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Multi-Drug Resistant (MDR) Bacterial Isolates on Close Contact Surfaces and Health Care Workers in Intensive Care Units of a Tertiary Hospital in Bulawayo, Zimbabwe

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Authors' contributions

This work was carried out in collaboration between all authors. Author JM designed the study, and carried out experimental work on the project. Authors AS, FB and KM carried out experimental work on the project. Author SR assisted in sampling and project design. Authors JM and AS developed the manuscript and all authors approved the final manuscript.

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ABSTRACT

Aims: A cross sectional study was conducted to determine the degree of microbial contamination of environmental surfaces and health-care workers (HCWs) in two multi-bed ICUs at a tertiary-referral hospital in Bulawayo, Zimbabwe.
Place and Duration of Study: Mpilo Hospital, National University of Science and Technology Microbiology Department; between January 2017 and August 2017.
Methodology: In total 64 surfaces were swabbed in two ICUs; inclusive of 6 hand swabs obtained from on-duty nurses. Fungal and bacterial isolates were identified using standard microbiological methods. Bacterial antibiogram profiles were determined by the Kirby-Bauer disc diffusion method.

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PCR was used to determine the presence of Extended Spectrum Beta Lactamase (ESBL) genes in isolated Enterobacteriaceae and the presence of the *mec*A gene in *Staphylococcus aureus* isolates.

Results: Out of the 58 fomites and medical devices swabbed 50 (86.21%) were positive for bacterial contamination, with coagulase negative Staphylococci (CoNS) (20.31%) and *Klebsiella* species (20.31%) being the most prevalent environmental isolates. All 6 (100%) hand swabs obtained from the HCWs were positive for microbial contamination. A total of 51 (75%) of the 68 bacterial isolates were resistant to at least 3 antibiotics and 39 multi-drug resistance patterns were exhibited by the MDR isolates. Combinations of the ESBL genes *bla*CTX-M, *bla*TEM and *bla*SHV were detected in *Escherichia coli*, *Klebsiella* spp and *Pseudomonas aeruginosa* isolates. Methicillin-resistant *Staphylococcus* isolates (MRSA) were detected using phenotypic and molecular methods.

Conclusion: ICU inanimate surfaces and medical equipment in the adult and paediatric ICUs of the referral hospital were heavily contaminated with MDR bacteria that could predispose critically ill patients to acquire nosocomial infections.

Keywords: Antibiotic resistance; intensive care units; multi-drug resistance; Zimbabwe.

1. INTRODUCTION

Frequently coined reservoirs of multi-drug resistant (MDR) pathogens, the intensive care unit (ICU) inanimate environment has warranted much attention for its probable contribution to the transmission of nosocomial infections [1,2]. A nosocomial infection also referred to as a health-care associated infection (HAI), is an infection occurring in a patient during the process of care in a hospital or other health-care facility, which was not present or incubating at the time of admission[3]. Due to their critical state, as well as other external risk factors, the rate of infection amongst ICU patients is several times higher than that of patients in other wards [4,5]. Several studies have determined environmental sources as focal points of outbreaks in critical care wards [6-8]. Although there is still much deliberation as to the actual role fomites (especially medical equipment) play in the transmission of HAIs in non-outbreak situations, a growing body of evidence highlights the significance of environmental disinfection in infection prevention [9-11]. The increased interest in the use of copper surfaces in ICUs to reduce surface microbial load is testament of the potential role the environment may play in cross-transmission of HAIs [12]. Furthermore, numerous studies have employed molecular characterisation techniques including whole genome sequencing and Pulsed-field gel Electrophoresis (PFGE) to determine definitive genomic similarities between pathogens isolated from the hospital environment, HCWs hands as well as clinical specimens from admitted patients [7,8,13,14]. It has also been extensively reported that patients admitted into rooms previously occupied by a patient infected by MRSA, *Clostridium difficile*, *Acinetobacter baumannii* or vancomycin-resistant enterococci (VRE) were more likely to acquire the infection of a previously admitted patient [15-17] . In cases where there is no direct contact between patients, the risk of infection is possibly associated with the environment. Hospital inanimate surfaces may harbour multi-drug resistant pathogens with the ability to synthesise biofilms, which serve as protective barriers against disinfectants or antimicrobial agents. This makes bacteria more resilient to desiccation, enabling them to persist in the environment for prolonged periods of time [18,19]. Although the ICU environment serves as a reservoir of MDR pathogens, the hands of healthcare workers have been identified as the vehicles or vectors through which cross infection occurs [2,4,20]. The main mechanism of transmission of infections within hospitals is via direct or indirect contact, in particular by the hands of health professionals which may either contaminate or be contaminated by hospital surfaces [21,22]. The shedding of endogenous bacteria from patients onto their nearby environment also contributes to the contamination of ICU surfaces [2,9,14]. Adhesion to hand hygiene protocols by ICU personnel is extremely crucial, and remains one of the most significant measures for the control and prevention of HAIs [15,20].

Infectious diseases continue to plague sub-Saharan Africa as a whole, where nosocomial infections contribute far more significantly to the disease burden than they do in developed countries [23-25]. A keen interest has been taken across Africa on the microbiological quality of

hospital surfaces [9,10,22,26,27]. However, in Zimbabwe there is still a severe dearth of information on the severity of HAIs in ICUs in general. The extent of the problem of HAIs in Zimbabwe, as well as the majority of African countries remains grossly under-estimated [24,28]. The interplay between the ICU environment, the hands of HCWs, and the transmission of MDR pathogens amongst patients in critical care wards is unfortunately not well documented in Zimbabwe [28]. It is estimated that in resource limited countries, as many as 50% or more of patients in ICUs will acquire a nosocomial infection [23,24]. With the dilapidated state of healthcare infrastructure accompanied by the declining healthcare standards in the Zimbabwean public health sector [29], it is imperative that more attention should be given to the role of the inanimate environment and HCWs hands in the transmission of MDR nosocomial infections. This study represents the first evaluation of the environmental microbial quality in intensive care units of a public hospital in Bulawayo, Zimbabwe.

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross-sectional, descriptive study carried out in two multi-bed ICUs (a paediatric ICU and an adult ICU) from January 2017 to August 2017 at a tertiary referral government hospital in Bulawayo, Zimbabwe. This study entailed the collection of 58 swab samples from high-contact environmental surfaces, as well as 6 swab samples from the hands of HCWs in two multi-bed ICUs.

2.2 Sampling and Isolation

Fomites and medical equipment were swabbed after the daytime cleaning of the ICU wards. No prior notice was given to ward staff prior to collection of hand swabs. The samples were obtained using an aseptic technique from surfaces that appeared visibly clean. Analysis of acquired swab specimens was conducted at the National University of Science and Technology (NUST) microbiology laboratories. A total of 72 isolates (64 environmental and 8 from HCWs' hands) were obtained in this study. A total of 37 swab samples were collected from a multi-bed adult intensive care unit (AICU); 34 inanimate high-contact surfaces and the hands of 3 AICU HCWs were swabbed. A total of 27 swab samples were collected from a multi-bed paediatric intensive care unit (PICU); 24 high-contact inanimate surfaces and the hands of 3 PICU HCWs were swabbed. Sterile swabs were moistened with sterile isotonic water, and then applied to defined areas by parallel spaced stripes by slight rotation, followed by perpendicular stripes on the same area. Once collected, swabs were immediately immersed in vials containing sterilised peptone water, sealed, labelled and transported to the NUST laboratory. Upon arrival at the laboratory, the specimens were incubated at 37°C for 18 hours. Subsequently, loopfuls of the incubated culture broth were streaked onto Blood agar, chocolate agar and MacConkey agar (New England Biolabs, Midrand, South Africa) for the initial screening of suspected pathogens. Cultured blood agar and MacConkey agar plates underwent aerobic incubation at 37°C ± 1°C for 24 hours, whilst chocolate agar plates were incubated in a 5% CO_2 atmosphere at 37°C ± 1°C for 24 hours. Subcultures were then prepared so as to obtain pure single colonies. Yeast cultures were obtained by initially culturing on Blood agar and incubating at 35°C for 24 hours. After 24 hours of incubation, colonies with distinct yeast like smells and colonial morphology resembling that of *Candida* spp were picked from the plates and subcultured onto Sabouraud Dextrose agar (New England Biolabs, Midrand, South Africa) prior to a germ tube test being done. Samples that showed germination after 4 hrs as viewed under the microscope were identified as *Candida albicans.*

2.3 Identification and Drug Susceptibility Testing

Positive cultures were identified by their Gram staining reaction, characteristic appearance on selective and differential media, as well as by the patterns of biochemical profiles using standard procedures [30]. Biochemical tests used in the identification of pathogenic isolates were selected and performed as per standard clinical methodology [31]. Antibiotic susceptibility testing was performed in accordance with the guidelines of the clinical and laboratory standards institution, employing a modified Kirby Bauer disc diffusion method [32]. Isolates were tested on Mueller Hinton agar (New England Biolabs, Midrand, South Africa). The following antibiotics were used in the assay: Amikacin (30ug), Ampicillin (10ug), Chloramphenicol (30ug), Ciprofloxacin (5ug), Ceftriaxoine (30ug), Ceftazidime (30ug), Erythromycin (5ug), Gentamicin (10ug), Imipenem (10ug),

Piperacillin/Tazobactum (100ug/10ug), Carbenicillin (100ug), Trimethoprim/Sulfamethoxazol (1.25ug/23.75ug), Oxacillin (1ug), and Vancomycin (30ug) (Mast group, UK). *E. coli* ATCC 25922 was used as a reference strain in all tests.

2.4 DNA Isolation

A half loopful of bacterial culture (approximately 1 cm sweep across the agar culture) was inoculated into 200 µl sterile TE buffer and boiled for 20 minutes in a water bath. The solution was allowed to cool on ice prior to centrifugation for 8 minutes at 9000 rpm. Twenty microlitres of supernatant was added to 80 µl of clean TE buffer. Quantification of crude DNA was done on the supernatant using a BioDoc analyse (Biometra, Germany). Crude DNA between 5-10 ng/ µl was used for PCR reactions.

2.5 Polymerase Chain Reaction for Detection of ESBL Genes

Conventional PCR was used for the genetic detection of three major ESBL genes namely; *bla*TEM, *bla*SHV and *bla*CTX-M from the *E. coli* (11), *Klebsiella pneumonia* (13) and *Pseudomonas aeruginosa* (8) isolates obtained in the study. The primers used for each gene have been described in previous studies [33]. One microliter of each of the DNA samples was mixed with all the necessary components for amplification in a 0.2ml PCR tube (Perkin-Elmer, USA) in a 10µl reaction volume and run on a Gene Amp PCR system 9700 thermocycler (Applied Biosystems, USA). The reaction mixture included 1µl Dream Taq buffer (10X concentration) (Thermo Scientific, USA), 0.2µl of deoxyribonucleotide triphosphate (dNTP) mix, 10mM (Thermo Fisher scientific, USA), 0.16µl of each of the forward and reverse primers, 0.4µM (Inqaba Biotech, South Africa) and 0.08 µl of Dream Taq DNA polymerase (Thermo Fisher Scientific, South Africa) and made up to 10 µl with nuclease free water. A *Klebsiella pneumoniae* isolate known to be positive for the assayed ESBL (*bla*TEM, *bla*SHV and *bla*CTX-M) genes was used as a positive control *bla*TEM (Genbank accession number KT818790), *bla*SHV (KT818791) and *bla*CTX-M (KT818792). The PCR profile was as follows: for *bla*CTX-M, *bla*SHV and *bla*TEM; denaturation 95°C for 2 minutes, 35 cycles (denaturation 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension 72°C for 1 minute) and the final extension at 72°C for 10 minutes. The amplified products were run on a 1% ethidium bromide stained agarose gel with a 100 bp DNA ladder (Thermo Scientific, USA) in TBE buffer for 1hr at 100V and then viewed using Uvipro-Silver Gel Documentation System (Uvitec, UK).

2.6 Polymerase Chain Reaction for Detection of MEC A Gene

The *S. aureus* isolates were all resistant to oxacillin (1µg) and were thus assayed for the presence of the *mec* A gene. One microliter of each of the DNA samples were mixed with all the necessary components for amplification in a 0.2ml PCR tube (Perkin-Elmer, USA) in a 10µl reaction volume and run on a Gene Amp PCR system 9700 thermocycler (Applied Biosystems, USA). The reaction mixture included 1µl Dream Taq buffer (10X concentration) (Thermo Scientific, USA), 0.2µl of deoxyribonucleotide triphosphate (dNTP) mix, 10mM (Thermo Fisher scientific, USA), 0.16µl of each of the forward (5'-GTAGAAATGACTGAACGTCCGATAA'-3) and reverse (5'-CCAATTCCACATTGTTTCGGTCTAA-3') primers, 0.4 µM (Inqaba Biotech, South Africa) and 0.08 µl of Dream Taq DNA polymerase (Thermo Fisher Scientific, South Africa) and made up to 10 µl with nuclease free water. The PCR profile for the *mec*A gene was as follows: denaturation 95°C for 2 minutes, 30 cycles (denaturation 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension 72°C for 30s) and the final extension at 72°C for 7 minutes. The amplified products were then run along a 1% ethidium bromide stained agarose gel with a 100 bp DNA ladder (Thermo Scientific, USA) in TBE buffer for 1hr at 100V and then viewed using a Uvipro-Silver Gel Documentation System (Uvitec, UK).

3. RESULTS AND DISCUSSION

In total 50 (86.21%) out of the 58 inanimate surfaces swabbed, and all 6 (100%) of the hand swabs obtained from the HCW were positive for microbial contamination. Poly-microbial growth was observed on 12 (20.69%) of 58 environmental surfaces, and 2 out of the 6 (33.33%) hand swabs. The acquisition or presence of MDR pathogens on HCWs hands or hospital surfaces reduces the quality of healthcare services provided at hospitals [26]. The elevated contamination rate ascertained in this study concurs with the 96.30% observed in a study from Morocco [10]. In contrast to our results, studies carried out in Iraq [34] and

Uganda [9] reported far lower surface contamination rates of 17.80% and 57.59% respectively. Variation in hand hygiene and sterilisation procedures could account for the differences. In our study we observed that hypochlorite based disinfectant diluted to as low as 1% was used to sterilize fomites in the adult ICU. Higher levels of microbial contamination observed in our study could be attributed primarily to the use of ineffective disinfectants during ward cleaning and sterilisation of shared medical devices. Consequently, we recommend the use of higher concentrations of the hypochlorite solution, or substituting it with an alternative alcohol based disinfectant. Infrequent cleaning of inanimate ICU surfaces and poor hand hygiene compliance by HCW could also contribute to poor microbial quality of the ICU surfaces. This places high risk patients in further jeopardy, by exposing them to even higher inoculums of bacteria capable of lingering on the environment for significant time periods. Gram negative bacteria (66.18%) comprised the greater percentage of the bacterial isolates as compared to Gram-positive bacteria (33.82%). Similar reports were documented by several authors [9,34]. Contrary to our findings studies conducted in Iran, southwest Nigeria and northern Nigeria recognised Gram-positive bacteria as the predominant environmental isolates [11,22,35]. Variation in methodology, study setting and geographical location could account for these differences.

A total of 37 swab specimens were procured from the adult ICU, of which 34 were obtained from environmental surfaces (fomites and medical equipment) and 3 were obtained from the hands of HCW working in the adult ICU. From these 37 swabs a total of 46 isolates were obtained; 43 from the environmental surfaces and 3 from the hands of HCW. A total 27 swab specimens were procured from the paediatric ICU, of which 24 were obtained from environmental surfaces and 3 were obtained from the hands of HCWs in the paediatric critical care ward. From these 27 swabs a total of 26 isolates were obtained; 21 from the environment and 5 from the hands of HCW. The isolated microbes and the ICU surfaces from which they were procured from are shown in Tables 1 and 2 respectively.

Table 1. Microorganisms isolated from the respective inanimate objects and HCW hands in the adult ICU

Microorganism	Animate/Inanimate surface microbe was isolated from adult ICU
E.coli	Visitor chair handle, monitor pad1, monitor pad2, monitor pad3, handles defibrillator, computer keyboard, emergency drug trolley, drug compartment
Klebsiella species	Patient bed mattress, monitor sensor probe, patient bed base, infusion pump, floor, liquid hand soap 1, clean mop, ventilator machine, oxygen tank,
S. aureus	Emergency drug trolley handles, medical chart1, male HCW1 hand swab*
CoNS	Telephone handle, Patient bed rail1, female HCW1 hand swab*, bedside curtain1, medical chart2, suction tubing interior2, female HCW2 hand swab*, ventilator nebuliser
Proteus vulgaris	Medical chart 1
Enterococcus faecalis	Medical chart2
Pseudomonas aeruginosa	suction tubing interior1, ventilator tubing interior, patient bed rail1, door handle2, monitor pad3, sink basin1, suction tubing interior2
LFC	Sink handle1, sisters work station, floor, clean mop, cardiac table,
NLF	Floor, clean mop, cardiac table, cardiac table2
Candida albicans	Ventilator nebuliser
No growth observed	Door handle1, liquid hand soap2

*KEY: CoNS; Coagulase negative Staphylococci, LFC; Lactose fermenting coliform (other than Klebsiella species, Escherichia coli or Proteus species), NLFE; Non-lactose fermenting enterobacteriaceae, HCW; Health Care Worker hands**

CoNS were frequently isolated from high contact surfaces, as well as from the hands of HCWs (Tables 1 and 2). The high prevalence of CoNS on inanimate surfaces in our study is comparable to studies by Cordeiro et al. [36] from Brazil and Lalami et al. [10] from Morocco. CoNS particularly *S. epidermidis* constitute part of the normal human flora, inhabiting the skin and mucous membranes [37]. CoNS are regularly shed onto the hospital environment by patients and medical personnel, whereupon they persist [14,18]. The hands of HCW can contaminate or become contaminated by ICU fomites and medical equipment after direct contact with these surfaces, and serve as vectors for the transmission of nosocomial pathogens [6,38]. The frequent isolation of CoNS from the hands of nurses (50%) in our study, and the isolation of CoNS from high-contact surfaces such as patient bed-rails, door handles, stethoscopes, medical charts and the unit telephone (Table 1 and 2) is evidence of the important relationship between HCWs' hands and the inanimate environment in the transmission of nosocomial pathogens. Russotto et al. [2] documented similar findings from ICUs in their literature. However it is important to note that clonality of isolates from HCW's hands and the inanimate environment was not proved in our study. Long considered clinical contaminants, CoNS have emerged globally as major causes of several types of HAIs [36,39,40].

In our study, *K. pneumoniae* was isolated from the main antiseptic hand wash solution used by HCW in the adult ICU (Table 1). Re-contamination of HCW hands with *K. pneumoniae* may result during hand washing. This could potentially lead to cross-transmission after hand-contact is made with patients, or environmental surfaces. Similar findings were documented in previous studies, were bacterial contamination of liquid hand soap used by HCW was linked to hospital outbreaks [41,42]. We also observed that during the manual cleaning of the ward, the water containing the disinfectant used to mop the floor was changed infrequently. This potentially perpetuates the dissemination of *K. pneumoniae* throughout the ward during cleaning.

P. aeruginosa (PSA) was predominantly isolated from sink faucets, sink basins and the interior of a post-sterilised ventilator tubing (Table 1 and 2). These findings are consistent with several reports that associated outbreaks in critical care wards to PSA contaminated sinks [8,43,44]. *P. aeruginosa* is primarily an environmental microorganism, and is particularly well- adapted to thrive in wet or moist conditions present in sinks and ventilator circuits [43].

Table 2. Microorganisms isolated from the respective inanimate objects and HCW hands in the paediatric ICU

Microorganism	Animate/Inanimate surface microbe was isolated from paediatric ICU
E.coli	Door handle; cardiac table1; female HCW3 hand swab*; bed pan (clean)
Klebsiella species	Bedside rail1; floor (by entrance); patient bed base; oxygen tank
S. aureus	Medical chart1; visitors bench handle
CoNS	Main door handle; blanket (clean); sink handle1; incubator exterior; female HCW1 hand swab*; female HCW3 hand swab*; drinking cup interior (clean); bedside rail1; stethoscope2
Pseudomonas aeruginosa	Sink basin1
LFC	Female HCW1 hand swab*
NLF	Sink handle1; mop (clean)
Candida albicans	Incubator exterior; Female HCW2 hand swab*; visitors bench handle
Proteus vulgaris	-
Enterococcus faecalis	-
No growth obtained	Paediatric bed pen; PICU liquid hand soap; nurses' station desk; stethoscope1; stethoscope3; nurses' station computer keyboard

*KEY: CoNS; Coagulase negative Staphylococci, LFC; Lactose fermenting coliform (other than Klebsiella species, Escherichia coli or Proteus species), NLFE; Non-lactose fermenting enterobacteriaceae, HCW; Health Care Worker hands**

Medical personnel in ICUs continually touch; door handles, patient monitor pad screens, computer keyboards, emergency drug trollies and defibrillator handles. The isolation of *E. coli* from these surfaces (Table 1 and 2) in our study is highly suggestive of faecal contamination via the hands of HCW who practice poor hand hygiene after use of the lavatories. These results correlate with reports from studies conducted in other resource limited countries [9,22,35]. Hand hygiene is absolutely paramount in the control and prevention of nosocomial infections [4,25].

The prevalence of antibiotic resistance phenotypes of the 68 bacterial isolates is presented in Table 3. *Klebsiella* species in our study were completely susceptible (100%) to Trimethoprim/sulfamethoxazole (Table 3).

However *Klebsiella* spp demonstrated the highest resistance of all isolates (38.46%) to imipenem. Pathogens of the Enterobacteriaceae family, particularly *K. pneumoniae* have a particular proclivity to acquire conjugative plasmids that are responsible for plasmid-encoded antibiotic resistance phenotypes. The accumulation of resistance genes results in strains that contain multi-drug resistant plasmids responsible for ESBLs and other mechanisms of resistance [45]. Imipenem was the most effective antimicrobial against *E.coli*, with a resistance rate of 9.09%. Resistance rates between the ranges 0% to 10% by *E.coli* to imipenem are globally comparable with several studies [9,46-48]. Gentamicin proved to be the second most effective antimicrobial against both *Klebsiella* and *E. coli* isolates; including ESBL producers. These findings are comparable with several reports from other African countries, including Nigeria and Ghana [9,22,26].

Resistance profiles of *S. aureus* and CoNS are shown in Table 3. *S. aureus* and CoNS isolates from the ICUs demonstrated elevated resistance to ampicillin. Ampicillin is one of the more commonly prescribed antibiotics in Zimbabwe [49]. Broad-spectrum antibiotic therapy with ampicillin exerts a selective pressure towards pathogens, perpetuating acquired drug resistance [37]. These high levels of ampicillin resistance are in accordance with findings from a Libyan study which reported 97.6% of Staphylococcal isolates from hospital environmental surfaces to be resistant to ampicillin [50]. Maryam et al. [22] also observed 100% of *S. aureus* isolates from hospital fomites in Nigeria to be ampicillin resistant. Imipenem displayed the highest efficacy against Staphylococci isolates, with 100% and 94.12% susceptibility rates for *S. aureus* and CoNS isolates respectively. A meta-analysis estimating the pooled resistance of clinical *S. aureus* isolates in Ethiopia reported resistance patterns of clinical isolates that are comparable to results obtained in our study [51].

Of the 5 *S. aureus* isolates obtained in this study, all were resistant to oxacillin (1µg) (Table 3). However, 3 (60%) of these 5 isolates were positive for the *mec*A gene. Although a small number of *S. aureus* isolates were obtained and assayed in our study, it is still alarming that MRSA isolates were present on ICU surfaces. In a study from Brazil, Rocha et al. [52] ascertained 36.3% of *S. aureus* isolated from ICU equipment to be resistant to oxacillin (MRSA/ORSA).

Vancomycin resistance was demonstrated by 2 (40%) *S. aureus* isolates and 4 of the 17 (23.54%) CoNS isolates (Table 3). Vancomycin resistant Staphylococci were also reported in a study from Nigeria, where 9.09% of Staphylococci isolates were vancomycin resistant, despite its dearth in usage [53]. Imipenem and ciprofloxacin displayed high efficacy against *P. aeruginosa* with 100% of isolates being susceptible to these 2 antibiotics, however high levels of resistance were demonstrated against carbenicillin, chloramphenicol and gentamicin (Table 3). Noticeable differences in the resistance profiles of *P. aeruginosa* isolated in our study and that of other studies [22,26] were observed. Variability in prescription patterns, methodology or strains of *P. aeruginosa* could account for these differences.

Resistance to β-lactam antibiotics was frequent amongst *E. coli* and *Klebsiella* isolates (*K. pneumoniae* and *K. oxytoca*) (Table 3). Co-resistance to ceftazidime and ceftriaxone by Enterobacteriaceae was used as a phenotypic indicator for isolates capable of ESBL production. Out of the 13 *Klebsiella* isolates, 9 (69.23%) were phenotypically positive for ESBL production, and out of the 11 *E. coli* isolates, 6 (54.55%) were phenotypically positive for ESBL production (Table 3). Similar findings were obtained by Tajeddin et al. [11] from Iran who reported 51.5% of *Klebsiella* and 77.8% of *E.coli* isolates to be ESBL positive, as well as Ahmed et al. [47] from Qatar who ascertained 51.4% of *Klebsiella* isolates to be ESBL positive. In accordance with our findings, literature from

Table 3. Antibiotic resistance percentages of all isolates obtained from paediatric and adult ICUs

Antibiotic (concentration)	Frequency of antibiotic resistant isolates procured from ICUs n (%)								
	Klebsiella species (n=13)	*E. coli (n=11)*	*Proteus species (n= 1)*	*P. aeruginosa (n=8)*	*S. aureus (n= 5)*	*CoNS (n=17)*	*E. faecalis (n=1)*	*LFC (n = 5)*	*NLFE (n= 7)*
Imipenem (10ug)	5(38.46)	1(9.09)	0(0)	0(0)	0(0)	1(5.88)	0(0)	0(0)	0(0)
Ciprofloxacin (5ug)	7(53.85)	7(63.64)	0(0)	0(0)	1(20)	15(88.24)	1(100)	1(20)	3(42.86)
Gentamicin (10ug)	1(7.69)	2(18.18)	1(100)	5(62.50)	4(80)	4(23.53)	0(0)	3(60)	2(28.57)
Chloramphenicol (30ug)	6(46.15)	7(63.64)	1(100)	5(62.50)	3(60)	3(17.65)	-	4(80)	4(57.14)
Trimethoprim/sulfamethoxazol (1.25ug/23.75ug)	0(0)	3(27.27)	0(0)	1(12.50)	-	-	-	3(60)	1(14.29)
Ampicillin (10ug)	11(84.62)	9(81.82)	0(0)	-	4(80)	14(82.35)	1(100)	4(80)	6(85.71)
Ceftriaxone (30ug)	9(69.23)	10(90.91)	0(0)	-	-	-	0(0)	3(60)	0(0)
Ceftazidime (30ug)	10(76.92)	6(54.55)	1(100)	-	-	-	-	2(40)	3(28.57)
Piperacillin/tazobactum (100ug/10ug)	-	-	-	4(50)	-	-	0(0)	-	-
Carbenicillin (100ug)	-	-	-	6(75)	-	-	-	-	-
Amikacin (30ug)	-	-	-	1(12.50)	-	-	-	-	-
Vancomycin (30ug)	-	-	-	-	2(40)	5(29.41)	0(0)	-	-
Erythromycin (5ug)	-	-	-	-	4(80)	11(64.71)	0(0)	-	-
Oxacillin (1ug)	-	-	-	-	5(100)	-	-	-	-

KEY: LFC- lactose fermenting coliforms (other than Klebsiella species, Escherichia coli or Proteus species); NLFE- non-lactose fermenting enterobacteriaceae

Table 4. Distribution of TEM, SHV and CTX-M in isolates

Organism	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
Detected Gene(s)	**Number of isolates N (%)**	**Number of isolates N (%)**	**Number of isolates N (%)**
CTX-M & TEM	1 (9.1)	0 (0)	0
CTX-M & SHV	0 (0)	0(0)	0
SHV& TEM	1 (9.1)	0(0)	0
CTX-M & SHV & TEM	6 (54.5)	2(25)	4(40)
Noneb	3 (27.3)	6(75)	9(60)
Total number of isolates assayed	11	8	13

KEY:b negative for all assayed ESBL genes

Table 5. Multi-drug resistance patterns of bacterial isolates

Proteus	*Klebsiella*	PSA	*Sau*	CONS	*E.coli*	LFC	NLF	Resistance pattern
1	0	0	0	0	0	0	0	GM, C, CXM
0	4	0	0	0	0	0	0	IMI, CIP, AP, CRO, CXM
0	1	0	0	0	0	0	0	IMI, C, AP, CRO, CXM
0	1	0	0	0	0	0	0	CIP, CRO, CXM
0	1	0	0	0	0	0	0	C, CIP, AP
0	1	0	0	0	0	0	0	CIP, AP, CRO,CXM
0	1	0	0	0	0	0	0	C, AP, CXM
0	1	0	0	0	0	0	0	AP, CRO, CXM
0	1	0	0	0	1	0	0	C, AP, CRO, CXM
0	0	1	0	0	0	0	0	IMI, CIP, GM, PY
0	0	2	0	0	0	0	0	GM, C, PTZ
0	0	1	0	0	0	0	0	GM, PY, C
0	0	1	0	0	0	0	0	GM, PY, AK, PTZ
0	0	1	0	0	0	0	0	PY, C, PTZ
0	0	1	0	0	0	0	0	PY, C, TS
0	0	0	1	0	0	0	0	VAN, GM, C, E, AP, CIP, OX
0	0	0	1	0	0	0	0	VAN, GM, C, E, AP, OX

Proteus	*Klebsiella*	PSA	*Sau*	CONS	*E.coli*	LFC	NLF	Resistance pattern
0	0	0	1	0	0	0	0	GM, C, E, OX
0	0	0	1	0	0	0	0	GM, E, AP, OX
0	0	0	0	1	0	0	0	IMI, VAN, GM, E, AP, CIP
0	0	0	0	3	0	0	0	VAN, GM, AP, CIP
0	0	0	0	1	0	0	0	VAN, C, E, AP, CIP
0	0	0	0	1	0	0	0	C, E, AP
0	0	0	0	1	0	0	0	C, E, AP, CIP
0	0	0	0	6	0	0	0	E, AP, CIP
0	0	0	0	0	1	0	0	IMI, CIP, C, TS, AP, CRO, CXM
0	0	0	0	0	1	0	0	CIP, C, AP, CRO, CXM
0	0	0	0	0	2	0	0	CIP, GM, C, AP, CRO, CXM
0	0	0	0	0	1	0	0	CIP, AP, CRO
0	0	0	0	0	1	0	0	CIP, TS, AP, CRO, CXM
0	0	0	0	0	1	0	0	TS, AP, CRO
0	0	0	0	0	0	1	0	GM, C, TS, AP, CRO
0	0	0	0	0	0	1	0	GM, C, AP, CRO, CXM
0	0	0	0	0	0	1	0	C, TS, AP, CRO,CXM
0	0	0	0	0	0	1	0	C, TS, AP
0	0	0	0	0	0	0	1	CIP, C, AP, CXM
0	0	0	0	0	0	0	1	CIP, GM, AP, CXM
0	0	0	0	0	0	0	1	CIP, GM, C

KEY: AK: Amikacin, AP: Ampicillin, C: Chloramphenicol, CIP: Ciprofloxacin, CRO: ceftriaxone, CXM: Cefuroxime, E: Erythromycin, GM: Gentamicin, I: Imipenem, OX: Oxacillin, PTZ: Piperacillin/tazobactum, PY: Carbenicillin, TS: Trimethoprim/sulfamethoxazol , VAN: Vancomycin, PSA: Pseudomonasaeruginosa, Sau: S.aureus, CoNS: Coagulase negative staphylococci, LFC: Lactose fermenting coliform, NLFE: Non-lactose fermenting Enterobacteriaceae

Uganda and the United Kingdom also established *Klebsiella* species as the most frequent ESBL producers [9,39]. In this study we also assayed ESBL genes using PCR in *Klebsiella* spp, *E. coli* and *P. aeruginosa*. Of the 11 *E. coli* isolates assayed 8 (72.7%) carried a combination of the *bla*TEM, *bla*SHV and *bla*CTX-M genes (Table 4).

Six isolates (54.5%) had the *bla*TEM, *bla*SHV and *bla*CTX-M combination, whilst the other 2 isolates had the *bla*TEM, *bla*SHV and the *bla*SHV and *bla*CTX-M combinations respectively. Four *Klebsiella* isolates (30.8%) and two *P. aeruginosa* isolates (25%) had the *bla*TEM, *bla*SHV and *bla*CTX-M combination (Table 4). The occurrence of ESBL genes in isolates obtained from both ICUs is a cause for concern. Molecular detection of ESBL producers is crucial as microbes confirmed positive for ESBL production are considered and reported as resistant to all extended spectrum beta-lactam antibiotics, regardless of their susceptibility test results [54]. The detection of ESBL genes in *E. coli* and *Klebsiella* isolates from ICUs is consistent with findings from other studies in Taiwan [55] and France [56]. Previous studies done in Zimbabwe have indicated that there are ESBL producing *E. coli* circulating in humans [29].

In this study, multi-drug resistance was defined as resistance to 3 or more antimicrobial classes [57]; where carbapenems, cephalosporin and penicillin were considered as separate classes. A total of 51 bacterial isolates (75%) were resistant to at least 3 antibiotics, and 39 multi-drug resistance patterns were observed (Table 5).

Klebsiella species demonstrated the highest rate of multi-drug resistance (84.62%). Of the 5 *S. aureus* isolates 3 were ascertained to be methicillin resistant *Staphylococcus aureus* (MRSA) through molecular detection of the *mec* A gene (results not shown). A significant proportion of clinically relevant bacterial isolates (75%) procured in this study were ascertained through AST to be multi-drug resistant (MDR) as shown in Table 5. Our findings are in accordance with a study by Tajeddin et al. [11] from Iran, who reported 79.38% of environmental isolates to be MDR, with the greater part of MDR isolates in their study being obtained from the critical care wards. Lower rates of multi-drug resistance were however, confirmed in other studies from Uganda and Libya with percentage rates of 16.17% and 59.5% respectively [9,50]. Intensive care units are the main source of upsurges in multi-drug resistant bacteria in hospitals [45]. Elevated levels of multi-drug resistance observed in our study could be attributed to the confinement of our study to ICU wards only. Higher rates of antibiotic administration to critical care patients repeatedly expose pathogens to antibiotics. This fosters antibiotic selective pressure, and the emergence of resistant microbes that persist in the ICU environment. Often times these ICU pathogens are embedded in complex biofilms, propagating the transfer of resistance plasmids [15].

5. CONCLUSION

To the best of our knowledge, this report serves as the first evaluation of the prevalence and antibiotic resistance patterns of clinically relevant pathogens isolated from fomites including shared medical equipment in ICUs in Zimbabwe. We conclude from this study that the burden of environmental microbial contamination and multi-drug resistance is alarmingly high. Neglect of effective surface sterilisation procedures, as well as hand hygiene non-compliance by HCWs potentially fuels cross-transmission of pathogens amongst critical patients, and could potentially lead to outbreaks in these ICUs. Our findings therefore indicate a dire need to drastically improve infection prevention control practices in intensive care wards.

6. LIMITATIONS

Our study had several limitations. Firstly, due to financial constraints our sampling was restricted to two ICUs of one major referral public hospital; these constraints also limited our sample size. Our results therefore might not be completely applicable to or representative of all ICUs across Zimbabwe.

7. RECOMMENDATIONS

Based on the results of this study, the IPC team at the hospital should be able to implement affordable and effective solutions to reduce the incidence of pathogenic bacteria on ICU surfaces and hence reduce the frequency of nosocomial infections acquired in the ICUs in the hospital. There is need to consider using higher concentrations of the cleaning solution or to consider changing it altogether. The IPC unit at the hospital should proceed to carry out an assessment of washing or cleaning procedures

(effectiveness of reagents used, adherence to hand washing practices and equipment sterilisation procedures).

CONSENT

Nurses sampled in the study gave written informed consent to participate in the study as required by the Medical Research Council of Zimbabwe (MRCZ/B/1340).

ETHICAL APPROVAL

Ethical approval was sought and granted by the Institutional review board of the Hospital were the study was done, the Ethics review board of the National University of Science and Technology and the Medical Research Council of Zimbabwe (MRCZ/B/1340).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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