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BACTERIAL PROFILE AND THEIR ANTIBIOGRAM ON COMPUTER KEY BOARDS AND OTHER FOMITES USED IN FEDERAL TEACHING HOSPITAL ABAKALIKI EBONYI STATE, NIGERIA

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ABSTRACT

The transmission of infection through inanimate objects constitutes a major threat to public health especially in the developing countries. This study was carried out to isolate and identify potential bacteria reservoirs that may be responsible for nosocomial infections, in Federal Teaching Hospital, Abakaliki, (FETHA). A total of 100 swabs was taken from computer keyboards, mouse, taps, bedrails, work benches, stethoscope, sphygmomanometer, thermometer, file folder and door handles. Four selective and differential media were used, Manitol salt agar, blood agar, *Salmonella- Shigella* agar and MacConkey agar. The isolates obtained were examined and identified by colonial morphology, Gram reaction and biochemical characteristics. Also antimicrobial susceptibility was performed on the isolates by paper disc diffusion method. A total of 279 bacteria were isolated. Gram negative bacteria had the highest prevalence of 174 (62.4 %) while Gram positive bacteria were 105 (37.6 %) in occurrence. *Staphylococcus aures* was the most predominant bacteria 72 (25.8 %) followed by *Salmonella* species 53(19.0 %) and the least *Proteus* species 17(6.1 %). Gram positive bacteria were highly susceptible to Erthromycin, Streptomycin, Ciprofloxacin, Levofloxacin and Gentamicin while Gram negative bacteria were mostly susceptible to Gentamincin, Cephalexin, Ciprofloxacin and Streptomycin. Their presence on the sampled surfaces may probably pose a significant public health risk to the hospital Community and its surrounding. Periodic cleaning of the fomites in use with appropriate disinfectants before an after each contact with patients is recommended.

Keywords: Bacterial profile, Antibiogram, Computer key boards, Formites and FETHA

INTRODUCTION

Bacteria that can cause infectious diseases are found every where. The environments where they are found include our body, house, food, soil, water, other organisms and other environmental objects [1]. These infectious disease caused by bacteria can be transmitted to humans through air, vectors and inanimate objects that called fomites [2]. From past research, it was revealed that bacteria contaminate and also colonize inanimate objects found in house, school premises, day care centres, office areas, and hospitals [3, 4, 5 and 6]. The presence of disease causing bacteria was also reported by these scientists [7, 8 and 9].

Furthermore, bacteria that contaminate fomites have been reported to remain on inanimate surfaces for a long period of time which may stretch from hours to months [10]. There is also the report of cross infection of bacteria between fomites found in the environment and their hosts [11]. The common objects that are contaminated by bacteria include currency notes, office desks, kitchen sinks, door handles, computer keyboards and mouse, cell phones, automated teller machine and elevator bottons [12 and 13].

Computer keyboards and mouse are components of computer in use with the increase in demand of computer system application. Computer keyboards and mouse are component of a computer that is used every day in performing different computer tasks in various parts of the society. There is now high demand and use of computer in cybercafés, banks, schools, offices, research centres and hospitals [14]. The ability of computer keyboards and mouse to act as a fomite have been documented in hospital and Health care environment [15 and 16]. Also in none hospital environment [17]. In addition to computer keyboards and mouse being documented as fomites, other hospital equipment and instrument such as thermometer, sphygmomanometer, stethoscope, folder, bedrail, work benches and taps have also been reported as being contaminated with pathogenic bacteria [18].

The main cause of bacterial contamination of keyboard and other fomites in hospital settings may be eating lunch while working so crumbs and spills can fall on the fomites. The food deposit encourages the growth of bacteria. Another possible cause may be from gloves that have been contaminated and poor hygiene of health care specialist due to their neglecting to wash hands after going to the bathroom and examining patients. Dust can also trap moisture and enable any bacteria that are already on the keyboards to multiply. Another potential cause of keyboards and other fomites contamination is sharing it among workers one of when may have coughed and sneezed. Also because of the great number of people that visits the hospital, majority of them may be sick and from them comes new bacteria that will be transferred to keyboards and other fomites through air or from physical contact [19 and 20].

Many factors have been shown to influence bacterial contamination of fomites. These include temperature, moisture levels, pH, pressure and friction between surfaces [21 and 22].

Aim of Study

The aim of this study is to evaluate the degree of bacterial profile and their anti-biogram on computer keyboards and other fomites used in Federal Teaching Hospital, Abakaliki Ebonyi State.

Specific Objectives

1. To isolate and characterize bacteria spp. associated with keyboards and fomites
2. To determine the frequency of distribution of the bacterial isolates.
3. To determine the antimicrobial susceptibility pattern of the isolates against different classes of antibiotics.
4. To determine the multiple antibiotic resistance index of the bacterial isolates.

MATERIALS AND METHODS

Materials:

Media

Media that were used include: Mueller Hinton agar, Blood agar, manitol salt agar, S*almonella-shigella* agar, Nutrient agar (Oxoid, Uk), MacConkey agar (Fluka, Chemie, India) and Simmons Citrate agar (BIOTECH Laboratories Ltd, UK). Broth media that were used include Bacteriological peptone water (Oxoid, UK).

Instruments

Instruments that were used include: wire gauze, tripod stand, inoculating loop, test tube rack, swab stick, spatula, laboratory forceps, meter rule, electronic weighing balance (Scout Pro, China, model No: SPU401), conical flask, beaker, Petri dish, stirring rods, Whatman No.1 filter papers, glass slides, aluminum foil, injection syringes, gloves, marker pen, cotton wool, masking tapes, noise masks, bijou bottles, folder, tap, work benches, sphygmomanometer, stethoscope, bedrail, door handle, thermometer, computer keyboard and mouse.

Chemicals

Chemicals that were used include: methanol, distilled water, normal saline, immersion oil, Lugol's iodine, ethanol, safranin, crystal violet, Hydrogen peroxide, tetramethyl-p-phenylenediamine, sodium chloride, dimethylsulphoxide (DMSO), kovac's reagent (Fisher Scientific Company, USA).

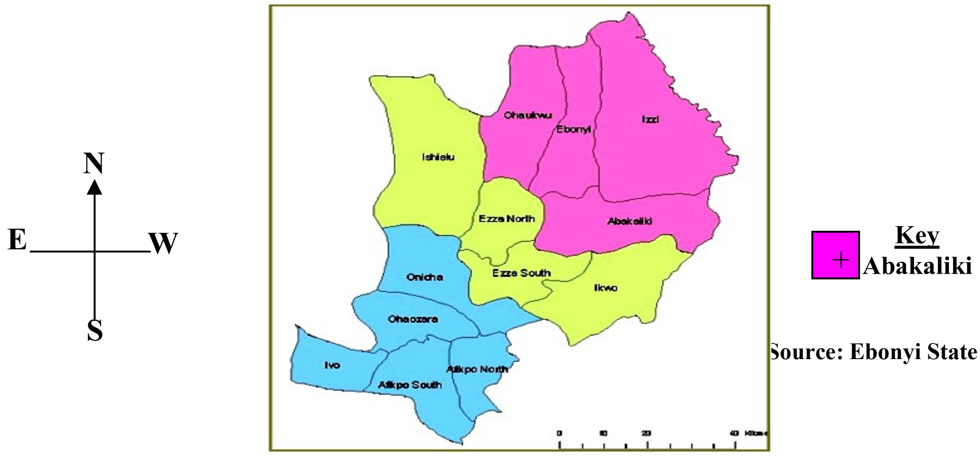
Equipment

Equipment that were used include refrigerator (Samsung, China, model No: GC-051SA), incubator (Merck, Germany, MIN1/50), bunsen burner (SEDI, Akuke, Nigeria), microscope (Olympus, Germany,) autoclave (Medica Instrument MFG. Co), computer keyboards and mice, colony counter machine (Rachert, USA).

Methods

Study Area

This study was carried out in the Federal Teaching Hospital, Abakaliki which is located at the centre of Abakaliki town behind Nigerian prisons and the annex along Abakaliki –Enugu express way formally known as Ebonyi State University Teaching Hospital (EBSUTH). It is the major Hospital in Abakaliki metropolis. Abakaliki is the capital city of Ebonyi State, with an estimated populate of 113,130 (National Population Commission, 2006). Abakaliki is located at the North Central Zone of Ebonyi State. Ebonyi State is located in the Eastern past of Nigeria and is bound to the North by Benue State, to the east by cross River Stape, to the South by Abia State and to the West by Enugu State. The State lies between longitude 7^0 35N and latitude 6^0 45'E. It experiences two seasons, rainy season (between April –October) and dry season (between November to March).The maximum temperature during the dry season is 37^0C while the minimum temperature is 27^0C. The period of rainy season has a maximum temperature of 32. 9^0C and a minimum of temperature of 24.1^0C. The relative humidity is between 60-80 %. The research was carried out from March 2016 to september 2016. During this period, Abakaliki is usually dusty when it is not raining and together with the temperature, relative humidity and the great number of people that patronize the hospital, contamination and multiplication of bacteria on computer keyboards, mouse and other fomites is likely possible.



Source: Ebonyi State Map Google Search

Fig. 1: Map of Ebonyi State Showing Abakaliki, the Capital of Ebonyi State.

Sample Collection Procession

Sterile cotton swab was used to collect samples. One hundred samples were collected. (10 each from door handles, mouse, computer keyboards, work benches, taps, thermometer, stethoscope, sphygmomanometer, folder and bed rails). The samples were collected in morning from wards, record and administrative block. Following swab taken, each swab was placed back into the casing to avoid contamination and was labeled appropriately. All the samples collected was transported with in an hour for a culture and treated according to standard method [20]. The samples were aseptically inoculated into bacteriological peptone (10 mls) shaken and incubated over night at 37^{0}C.

All laboratory work was undertaken in the Applied Microbiology, complex laboratory, faculty of science Ebonyi State University, Abakaliki.

Preparation of Culture Media

Preparation of Peptone water

Peptone water was prepared by dissolving 2.8g of peptone water powder (Oxoid, Uk) in. 100 ml of distilled water according to the manufacturer's instructions. Then, 5 ml each was dispensed aseptically into capped test tubes which were sterilized by autoclaving at 121 °C for 15mins at 15 psi [23].

Preparation of Agar media,

Agar plates was prepared by dissolving 2.8 g, 3.8 g, 4 g, 5.35 g, 11.1 g respectively of nutrient agar, Mueller-Hinton, Blood agar, MacConkey agar and Manitol salt agar in 100 ml of distilled water each according to the manufacturer's instructions. This was heated over a Bunsen burner flame to dissolve and was sterilized by autoclaving at 121°Cfor 15mins at 15 psi. Twenty milliliters each was dispensed aseptically into Petri dishes.

Salmonella – Shigella agar: 63 grams of the medium was weighed and suspended in 1 liter of distilled water, mix well and heated over Bunsen burner to boil and allowed to cool to 50^0C. Thereafter, pour into sterile Petri dishes.

Isolation of Organisms:

The swab sticks were pre-enreched in bacteriological peptone water and incubated at 37^OC for 24 hours. A loop full was subcultured by streaking into *salmonella-shigella* agar, MacConkey agar, blood agar and Manitol salt agar. The culture plates were in cubated at 37^OC for 24 hours.

Identification of Organisms:

Pure isolated colonies were Gram stained and then identified biochemically by catalase, coagulase, indole, urease, oxidase, citrate, Methyl red, voges proskauer and sugar fermentation tests [23].

Gram Staining

The bacteria isolate was picked using a sterile wire loop and emulsified in a drop of sterile distilled water placed on a glass slide, to make a thin smear. This was air dried and heat fixed by passing the slide over Bunsen burner flame to prevent the smear from being washed off during staining. The fixed smear was flooded with crystal violet stain for 60 seconds, and was washed off with clean water, Lugol's iodine was then be poured over the smear and allowed to stand for 60 seconds and was washed off with clean water. Acetone-alcohol was used to decolorize the smear and was washed off immediately. Then neutral red was used to counter stain the smear and washed off after about 2 minutes. The slides was placed on a draining rack to air dry before being examined under the microscope using the x40 and oil immersion x100 (to confirm bacteria and cell;) [23].

Oxidase Test

Oxidase reagent was freshly prepared. Two drops was placed on a filter paper in clean Petri dish. Then a colony of the bacteria isolate was smeared on the filter paper. Development of a blue-purple colour within a few seconds was an indication of a positive oxidase test [23].

Sugar fermentation using Triple Sugar and Iron (TSI) agar

Using a .sterile straight inoculation needle, a colony of the test isolate was collected and the center of the TSI agar (made of lactose, sucrose and glucose in ration 10:10:1) in a tube was stabbed and also the surface of the agar was streaked. The tube was incubated at 37^0C for 24 hours with the cap of the tube loosely fitted. If the medium remains red, this was indicated that there was no fermentation. If the slant is red and butt yellow, this indicates that only glucose has been fermented. If the slant and butt turn yellow, this will indicate that all 3 were fermented. And if there is black colouration, H_2S has been produced [23].

Catalase Test

Three percent hydrogen peroxide (2 - 3 ml) was put in a clean test tube. A sterile wooden stick or glass rob was used to pick the test organism and then immersed in the hydrogen peroxide solution. Production of bubble was indicative of catalase production [23].

Coagulase Test

Thick emulsions of a colony of the test isolates was made with a drop of distilled water each, on two separate slides. A loopful of plasma was added to one of the slides and mixed. Observation of clumping with 10 seconds was a positive coagulase production test [23].

Simmon's Citrate Test

The medium was inoculated with a straight wire from a light suspension of the organism prepared in sterile saline, incubated for 7 days at the optimum temperature of 37^0C and observed for colour change of blue coloration and growth for positive test [24].

Methyl Red (MR) Tests

A few drops of methyl red were added to the culture of isolated organism in glucose phosphate peptone water. The culture of the organism in glucose phosphate peptone water was incubated for 4 to 5 days at 37^0C and observed for the colour change where red colour is positive and yellow is negative [24].

Voges Proskauer (VP):

A knife cut of creatine was added to a 24 hours culture, followed by 5ml of 40 percent NaOH, The shake vigorously for several minutes and was observed for pink colour production within 2 - 5 minutes [24].

Preparation of 0.5 Mcfarland Turbidity Standard

A ml of concentrated H_2S04 was added to 99 ml of distilled water in flask. 0.5 g of 0.5g of dehydrated barium chloride

($BaCl_2.2H_20$) was dissolved in 50 ml of distilled water in other flasks respectively. Barium chloride solution (0.6 ml) was added to 99.4 ml of the H_2SO4 solution in a separate tube, and then mixed well to get 0.5 McFarland turbidity equivalents standard. The turbidity standards were transferred in small portions to capped test tubes and stored at room temperature (28°C). This was used to adjust and to compare the turbidity of the test bacteria in order to get a confluent growth on a growth or .culture plate [23], when performing Antimicrobial Susceptibility Testing (AST).

Standardization of Test Bacteria

Each of the bacteria to be tested was standardized individually before use by inoculating a 5 ml normal saline in sterile test tubes with loopful of a 24hr young culture of the test organism from -a nutrient agar slant. Afterwards, dilutions using loopful of the test bacterium and sterile water was carried out in order to get microbial population of 10^5 colony forming unit per milliliter/ (CFU/ml) by comparing it with 0.5 McFarland turbidity standards [25].

Antimicrobial Susceptibility Pattern of Bacterial Isolates:

The antimicrobial pattern of the isolates were determined using the disk diffusion method of Mueller - Hinton agar. The bacteria isolates were emulsified in sterile Nacl (normal saline). The turbidity formed were compared to 0.5 MacFarland standard and then inoculated on Muller Hinton agar (MHA) using sterile swab sticks according to modified Kirby-Bauer - diffusion method [26 and 27]. The antibiotic paper discs were placed aseptically on the surfaces of Muller Hinton agar with aid of sterile forceps. The plates was then be incubated at 37^0C for 24 hours. The antibiotics that was used include discs for Gram positive bacteria containing ciprofloxacin (5 µg); Norfloxacin (10 µg), Gentamicin (10 µg). Amoxillin (25 µg), Streptomycin (30 µg), Rifampcin (5µg), Erythronycin (15 µg), Chloramphenicol (30 µg), Ampicillin / Cloxcicillin (30 µg) and Levofloxacin (5 µg). Discs for Gram negative bacteria contains Ofloxacin (10 µg), Peflacine (10 µg), Ciprofloxacin (5 µg), Augmentin (30 µg), Gentamicin (10 µg), Streptomycin (30µg), Sulphamethazole/Trimethoprim (30 µg), Ampicillin (30 µg), Cephalexin.(30 µg), Cephalexin.(30 µg) and Nalidixic acid (30 µg). (Optum Lab Nig.) The of susceptibility of the bacteria isolates to antibiotics were interpreted according to the guidelines established by Clinical and Laboratory

Standard Institute (CLSI) as either susceptible(s) or resistant (R) by measuring the diameters of zones of inhibition in millimeter using ruler [27].

Determination of Multiple Antibiotic Resistant Index (MARI)

Multiple antibiotic resistance index (MARI) was determined to ascertain the number of antibiotics the isolates were resistant to.

MARI = 1/b.

Where a = number of antibiotics to which the isolate was resist to; b = total number of antibiotics to which the isolates was subjected to; according to [28].

Statistical Data Analysis

Data Analysis and Interpretation: Statistical software package (SPSS) (version 20) was used to analyze the data collected. Findings from the study were explained in words and summary analysis was performed using the analysis of variance (ANOVA). P-values less than 0.05 was regarded as statistical significant in all the cases, which implies rejection of the null hypothesis as the case may be.

Ethical Consideration:

The Ethical clearance was obtained from the Ethics Committee of Federal Teaching Hospital, Abakaliki, Ebonyi State. Permission was also obtained from Medical Director and heads of department of wards and offices of Federal Teaching Hospital Abakaliki.

RESULTS

The Table 1 above revealed that a total of eight bacteria were isolated. Out of these eight bacteria, two are cocci while six are Rod Shaped.

Table 2 revealed that a total of 279 bacteria were isolated, Gram negative bacteria have the highest prevalence of 174, while Gram positive bacteria were 105. *Staphylococcus aureus* (72) was the most predominant bacteria, followed by *Salmonella* species (53) and the least *Proteus* species (17).

The result from table 3 revealed that Gram negative bacteria isolated are 100% susceptible to Gentamicin, They were also highly resistant to Amplicillin (92.5%), Nalidixic acid and sulphametha zole / trimethoprim (78.7%).

The result from table 4 revealed that both *S. aureus* and CoNs are highly susceptible to Erythromycinn, Ciprofloxacin; Gentamicin and Levofloxacin. The result also showed that both organisms were 100% resistant to Norfloxacin, Amoxcillin and Ampicillin / Cloxacillin.

Table 5 revealed that *shigella* species are resistant to more class of antibiotics (0.8) followed by *Proteus* species (0.68), *Salmonella* species (0.53), *E. coli* (0.48), *Pseudomonas aeruginosa* (0.33) and the least *Klebsiella* species (0.25).

Table 1: Morphology and Biochemical Characterization of Bacterial Isolates from Computer Keyboard and other Fomites.

Shape	Gram	Motility	Catalase	Coagulase	Indole	Oxidase	Methyl Red	Voges Proskauer Auer	Citrate	Urease	Lactose	Manitol	Glucose	Sucrose	Gas	H_2S	Butt	Slope	Suspected Organisms
ROD	-	+	-	-	+	-	+	-	-	-	+	+	+	+	+	-	Y	Y	*Escherichia coli*
COCCI	+	-	+	+	-	-	+	+	+	+	+	+	+	+	-	-	Y	Y	*Staphylococcus aureus*
COCCI	+	-	+	-	-	-	+	+	+	+	+	+	+	+	-	-	Y	Y	*CONS*
ROD	-	+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	R	R	*Pseudomonas aeruginosa*
ROD	-	+	+	-	+	-	+	-	+	+	-	+	+	-	+	+	Y	R	*Proteus* species
ROD	-	-	+	-	-	-	-	+	+	-	+	+	+	+	-	-	Y	Y	*Shigella* species
ROD	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	Y	R	*Klebsiella* species
ROD	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+	Y	R	*Salmonella* species

Keys:
+ = Positive, – = Negative, Y = Yellow (acid reaction), R = Red – (alkaline reaction),
H_2S = hydrogen sulphide (blackening), CONS = Coagulase negative staphylococci

Table 2: Frequency of Bacterial Isolates Obtained from Computer Keyboards and other Fomites from Federal Teaching Hospital, Abakaliki.

Bacterial Isolates	Frequency	Percentage Frequency (%)
Gram Positive Bacteria		
Staphylococcus aureus	**72**	**25.8**
Coagulase negative staplylococci	**33**	**11.8**
Total	**105**	**37.6**
Gram Negative Bacteria		
Salmonella species	**53**	**19.0**
Pseudomonas aeruginosa	**34**	**12.2**
Escherichia coli	**25**	**9.0**
***Klebsiella* species**	**23**	**8.2**
***Shigella* species**	**22**	**7.9**
***Proteus* species**	**17**	**6.1**
Total	**174**	**62.4**
Grand total	**279**	**100**

Table 3: Antibiotic Susceptibility Pattern of Gram Negative Bacteria Isolated from Fomites in Federal Teaching Hospital, Abakaliki

Isolates	No. Tested	Observation	Number and proportion of isolates susceptible to (%)									
			S	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
E coli	25	S	10(40.0)	13(52.0)	25(100)	14(56)	0(0.0)	15(60.0)	25(100)	14(56.0)	13(52.0)	0(0.0)
		R	15(60.0)	12(48.0)	0(0.0)	11(44.0)	25(100)	10(40.0)	0(0.0)	11(44.0)	12(48.0)	25(100)
Proteus species	17	S	17(100)	0(0.0)	11(65.0)	0(0.0)	0(0.0)	0(0.0)	17(100)	0(0.0)	0(0.0)	9(53.0)
		R	0(0.0)	17(100)	6(35.0)	17(100)	17(100)	17(100)	0(0.0)	17(100)	17(100)	8(47.0)
Klebsilella species	23	S	13(57.0)	0(0.0)	23(100)	20(87.0)	23(100)	23(100)	23(100)	23(100)	23(100)	0(0.0)
		R	10(33.0)	23(100)	0(0.0)	3(13.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	23(100)
P aeruginosa	34	S	18(52.9)	0(0.00)	23(67.6)	32(94.1)	0(0.0)	29(85.3)	34(100)	34(100)	31(91.2)	28(82.4)
		R	16(47.0)	34(100)	11(32.4)	1(5.9)	34(100)	5(14.7)	0(0.0)	0(0.0)	3(8.8)	6(17.6)
Shigella species	22	S	22(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	22(100)	0(0.0)	0(0.0)	0(0.0)
		R	0(0.0)	22(100)	22(100)	(22(100)	22(100)	22(100)	0(0.0)	22(100)	22(100)	22(100)
Salmonella species	53	S	26(49.0)	0(0.0)	40(75.4)	30(56.6)	0(0.0)	25(47.0)	53(100)	23(43.0)	40(75.0)	0(0.0)
		R	27(51.0)	53(100)	13(24.6)	23(44.4)	53(100)	28(53.0)	0(0.0)	30(57.0)	13(24.6)	53(100)
Total	174	S	106(60.9)	13(7.5)	122(70.1)	96(55.2)	23(13.2)	92(52.9)	174(100)	94(54.0)	107(61.5)	37(21.3)
		R	68(39.1)	161(92.5)	52(29.9)	78(43.4)	151(86.8)	82(47.1)	0(0.0)	80(46.0)	67(38.5)	137(78.7)

Key: S: Streptomycin; PN: Ampicillin; CEP: Cephalexin ; OFX; Ofloxacin ; NA: Nalidixic acid ; PEF: Peflacine ; CN: Gentamicin; AU: Augumentin; CPX: Ciprofloxacin; SXT: Sulphamethoxazole/Trimethoprim. S: susceptible; R: Resistance.

Table 4: Antibiotic Susceptibility Pattern of Gram Positive Bacteria Isolated from Computer Keyboard and other Fomites in Federal Teaching Hospital, Abakaliki

Isolates	No. Tested	Observation	S	NB	CH	CPX	E	LEV	CN	APX	RD	AML
			Number and proportion of isolates susceptible to (%)									
S. aureus	72	S	65(90.3)	0(0.0)	40(56.0)	72(100)	70(91.2)	70(91.2)	60(83.3)	0(0.0)	24(33.3)	0(0.0)
		R	7(9.7)	72(100)	32(44.0)	0(0.0)	2(2.8)	2(2.8)	12(16.7)	72(100)	48(66.7)	72(100)
CONS	33	S	0(0.0)	0(0.0)	11(33.3)	30(90.9)	33(100)	17(51.5)	31(91.0)	0(0.0)	0(0.0)	0(0.0)
		R	33(100)	33(100)	22(66.7)	3(9.1)	0(0.0)	16(48.5)	2(9.0)	33(100)	33(100)	33(100)
Total	105	S	65(61.9)	0(0.0)	51(48.6)	102(97.1)	103(98.1)	87(82.9)	91(86.7)	0(0.0)	24(22.9)	0(0.0)
		R	40(38.1)	105(100)	54(31.4)	3(2.9)	2(1.9)	18(17.1)	14(13.3)	105(100)	81(77.1)	105(100)

S: Streptomycin; NB: Norfloxacin; CH: Chloramphenicol; CPX: Ciprofloxacin; E: Erythromicin; Lev: Levofloxacin; CN: Gentamicin; APX: Ampicillin / Cloxacillin; RD: Rifampcin; AML: Amoxcillin; CONS – Coagulase Negative Staphylococci; S: susceptible; R: Resistance

Table 5: Multiple Antibiotic Resistance Index of Gram negative Bacterial Isolates from computer Keyboards and other Fomites

Escherichia coli

Organism	E_1	E_2	E_3	E_5	E_6	E_7	E_8	E_9	E_{10}	E_{11}	E_{12}	E_{13}	E_{14}	E_{15}	E_{16}	E_{17}	E_{18}	E_{19}	E_{20}	E_{21}	E_{22}	E_{23}
MARI	0.40	0.30	0.50	0.50	0.40	0.30	0.30	0.60	0.50	0.80	0.30	0.60	0.50	0.50	0.70	0.50	0.50	0.80	0.80	0.30	0.50	0.20

E_{24}	E_{25}	Total	Average
0.30	0.40	12.0	0.48

***Proteus* species**

Organism	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	P_{13}	P_{14}	P_{15}	P_{16}	P_{17}	Total	Average
MARI	0.60	0.70	0.70	0.70	0.70	0.60	0.70	0.60	0.60	0.70	0.60	0.70	0.70	0.70	0.70	0.80	0.80	11.6	0.68

***Klebsiella* species**

Organism	K_1	K_2	K_3	K_4	K_5	K_6	K_7	K_8	K_9	K_{10}	K_{11}	K_{12}	K_{13}	K_{14}	K_{15}	K_{16}	K_{17}	K_{18}	K_{19}	K_{20}	K_{21}
MARI	0.20	0.20	0.20	0.20	0.20	0.20	0.40	0.20	0.20	0.30	0.20	0.40	0.30	0.30	0.30	0.30	0.20	0.30	0.20	0.20	0.20

K_{22}	K_{23}	Total	Average
0.30	0.30	5.80	0.25

Pseudomonas aeruginosa

Organism	Ps_1	Ps_2	Ps_3	Ps_4	Ps_5	Ps_6	Ps_7	Ps_8	Ps_9	Ps_{10}	Ps_{11}	Ps_{12}	Ps_{13}	Ps_{14}	Ps_{15}	Ps_{16}	Ps_{17}	Ps_{18}	Ps_{19}	Ps_{20}	Ps_{21}
MARI	0.40	0.20	0.80	0.20	0.40	0.30	0.20	0.50	0.20	0.20	0.60	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.50	0.30	0.40

Ps_{22}	Ps_{23}	Ps_{24}	Ps_{25}	Ps_{26}	Ps_{27}	Ps_{28}	Ps_{29}	Ps_{30}	Ps_{31}	Ps_{32}	Ps_{33}	Ps_{34}	Total	Average
0.30	0.30	0.30	0.20	0.20	0.20	0.50	0.20	0.40	0.30	0.20	0.30	0.40	11.30	0.33

***Shigella* species**

Organism	Sh_1	Sh_2	Sh_3	Sh_4	Sh_5	Sh_6	Sh_7	Sh_8	Sh_9	Sh_{10}	Sh_{11}	Sh_{12}	Sh_{13}	Sh_{14}	Sh_{15}	Sh_{16}	Sh_{17}	Sh_{18}	Sh_{19}	Sh_{20}	Sh_{21}	Sh_{22}	Total	Average
MARI	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	17.60	0.8

***Salmonella* species**

Organism	Sm 1	Sm 2	Sm 3	Sm 4	Sm 5	Sm 6	Sm 7	Sm 8	Sm 9	Sm 9	Sm 10	Sm 11	Sm 12	Sm 13	Sm 14	Sm 15	Sm 16	Sm 17	Sm 18	Sm 19	Sm 20	Sm 21	Sm 22	Sm 23	Sm 24
MARI	0.40	0.70	0.30	0.60	0.30	0.60	0.40	0.40	0.60	0.50	0.50	0.50	0.5-	0.30	0.40	0.50	0.60	0.50	0.50	0.70	0.60	0.70	0.70	0.30	0.50

Sm 25	Sm 26	Sm 27	Sm 28	Sm 29	Sm 30	Sm 31	Sm 32	Sm 33	Sm 34	Sm 35	Sm 36	Sm 37	Sm 38	Sm 39	Sm 40	Sm 41	Sm 42	Sm 43	Sm 44	Sm 45	Sm 46	Sm 47	Sm 48	Sm 49	Sm 50
0.60	0.40	0.60	0.30	0.70	0.40	0.70	0.60	0.70	0.60	0.50	0.60	0.60	0.40	0.70	0.60	0.80	0.80	0.80	0.80	0.60	0.50	0.50	0.50	0.60	0.30

Sm 51	Sm 52	Sm 53	Total	Average
0.50	0.70	0.50	28.10	0.53

Key: E = ***Escherichia coli***, P = ***Proteus*** **species**, K = ***Klebsiella*** **species**, Ps = ***Pseudomonas aeruginosa***, Sh = ***Shigella*** **species**, Sm = ***Salmonella*** **species**

Table 6: Multiple Antibiotic Resistance Index of Gram Positive Bacterial Isolates from Computer Keyboard and other Fomites

Coagulase Negative Staphylococci

Organism	Co 1	Co 2	Co 3	Co 4	Co 5	Co 6	Co 7	Co 8	Co 9	Co 10	Co 11	Co 12	Co 13	Co 14	Co 15	Co 16	Co 17	Co 18	Co 19	Co 20	Co 21	Co 22
MARI	0.50	0.60	0.50	0.80	0.50	0.50	0.70	0.50	0.70	0.60	0.50	0.80	0.69	0.70	0.70	0.60	0.70	0.50	0.70	0.60	0.70	0.60

Co 23	Co 24	Co 25	Co 26	Co 27	Co 28	Co 29	Co 30	Co 31	Co 32	Co 33	Total	Average
0.70	0.70	0.60	0.50	0.70	0.70	0.50	0.70	0.70	0.60	0.70	20.70	0.63

Staphylococcus aureus

Organism	Sa 1	Sa 2	Sa 3	Sa 4	Sa 5	Sa 6	Sa 7	Sa 8	Sa 9	Sa 10	Sa 11	Sa 12	Sa 13	Sa 14	Sa 15	Sa 16	Sa 17	Sa 18	Sa 19	Sa 20	Sa 21	Sa 22
MARI	0.30	0.30	0.30	0.60	0.50	0.60	0.40	0.50	0.30	0.40	0.40	0.40	0.40	0.50	0.70	0.40	0.40	0.40	0.50	0.60	0.60	0.50

Sa 23	Sa 24	Sa 25	Sa 26	Sa 27	Sa 28	Sa 29	Sa 30	Sa 31	Sa 32	Sa 33	Sa 34	Sa 35	Sa 36	Sa 37	Sa 38	Sa 38	Sa 49	Sa 41	Sa 42	Sa 43
0.30	0.40	0.40	0.40	0.50	0.50	0.30	0.40	0.30	0.40	0.40	0.30	0.30	0.50	0.50	0.40	0.40	0.50	0.50	0.60	0.50

Sa 44	Sa 45	Sa 46	Sa 47	Sa 48	Sa 49	Sa 50	Sa 51	Sa 52	Sa 53	Sa 54	Sa 55	Sa 56	Sa 57	Sa 58	Sa 59	Sa 60	Sa 61	Sa 62	Sa 63	Sa 64	Sa 65	Sa 66
0.50	0.40	0.60	0.30	0.40	0.40	0.40	0.50	0.40	0.50	0.50	0.60	0.60	0.60	0.40	0.40	0.40	0.50	0.40	0.50	0.50	0.50	0.50

Sa 67	Sa 68	Sa 69	Sa 70	Sa 71	Sa 72	Total	Average
0.40	0.40	0.30	0.40	0.60	0.50	32.20	0.45

Key: Sal = *Staphylococcus aureus*, Co = Coagulase negative staphylococci

Table 6 revealed that CoNs (0.63) are resistant to more class of antibiotics than *S aureus* (0.45).

Fig. 2 above revealed that computer keyboards and mouse were the most contaminated (35), each followed by folder and door handle (30), Thermometer (29), Stethoscope (26), Tap and Sphygmomanometer (25), Bedrail (23) and the least work benches (21).

From figure 3, children emergency unit (33) most contaminated followed by Pediatric ward (28), the least being research and ethics (5).

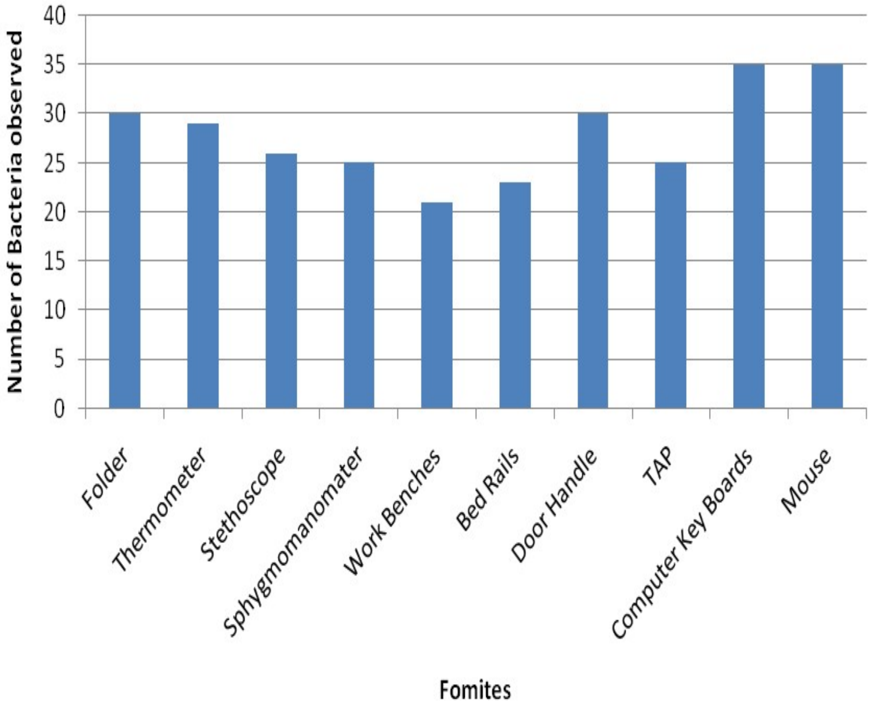


Fig 2: Frequency Distribution of Bacteria Isolates among fomites.

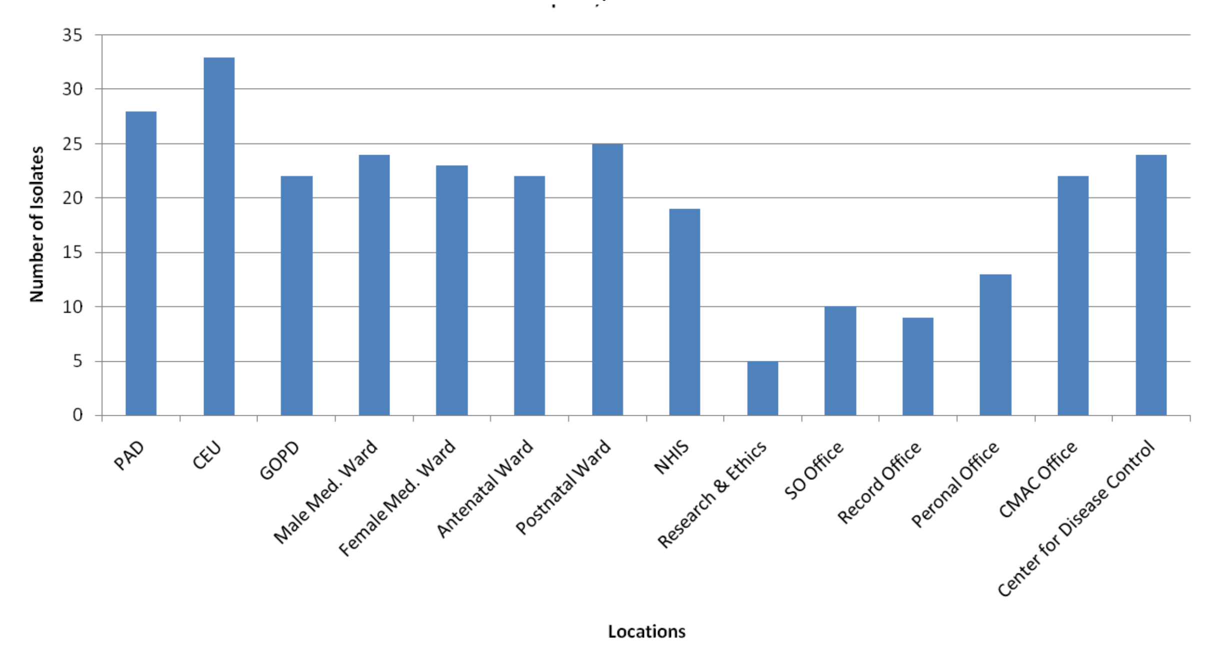


Fig. 3: **Distribution of Bacterial Isolates with reference to specific location in Federal Teaching Hospital, Abakaliki.**

Key: PAD – Pediatric Ward
CEU - Children Emergency Unit
SO - Secretary Office
NHIS – National health Insurance Scheme
CMAC – Chairman Medical Advisory Committee

DISCUSSION

Results from this study confirmed that various fomites sampled in Federal Teaching Hospital, Abakaliki were contaminated with known bacterial pathogens although the rate of contamination varied ($P < 0.05$). Direct involvement of these fomites in disease transmission was not investigated in this study, however, the isolation of *Staphylococcus aureus*, Coagulase negative Staphylococcs, *Pseudomonas aeroginosa*, *Escherichia coli*, *Klebsiella* species, *Shigella* species and *Proteus* species present a significant public health risk ($P < 0.05$).

The health risk associated with majority of these bacterial isolates has been reported by [11]. The bacteria isolated in our study are similar to those that have been isolated from surfaces and objects by [29, 30 and 31].

Furthermore 8 genera of bacteria totaling 279 isolates were isolated. Gram negative bacteria isolate were more prevalent (n = 174, 62.3 %) than Gram positive bacteria (n = 105, 37.6 %). This is in agreement to the result obtained by Nwankwo *et al* (2012), [31], who isolated 60.8 % and 39.2 % in Nigeria, but contrary to the result of Srikanth *et al*. (2012), [32], who isolated 25.0 % and 75.5 % in India and Agresew *et al* (2015), [33], in Ethiopia who had 39.5 and 60.5%. There was a lower prevalence of Gram negative bacteria and Gram positive bacteria, 6.3% and 13.8% in China [34]. Among the Gram positive bacterial isolates and over all the isolates, *S. aureus* was the most prevalent. Interestingly this is similar to the results of [35 and 20], where *S aureus* showed the highest prevalence but contrary to the work of Nwankwo *et al.* (2012), [31], where coagulase negative staphylococci was the highest. *Staphlococcus aureus* is one of the major organisms found in the skin and nostrils. This probably supports the high prevalence as seen in this study and the report that they can be discharged through contact with skin, talking and sneezing [36].

Thirty three Coagulase negative staphylococci were isolated in this study. This is in agreement to the work of Srikanth *et al* (2012), [32], in India who had 33 and Nwankwo *et al*. (2012), [31], in Nigeria who had 34 but contrary to the result of Das *et al*, (2011), [37], in USA who isolated 25. CoNs is also a normal flora of the skin which sometimes assume an opportunistic tendency in the spread of infections like endocarditis; urinary tract infections and bacteriamia, [35]. Congulase negative staphylococci have also been isolated from hospital surfaces and objects and have been implicated in causing noscomial infections especially in immuno compromised individuals [36].

Among the Gram negative bacterial isolates, *Salmonella* species showed the highest prevalence (n = 53, 19 %). In terms of overall contamination as seen in this study, it ranked second. This is contrary to the result obtained by Nwankwo *et al.,* (2012), [31], who had lower prevalence of this organism from fomites. The presence of these organisms probably suggests fecal contamination and may reflect the degree of personal and environmental hygiene of the hospital community.

Pseudomonas aeruginosa was isolated from tap, computer key boards and mouse. This confirms the report of Penagea *et al*. (2005), [38], who stated that *P aeruginosa* is mostly isolated from sinks and moist sites. The isolation of *P aeruginosa* from computer key boards and mouse may be as a result of the use of water contaminated with the organism to clean the computer key boards and mouse by computer operators in the hospital.

E coli is another Gram negative bacterial isolate recovered in our study. It is an entric bacterium that spread diseases through touch, and poor sanitary activities. The isolation rate of *E coli* in this present study was 9.0 and it was commonly isolated from inanimate objects. These findings are in conformity to the research work done in Nigeria by Nwankwo *et al.* (2012), [31], in Ghana by [30] and in Ethiopia by [33]. Isolation of *E coli* from these fomites also reflects the extent of purity or signals faecal contamination of health workers [39]. The isolation of other Gram negative bacterial isolates like *Klebsiella* species, *Shigella* species and *Proteus* species in our study is also a cause for alarm as they have been shown to posses the potential to cause infections especially in a hospital environment as recorded by [40] and by [41].

Our study revealed that computer key boards and mouse were the most contaminated. This is in agreement with the work of Harmatan *et al.* (2004), [42], where computer keyboards and mouse were most contaminated.

Contamination of the computer keyboards, mouse, and other fomites sampled in this study is a clear indication that proper sterilization method was not employed by the health workers in the hospital [43].

Distribution of bacterial isolates between the wards / offices from where the swab samples were collected showed that children emergency unit (33) and Pediatric ward (28) were the most contaminated. This poses a serious public health risk as children are readily infected and can therefore easily contact nosocomial infections as observed by [44]. The number of bacteria recovered in postnatal ward (25), Centre for Disease Control Unit (24), Male Medical Ward (24), Female Medical Ward (23), Antenatal Ward (22), Chairman Medical Advisory Committee Office (22) and National Health Insurance Scheme Office (19) are also of serious public health risk ($P < 0.05$) [45].

Also, in this work, the antibiotic susceptibility profile of Gram positive bacteria revealed that *S aureus* and CoNs showed high sensitivity to streptomycin ciprofloxacin, levofloxacin, Gentamicin with Erythromycin being the most effective. Tula and Iyoha, in 2014 used the same antibiotics but the difference between their result and our findings is that their isolates were highly susceptible to all the antibiotics but our own were susceptibility to five of the antibiotics and highly resistance to the other five antibiotics. This may be due to genetic nature of the organisms, environmental conditions, geographical locations and improper use of antibiotics within the locality [46]. *S aureus* and CoNs were more resistant to Amoxicilin / cloxacillin, Amoxcillin, Norfloxacin (100 %) and Rifampicin 78.1 % with MAR index of 0.45 and 0.63 respectively revealing that CoNs were resistant to more class of antibiotics than *S aureus*.

The antibiotic susceptibility profile of Gram negative bacterial in our study revealed that the they were mostly susceptible to Gentamicin (100 %), Cephalexin (70.1 %), Ciprofloxacin (61.5 %), Streptomycin (60.9 %), Ofloxacin (55.2 %), Augmentin (54.0 %) and Peflaxacin (52.0 %). This is contrary to report of Tula and Iyoha, (2014) where most of their isolates were resistance to the same antibiotics. The resistance profile of Gram negative bacteria revealed that the isolates were highly resistance to Ampicillin (92.5 %), Nalidixic acid (87.8 %) and sulphamethoxazole / trimethoprim (78.7 %). This result is similar to that of Manikandan *et al,* (2011), [47], which showed 83.3 % and 80.6 % of their bacterial isolates were resistance to sulphamethoxazole/trimethoprim and Nalidixic acid respectively. Our findings that 92.5% of the isolates were resistance to Ampicillin confirms the report of Sahm *et al* (2001), [48], that Ampicillin is not effective in treating infections caused by Gram negative bacteria . This may be due to frequent and improper use of Ampicillin. This is also supported by the report of Rhan and Zaman (2006), [49], which showed that

90 % of their isolates were resistant to Ampicillin.

In this study also Gentamicin was highly effective in both Gram negative (100 %) and Gram positive bacteria (86.7 %). This confirms earlier research on bacterial isolates from drinking water and milk by [50 and 51] respectively.

The multi drug resistance index of Gram negative bacterial revealed that *Shigella* species has the highest MAR index of 0.8, followed by *Proteus* species (0.68), *Salmonella* species (0.53), *E. coli* (0.48), *Pseudomonas aeruginosa* (0.33) and the least *Klebsiella* species (0.25). Multi drug resistance to antimicrobial agents used in treating *Shigella* species, *Proteus* species, *Salmonella* species, *E coli, P aeruginosa* infections have been reported in many parts of the world including Nigeria. The increasing relative prevalence of these species of multi resistance organisms pose a clear public health problem as observed by [52 and 53].

RECOMMENDATIONS

The following measures are recommended:

1) Periodic cleaning of the fomites in use with appropriate disinfectants, before an after each contact with patients.
2) Frequent hand washing should be encouraged among health workers, patients and the entire hospital community.
3) There should also be periodic monitoring of antimicrobial susceptibility both in the community and the hospital environment.

CONCLUSION

This study revealed bacterial contamination of sampled surfaces in Federal Teaching Hospital, Abakaliki. The bacterial isolates, *S aureus*, coagulase negative staphylococci, *E. coli*, *P aeruginosa*, *Klebsiella* species, *Shigella* species and *Proteus* species, were found to be resistant to some commonly used antibiotics. The presence of these bacteria contaminants are reservoir for bacteria disease spread in the hospital and its environs.

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