers, the patients and the hospital environment. However, data on bacteria contaminating hospital surfaces and their antibiotic susceptibility patterns in Tanzania, after hospital routine cleaning is limited. Findings from this study conducted in a national hospital in Tanzania will enrich data on understanding the sources of HAI. The study aimed to determine proportion of bacteria isolated from hospital surfaces and their antimicrobial susceptibility patterns.

Methods

A cross sectional study was conducted at Muhimbili National Hospital (MNH), Dar es Salaam from August to October 2020. A total of 271 surface samples were collected from MNH surgical wards and surgical intensive care unit after cleaning and changing of bedsheets. The samples were then inoculated on culture media and thereafter antimicrobial susceptibility test was done. Data was analyzed in Statistical Package for Social Sciences (SPSS) version 20.0. Frequencies and proportions of bacteria isolated and their antibiogram were determined.

Results

Out of the 271 surface swabs collected, 131/271(48.3%) yielded positive culture results. Swabs from bedsheets, bed-sides, door-handles and sink-tap were 112, 112, 29 and 18, respectively, and the positive yield was the highest (59) in bed-sheets. Gram-positive bacteria (GPB) were the most prevalent accounting for 69.5% (91/131). Gram-negative bacteria (GNB) isolated accounted for 30.5% (40/131). GBP resistance to different antibiotics such as azithromycin, clindamycin and vancomycin among others ranged from 16.9%-100%. GNB isolated were resistant to amoxicillin clavulanic acid, meropenem, cefotaxime and ceftazidime. The prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) was 75%(12/16). Resistance of Coagulase Negative Staphylococci (CoNS) ranged from 16.7%- 87.5%. Extended Spectrum Beta Lactamase producing Enterobacteriaceae (ESBL-PE) accounted for 55.5% (5/9).

Conclusion

A high proportion of both gram positive and gram-negative bacteria were isolated from hospital surfaces. CoNS were the most common bacteria followed by *P. aeruginosa and S. aureus.* Of the GPB, the prevalence of MRSA and CoNS was high and more than half of the GNB were ESBL-PE. Most of the bacteria isolated showed MDR. The findings support the need for enhanced efforts for infection and prevention control in the local setting to combat antimicrobial resistance.

Keywords: *Bacterial Surface Contamination, MRSA, ESBL-PE, Intensive Care Unit, Tanzania.*

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Introduction

Hospital Associated Infection (HAI) refers to infection acquired within the hospital by a patient who was initially admitted for reasons other than that infection (1). HAI are increasingly becoming a concerning threat for the health care system (2) as they contribute to high morbidity especially among hospitalized patients (3). Moreover, they also increase hospital costs and length of stay in the hospital impairing economic and social affairs of the patient (3).

Recent evidence has shown that HAI pathogens including Methicillin-resistant *Staphylococcus aureus* (MRSA), norovirus, and Vancomycin-resistant *Enterococci (VRE)* species are being shed by patients and can contaminate hospital surfaces at concentrations sufficient for transmission while surviving for extended periods despite attempts to remove them (4). Transmission of bacteria from the contaminated environment to the patient occurs when there is non-compliance to infection control process, hence cleaning is becoming a key intervention in the infection prevention control (5). Although cleaning is performed to ensure that hospital surfaces remain clean and free from infectious bacteria (6), the majority of routine cleaning are sub-optimal leaving surfaces to be reservoirs for transmission of pathogens (5) either directly through patient contact with the environment or indirectly through the contamination of healthcare workers' hands and gloves (7). Bacterial surface contamination serves as a source of multidrug resistance (MDR) for both Gram positive and Gram-negative bacteria (GPB/GNB). This is evidenced by Stiller et al who showed resistant bacteria such as MRSA and VRE as well as MDR among GNB (8).

Therefore, generating evidence for suboptimal cleaning, by investigating bacteria contamination on hospital surfaces after routine cleaning is important for the sake of intervention as well as prevention of HAI in the hospital setting. Hence the aim of the current study was to determine proportion of bacteria isolated from contaminated hospital surfaces and their antimicrobial susceptibility testing patterns.

Materials and Methods

Study design

This was a hospital based cross sectional study conducted at Muhimbili National Hospital (MNH) in Dar es Salaam.

Study settings

Eight General Surgical wards namely ward 9, 10, 11,12,13,14, 15 and 16 and one Surgical Intensive Care Unit (SICU) at MNH were included in the study. MNH wards are cleaned by a private cleaning company and facilities for washing hospital linen are also available.

Study population

Highly touched areas were bedsides, bed sheets, door handles and sink taps. The study included the General Surgical wards and SICU. Surgical wards and SICU were selected because they admit patients that are highly prone to infection as a result of open wounds or severe infection, respectively, and are on antibiotics as a form of prophylaxis.

Study duration

This study was conducted for three months from August to October 2020.

Sample size estimation

The minimum required sample size was 270 samples. These were obtained and distributed in each category involved in the study as further explained in the sampling method.

Sampling procedure

Systematic sampling technique was used to obtain the required sample size. Swab surface samples were collected systematically after every second surface. The 270 samples were distributed as follows: 18 out of 43 from sink taps, 29 out of 66 from door handles, and 224 out of 245 from bedsides and bed sheets based on the quantity and representation of contaminated hospital surfaces. The remaining 224 samples were divided equally to the number of wards from ward number 9 to 16.

Laboratory investigations

Sample were collected once a day i.e. every morning after cleaning and bedsheets change. Swabbing was done using a sterile cotton swab. Collected surface swabs were then transferred directly into Amies transport media and subsequently transported to Microbiology Section, Central Pathology Laboratory (CPL) at MNH. Laboratory processing was done following relevant Standard Operating Procedures (SOP) for culture and further testing. Samples were then cultured on blood agar and MacConkey Agar media with sorbitol and were incubated at 37°C for 18-24 hours. Bacterial growth was observed in the following morning and all plates with bacterial colonies were subjected to Gram staining and biochemical tests for identification. A quantitative aerobic colony count of <5 cfu/cm^2 on frequent hand touch surfaces was regarded as hospital surface contamination. A plate which had no growth was reported as no bacteria growth after 24 hours of incubation. In case of a mixed bacterial growth, the isolates were sub-cultured on Nutrient Agar plate and incubated at 37°C for 24 hours to get pure isolates for biochemical identification and antimicrobial susceptibility testing (AST). Gram staining was performed for all isolated bacteria. GNB were tested for bacterial identification tests which included oxidase, citrate utilization, urease, SIM (Sulphur Indole Motility) and KIA (Kligler Iron Agar). GPB were further subjected to biochemical identification tests which included catalase, coagulase and DNase.

AST

AST was performed for each set of bacterial isolates using Mueller–Hinton Agar (MHA) (Oxoid, England) by the Kirby–Bauer disc diffusion method following SOP. Antibiotic discs were added based on Clinical Laboratory Standard Institute (CLSI) 2019 guidelines and incubated at 37°C for 24 hours. For GPB, seven antibiotics discs were used which included cefoxitin, azithromycin, ciprofloxacin, gentamicin, clindamycin, doxycycline and vancomycin. For GNB, (enterobacteriaceae), seven antibiotic discs were also used which included ciprofloxacin, gentamicin, amikacin, amoxicillin clavulanic acid, cefotaxime, meropenem and imipenem. Only gentamicin, ciprofloxacin, cefepim and aztreonam were tested for *P. aeruginosa* based on available discs and CLSI 2019 guidelines.

Cefoxitin was used to screen for MRSA and confirmed if zone of inhibition was 21 mm or less around cefoxitin disc. Extended Spectrum Beta Lactamase producing Enterobacteriaceae (ESBL-PE) was tested using double disk synergy between amoxicillin clavulanic acid, cefotaxime and ceftazidime. It was confirmed when there was

≥5 mm increase in a zone diameter for either antibiotic tested in combination with clavulanic acid versus when tested alone.

Quality Assurance in Laboratory Testing

All stains and reagents were clearly labeled, dated and stored correctly. The operating temperature of the refrigerator and incubator were also daily monitored and documented. All culture media were prepared according to the manufacturers' instructions and were tested for performance and sterility. Incubation period of 24 hours to support interpretation of VRE control organisms such as *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29212 was used in all testing procedures.

Data collection

Data were collected using swabs from sampled hospital surfaces. Surface swab was taken to obtain data on surface contamination.

Statistical Analysis

Data on laboratory results of bacteria isolated from contaminated hospital surfaces were checked for accuracy, coded, entered into Microsoft Excel and were then exported to Statistical Package for Social Sciences (SPSS) version 20.0 for analysis. Frequencies and proportions of outcome variables (bacteria isolated and their antibiogram) were determined. Contaminated surfaces of General Surgical wards and SICU were also determined. A p-value of < 0.05 was considered as statistically significant.

Ethical considerations

Ethical approval for this study was obtained from MUHAS Research and Ethics Committee (IRB No. MUHAS-REC-07-2020-385). Permission to conduct the study was granted by MNH Management.

Results

Bacteria Isolated from Hospital Surface Contamination

Of 271 surface swab samples that were collected and subjected to culture, bacteria were isolated from 131 samples giving overall proportion of 48.3%. Isolated microorganisms were both GPB (69.5%, 91/131) and GNB (30.5%, 40/131). Coagulase-negative Staphylococci (CoNS) was the most common bacteria isolated from contaminated hospital surfaces followed by *P. aeruginosa and S. aureus. P. mirabilis* was the least isolated bacteria (Table 1).

Proportion of bacteria isolated in each site of sample collection

Table 2 presents the bacteria isolated from various sites of collection. The majority of the isolated bacteria were obtained from bedsheets 52.7% (59/112) and bedsides 50.9% (57/112). Most of the isolated bacteria from the door handles and sink taps were CoNS*, S. aureus and E. coli.*

Table 1: Bacteria Isolated from Hospital Surface Contamination (N=131) at MNH

Bacterial Isolate	Frequency	%
CoNS	72	55.0
S. aureus	16	12.2
E. faecalis	3	2.3
P. aeruginosa	28	21.4
A. baumanii	1	0.76
A. hydrophila	1	0.76
K. oxytoca	1	0.76
K. pneumoniae	1	0.76
P. mirabilis	1	0.76

Table. 2: Proportion of Bacteria Isolated in Each Site of Sample Collection

Sample collection sites	Bacterial Isolates									
	S. aureus n (%)	CoNS n (%)	*P. aeruginosa n* (%)	*E. coli* n (%)	*E. faecalis* n (%)	*A. hydrophila*n n (%)	*A. baumanii n* (%)	*K. pneumoniae* n (%)	*P. mirabilis*n n (%)	*K. oxytoca* n (%)
Bedsheets (n = 112)	4 (3.6)	38 (33.9)	14 (12.5)	1 (0.9)	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)
Bedsides (n = 112)	10 (8.9)	27 (24.1)	14 (12.5)	3 (2.7)	2 (1.8)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)
Door handles (n = 29)	2 (6.9)	6 (20.7)	0 (0.0)	2 (6.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sink tap (n = 18)	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)

Antimicrobial Resistance Patterns of GPB (n=91) Isolated from Contaminated Hospital Surfaces at MNH

S aureus showed antimicrobial resistance to azithromycin (68.8%), ciprofloxacin (31.3%), gentamicin (25.0%), clindamycin (87.5%), vancomycin (87.5%) and doxycycline (25.0%). The prevalence of MRSA was 75% (12/16) and VRE accounted for 33%. Resistance of CoNS ranged from 16.7% - 87.5% with the highest resistance being observed with clindamycin (Table 3).

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Table 3: Antimicrobial Resistance Patterns of GPB (n=91) Isolated from Contaminated Hospital Surfaces at MNH

Bacterial Isolate	Antimicrobial agent tested (%)					
	AZM	**CPFX**	**Gen**	**CLN**	**Van**	**DOX**
S. aureus (n = 16)	68.8	31.3	25.0	87.5	87.5	25.0
CoNS (n = 72)	76.4	16.7	30.6	87.5	75.0	50.0
E. faecalis (n = 3)	33.3	66.7	33.3	100	33.3	33.3

AZM = Azithromycin, CPFX = Ciprofloxacin, Gen = Gentamicin, CLN= Clindamycin, Van = Vancomycin, DOX = Doxycycline

Antimicrobial Resistance Patterns of GNB (n=40) Isolated from Contaminated Hospital Surfaces at MNH

Table 4 presents antimicrobial resistance pattern of GNB isolated. Isolated GNB were found to be resistant to amoxillin clavulanic acid, cefotaxime, ceftazidime meropenem, cefepime and aztreonam. *P. aeruginosa* was highly resistant to cefotaxime (96.4%), aztreonam (87.5%), cefepime (71.4%) and ceftazidime (53.6%). *E. coli* isolated showed resistance to cefotaxime (100%), amoxillin clavulanic acid (71.4%) and ceftazidime (57.1%). ESBL-PE isolated were *E. coli (3/7; 42.9%), K pneumoniae (1/1; 100%)* and*) P. mirabilis* (5/9; 55.6%).

Table 4: Antimicrobial Resistance Patterns of GNB (n=40) Isolated from Contaminated Hospital Surfaces at MNH

Bacterial Isolate	Antimicrobial agent tested (%)								
	CPFX	**GEN**	**AMK**	**AMC**	**MEM**	**CTX**	**CAZ**	**CFPM**	**ATM**
P. aeruginosa (n = 28)	7.1	7.1	0.0	35.7	3.6	96.4	53.6	71.4	89.3
E. coli (n = 7)	14.3	14.3	14.3	71.4	14.3	100	57.1	-	-
A. baunamii (n = 1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-
P. mirabilis (n = 1)	100	0.0	0.0	0.0	0.0	0.0	100	-	-
K. pneumoniae (n = 1)	0.0	0.0	100	100	100	100	100	-	-
K. oxytoca (n = 1)	0.0	0.0	0.0	0.0	100	100	100	-	-
A. hydrophila (n = 1)	0.0	0.0	0.0	100	0.0	100	100	-	-

***Key:** CPFX = Ciprofloxacin, GEN = Gentamycin, AMK = Amikacin, AMC = Amoxicillin clavulanic acid, MEM = Meropenem, CTX = Cefotaxime, CAZ = Ceftazidime, CFPM = cefepime, ATM = Aztreonam.*

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MDR Bacteria isolated

MDR was shown by *S. aureus* by 62.5% (10/16) and 86.1% (62/72) of CoNS. Induced clindamycin and vancomycin resistance was 12.5% (2/16), respectively. *P. aeruginosa (96.3%), E. coli (71.4%), K. pneumoniae (71.4%), K oxytoca (42.9%), A. hydrophila (42.9%) and P. mirabilis (28.6%),* respectively, showed MDR. *A. baunamii* did not show MDR.

Discussion

The current study isolated different species of bacteria from contaminated hospital surfaces. CoNS and *P. aeruginosa* were the most predominant bacteria isolated from contaminated hospital surfaces. AST patterns showed MRSA, VRE and ESBL-PE amongst the isolated organisms on hospital surfaces.

The proportion of bacteria isolated from hospital surfaces at MNH General Surgical wards was found to be 45%. The proportion of hospital surface contamination reported in this study is relatively higher than that reported in Mulago Hospital (31.6%) in Uganda and Bugando Medical Centre in Tanzania (33.5%) (10,11). The difference in proportions observed might be due to the difference in sample size and sources of surface swabs collected. In the study conducted in Uganda, a total of 100 samples were collected from patients [25], health care workers [36], and environment/burns unit [39]. In the current study, 271 surface swabs were collected from bedsheets, bed sides, door handles and sink taps and about half yielded positive culture. Moreover, our finding concurs with another study conducted at MNH in Dar es Salaam, Tanzania where hospital surface contamination was found to be 43.7% (12). Since the sample for current study was taken after cleaning, the study findings emphasize on importance of adherence to effectiveness of cleaning process, infection prevention and control. Effective hospital cleaning is a key aspect in successful infection prevention and control. The findings in the current study could be a result of inadequate compliance to cleaning regimes irrespective of whether cleaning is conducted by hospital staff or outsourced to non-medical professional cleaners.

In this study, CoNS were the most common cause of hospital surface contamination in surgical wards, a finding which is similar to a previous study in Ethiopia on bacteria isolated and their antibiogram (13). Current literature by Franca A et al has shown CoNs to be ably resistant to antimicrobial agents and more recently found to have an array of potent virulence factors. Hence, there is a need to evolve accordingly and conduct AST especially when dealing with highly susceptible populations like post-surgery patients (14). Apart from

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CoNS, other organisms such as *P. aeruginosa, E. coli, E. faecalis, A. baumannii, A. hydrophila, S. aureus and P. mirabilis* were also isolated from surfaces in this study which is similar to previous studies conducted in Tanzania and Europe (15,16).

In the current study, GPB isolated were mostly resistant to azithromycin, clindamycin and vancomycin while GNB isolated were mostly resistant to amoxillin clavulanic acid, meropenem, cefotaxime and ceftazidime. The high levels of resistance to commonly used antibiotics has also been reported in previous studies conducted in Mwanza, Dar es Salaam – Tanzania, India, Ethiopia and Uganda (10,11,13,16,17).

The prevalence of MRSA was found to be 75%. The prevalence is higher compared to that of another study conducted in Tanzania on MRSA contamination (19.5%) and distribution (12). In the previous study that focused on MRSA contamination and distribution, sources of samples were 36 general wards, seven ICUs and seven operating rooms. In the current study, a total of 271 surface samples were collected from eight General Surgical wards and one SICU. The finding suggests that the resistant strains continue to increase rapidly on the surfaces and impose high risk to healthcare providers, patients and visiting persons.

The proportion of vancomycin resistance ranged from 33.3-87.5% and it was found to be the highest with *S. aureus*. Vancomycin is the antimicrobial agent of choice for treatment of MRSA infection. Very high resistance of vancomycin can lead to limited options to treat MRSA infection (18) hence urgent measures are needed to prevent cross-transmissions.

Although few, VRE were also isolated. VRE falls under the 'high priority' category in the WHO list of organisms that requires urgent research effort to develop active drugs (18). According to Remschmidt et al, the rate of acquiring VRE is up to 28% for admitted patients, and antibiotic use is the most significant risk for acquiring VRE especially if one is taking carbapenem and glycopeptides (19). Surgical patients are usually kept under antibiotic prophylaxis to prevent occurrence of infection and hence are at a high risk of acquiring resistance. Moreover, *VRE* species when shed by patients can survive for extended periods of time to facilitate transmission (20) and hence they pause a significant risk for infection among hospitalized patients. Therefore, prudent use of antibiotic, contact precaution and intensive disinfection strategies are recommended to halt the spread of these organisms.

This study found surfaces of SICU, General Surgical wards 14 and 16 were more likely to be contaminated by bacteria compared to ward 11. These findings agree with findings from Dar es Salaam Tanzania (12). The high rate of surface contamination is possibly explained by the facts that surgical patients may have open wounds that can leak body fluids which

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increases the risk of infection transmission for the incoming new patient (20). In the current study, bedsheets and bedsides were more likely to be contaminated with pathogens compared to sink taps. These findings are in agreement with those from Tanzania, India, and Europe (12,13,15). Bedsides and bedsheets are patient contact surfaces and therefore isolated pathogens could be due to improper handling of cleaned bedsheets and ineffective cleaning. Since this study was conducted at General Surgical wards and SICU at MNH, these findings may not be generalizable. Considering these findings, it is concluded that the proportion of surface contamination in the investigated SICU and general surgical wards at MNH is high. CoNS were the most common bacteria followed by *P. aeruginosa and S. aureus.* Of the GPB, the proportions of MRSA and CoNS were high and more than half of the GNB were ESBL supporting the enhanced efforts for infection and prevention control in the local setting. Most of the bacteria isolated showed MDR. This has implications of potential risk of HAI to hospital staff, patients, caretakers and visiting persons. It is therefore recommended to strengthen the implementation of infection prevention and control to combat the spread of AMR. It is also recommended to conduct further molecular characterization studies on *P. aeruginosa* isolated to determine Metallo-beta-lactamase *Pseudomonas aeruginosa* (MBL-PA) strain. Further studies are also recommended to be conducted at hospital laundry unit to determine effectiveness of cleanliness and handling of cleaned bedsheets.

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Author's contribution

SA contributed to the conception and design of the study, interpretation of data, writing and critical revision of manuscript. TNM contributed to the conception and design of the study, data collection, analysis of laboratory samples and manuscript writing. LN contributed to the review of manuscript. All authors approved the final version of the manuscript.

Availability of data and material

All data used to for manuscript writing is available.

Conflicts of interest

The authors declare that they have no competing interest.

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