Antibiogram Profile of Enteric pathogens Isolated from Fomites in Cross River University of Technology Medical Centre, Calabar, Nigeria

ABSTRACT

Background: Healthcare environments are considered as potential reservoirs for pathogenic microorganisms especially those responsible for nosocomial infections. Such microorganisms often present with varying degrees of drugs resistance. This study was aimed at evaluating the Cross River University of Technology (CRUTECH) Medical Center Environment for the presence of pathogenic bacterial contaminants and antibiotics susceptibility profile of such isolates. **Materials and Methodology**: A total of 72 swab samples were collected from nineteen frequently touched hospital surfaces and processed using the standard bacteriological procedures. The emergent bacterial colonies were identified using phenotypic and biochemical tests. Antibiotic susceptibility testing of the presumptively identified isolates was carried out using Kirby-Bauer's method. **Results**: Out of 72 swab samples collected, 44 (61.1%) were positive for Enteric bacterial pathogens. The mean viable count ranged from 1.8×10^6 Cfu/cm^2 (weighing scales) to 2.41×10^7 Cfu/cm^2 (wash sinks). The most prevalent isolate was *E. coli* (48 of 127, 37.8%) followed by *Klebseilla* sp (27 of 127, 21.3%), *Salmonella* sp (19 of 127, 14.9%), *Proteus* sp (12 of 127, 9.4%), *Citrobacter* sp (11 of 127, 8.7%), *Enterobacter* sp (7 of 127, 5.5%) while *Shigella* sp (3 of 127, 2.4%) was least isolated. Isolates demonstrated high level of susceptibility to Norfloxacin 124 (97.6%), Imipenem 116 (91.3%) and Chloramphenicol 105 (82.7%). Resistance to Erythromycin was 95 (74.8%), Gentamycin 84 (66.1%) and Amikacin 82 (64.6%). **Conclusion**: Contamination of healthcare surfaces by multi-drugs resistant pathogens is a potential risk, especially to hospitalized patients and health care workers. Thus, it is therefore imperative that appropriate hygienic measures be implemented to suppress any potential microbial cross-contamination.

KEYWORDS: Antibiogram, Enteric pathogens, Fomites, CRUTECH, Medical Centre, Calabar.

INTRODUCTION

Enterobacteriaceae are a large, heterogeneous group of medically important bacteria. Commonly, they are found in the soil, water, decaying matter, but their natural habitat is the intestinal tract of humans and animals [1,2]. This family of bacteria consists of Gram-negative, facultative anaerobic rods, which are oxidase-negative, and catalase positive [3]. Often referred to as enteric bacilli, some strains are metabolically important [4,5], while others such as *Salmonella*, *Shigella*, and *Yersinia* sp. are obligate human pathogens [1,6].

Essentially, disinfection keeps surfaces in hospital environments void of pathogens from the surrounding environments [7]. However, despite the various efforts made by healthcare workers to keep these environments sterile [8,9], such surfaces, whether constantly touched or not, are often contaminated. Reports have revealed the presence of such bacterial organisms as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, Acinetobacter sp, *Escherichia coli*, *Serratia marcescens*, *Shigella* sp, *Klebsiella pneumonia*, *Salmonella* sp, *Citrobacter* sp and *Yersinia* sp. [10,11,12,13], as well as fungi (*Candida* sp, *Aspergillus* sp etc) and viruses (adenoviruses, noroviruses, rotaviruses, influenza, parainfluenza, hepatitis B viruses and severe acute respiratory syndrome (SARS)-associated coronaviruses) [7,10,14] on these surfaces. Therefore, inanimate objects (fomites) have become potential reservoirs for the transmission of nosocomial (healthcare associated infections' (HCAI)) infections [15,16]. These are infections acquired by patients under medical care in the hospital or other healthcare facility which were absent at the time of admission [17,18,19]. The ability of microorganisms to survive on surfaces is enhanced by the production of adhesion molecules and biofilms [20].

Despite the available evidences that fomites harbor nosocomial causing pathogens [21], studies still assert that transmission of nosocomial infections is largely via hand contact with surfaces [22,23,24] as well as through direct contact from the healthcare workers' bacterial contaminated hands to patients [23,25]. Adherence to good hand hygiene would reduce the transmission chain [25,26,27], but the level of compliance to these measures by healthcare workers cannot be ascertained [28,29,30].

The discovery of antibiotics was a success in the fight against infectious diseases. However, emergence of resistance to the available antibacterial products remains a global concern in disease management [31]. This resistance could be as a result of indiscriminate, widespread and lengthy antibiotics usage [32,33]. Hence, it became imperative that regularly, the evaluation of the resistance trend among pathogenic bacteria isolated from both clinical and environmental sources be done.

Multiple drug resistance to many available antibiotics including fluoroquinolones and carbapenems have been demonstrated against Enterobacteriaceae isolated from Calabar metropolis [34,35]. The production of extended β-Lactamases among Enterobacteriaceae groups, which impede the potencies of ESBL drugs has generated global concern [3,36]. The situation in Nigeria is more worrisome because most health care settings in Nigeria are poorly funded [37], hence there is no proper antibiotic surveillance before administration.

According to Bereket et al. nosocomial infections cause about 5%-10% of all hospitalized cases in Europe and North America and in more than 40% of those in parts of Asia, Latin America, and sub-Saharan Africa [38].

Little is known about the antibiotic susceptibility profile of Enterobacteriaceae isolated from inanimate hospital surfaces in Calabar, and Cross River University of Technology Medical Centre in particular. This study therefore, seek to evaluate the prevalence and antibiotic susceptibility profile of Enterobacteriaceae from fomites in patients' wards of the Cross River University of Technology (CRUTECH) Medical Centre, Calabar campus, Nigeria.

METHODOLOGY

DESCRIPTION OF STUDY SITE:

The study was conducted in the Cross River University of Technology (CRUTECH) Medical Centre, Calabar campus. The school is located within Calabar South Local Government Area of Cross River State, Nigeria. The people of Calabar are mostly the Efik speaking people. The city has an area of 406 square kilometers and in 2006 census, the population of the city of Calabar was estimated to be approximately 371, 022**.** Calabar has a distinct tropical climate marked by dry and wet seasons with temperature and humidity varying with seasons [39, 40]. The study was carried out following the approval by the hospital management.

SAMPLING TECHNIQUE:

A total of 72 swab samples were collected from different constantly touched fomites within the medical center wards, between the periods from November 2019 to March 2020. The sampled fomites include: Stethoscopes (ST) 3, Wash sinks (WS) 2, Bedrails (BR) 4, Bedside tables (BST) 3, Sink knobs (SK) 4, Patients beds (PB) 6, Door handles (DH) 6, Ward walls (WW) 4, Floors (FL) 5, Staff phones (STP) 4, Dressing trollies (DTR) 3, Chairs (CH) 5, Pillow cases (PC) 5, Bathroom door knobs (BRDK) 3, Forceps (FC) 3, Fan switches (FSW) 4, Benches (BH) 2, Medical charts (MCH) 5 and Weighing scales (WGS) 1. The samples were obtained by rubbing sterile cotton wool swabs pre-moistened with 8.5% physiological saline on the various fomites until the swabs were visibly stained. The swabs were then put into sterile tubes, closed tightly, labeled appropriately and then transported immediately to the Microbiology Laboratory of the Cross River University of Technology, Calabar for onward processing and culture.

PREPARATION OF CULTURE MEDIA

Nutrient agar and MacConkey agar were weighed according to the manufacturer's specification into clean conical flasks containing the appropriate volume of distilled water. The flasks were plugged with aluminum foil and heated over a Bunsen flame to dissolve the agar completely. They were then sterilized by autoclaving at 121°C for 15 minutes, allowed to cool to about 45°C-50°C, aseptically poured into sterile Petri dishes and allowed to solidify.

CULTURE AND ISOLATION OF ENTEROBACTERIACEAE

The samples were processed and cultured according to the method described by [37]. Each batch of samples was cultured within 1 hour of collection. The collected swabs were aseptically placed inside sterile test tubes containing nutrient broth and incubated at 37^{o}C for 24 hours. Thereafter, the swab sticks were removed from the incubated tubes and streaked onto the surfaces of sterile pre-poured MacConkey agar plates and incubated at 37^{o}C for 18-24 hours. After 24 hours of incubation, the morphological appearances of the emergent

discrete colonies were noted. These discrete pink or pale colonies were purified by three successive sub-culture and re-isolation onto freshly prepared MacConkey agar plates.

The total heterotrophic viable counts were taken by serial dilution of the broth culture from 10^{-1} to 10^{-6}. Then about 0.5 mL aliquot from the 10^{-5} dilution was platted in triplicate into freshly prepared nutrient agar plates using spread plate method. The plates were incubated at 37°C for 24 hours. Colonies in the range of 30 to 300 were counted, and the average of colonies counted in each sample was multiplied by the reciprocal of the dilution factor to obtain the total viable bacterial count of the sample.

BIOCHEMICAL CHARACTERIZATION OF ISOLATES

The isolates were identified using standard microbiological protocols [41].

GRAM STAINING

A loopful of distilled water was placed on a grease-free slide, and used to make a smear of the test isolate. It was then allowed to air dry and heat fixed. The smear was flooded with crystal violet, allowed for 60 seconds and washed off with water. It was also flooded with Lugol's iodine for 60 seconds and rinsed off. The smear was decolorized with acetone-alcohol for 3 seconds and immediately rinsed with water. It was counter stained with Safranin for 30 seconds, rinsed and blotted dry. The preparation was then examined under a microscope using x100 oil immersion objective.

CATALASE TEST

Two drops of 3% freshly prepared hydrogen peroxide solution were placed on a grease-free slide, and a 24 hours test isolate was transferred onto the slide and observed immediately for gas bubbles.

MOTILITY TEST

Semi-solid nutrient agar was used. With the aid of a sterile inoculating needle, the medium was inoculated with the test isolate by making a fine stab to a depth of about 1-2 cm short of the bottom of the tube. The tubes were then incubated at 37^{o}C for 24 hours.

METHYL-RED TEST

Methyl red-Voges-Proskauer (MR-VP) broth was inoculated with the test isolate and incubated at 37^{o}C for 24 hours. About 5 drops of methyl-red reagent was added to the broth culture and observed for colour change.

VOGES-PROSKAUR TEST

MR-VP broth was inoculated with test isolate and incubated at 37°C for 24 hours. 3 ml of 5% α-naphthol was added, followed by 0.5 ml of KOH. The tube was shaken gently and remained undisturbed for 5 minutes and observed for a red surface layer within 2-5 minutes.

OXIDASE TEST

2 to 3 drops of oxidase reagent was added to a piece of filter paper in a Petri dish. The filter paper was allowed to absorb the reagent, and a sterile wooden applicator stick was used to pick a test isolate for smearing onto the moistened filter paper. A purple colour formation at the region of bacteria smear within 10 seconds was observed for.

SIMMON CITRATE TEST

A loopful of the test organism from nutrient agar was inoculated onto Simmons' citrate agar slant in test tubes, and incubated at 37^{o}C for 24 hours. The development of a deep blue colour was observed for.

INDOLE TEST

The test isolate was grown in 5ml sterile peptone water at 37^{o}C for 48 hours. After incubation, 0.5 ml of Kovac's reagent was added. The broth was observed for the development red colour layer.

TRIPLE SUGAR IRON (TSI) TEST

The surface of TSI agar was streaked with the test isolate and the butt was stabbed before being incubated at 37^{o}C for 24 hours. The TSI tubes were observed for the production of gas, acid and hydrogen sulphide.

UREASE TEST

A little of the culture of the test bacteria was streaked over the surface of the agar slant of urease test medium with phenol red as indicator and incubated at 37°C for 7 days. A control of the basal medium containing no added urea was equally inoculated. A colour change of the medium from yellow to pink or red was an indication of a positive result and no colour change indicate a negative result.

SUGAR FERMENTATION TEST

1ml of 10% sugar (maltose, lactose, mannitol, glucose, sucrose, xylose and sorbitol) solution was added to 10ml of the basal medium containing the indicator phenol red and Durham tube. The media were inoculated with test isolates and incubated at 37^{o}C for 2-5days and observed daily for color change. Acid and gas production were observed for.

PREPARATION OF 0.5 MCFARLAND

McFarland standard was prepared by mixing 1% solution (0.5 ml) of Barium chloride ($BaCl_2$) and 1% solution of (99.5 ml) of Sulphuric acid (H_2SO_4). The mixture was placed in a screw-capped bottle and stored at room temperature.

STANDARDIZATION OF THE INOCULUM

The test isolates were first grown in nutrient broth for 18-24 hours. An appropriate quantity for each test isolate was mixed into 4-5 ml of physiological saline. The suspension was diluted until it becomes slightly turbid to match the already prepared 0.5 McFarland standard.

ANTIBIOTIC SUSCEPTIBILITY TESTING (ATS)

Antibiotic susceptibility patterns of the isolated Enterobacteria were determined by Kirby-Bauer method [33], using 10 commercial antibiotics, according to the Clinical Laboratory Standards Institute guideline [42]: Nalidixic acid (NA, 30µg), Chloramphenicol (CH, 30µg), Imipenem (IPM, 10µg), Norfloxacin (NB, 10µg), Ciprofloxacin (CPX, 10µg), Augmentin (AU, 30µg), Erythromycin (E, 30µg), Gentamycin (CN, 10µg), Rifampicin (RX, 20µg) and Amikacin (AMK, 30µg).

The 24 hours broth culture of each test organism was suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 McFarland Standard. The standardized inoculums were seeded on to the surfaces of already prepared Mueller Hinton agar plates using sterile cotton swabs. The seeded plates were left to stand for about 30 minutes then the antibiotic disks were aseptically placed on the surfaces of the seeded plates with the aid of a sterile forceps. They were then inverted and incubated at 37°C for 24 hours. After incubation, any clear circular zones of growth inhibition around the immediate vicinity of any disk

indicated susceptibility to that antibiotic agent. These inhibition zone diameters were measured, and the results interpreted based on the CLSI recommendation [42, 43].
All of the tests were performed in triplicates and the resulting values of the IZDs recorded.

RESULTS

Mean Counts (CFU/mL) of Bacteria Obtained from Fomites in CRUTECH Medical Centre

The study revealed that the sampled fomites were contaminated. Out of 72 swabs samples collected from CRUTECH medical center wards, 44 (61.1%) were positive for Enterobacterial growth. WS recorded the highest mean viable counts (2.41×10^7CFU/mL) while WGS had the lowest counts (1.8×10^6CFU/mL) (Table 1).

Occurrence of Bacterial Species in the Sampled Fomites

Table 2 shows the morphological and biochemical characteristics of the isolates. Of 127 bacterial species isolated from the samples, *E. coli* had 48 out of 127 (37.8%), the most predominant member of Enterobacteria, followed by *Klebseilla* sp with 27 out of 127 (21.3%), then by *Salmonella* sp 19 out of 127 (14.9%), *Proteus* sp 12 out of 127 (9.4%), *Citrobacter* sp 11 out of 127 (8.7%), *Enterobacter* sp 7 out of 127 (5.5%) while *Shigella* sp 3 out of 127 (2.4%) was least isolated (Figure 1).

Antibiogram of Enterobacteria Obtained from Fomites in CRUTECH Medical Centre

Table 3 shows the results of the antibiogram obtained from the of Enterobacteria isolated from fomites in CRUTECH medical centre. The Enterobacteria demonstrated varying degrees of susceptibility and resistance to the test antibiotics. Among the antibiotics, susceptibility to Norfloxacin was 124(97.6%), Imipenem 116(91.3%) and Chloramphenicol 105(82.7%).

The level of resistance to Erythromycin was 95(74.8%), Gentamycin 84(66.1%) and Amikacin 82(64.6%).

As depicted in Table 3, all (100%) of *Enterobacter* sp were susceptible to Ciprofloxacin, Norfloxacin, Chloramphenicol and Imipenem. Susceptibility to Nalidixic acid and Rifampicin was 85.7% each. Lastly to Erythromycin and Augmentin (42.9%) each (Table 3).

The most effective drugs against *Shigella* sp were Nalidixic acid, Norfloxacin, Chloramphenicol and Imipenem with growth inhibition of 100%. However, the results revealed that *Shigella* sp was completely (100%) resistant to Amikacin, Gentamycin and Erythromycin (Table 3).

All 11 (100%) of *Citrobacter* sp showed susceptibility to Norfloxacin and Imipenem respectively, followed by Ciprofloxacin and Nalidixic acid with 10 out of 11 (90.9%) each. While 9 out of 11 (81.8%,) were susceptible to Augmentin (Table 3).

Klebseilla sp was (100%) susceptible to Nalidixic acid and Norfloxacin, while 92.6%, 85.2% and 81.5% susceptibility was observed to Ciprofloxacin, Imipenem and Rifampicin respectively.

However, 92.6%, 85.2% and 55.6% of *Klebeilla* sp was resistant to Amikacin, Erythromycin and Gentamycin respectively (Table 3).

Salmonella spp was (100%) susceptible to Norfloxacin and Imipenem, followed by Chloramphenicol (89.5%), Rifampicin (84.2%) and Ciprofloxacin (78.9%). High resistance was shown against Gentamycin (73.7%) and Erythromycin (68.4%).

The most effective drugs against *E. coli* were Norfloxacin (100%), followed by chloramphenicol (95.8%) and Imipenem (85.4%). However, *E. coli* isolates demonstrated high resistance against Erythromycin (79.2%), Gentamycin (72.9%), Amikacin (77.1%) and Rifampicin (75.0%) (Table 3).

Contrastingly, *Proteus* sp showed significant susceptibility to the test antibiotics except for Augmentin and Gentamycin which showed 58.3% and 50.0% resistance respectively (Table 3).

Table 1: Samples and Occurrence of Bacterial Isolates from Fomites at CRUTECH Medical Centre

Fomite	No of sample	No of positive growth	Mean viable count (Cfu/cm^2)	No of isolate	Total % of isolate	Organisms isolated
ST	3	3	5.8×10^6	5	3.94	*E. coli*; *Citrobacte*r sp; *Klebseilla* sp
WS	2	2	2.41×10^7	7	5.51	*Shigella* sp; *Salmonella* sp; *E. coli*; *Proteus* sp; *Klebseilla* sp
BR	4	1	1.9×10^6	3	1.38	*Salmonella* sp; *E. coli*; *Proteus* sp
BST	3	1	6.8×10^6	6	4.72	*Salmonella* sp; *E. coli*; *Klebseilla* sp
SK	4	2	1.11×10^7	5	3.94	*E. coli*; *Citrobacter* sp; *Klebseilla* sp
PB	6	1	7.8×10^6	4	3.15	*Enterobacter* sp; *E. coli*
DH	6	6	1.68×10^7	12	9.45	*Salmonella* sp; *E. coli*; *Citrobacter* sp; *Klebseilla* sp
WW	4	1	2.1×10^6	5	3.94	*Enterobacter* sp; *Proteus* sp; *Klebseilla* sp
FL	5	5	1.24×10^7	10	7.87	*Shigella* sp; *Salmonella* sp; *E. coli*; *Citrobacter* sp; *Klebseilla* sp
STP	4	4	9.8×10^6	9	7.09	*Enterobacter* sp; *E. coli*; *Citrobacter* sp; *Proteus* sp; *Klebseilla* sp
DTR	3	2	5.3×10^6	6	4.72	*Salmonella* sp; *E. coli*; *Klebseilla* sp
CH	5	2	1.32×10^7	8	6.30	*Salmonella* sp; *E. coli*; *Proteus* sp; *Klebseilla* sp
PC	5	1	4.1×10^6	5	3.94	*Salmonella* sp; *E. coli*; *Proteus* sp; *Klebseilla* sp
BRDK	3	3	1.72×10^7	7	5.51	*Salmonella* sp; *E. coli*; *Proteus* sp; *Klebseilla* sp
FC	3	1	5.3×10^6	3	1.38	*Salmonella* sp; *Proteus* sp
FSW	4	3	2.7×10^6	8	6.30	*Enterobacter* sp; *Salmonella* sp; *E. coli*; *Citrobacter* sp *Klebseilla* sp
BH	2	1	4.3×10^6	7	5.51	*E. coli*; *Citrobacter* sp
MCH	5	4	3.4×10^6	11	8.66	*E. coli*; *Proteus* sp; *Klebseilla* sp
WGS	1	1	1.8×10^6	6	4.72	*E. coli*; *Klebseilla* sp

KEY: ST= Stethoscope; WS = Wash sink; BR= Bedrails; BST= Bedside tables; SK= Sink knobs; PB= Patient bed; DH= Door handles; WW= Ward walls; FL= Floor; STP= Staff phones; DTR= Dressing trolley; CH= Chairs; PC= Pillow case; BRDK= Bathroom door knobs; FC= Forceps; FSW= Fan switches; BH= Benches; MCH= Medical chart; WGS= Weighing scale.

Table 2: Morphological and Biochemical Characteristics used in Identification of Isolates Obtained from Fomites

Character							
Cell's shape	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Gram stain	-	-	-	-	-	-	-
Motility	+	-	+	-	+	+	+
Catalase	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-
Indole	-	v	V	-	-	+	v
Methyl-red	v	+	+	-	+	+	+
Voges-proskaur	+	-	-	+	-	-	v
Simmon citrate	+	-	+	+	+	-	v
Urease	-	-	+	v	-	-	+
Glucose	+	+	+	+	+	+	+
Sucrose	+	-	+	v	-	+	+
Lactose	+	v	+	+	-	+	-
Maltose	+	+	+	+	+	+	+
Mannitol	+	-	+	+	+	+	-
Sorbitol	+	+	+	+	-	+	+
Xylose	+	-	+	+	-	+	+
TSI	A/A, G	K/A	K/A, G+H_2S	A/A, G	K/A, G+H_2S	K/A, G	K/A, G+H_2S
Probable organism	*Enterobacter* sp	*Shigella* sp	*Citrobacter* sp	*Klebseilla* sp	*Salmonella* sp	*E. coli*	*Proteus* sp
No isolated	7	3	11	27	19	48	12

KEY: A= Acid; K= Alkaline; G= Gas; H_2S= Hydrogen sulphide; TSI= Triple sugar iron agar test; - = Negative; + = Positive; v = Different strains give different results.

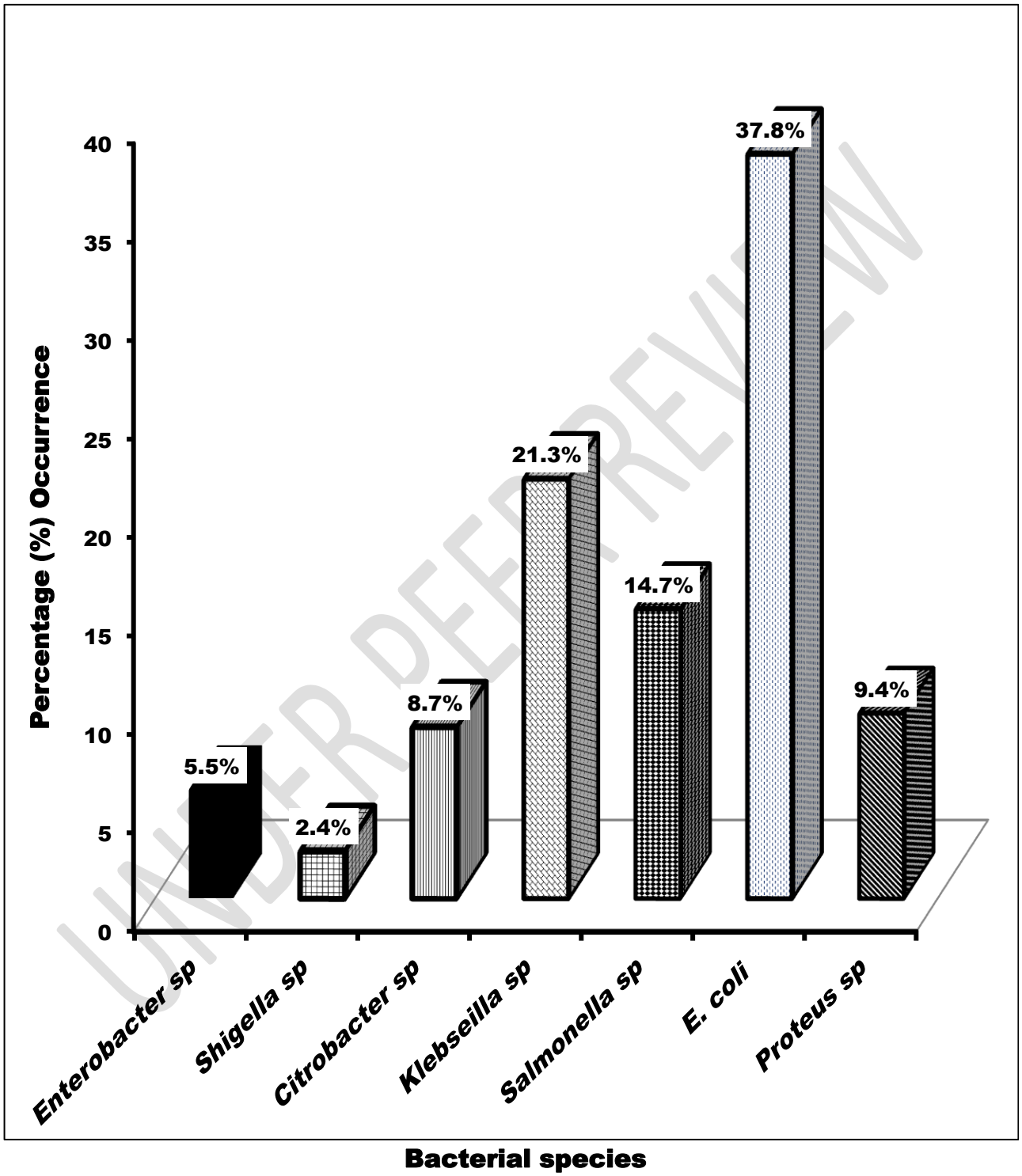

Figure 1: Prevalence of bacteria isolated from fomites at CRUTECH medical centre

Table 3: Antibiogram of Enterobacteriaceae Isolated from Fomites in CRUTECH Medical Centre

			***Enterobacter* sp (N=7)**		***Shigella* sp (N=3)**		***Citrobacter* sp (N=11)**		***Klebseilla* sp (N=27)**	
Antimicrobial Category	**Antimicrobial Tested**	**Disc (µg)**	**S n(%)**	**R n(%)**	**S n(%)**	**R n(%)**	**S n(%)**	**R n(%)**	**S n(%)**	**R n(%)**
Aminoglycosides	Amikacin (AMK)	30	4 (57.1)	3 (42.9)	0 (0.0)	3 (100)	7 (63.6)	4 (36.4)	2 (7.4)	25 (92.6)
	Gentamycin (CN)	10	4 (57.1)	3 (42.9)	0 (0.0)	3 (100)	3 (27.3)	8 (72.7)	12 (44.4)	15 (55.6)
Macrolides	Erythromycin (E)	30	3 (42.9)	4 (57.1)	0 (0.0)	3 (100)	2 (18.2)	9 (81.8)	4 (14.8)	23 (85.2)
Fluoroquinolones	Ciprofloxacin (CPX)	10	7 (100)	0 (0.0)	2 (66.7)	1 (33.3)	10 (90.9)	1 (9.1)	25 (92.6)	2 (7.4)
	Nalidixic acid (NA)	30	6 (85.7)	1 (14.3)	3 (100)	0 (0.0)	10 (90.9)	1 (9.1)	27 (100)	0 (0.0)
	Norfloxacin (NB)	10	7 (100)	0 (0.0)	3 (100)	0 (0.0)	11 (100)	0 (0.0)	27 (100)	0 (0.0)
Phenicols	Chloramphenicol	30	7 (100)	0 (0.0)	3 (100)	0 (0.0)	9 (81.8)	2 (18.2)	13 (48.1)	14 (51.9)
Carbapenems	Imipenem (IPM)	10	7 (100)	0 (0.0)	3 (100)	0 (0.0)	11 (100)	0 (0.0)	23 (85.2)	4 (14.8)
Ansamycin	Rifampicin (RX)	20	6 (85.7)	1 (14.3)	2 (66.7)	1 (33.3)	8 (72.7)	3 (27.3)	22 (81.5)	5 (18.5)
β-Lactams	Augumentin (AU)	30	3 (42.9)	4 (57.1)	2 (66.7)	1 (33.3)	9 (81.8)	2 (18.2)	15 (55.6)	12 (44.4)
	Total %		**54 (77.1)**	**16 (22.9)**	**22 (73.3)**	**8 (26.7)**	**80 (72.7)**	**30 (27.3)**	**170 (63.0)**	**100(37.0)**

KEY: N = Number of isolates, % = Number of the organism isolated/total number of organisms isolated x 100, n(%)= Number of isolates susceptible/resistant and their percentage, R-Resistant, S-Susceptible.

Table 3: Antibiogram of Enterobacteriaceae Isolated from Fomites in CRUTECH Medical Centre Continue

			***Salmonella* sp (N=19)**		***E. coli* (N=48)**		***Proteus* sp (N=12)**	
Antimicrobial Category	**Antimicrobial Tested**	**Disc (µg)**	**S n(%)**	**R n(%)**	**S n(%)**	**R n(%)**	**S n(%)**	**R n(%)**
Aminoglycosides	Amikacin (AMK)	30	11 (57.9)	8 (42.1)	11 (22.9)	37 (77.1)	10 (83.3)	2 (16.7)
	Gentamycin (CN)	10	5 (26.3)	14 (73.7)	13 (27.1)	35 (72.9)	6 (50.0)	6 (50.0)
Macrolides	Erythromycin (E)	30	6 (31.6)	13 (68.4)	10 (20.8)	38 (79.2)	7 (58.3)	5 (41.7)
Fluoroquinolones	Ciprofloxacin (CPX)	10	15 (78.9)	4 (21.1)	23 (47.9)	25 (52.1)	9 (75.0)	3 (25.0)
	Nalidixic acid (NA)	30	9 (47.4)	10 (52.6)	24 (50.0)	24 (50.0)	8 (66.7)	4 (33.3)
	Norfloxacin (NB)	10	19 (100)	0 (0.0)	48 (100)	0 (0.0)	9 (75.0)	3 (25.0)
Phenicols	Chloramphenicol	30	17 (89.5)	2 (10.5)	46 (95.8)	2 (4.2)	10 (83.3)	2 (16.7)
Carbapenems	Imipenem (IPM)	10	19 (100)	0 (0.0)	41 (85.4)	7 (14.6)	12 (100)	0 (0.0)
Ansamycin	Rifampicin (RX)	20	16 (84.2)	3 (15.8)	12 (25.0)	36 (75.0)	7 (58.3)	5 (41.7)
β-Lactams	Augumentin (AU)	30	9 (47.4)	10 (52.6)	20 (41.7)	28 (58.3)	5 (41.7)	7 (58.3)
	Total %		**126 (66.3)**	**64 (33.7)**	**248 (51.7)**	**232 (48.3)**	**83 (69.2)**	**37 (30.8)**

KEY: N = Number of isolates, % = Number of the organism isolated/total number of organisms isolated x 100, n(%)= Number of isolates susceptible/resistant and their percentage, R-Resistant, S-Susceptible.

DISCUSSION

Multi-drug resistance (MDR) pathogens are constantly evolving, especially in healthcare environment. These organisms are often transmitted via hospital surfaces and healthcare worker's hands leading to widespread of nosocomial infections [44, 45]. In light of this, inanimate surfaces in CRUTECH medical centre were investigated for the presence of Enterobacteriaceae. Also, susceptibility pattern of isolated organisms to commonly used antibiotics was determined.

In this study, out of 72 swab samples collected, 44 (61.1%) were contaminated with Enterobactericeae. The contamination of fomites observed in this study could be attributed to non-compliance to healthcare associated infections guidelines, overcrowding, and inadequate surveillance system [30, 28, 46]. In other study, similar contamination rate was observed [47] but lower than 71.7% (71 of 99) reported by Birru *et al*. [48]. The differences in contamination rate observed could be attributed to magnitude of samples and population density of visitors and patients in the facility. The study revealed that potentially pathogenic Enterobacteriaceae are present in different sampled fomites. This findings affirmed earlier report that inanimate hospital surfaces harbors pathogenic organisms [44, 49, 50].

The predominant member of Enterobacteriaceae in this study was *E. coli* with prevalent rate of 37.8%, followed by *Klebseilla* sp (21.3%). This is in line with other report [33]. Others are *Salmonella* sp (14.9%), *Proteus* sp (9.4%), *Citrobacter* sp (8.7%), *Enterobacter* sp (5.5%) and *Shigella* sp (2.4%). The bacterial species isolated in this study has been reported previously from hospital surfaces [51, 45, 12, 52]. Occurrence and survival of organisms on inanimate surfaces is greatly influenced by their ability to inhabit dry surfaces [45]. This intrinsic feature is attributed to the presence of surface molecules (flagella, pili and polysaccharide capsule) and the production of extracellular matrix (adhesion molecules and biofilms) [17, 51]. The prevalence of *E. coli* as the predominate member of Enterobacteriaceae in this study conform to other investigations. For instance, a research conducted in teaching hospital in northern Nigeria, reported *E. coli* (26.1%) as the most predominant Gram negative bacteria followed by *Klebseilla* sp (13.0%) [53]. Elsewhere in Kuwait, it was found that, of 20% gram negative bacteria isolated from inanimate objects, *E. coli* (10%) had the highest degree of occurrence [51].

However, when compared with other studies, there exist variations in terms of prevalent rate of isolated bacteria. These differences could be attributed to a number of factors including: sanitary measures adopted by individuals hospitals, nature and location of fomites sampled, and type of healthcare facility under investigation. For instance, Segujja *et al*. [13] argued that prevalence rate is higher in teaching hospitals compared to non-teaching hospitals. Contamination of sampled fomites with pathogenic bacteria observed in this study corroborate previous report on the linked between hospital fomites and nosocomial infections [21, 45, 54, 55, 22]. Occurrence of enteric pathogens [3, 56, 2] in the analysed fomites pose a high risk to patients and is an indication of feacal contamination and inadequate hygiene [12, 13].

Most of the isolates demonstrated varying degree of susceptibility and resistant to the tested antibiotics (Table 3). In general, Enterobacteriaceae demonstrated highest level of susceptibility to Norfloxacin 124(97.6%), Imipenem 116(91.3%), Chloramphenicol 105(82.7%), and Ciprofloxacin 91(71.7%) and high level of resistance to Erythromycin 95(74.8%), Gentamycin 84(66.1%) and Amikacin 82(64.6%).

Among the antibiotics tested, Ciprofloxacin, Norfloxacin, Chloramphenicol and Imipenem were the most active drugs against *Enterobacter* sp (100%) followed by Nalidixic acid and Rifampicin (85.7% each). On the other hand, the isolate showed resistance to macrolides (Erythromycin), aminoglycosides (Amikacin and Gentamycin), and β-Lactams (Augumentin)

at 57.1%, 42.9%, 42.9% and 42.9% respectively. Resistance of *Enterobacter* sp to macrolides, aminoglycosides and β-Lactams has previously been reported [48, 57, 34]. Resistance of *Enterobacter* sp as observed above may be mediated by the production β-Lactamses [58]. However, there exists variation in terms of percentage of resistance of *Enterobacter* sp to Gentamycin and Amikacin recorded in our study compared to 66.7% and 33.3% report by Birru *et al*. [48] respectively. These differences may be due to abuse of drugs. 100% of *Shigella* sp was resistance to Amikacin, Gentamycin and Erythromycin. Resistance of organisms to macrolides and aminoglycosides is chiefly mediated by efflux protein and ribosomal mutation [59, 60]. The high resistance against aminoglycosides and macrolides in our study could be attributed to misuse of these classes of antibiotics [61].

E. coli showed highest resistance rate to all the tested antibiotics (48.3%). *E. coli* is known to produce Extended Spectrum β-Lactamses [57, 62, 63]. These enzymes inactivate the potencies of antibiotics; this explains its exceptional insensitivity to classes of antibiotics as seen in this study. However, Norfloxacin (100%), Chloramphenicol (95.8%) and Imipenem (85.4%) were effective against *E. coli* isolates. *E. coli* isolates from hospital environment has been shown to resist a number of antibiotics [13, 34]. The 79.2% resistance to gentamycin recorded in our study disagrees with other findings [53] and [64] who reported 100% and 75% sensitivity of *E. coli* isolates to gentamycin respectively. Resistance to Erythromycin as observed in this study corroborates [55] who reported 60% resistance of *E. coli* to Erythromycin. Also, 83.3% susceptibility of *E. coli* to ciprofloxacin was published [53] while in our study 47.9% was recorded.

Similarly, *Klebseilla* sp showed complete (100%) susceptibility to Nalidixic acid and Norfloxacin. However, 92.6%, 85.2% and 55.6% of *Klebeilla* sp was resistance to Amikacin, Erythromycin and Gentamycin respectively. *Klebseilla pneumoniae* and *Klebseilla oxytoca* isolated from fomites in intensive care unit of university of Maiduguri teaching hospital demonstrated resistance to macrolides, aminoglycosides, beta-lactams and fluoroquinolones [64]. However, in this study, 2(7.4%) *Klebseilla* sp resisted antibacterial effect of Ciprofloxacin compared to 20% and 33% reported against *Klebseilla pneumoniae* and *Klebseilla oxytoca* respectively [64]. The 51.9% and 55.6% resistance against Chloramphenicol and Gentamycin recorded in our study is higher than 48.1% and 29.6% reported by Ayalew *et al*. [44] in Ethiopia. Also, *Citrobacter* sp was 100% susceptible to Norfloxacin and Imipenem, followed by Nalidixic acid and Ciprofloxacin (90.9%). This is in line with [44] but disagrees with 100% susceptibility to Gentamycin reported by the same authors. Contrastingly, 81.8% of *Citrobacter* sp were sensitive to beta-lactams (Augumentin). *Salmonella* sp demonstrated similar trend. Meanwhile, high resistant was showed against Gentamycin (73.7%) and Erythromycin (68.4%). *Proteus* sp showed significant susceptibility to the tested antibiotics except for Augumentin and Gentamycin were (58.3%) and (50.0%) resistance was recorded respectively. This results corroborate 100% sensitivity to Imipenem recorded previously [13]. The high resistance rate observed among members of Enterobacteriaceae support earlier assertion that majority of multidrug resistant isolates in clinical and environmental samples are Gram negative bacteria [65, 66, 67]. Gram negative bacteria possessed outer membrane in addition to cell wall. This membrane prevents many substances from entering into the cell [3, 68]. Multi-drugs resistant strains pose serious health effects with attendant treatment failure, prolong hospital stay and increase cost of treatment [66].

CONCLUSION

In conclusion, results from our study have revealed that inanimate surfaces from CRUTECH medical centre habour member Enterobacteriaceae most of which demonstrated multidrug resistant to commonly used antibiotics. The most effective drug against the isolated organisms was Norfloxacin 124(97.6%), Imipenem 116(91.3%), Chloramphenicol

105(82.7%), and Ciprofloxacin 91(71.7%). However, high levels of resistance to Erythromycin 95(74.8%), Gentamycin 84(66.1%) and Amikacin 82(64.6%) were recorded. Contamination of healthcare surfaces with multi-drugs resistance pathogens is a potential risk, especially to hospitalized patients. Thus, appropriate hygienic measures to suppress any potential microbial cross-contamination are therefore needed.

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SUPPLEMENTARY DATA

APPENDIX 1: Samples and Occurrence of Bacterial Isolates from Fomites at CRUTECH Medical Centre

Fomite	No of sample	Positive growth	Mean viable count (Cfu/cm^2)	No of isolate	Total % of isolate	*Enterobacter* sp n(%)	*Shigella* sp n(%)	*Citrobacter* sp n(%)	*Klebseilla* sp n(%)	*Salmonella* sp n(%)	*E. coli* n(%)	*Proteus* sp n(%)
ST	3	3	5.8×10^6	5	3.94%	0 (0.0)	0 (0.0)	1 (0.79)	3 (2.36)	0 (0.0)	1 (0.79)	0 (0.0)
WS	2	2	2.41×10^7	7	5.51%	0 (0.0)	1 (0.79)	0 (0.0)	1 (0.79)	1 (0.79)	3 (2.36)	1 (0.79)
BR	4	1	1.9×10^6	3	1.38%	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.79)	1 (0.79)	1 (0.79)
BST	3	1	6.8×10^6	6	4.72%	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.57)	1 (0.79)	3 (2.36)	0 (0.0)
SK	4	2	1.11×10^7	5	3.94%	0 (0.0)	0 (0.0)	1 (0.79)	3 (2.36)	0 (0.0)	1 (0.79)	0 (0.0)
PB	6	1	7.8×10^6	4	3.15%	2 (1.57)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.57)	0 (0.0)
DH	6	6	1.68×10^7	12	9.45%	0 (0.0)	0 (0.0)	1 (0.79)	2 (1.57)	1 (0.79)	8 (6.29)	0 (0.0)
WW	4	1	2.1×10^6	5	3.94%	2 (1.57)	0 (0.0)	0 (0.0)	1 (0.79)	0 (0.0)	0 (0.0)	2 (1.57)
FL	5	5	1.24×10^7	10	7.87%	0 (0.0)	2 (1.57)	1 (0.79)	1 (0.79)	5 (3.93)	1 (0.79)	0 (0.0)
STP	4	4	9.8×10^6	9	7.09%	1 (0.79)	0 (0.0)	3 (2.36)	1 (0.79)	0 (0.0)	3 (2.36)	1 (0.79)
DTR	3	2	5.3×10^6	6	4.72%	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.79)	2 (1.57)	3 (2.36)	0 (0.0)
CH	5	2	1.32×10^7	8	6.30%	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.57)	2 (1.57)	3 (2.36)	1 (0.79)
PC	5	1	4.1×10^6	5	3.94%	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.57)	1 (0.79)	2 (1.57)	0 (0.0)
BRDK	3	3	1.72×10^7	7	5.51%	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.57)	1 (0.79)	3 (2.36)	1 (0.79)
FC	3	1	5.3×10^6	3	1.38%	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.57)	0 (0.0)	1 (0.79)
FSW	4	3	2.7×10^6	8	6.30%	2 (1.57)	0 (0.0)	2 (1.57)	1 (0.79)	2 (1.57)	1 (0.79)	0 (0.0)
BH	2	1	4.3×10^6	7	5.51%	0 (0.0)	0 (0.0)	2 (1.57)	0 (0.0)	0 (0.0)	5 (3.93)	0 (0.0)
MCH	5	4	3.4×10^6	11	8.66%	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.57)	0 (0.0)	5 (3.93)	4 (3.15)
WGS	1	1	1.8×10^6	6	4.72%	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.36)	0 (0.0)	3 (2.36)	0 (0.0)

KEY: ST= Stethoscope; WS = Wash sink; BR= Bedrails; BST= Bedside tables; SK= Sink knobs; PB= Patient bed; DH= Door handles; WW= Ward walls; FL= Floor; STP= Staff phones; DTR= Dressing trolley; CH= Chairs; PC= Pillow case; BRDK= Bathroom door knobs; FC= Forceps; FSW= Fan switches; BH= Benches; MCH= Medical chart; WGS= Weighing scale.