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Characterization and Antimicrobial Susceptibility Patterns of Isolates from Ward Fomites

Farouk Segujja[1,2*], Bashir Mwambi[2], Charles Kato Drago[1], Nathan Musisi Lubowa[1], Joseph Mugambwa[3] and Patrick Wabuyi[2]

[1]Department of Biomolecular Resources, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, P.O.Box 7062, Kampala, Uganda.
[2]Department of Medical Microbiology, International Health Sciences University, P.O.Box 7782, Kampala, Uganda.
[3]Department of Medical Microbiology, Faculty of Health Sciences, Habib Medical School, Islamic University in Uganda, Kampala Campus, P.O.Box 7689, Kampala, Uganda.

Authors' contributions

This work was carried out in collaboration between all authors. Author FS designed the study, wrote the protocol, participated in the data collection and wrote the first draft of the manuscript. Author BM performed the data entry and statistical analysis. Authors CKD and NML supervised and conceptualized the project. Authors JM and PW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was conducted to determine antimicrobial susceptibility patterns among isolates from ward fomites at Kiwoko Hospital and to detect resistances in the form of Macrolide Lincosamide StreptograminB (MLS_B), Methicillin Resistant *Staphylococcus aureus* (MRSA), Extended Spectrum β Lactamases (ESBLs), *Amp*C, and Multi Drug Resistant (MDR) pathogens.
Study Design: Laboratory based cross-sectional study.
Place and Duration of Study: The study was conducted in various wards and sections at Kiwoko Hospital, a rural setting in the central region of Uganda, between January and June 2015.
Methodology: We recruited 290 samples from the Surgical, Medical, Maternity and Pediatric

**Corresponding author: E-mail: segujjaf@gmail.com;*

wards as well as the Out Patient Department (OPD) at Kiwoko Hospital for the study. Samples were taken by swabbing the different surfaces and instruments which included; sphygmomanometers, stethoscopes, beds, nurses' stations, staff/visitors' chairs, door handles, patients' crepe bandages, curtains, switches, and sink handles among others. Susceptibility testing was done using the disc diffusion methods by Kirby Bauer for phenotypic expression of MLS_B resistances, MRSA, MSSA, ESBL, MDR and *Amp*C. Co-resistances exhibited by isolated ESBL producers were also phenotypically tested.

Results: Of the 290 surfaces and instruments swabbed, 57.59% (CI= 49.18 - 67.01) carried bacterial pathogens and by using standard surface agar plating methods, *Staphylococcus aureus* was the mostly isolated pathogen 43 (25.75%), followed by *Klebsiella pneumoniae* 35 (20.96%), *Escherichia coli* 31 (18.55%), *Pseudomonas aeruginosa* 20 (11.98%), *Enterococcus faecalis* 12 (7.19%), *Staphylococcus epidermidis* 10 (5.98%), *Proteus mirabilis* 9 (5.39%), *Bacillus* spp. 4 (2.40%), and *Staphylococcus saprophyticus* 3 (1.80%). Among enterobacteriaceae, 5 (6.67%, CI= 2.16 – 15.56) were identified as *Amp*C producers and 16 (21.33%, CI= 12.19 - 34.64) as ESBL producers out of which 4/16 (25.00%, CI = 6.81 – 64.01) showed ESBL co-resistance. Of the 43 *Staphylococcus aureus* isolates, 9.30% were MRSA (CI = 2.53 - 23.82) and 90.70% MSSA (CI = 64.49 - 100). In MLS_B resistance patterns, 23.26% of the total *S. aureus* isolates were constitutive MLS_B while 6.98% showed inducible MLS_B as 27.91% exhibited an MS phenotype. Out of all the isolates recovered from fomites, 27/167 (16.17%, CI = 10.65 – 23.52) were identified as Multi Drug Resistant (MDR).

Conclusion: Hospital fomites harbored resistant pathogens that could well persist for a long period of time thereby predisposing patients to Hospital acquired infections. Therefore, routine screening of clinical samples for MLS_B, ESBL, *Amp*C, MRSA and MDR could significantly monitor potential treatment failures in the management of resistant bacterial infections spread by pathogens on ward items and surfaces at Kiwoko Hospital, Uganda.

Keywords: Nosocomial infections; Macrolide Lincosamide StreptograminB; phenotypic; ward; fomites.

ABBREVIATIONS

ATCC : American Type Culture Collection.
CDH : Central Drug House.
CLSI : Clinical and Laboratory Standard Institute.
$cMLS_B$: Constitutive Macrolide-Lincosamide-StreptograminB.
ESBL : Extended Spectrum β Lactamase.
HAIs : Hospital Acquired Infections.
$iMLS_B$: Inducible Macrolide Lincosamide-StreptograminB.
MDR : Multi-Drug Resistant.
MRSA : Methicillin-Resistant Staphylococcus aureus.
MS : Macrolide-Streptogramin B resistant phenotype.
MSSA : Methicillin-Sensitive Staphylococcus aureus.
AMPC : This is a chromosomal mediated, inducible mechanism of resistance to Cephamycins and 3rd generation spectrum as a result of acquisition of AMPC gene or depression of a chromosomal AMPC gene.
AMP C Inducer : An antimicrobial that switches on the AMPC resistance gene to render treatment with cephamycins and 3rd generation cephalosporin inappropriate.
ESBL : Extended spectrum β-lactamase (ESBL) is an enzyme whose coding gene is a bacterial Plasmid and confers resistance to β-lactam antibiotics up to the 3rd generation cephalosporins, penicillins and aztreonams thereby rendering them inactive.
FOMITES : Objects or materials which are likely to carry or harbour pathogens that cause infections in a healthcare setting/hospital.
MLS resistance : Resistance to macrolides such as erythromycin, and lincosamides such as clindamycin, usually is due to an erm gene. These erm genes code for production of an RNA methylase enzyme that modifies the ribosomal binding site of macrolides, lincosamides, and StreptograminB.

1. INTRODUCTION

Although its clearly evident that several control measures including use of checklists, effective handwashing, surveillance and setting up infection control units have been adopted in many healthcare settings over the years to help reduce rates of contamination [1,2], hospital acquired infections also known as nosocomial infections remain a major cause of increased patient management costs [3,4], prolonged hospital stays and death among hospitalized patients worldwide [5-8].

Nosocomial infections are infections occurring in hospitalized patients in whom the infection was not present or incubating at the time of admission, these encompass infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility [7]. At any one moment, approximately 5% to 10% of hospitalized patients have an infection acquired after admission [9]. Higher nosocomial infection rates have been reported in developing countries more often than those documented from developed countries [4,6,7,10,11].

Recent studies suggest that contaminated environmental surfaces may play a major role in the transmission of nosocomial infections [12-15]. Harbored organisms may well survive for long periods on items or surfaces thereby presenting as a major health concern [5,6,10,16,17]. Such inanimate surfaces or objects are often known as fomites and are greatly associated with detrimental infection outbreaks [18-20].

Despite undoubtable proof that fomites do harbor nosocomial causing pathogens [5,12,13,16], there still remains substantial controversy on whether or not some healthcare personnel contribute to the transmission of these infections in hospital settings. Several studies have however indicated that healthcare workers are equally involved and that bacterial contamination of their hands is likely to result into transmission to patients [14,21-23]. Without contact, pathogens can still find their way to a new host via contaminated air by patients carrying airborne infections commonly through coughing and sneezing [24].

In an attempt to treat nosocomial infections, there has been increased use of first-line antibiotics where appropriate second-line drugs are not readily available or affordable, this has eventually led to the emergence of quite a number of resistant bacteria that have subsequently persisted in the hospital environment thereby becoming endemic [7]. Many resistant strains including ESBL producers [25], AmpC producers [26,27], multidrug resistant pathogens [28,29], *Pseudomonas aeruginosa* [30], *Enterococcus* spp. [5,16,17] and MLS_B resistant isolates [31-33], have been prevalently reported in many hospital settings as potential life threating pathogens worldwide.

Little is known about bacterial contamination of the environment around patients admitted to local hospitals in Uganda. This study therefore aimed at investigating the presence, distribution, rate and antibiogram of pathogens isolated from ward items and surfaces of Kiwoko Hospital that could potentially predispose patients to hospital acquired infections.

2. MATERIALS AND METHODS

2.1 Study Design

This was a laboratory based descriptive cross sectional study conducted between January and June of 2015 at Kiwoko Hospital and Medical Microbiology laboratories of Makerere University, International Health Sciences University and Habib Medical School, Islamic University In Uganda, Kampala Campus.

2.2 Study Samples

These were swabs taken from all items and surfaces in surgical, medical, pediatric and maternity wards as well as OPD sections at Kiwoko Hospital. Disinfection was aways done at the start of the day and samples were collected during work. Fomites included; thermometers, sphygmomanometer, stethoscopes, visitors' chairs, intravenous poles, beds, bedside tables, hand towels, kidney dishes, patients' crepe bandages, curtains, telephones, dressing trolleys, light switches, door handles, record books, recording pens, examination bed area, pediatric weighing scale, pulsometer control panels, cupboard shelves, drug locks, screens, cardiotocograph control panels, counter tops (Nurses and doctors' stations) and water sinks (Patients' sinks, Doctors' sinks, Nurses' sinks).

2.3 Laboratory Procedures

2.3.1 Specimen collection and identification of pathogen

Specimen collection involved using a sterile cotton swab stick moistened with 0.9% physiological saline. The swab was then pressed and rolled several times over the entire surface of a selected item. With care not to touch the swab, it was then immediately placed into an appropriate bijou bottle containing Brain heart infusion broth and aerobically incubated overnight at 37°C as published by Bauer et al [34].

On suspected growth exhibited by turbidity in the broth, specimens were subcultured on Blood agar (Central Drug House (CDH), India) and MacConkey agar (CDH, India) using aseptic streaking techniques followed by 24 hours of incubation at 37°C aerobically. Plates were then read for bacterial growth, and organisms examined for their characteristic colonial appearance, hemolysis, swarming, and/or pigmentation on the different media, before subsequent follow-up for identification and confirmation through gram-staining, sugar fermentation, and biochemical reactions. On failure to grow within 24 hours, plates were further re-incubated for the same time under similar conditions before discarding them and recording their results as negative.

Members of the family Enterobacteriaceae were identified by indole production, Hydrogen Sulphide (H_2S) production, citrate utilization, gas production, motility tests, urease test, oxidase, and carbohydrate utilization. For gram-positive bacteria identification and confirmation, coagulase, DNase, catalase, mannitol fermentation, CAMP (Christie, Atkins, and Munch Peterson) test, esculin bile test, bacitracin, optochin and novobiocin susceptibility tests were all used.

2.3.2 Antimicrobial susceptibility testing

Antimicrobial susceptibilities were studied by modified Kirby-Bauer's agar disc diffusion methods [34] and according to guidelines from Clinical and Laboratory Standards Institute [35]. Susceptibility testing was done in categories for Enterobacteriaceae (*Klebsiella pneumoniae*, *E. coli* and *P. mirabilis*), non-enterobacteriaceae (*P. aeruginosa*), *S. aureus* and *E. faecalis*. Testing was not done for isolated *S. epidermidis*, *S. saprophyticus*, and *Bacillus* spp.

Among the enterobacteriaceae isolates, antibiotics tested included; Augmentin (AUG, 30 µg), Ceftriaxone (CRO-30 µg), Cefoxitin (FOX, 30 µg), Ertapenem (ETP, 10 µg), Imipenem (IPM, 10 µg) and Piptazocin (PTZ, 110 µg) a drug combination of piperacillin and tazobactam.

These six discs phenotypically identified ESBL and *Amp*C producing enterobacteriaceae by positioning the Augmentin disc in the centre of the media plate at a distance of 20 mm away from a 3rd generation cephalosporin (Ceftriaxone, 30 µg) on one side, and Cefoxitin (acting as a strong labile *Amp*C inducer) on the other (double synergism method). As a last resort treatment option for ESBLs and AMPC pathogens, imipenem (IPM, 10 µ) was included to ascertain whether or not the isolate was Carbapenem Resistant Enterobacteriaceae (CRE), this imipenem also acted as another AMPC inducer. To report CRE results correctly, another stronger carbapenem drug in the form of Ertapenem (ETP, 10 µg) was included as per the CLSI defining guidelines [35].

Potential ESBL producers showed synergism between clavulanic acid (AUG, 30µg) and ceftriaxone (3rd Generation cephalosporin), resistance to Piptazocin (PTZ, 110µg) and sensitivity to Cefoxitin (FOX, 30µg), a cephamycin [26,35,36].

*Amp*C gene producing enterobacteriaceae exhibited resistance to all Augmentin (AUG, 30 µg), Ceftriaxone (CRO, 30 µg), Cefoxitin-30 µg, and also showed a flattening (blunting) of the zone size of Ceftriaxone at its junction with the zone edges of both Cefoxitin and Augmentin that were adjacently placed 20 mm away from it on either sides [37].

Sensitivity testing was further done on pathogens that showed ESBL and *Amp*C production to determine which drugs would be used as treatment options and to ascertain whether they still showed co-resistance patterns against such alternatives. These drug categories included; an aminoglycoside (Gentamycin, 10 µg), Fluoroquinolones (Ciprofloxacin, 10 µg), Chloramphenicol (Chloramphenicol, 30 µg), Folic acid inhibitor (Sulphamethazole-Trimethoprim, 1.25 µg), or Tetracyclines (Tetracycline, 30 µg) [25,36]. An ESBL producer that showed

resistance to at least one agent in three or more antimicrobial categories for which it did not have known intrinsic resistance was defined as a co-resistant pathogen [29].

Among the non-enterobacteriaceae (particularly *P. aeruginosa*), antimicrobial agents tested included; Ceftazidime (CAZ, 30 µg), Meropenem (MEM, 10 µg), Gentamycin (CN, 10 µg), Ciprofloxacin (CIP, 30 µg) and Piptazocin (PTZ, 110) a drug combination of piperacillin and tazobactam. These were kept five to limit synergism that would falsely show increased zone size diameters between Piptazocin and ciprofloxacin, or piptazocin and Gentamycin [38].

For *Enterococcus faecalis*, antibiotic discs tested were; Gentamycin (CN, 10 µg), Vancomycin (VA, 30 µg) to detect Vancomycin Resistant *Enterococcus* (VRE), Clindamycin (DA, 2 µg), Erythromycin (E, 15 µg), Ceftriaxone (CRO, 30 µg), and Ciprofloxacin (CIP, 30 µg).

In testing susceptibility patterns of *S. aureus*, six (6) discs were set including Cefoxitin - FOX, 30 µg (as a stronger surrogate to oxacillin or methicillin), Gentamycin (CN, 10 µg), Vancomycin (VA, 30 µg) to detect Vancomycin Resistant *S. aureus* (VRS) and as a treatment option for MRSA, Ciprofloxacin (CIP, 10 µg), Erythromycin (E, 15 µg) and Clindamycin (DA, 2 µg).

To detect Macrolide-Lincosamide-StreptograminB (MLS_B) resistances, Erythromycin and Clindamycin discs were place at a distance of 15mm edge to edge from each other [39]. A positive "D test" showed flattening of the zone of inhibition of Clindamycin (D-shaped inhibition zone ≥21 mm) at the area besides which it was adjacent to Erythromycin (zone size ≤ 13 mm) and was defined as an inducible MLS_B ($iMLS_B$) phenotype. *S. aureus* strains that were resistant to both Erythromycin and Clindamycin with a perfect circular-like inhibition zones were defined as constitutive MLS_B ($cMLS_B$) phenotype. Isolates that were resistant to Erythromycin (zone size ≤13 mm) but sensitive to Clindamycin (zone size ≥21 mm) without D-shaped zones around Clindamycin were defined as MS phenotypes [32].

A multidrug resistant (MDR) phenotype of isolates was identified as an expression of resistance to at least one agent in three or more different antimicrobial categories to which these isolates did not have known intrinsic resistances. These MDR defining categories included: cephamycin, cephalosporins, carbapenems, glycopeptides, aminoglycosides, macrolides, lincosamides, fluoroquinolones, folate pathway inhibitors (sulfamethazole-trimethoprim), antipseudomonal Penicillin-β-lactamase inhibitors (piptazocin), tetracycline, and chloramphenicol. All MRSA were defined as MDR by virtue of being MRSA as this predicted resistance to cephamycin (Cefoxitin, a surrogate marker for Methicillin), ciprofloxacin, and all β lactam antibiotics [29].

2.4 Quality Control

Reference strains used as controls were: *E. coli* (ATCC 25922), *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923), Methicillin Resistant *S. aureus* (ATCC 43300), *Escherichia coli* ATCC 35218 (ESBL producer), *K. pneumoniae* ATCC 700603 (ESBL producer), and *P. aeruginosa* (ATCC 27853).

2.5 Data Analysis

Data was entered in Microsoft excel, cleaned and imported to Stata version 13 statistical package for analysis. Results were then presented in form of graphs and tables for proportions, absolute values, percentages, and confidence intervals calculated by Poisson's test for point estimates at 95% level of confidence with a *P*-value of 0.05 considered as statistically significant.

3. RESULTS AND DISCUSSION

During the study period, 290 items and surfaces from across all wards were recruited. Among these, 167 (57.59%, CI = 49.18 - 67.01) harbored bacterial pathogens. *S. aureus* (25.75%) and *K. pneumoniae* (20.96%) were the most frequently isolated bacteria, followed by *E. coli* (18.55%), *P. aeruginosa* (11.98%), *E. faecalis* (7.19%), *S. epidermidis* (5.98%), *P. mirabilis* (5.39%), *Bacillus* species (2.40%) and *S. saprophyticus* (1.80%) (Table 1).

3.1 Antimicrobial Susceptibility Profiles of Isolates from Ward Fomites

3.1.1 Antibiogram of enterobacteriaceae isolates

Amongst Enterobacteriaceae, different isolates showed varying susceptibility patterns to the set of first antibiotics that included: Augmentin (AUG, 30 µg), Ceftriaxone (CRO, 30 µg), Imipenem

(IPM, 10 µg), Ertapenem (ETP, 10 µg), Cefoxitin (FOX, 30 µg), and Piptazocin (PTZ, 110 µg). The most effective antibiotics against enterobacteriaceae (*P. mirabilis*, *E. coli* and *K. pneumoniae*) were imipenem and Ertapenem with a sensitivity rate of 100% (Table 2).

*3.1.1.1 Phenotypic detection of Amp*C *and ESBL among enterobacteriaceae*

With susceptibility patterns exhibited by enterobacteriaceae (Table 2), ESBL and *Amp*C producers were phenotypically detected (Table 3). ESBL producing enterobacteriaceae showed synergism between clavulanic acid (AUG, 30 µg) and ceftriaxone (3rd Generation cephalosporin). They also expressed resistance to Piptazocin (PTZ, 110 µg) and sensitivity to Cefoxitin (FOX, 30 µg) a cephamycin.

*Amp*C gene producing enterobacteriaceae exhibited resistance to Augmentin (AUG, 30 µg), Cefoxitin-30 µg.

Out of the 75 enterobacteriaceae isolates, 16 (21.33%, CI= 12.19 - 34.64) were identified as ESBL producers while 5 (6.67%, CI= 2.16 - 15.56) phenotypically emerged as *Amp*C producers. *K. pneumoniae* and *P. mirabilis* expressed the highest resistance genes (ESBL and *Amp*C) amongst all enterobacteriaceae (Table 3).

Table 1. Bacterial profile of ward fomites at Kiwoko Hospital

Isolate	Frequency	Percentage	95% CI
S. saprophyticus	3/167	1.80%	0.37 - 5.25
Bacillus species	4/167	2.40%	0.65 - 6.13
P. mirabilis	9/167	5.39%	2.46 - 10.42
S. epidermidis	10/167	5.98%	2.87 - 11.01
E. faecalis	12/167	7.19%	3.71 - 12.55
P. aeruginosa	20/167	11.98%	7.32 - 18.50
E. coli	31/167	18.55%	12.61 - 26.35
K. pneumoniae	35/167	20.96%	14.60 - 29.15
S. aureus	43/167	25.75%	18.63 - 34.68
Total	167/290	(57.59%)	49.18 - 67.01

**Bacterial pathogen recovered from ward items, CI - Confidence Interval*

Table 2. Antibiogram of isolated enterobacteriaceae

Antibiotics	Isolates							
	Escherichia coli **n= 31**		***Proteus mirabilis*** **n= 9**		***Klebsiella pneumoniae*** **n= 35**		**Total n= 75**	
	R (%)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Cefoxitin	3 (9.68)	28 (90.32)	1 (11.11)	8 (88.89)	5 (14.29)	30 (85.71)	9 (12)	66 (88)
Augmentin	13 (41.94)	18 (58.06)	5 (55.56)	4 (44.44)	16 (45.71)	19 (54.29)	34 (45.33	41(54.67
Imipenem	0 (0.00)	31 (100)	0 (0.00)	9 (100)	0 (0.00)	35 (35)	0 (0.00)	75 (100)
Ertapenem	0 (0.00)	31 (100)	0 (0.00)	9 (100)	0 (0.00)	35 (100)	0 (0.00)	75 (100)
Ceftriaxone	13 (41.94)	18 (51.43)	4 (44.44)	5 (55.56)	18 (58.06)	17 (48.57)	35 (46.67	40(55.33
Piptazocin	14 (45.16)	17 (54.84)	4 (44.44)	5 (55.56)	15 (42.86)	20 (57.14)	33 (44)	42 (56)
Total (%)	43 (23.12)	143(76.88)	14(25.93)	40(74.07)	54 (25.71)	156(74.29)	(24.67)	(75.33)
CI (%) =	16.73-31.14	64.80-90.57	14.17-43.5	52.92-100	19.32-3.55	63.06- 86.90	20.29-29.7	67.53-83.8

** R – Resistant, S – Sensitive, CI – Confidence Interval*

Table 3. Prevalence of ESBL and *Amp*C producing enterobacteriaceae

Resistance definition	Isolates			Total
	Klebsiella pneumoniae **n = 35**	***Escherichia coli*** **n = 31**	***Proteus mirabilis*** **n = 9**	**Enterobacteriaceae n = 75, (%)**
ESBL producer	8 (25.81%)	5 (16.13%)	3 (33.33%)	16(21.33%)CI=12.19-34.64
*Amp*C producer	3 (8.57%)	2 (6.45%)	0 (0.00%)	5 (6.67%) CI= 2.16 -15.56
Total (%)	11 (31.43)	7 (22.58)	3 (33.33)	21 (28.0)
CI (%) =	CI=15.69 – 56.23	CI=9.08– 46.52	CI=6.87-97.41	CI= 17.33 - 42.80

ESBL – Extended spectrum β lactamase, CI – Confidence Interval

3.1.1.2 Co-resistance among ESBL enterobacteriaceae

Out of the 16 ESBL producing enterobacteriaceae isolated, 4 (25%, CI= 6.81 - 64.01) showed co-resistance. Among these, 2 were identified as *K. pneumoniae* 2(12.5%, CI= 1.51- 45.15) was the most frequently isolated co-resistant pathogen followed by *E. coli* (6.25%) and *P. mirabilis* (6.25%) (Fig. 1).

The most effective drugs for ESBL producing enterobacteriaceae were carbapenems (imipenem and ertapenem) as shown in Table 2, followed by Ciprofloxacin at a low resistance rate of 6.25% (CI= 0.16 – 34.82), Gentamycin (12.5%) and Chloramphenicol (12.5%). The most antimicrobial resistances in ESBL producers were reported with Tetracycline (37.5%, CI= 13.76 - 81.6) and Sulphamethazole-Trimethoprim (31.25%, CI= 10.15 - 72.93) (Table 4).

3.1.2 Antibiogram of non enterobacteriaceae isolates

The susceptibility rate of *P. aeruginosa* was highest for Piptazocin (100%), a drug combination of piperacillin and tazobactam. It showed utmost resistance to ciprofloxacin (90%) (Table 5).

3.1.3 Antibiogram of *Enterococcus faecalis*

E. faecalis showed 100% resistance to 50% of the set antibiotics leaving Vancomycin and Gentamycin as the only treatment options at sensitivity levels of 100% and 75% respectively (Table 6).

3.1.4 Antimicrobial susceptibility pattern of *Staphylococcus aureus*

The susceptibility rate of all *S. aureus* isolates was highest for Vancomycin (100%) irrespective of whether they were identified as Methicillin resistant or Methicillin Sensitive *S. aureus* (Table 7).

3.1.4.1 Phenotypic detection of MLS_B resistances and MRSA

Out of all the 43 *S. aureus* pathogens isolated from ward fomites, 39 (90.70%) were detected as Methicillin Sensitive *S. aureus* (MSSA) while 4 (9.30%, CI= 2.53 - 23.82) were Methicillin Resistant *S. aureus* (Table 8).

Table 4. Antibiogram showing treatment options for ESBL enterobacteriaceae

Antibiotics	ESBL producing enterobacteriaceae (n=16)					
	Escherichia coli n=5		*Proteus mirabilis n=3*		*Klebsiella pneumoniae n=8*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Tetracycline	1(20.00)	4(80.00)	3(100.0)	0(0.00)	2(25.00)	6(75.00)
Gentamycin	1(20.00)	4(80.00)	0(0.00)	3(100.0)	1(12.50)	7(87.50)
Chloramphenicol	0(0.00)	5(100.00)	1(33.33)	2(66.67)	1(12.50)	7(87.50)
Ciprofloxacin	0(0.00)	5(100.00)	0(0.00)	3(100)	1(12.50)	7(87.50)
Sulpha-trimethoprim	1(20.00)	4(80.00)	1(33.33)	2(66.67)	3(37.50)	5(62.50)
Total (%)	3(12.00)	22(88.00)	5(33.33	10(66.67	8(20.00)	32(80.00)
CI (%) =	2.47-35.07	55.15-100	10.82-7.79	31.97-100	8.63-39.41	54.72-100

R – Resistant S – Sensitive CI – Confidence Interval, ESBL – Extended spectrum β lactamase, CI – Confidence Interval

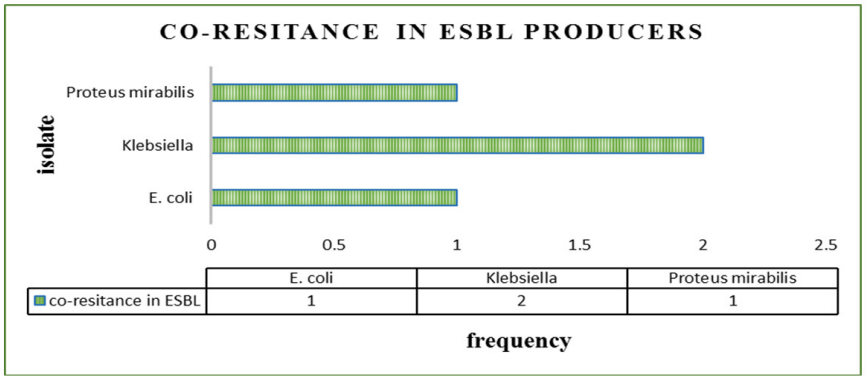

Fig. 1. Co-resistance among ESBL enterobacteriaceae

ESBL – Extended spectrum β lactamase

Table 5. Susceptibility pattern of isolated non-enterobacteriaceae

Antibiotics	Isolate	
	Pseudomonas aeruginosa n = 20	
	R (%)	S (%)
Ceftazidime (CAZ, 30 µg)	6 (30.00)	14 (70.00)
Meropenem (MEM, 10 µg)	7 (35.00)	13 (65.00)
Gentamycin (CN, 10 µg)	14 (70.00)	6 (30.00)
Ciprofloxacin (CIP, 30 µg)	18 (90.00)	2 (10.00)
Piptazocin (PTZ, 110 µg)	0 (0.00)	20 (100.00)
Total (%)	45 (45.00)	55 (55.00)
CI (%) =	32.82 - 60.21	41.43 – 71.60

R – Resistant S – Sensitive CI – Confidence Interval

Out of the 25 (58.14%, CI= 37.62 – 85.83) erythromycin resistant *S. aureus* isolates, 3 (12%, CI= 2.47 – 35.07) expressed an inducible MLS_B phenotypic resistance pattern in which D-shaped inhibition zones of Clindamycin placed 15mm away from erythromycin were observed. A total of 10 (40%, CI= 19.18 – 73.56) *S. aureus* isolates were identified as constitutive MLS_B with resistances to both erythromycin and clindamycin. All the 4 isolated MRSA exhibited a constitutive MLS_B pattern while 12 (48%, CI= 24.86 – 83.85) isolates presented with an MS resistant phenotype (Table 8).

Table 6. Antimicrobial susceptibility profile of *Enterococcus faecalis*

Antibiotics	Isolate	
	Enterococcus faecalis n = 12	
	R (%)	S (%)
Vancomycin (30 µg)	0 (0.00)	12 (100.00)
Clindamycin (2 µg)	12 (100.00)	0 (0.00)
Erythromycin (15 µg)	12 (100.00)	0 (0.00)
Gentamycin (10 µg)	3 (25.00)	9 (75.00)
Ceftriaxone (30 µg)	12 (100.00)	0 (0.00)
Ciprofloxacin (30 µg)	5 (41.67)	7 (58.33)
Total (%)	44 (61.11)	28 (38.89)
CI (%) =	44.40 - 82.04	25.84 - 56.21

R – Resistant S – Sensitive CI – Confidence Interval

Table 7. Antibiogram of *Staphylococcus aureus* isolated from hospital fomites

Antibiotic	Isolate	
	Staphylococcus aureus n = 43	
	R (%)	S (%)
Cefoxitin (30 µg)	4(9.30)	39(90.70)
Clindamycin (2 µg)	13(30.23)	30(69.77)
Erythromycin (15 µg)	25(58.14)	18(41.86)
Gentamycin (10 µg)	18(41.86)	25(58.14)
Ciprofloxacin (30 µg)	12(27.91)	31(72.09)
Vancomycin (30 µg)	0(0.00)	43(100.00)
Total (%)	72 (27.91)	186 (72.10)
CI (%) =	21.84 - 35.14	62.10 - 83.23

R – Resistant, S – Sensitive, CI – Confidence Interval

Table 8. Distribution of MRSA and MLS_B resistances among *S. aureus*

Resistance definition	MSSA (%)	MRSA (%)	Total (%)	Antibiotic agent	
				E, 15 µg	DA, 2 µg
$cMLS_B$	6 (15.38)	4 (100.00)	10 (23.26)	R	R
$iMLS_B$	3 (7.69)	0 (0.00)	3 (6.98)	R	S
MS phenotype	12 (30.77)	0 (0.00)	12 (27.91)	R	S
No DA resistance	18 (46.15)	0 (0.00)	18 (41.86)	S	S
Total (%)	39 (90.70)	4 (9.30)	43		
CI (%) =	64.49 – 100	2.53 - 23.82			

R- Resistant, S- Sensitive, E- Erythromycin, DA- Clindamycin, MRSA - Methicillin Resistant Staphylococcus aureus MSSA - Methicillin Sensitive Staphylococcus aureus, MLS_B - Macrolide Lincosamide StreptograminB $cMLS_B$ - constitutive MLS_B; $iMLS_B$ - inducible MLS_B, MS - Macrolide Streptogramin B; CI - Confidence interval

Table 9. Prevalence of multi-drug resistance among isolates

Isolate n = 167	Antimicrobial categorical resistance definition, MDR (%)	Confidence interval (%)
P. aeruginosa (n=20)	8 (40.00)	17.27 - 78.81
K. pneumoniae (n=35)	6(17.14)	6.29 - 37.31
P. mirabilis (n=9)	3(33.33)	6.87 - 97.41
E. coli (n=31)	4(12.90)	3.51 - 33.04
S. aureus (n=43)	6(13.95)	5.12 - 30.37
Total (%)	27/167 (16.17)	10.65 - 23.52

MDR - Multidrug resistant

3.1.5 Multi-drug resistance among isolates on ward fomites

The overall Multi-drug resistance rate was 16.17% (CI= 10.65 – 23.52) with *P. aeruginosa* singly accounting for 29.63% (8/27, CI= 12.79 – 58.38) of the total MDR prevalence.

3.2 Discussion

Our study found an overall bacterial contamination rate of 57.59% (167/290, 95%CI= 49.18 - 67.01%) that was obtained from all the fomites swabbed. The contamination of fomites in this study could be attributed to irregular disinfection, the types of disinfectants used, hygienic conditions, overcrowding and lack of surveillance and implementation of HAI control protocols [4,10]. Elsewhere, contamination has been documented to occur on fomites but in varying degrees. The contamination rate from our study was similar to the 44% Obtained at Cleveland Veterans Affairs Medical Centre, USA [12]. This could be attributed to the similarities of sampled items.

When compared to other studies elsewhere, the prevalence rate in our study was higher than that reported at Murtala Mohammed Specialist Hospital in Nigeria 23.33% (95% CI= 20.28 - 26.71%) [40], and at Al Imam Ali Hospital in Bagdad 4% [41]. However, it was significantly lower than what was reported at San Vicente Foundation, a teaching hospital in Columbia (98.7%; 157/159) [13], and Gondar University Hospital in which 83.1% (95% CI = 68.78 – 99.52%) of inanimate objects were contaminated [42].

The items swabbed at Murtala Mohammed Specialist Hospital were from theatre [40] contrary to our study that involved samples from various general wards. There are always high safety precaution measures taken to control infection transmission from theater fomites compared to general wards [43,44]. In theaters, there is always limited access to only authorized health workers, strict handwashing and wearing of sterile theater attires (gowns, surgical masks, surgical boots and gloves) [21,43,44]. This is contrary to the practice in general wards. This could be the reason for the higher rates of contamination in our study compared to what Nwankwo and colleagues reported in 2012 [40]. On the other hand, the lower prevalence rate in our study compared to what was documented at San Vicente [13] could be attributed to the difference in settings. San Vicente is a teaching hospital, up till now several authors have reported that bacterial contamination is more common in teaching hospitals than in non-teaching [4,10,41]. In teaching hospitals, students are normally being introduced to ward sections and know less about safety measures taken to control bacterial contamination [45,46].

The obtained fomite contamination rate highlighted a major role of ward items as potential vehicles and reservoirs of nosocomial infections, this accords with findings of several previous bacterial investigations of fomites [5,16, 21-23,47]. We never assessed for the relatedness of clinical isolates to fomites in this study, however, numerous authors have reported HAIs from various global regions [4,6,10, 20,48,49] with some documenting genotypic similarities of clinical isolates to hospital surfaces [50,51]. Therefore, bacterial contamination of fomites in our study reflects a regular daily risk of exposure to several hospital-acquired infections.

Staphylococcus aureus was the most frequently isolated pathogen across all wards, a result consistent with findings from different studies across the globe [15,52]. This is attributed to the fact that it is a normal flora of the skin [53-55] that subsequently gets into contact with ward surfaces from which it eventually invades patients thereby causing infections especially on surgical sites and other life-threatening diseases [55,56]. Previous studies have documented nasal carriage of *S. aureus* with or without any pathogenic events [42,57-59]. In health adults, the percentage carriage of *S. aureus* in nostrils is estimated to be 40% [53]. The presence of *Proteus*, *K. pneumoniae and E. coli* which are enteric bacteria [53,55], was indicative of feacal pollution and poor personal hygiene especially irregular handwashing practice. As a normal flora, *P. aeruginosa* has been documented to inhabit nonsterile areas on healthy individuals, however, it is able to infect any tissues especially in immune-compromised patients [54,56], its presence on various ward surfaces and instruments could therefore provoke severe infections in the form of wound contamination following surgical procedures, or those associated with catheterization such as urinary tract complications that may well persist as a result of bio-film formations in urogenital organs [30].

Vancomycin was the most effective antimicrobial agent against *S. aureus* followed by Cefoxitin,

Ciprofloxacin, Clindamycin, Gentamicin and Erythromycin with susceptibility rates of 100%, 90.70%, 72.09%, 69.77%, 58.14% and 41.86% respectively as seen in Table 7. This pattern is consistent with susceptibilities of clinical isolates elsewhere [58,60]. Several Clindamycin resistances were identified in our study (Table 8) a finding quite comparable with a number of previous investigations that have highlighted their existence in hospital settings, however, clindamycin that showed a moderate sensitivity level (69.77%) in our study, remains highly recommended in the treatment of various staphylococcal resistant strains today because of its better oral bioavailability, low cost, its importance as an alternative antibiotic in penicillin-allergic patients, excellent tissue penetration, and the fact that it accumulates in abscesses [33,39] than the Cefoxitin and Ciprofloxacin (which performed better in this study). Vancomycin was remarkably excellent, however, it should be given when therapeutic options are limited due to severe renal impairment associated with its prolonged administration [61,62].

Our study demonstrated presence of $cMLS_B$ resistant phenotypes in *S. aureus*, and therefore should a patient pick a *Staphylococcus aureus* pathogen from the hospital, then chance was 30.23% (95% CI= 16.10 – 51.70) that it would be resistant ($cMLS_B$ and iMLSB) to clindamycin a commonly used antibiotic in this setting. Of all the MLS_B phenotypic resistances exhibited by *S. aureus* (25/43), clindamycin would actually treat 48% (12/25). This accords with several other scientists who have continuously recommended that clindamycin should be cautiously used in the treatment of MLS_B resistances because of its increasing failures [39,63,64]. The fact that clindamycin is bacteriostatic, many studies have disapproved of its use in the treatment of endovascular infections like endocarditis or septic thrombophlebitis all pointing to prescription issues [63].

This study shows that out of the 43 isolates of *S. aureus*, 4 (9.03%) were MRSA and these were only sensitive to Vancomycin (100%). Such a finding is empirically suggestive of treatment with Vancomycin in case of suspected MRSA as previously recommended by other scientists [7,35,60,63,65]. However, recent reviews report increasing Vancomycin Resistant *Staphylococcus aureus* (VRSA) strains [66]. Therefore, this does not only necessitate the need to seek other VRSA therapeutic options (Linezolid, daptomycin or ceftaroline), but also routine screening of clinical samples for staphylococcal antimicrobial resistance to these options as a better patient management strategy in rural hospitals.

E. faecalis showed the highest resistance rate of all isolates 61.11% (95% CI= 44.40 - 82.04%) as seen in Table 6. *E. faecalis* is known to be intrinsically resistant to a number of antimicrobial classes [29,67] and this therefore explains its exceptionally high insensitivity to the antimicrobial agents in this present study. Vancomycin was the most effective antibiotic against *E. faecalis* with a sensitivity rate of 100%, a finding similar to several earlier studies that have endorsed it as the best therapeutic drug of choice against *Enterococcus* species [7,35]. However, transferable resistance to vancomycin is now prevalently common in *Enterococcus* and has found its way into MRSA strains [66,68], thereby making treatment of Vancomycin Resistant *Staphylococcus aureus* infections complex [17].

Of all the enterobacteriaceae isolates, 21.33% (95% CI= 12.19 - 34.64) were identified as Extended spectrum β-lactamase (ESBL) producing pathogens (Table 3). These findings are comparable with several other studies that have reported ESBL prevalence rates in a range of 4 to 46% [10,25]. Co-resistances against therapeutic options for ESBL enterobacteriaceae were reported in 25% (4/16) of all ESBL producers. This translates to the possibility that there is 5% chance that an enterobacteriaceae picked from ward items and surfaces would be an ESBL co-resistant pathogen, nevertheless this slight chance still indicates the existence of ESBL producers in this hospital that would surely present with quite a great patient management problem especially unresponsiveness to what could be used as ESBL treatment alternatives [25,26,28,69].

The occurrence of co-resistance patterns among ESBL producers in the present study is quite consistent with numerous earlier findings that have documented similar co-resistances to non-β-lactam antibiotics [25,36]. Their presence could be attributed to chromosomal mutations conferring resistance to β-Lactam antibiotics and large plasmids that mediate multiple resistance to other antimicrobial classes [67,70,71], that would be used as alternatives against ESBL producers [36,72-74]. In our study, carbapenems

were the most effective antibiotics against enterobacteriaceae including all ESBL and AmpC producers (Table 2), registering a susceptibility rate of 100%, a finding that highlights their importance in the management of ESBL and AmpC producing enterobacteriaceae at Kiwoko Hospital. This is in agreement with several other studies that have recently demonstrated that Carbapenems remain the preferred treatment option against such resistant enterobacteriaceae [28,29,75]. All ESBL producers were sensitive (100%) to Cefoxitin, a common cephamycin. This accords with earlier recommendations of its use as an alternative antimicrobial agent against β-Lactamase producing enterobacteriaceae [28,73].

Out of all the 167 isolates, 25 (16.17%) were detected as Multi-drug resistant species (Table 9). The presence of MDRs on hospital items possibly suggests pathogen shedding from patients who frequently misuse and overuse antibiotics [76,77]. In many countries antibiotics are available over counters without prescription or supervision [78-81]. Similarly, episodes of antimicrobial self-medication among Ugandans have also been reported over the years [82,83]. Such practices would subsequently increase patient management costs and prolonged hospital stays as observed by several earlier scientists [3,5,6,84]. Our findings on MDR pathogens therefore support numerous studies that have cited their alarmingly growing occurrence in Sub-Saharan Africa [85-89].

4. CONCLUSION

In conclusion, findings from our study have revealed the occurrence of resistant bacterial strains on hospital fomites for the first time in our region. In terms of diagnostic accuracy, it is therefore important that routine detection of resistant patterns in the form of MLS_B, ESBL, *Amp*C, MDR and MRSA from clinical samples is done together with the recommended WHO nosocomial infection control guidelines, to aid in the improvement of patient management strategies, and as a lift towards fighting increasing antimicrobial resistances here in Africa.

It remains unclear as to which extent bacterial contamination of ward surfaces/items contribute to the transmission of pathogens to hospitalized patients. However, evidence based on this study does indicate an urgent need to alert and educate hospital staff about the potential health risks associated with the use of fomites.

ETHICAL APPROVAL

The study was approved by the research and ethics review committee of Makerere University, school of Biotechnology and Laboratory Science College of Veterinary Medicine, Animal resources and Biosecurity. Permission was sought from the hospital and laboratory authorities. The ethical principles of a scientific research, related laws and regulations were strictly adhered to as well.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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