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Phenotypic Detection of Virulence Markers, Antibiotic and Disinfectant Susceptibility of Bacterial Isolates from Automated Teller Machine Keypads, Computer Keyboards and Mice in Uyo, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author OJA designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors OJA, OKF and ESU managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The occurrence and virulence markers of bacterial isolates from Automated Teller Machine Keypads (ATM), Computer Keyboards (CK) and Computer Mice (CM) were determined using standard bacteriological methods. The susceptibilities of the isolates to antibiotics and disinfectants (Savlon, Dettol and hydrogen peroxide) were determined by disc diffusion techniques. The bacterial isolates on the 12 CMs from cyber cafés were *Staphylococcus aureus* 7 (58.3%), *Bacillus* spp 5 (41.7%), *Staphylococcus epidermidis* 4 (33.3%), *Streptococcus* spp 4 (33.3%), *Escherichia coli* 2 (16.7%), *Enterococcus* spp 2 (16.7%) and *Pseudomonas aureginosa* 1(8.3%). *S. aureus* was

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the predominant bacterial isolate from CKs and ATM keypads, while *Bacillus* spp. had the lowest percentage of occurrence. A total of 23 (47.9%) CK swab samples had single bacterial growth, while 25 (52.1%) had mixed bacterial flora. Of the 12 CM from cybercafés, 3/12 (25.0%) showed growth of single bacterial isolate, while 9 /12 (75.0%) had mixed bacterial growth. More than 52% *S. aureus, Streptococcus* spp and *Enterococcus* spp were sensitive to ofloxacin and streptomycin, while ≥ 39.1% *E. coli* were resistant to ceftriaxone and ampicillin. The results showed that between 46/146 (31.5%) and 61/146 (41.8%) of the isolates produced DNase, TNase and amylase. Of the 73/146 (50.0%) lipase producing bacterial isolates, the widest clear zone was observed on the tributyrin agar plate containing *S. aureus* SA-C2. More than 21.9% bacterial isolates produced capsules, haemolysins, caseinase and gelatinase. Savlon showed the highest antibacterial activities than Dettol and hydrogen peroxide. The highest and lowest inhibitory zones were observed in the plates containing *P. aureginosa* PA-B2 and *S. aureus* SA-A6 having the mean ± SD of 14.0 ± 0.5 mm and 6.4 ± 1.2 mm, respectively. The ATM, computer keyboards and mice harboured multidrug resistant pathogenic bacteria that may be transferred to / among the users and Savlon could be first choice of disinfectant for the cleansing / disinfecting of these fomites.

Keywords: Bacteria; computer; automated teller machine; virulence; disinfectant; antibiotics; Uyo.

1. INTRODUCTION

Globally, the necessity of computer in numerous recognized specialties (Accounting, Medicine, Engineering and Microbiology etc) has been a fundamental purpose for the never-ending proliferation of computer usage. Computers have become a central component in business offices, cyber cafes, banks for improved and effective reports [1]. Providers of services in business offices, cybercafés and banks move back and forth, between computers and customers while serving the customers as part of daily routine [1].

In Nigeria and other parts of the world, the use of Automated Teller Machine (ATM), a self-service computerized telecommunication device, that dispenses cash and carry out some human teller functions such as balance enquiry and bills payments, has overwhelmingly increased [2,3]. The ability of computer key-boards, mice and other inanimate objects to support micro-organisms for prolonged period of time is well documented [4,5]. Scientific investigations have shown that frequently used interfaces such as computers, telephones and ATM keypads are potential sources of infectious bacteria such as *Staphylococcus aureus, Streptococci* spp. *Escherichia coli* and *Bacillus* spp [6,7]. The colonization of inanimate objects such as the ATM keypads, computer keyboards and computer mice by viable pathogenic micro-organisms have been reported [6,8,9]. Since these fomites are not routinely disinfected, they may serve as vehicles in transmission of pathogenic micro-organisms either directly by surface to mouth contact or indirectly by contamination of fingers and subsequent hand to mouth contact [6,10].

Antimicrobial resistance is a global phenomenon that has resulted in high morbidity and mortality as a result of treatment failures and increased health care costs [11]. Several studies have shown the occurrence of multidrug resistant bacterial isolates on external surfaces of computer keyboard, computer mice and ATM [6,9,12]. Some micro-organisms possess virulence factors that enhance or contribute to their pathogenicity [11,13]. These virulence factors such as toxins, cell surface protein and hydrolytic enzymes are frequently involved in the direct interaction with the host tissues or in concealing the bacterial surface from the host's defense mechanism [13,14,15]. The quality of cleaning services is a vital condition in the prevention and control of microbial spread, as well as the type of disinfectants used to reduce risks of infection [16].

This study aimed at determining the virulence markers and susceptibilities of bacterial isolates from Automated Teller Machine Keypads (ATM), Computer Keyboards (CK) and Computer Mice (CM) to antibiotics and some commercially-available disinfectants.

2. MATERIALS AND METHODS

2.1 Description of Study Area

Uyo is a city in South-Southern Nigeria and is the capital of Akwa Ibom State. Akwa Ibom State shares boundaries with Abia, Cross River and

Rivers States. The population is estimated to be about 451,128. Uyo is located between latitudes 5° 02' 37" North and longitudes 7° 54' 06" East. There are many hospitals, tertiary institutions, colleges, markets, restaurants, banks, cyber cafés and business centres in Uyo.

2.2 Ethical Permission

The consent and permission of the operators and managements of the business centres and cyber cafés were obtained in order to carry out this research work. Consequently, the confidentialities of the information obtained were kept.

2.3 Collection and Bacteriology of Samples

A total of seventy-two (72) swab samples consisting of computer mice swab samples (n=36) and computer keyboards (number / letter keys, spacebar key, enter key, control key and other keys) swab samples (n=36) were obtained from business centres, cyber cafés and those that were personally used (personal computers). Twelve (12) automated teller machines keypads (cancel key, enter key, clear key, number keys etc) were also aseptically swabbed with commercially available sterile swab sticks moistened with sterile normal saline solution (Figs. 1-3). Each swab obtained was inoculated onto each test tube containing 2 ml nutrient broth and all the samples were taken immediately to the microbiology laboratory for bacteriological analysis. Each swab sample in the tube containing nutrient broth was vortexed and 0.1 ml was inoculated onto each plate of blood agar, chocolate agar, MacConkey agar, mannitol salt agar, nutrient agar, eosine methylene blue agar and aerobically incubated at 37°C for 24 hr. Cultures were considered negative if no growth was detected within 24 - 48 hr of incubation. Bacterial colonies varying in shape, size and colour were picked from the different plates using wire loops, subcultured onto plates of nutrient agar and aerobically incubated at 37°C for 24 hr. After incubation, pure colonies of isolates obtained were streaked onto nutrient agar slants, incubated at 37°C for 24 hr and stored in the refrigerator at 4°C for characterization and identification. All isolates were subjected to Gram staining, motility test, biochemical tests (citrate utilisation, urease, coagulase, oxidase, indole, methyl red and Vogues Proskauer) and sugar fermentation tests (glucose, sucrose, mannitol and lactose) using standard techniques described by [17,18].

2.4 Antibiotic and Disinfectant Susceptibility of the Bacterial Isolates

The susceptibility of the bacterial isolates to antibiotics (10 µg Penicillin; 30 µg Ceftazidime; 30 µg Streptomycin; 10 µg Gentamycin; 5 µg Ofloxacin; 5 µg Nalidixic acid; 10 µg Ampicillin and 5 µg Ciprofloxacin) and disinfectants (Dettol, Savlon and hydrogen peroxide) were determined using disc diffusion method [19,20]. Zero point one (0.1) ml of each bacterial isolates prepared directly from an overnight agar plate adjusted to 0.5 McFarland Turbidity Standard was inoculated using sterile pipette onto each of the plates containing Mueller-Hinton Agar (MHA). The discs containing the antibiotics and sterile filter paper discs (6 mm diameter) impregnated with each disinfectant were aseptically placed onto the surfaces of the MHA plates using a sterile forceps and gently pressed to ensure even contact. All the plates were incubated at 37°C for 18 hr and the zones of inhibition after incubation were observed and the diameters of inhibitory zones were measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant to the antibiotic was made according to the manufacturer's standard zone size interpretative manual.

2.5 Detection of Deoxyribonuclease (DNase) Producing Bacterial Isolates

Production of DNase by bacterial isolates was determined using DNase agar [11,21]. The DNase agar plates were spot inoculated with bacterial isolates using sterilized wire loop and incubated for 24 hr at 37°C. The growth on the surface of the agar was flooded with 1N hydrochloric acid. Clear zones around the colonies showed the production of DNase.

2.6 Detection of Thermonuclease (TNase) Producing Bacterial Isolates

Production of TNase by bacterial isolates was determined using the method of [22]. Plates of toluidine blue-deoxynucleic acid agar were spot inoculated with bacterial isolates using sterilized wire loop and incubated for 24 hr at 37°C. Formation of a pink halo around the colonies showed the production of TNase.

Fig. 1. Automated teller machine

Fig. 2. Computer keyboard

Fig. 3. Computer mouse

2.7 Detection of Lipase Producing Bacterial Isolates

Detection of lipase producing bacterial isolates was carried out using tributyrin agar. The tributyrin agar plates were spot inoculated with bacterial isolates using sterilized wire loop and incubated for 24-48 hr at 37℃. Clear zones around the colonies indicated the production of lipase by the isolates [11,23,24].

2.8 Detection of Capsule Producing Bacterial Isolates

A 24 hr old colony was emulsified in sterile distilled water to make a thin smear on a clean scratch and grease free slide using sterilized wire loop. The smear was air dried, stained with crystal violet for 5-7 mins. The stain was washed off with 20% copper sulphate and air-dried. The smear was then examined microscopically with the 100 x oil immersion objective and bacterial capsule appeared as faint blue-violet zones around dark - blue bacterial cells [25].

2.9 Detection of Haemolysin Producing Bacterial Isolates

The production of haemolysins by bacterial isolates was determined using Columbia blood agar base supplemented with 5% human blood [11,18]. The bacterial isolates were streaked onto blood agar plates and incubated for 18 - 24 hrs at 37℃. The presence of greenish colouration halos around the colonies indicated production of *α*-haemolysin, while complete clear zone indicated production of *β*-haemolysin.

2.10 Detection of Gelatinase Producing Bacterial Isolates

Gelatinase producing bacterial isolates was detected using gelatin agar (2% bacteriological gelatin, 1% bacteriological agar and nutrient broth). The bacterial isolates were streaked on plates of gelatin agar and incubated for 24 - 48 hr at 37℃. Zone of clearance around the bacterial colonies indicated production of gelatinase [25].

2.11 Detection of Caseinase Producing Bacterial Isolates

Caseinase producing bacterial isolates was detection using skimmed milk agar (3% skimmed milk, 1% bacteriological agar and nutrient broth). The bacterial isolates were streaked on plates of skimmed milk agar and incubated aerobically for 24 hrs at 37℃. Transparent zones around the bacterial colonies indicated production of caseinase [23,24].

2.12 Detection of Amylase Producing Bacterial Isolates

Amylase producing bacterial isolates was detected using starch agar. The bacterial isolates were streaked onto plates of starch agar and incubated for 24-48 hr at 37℃. After incubation, 3 drops of 10% Lugol iodine was put on the culture plates and allowed to react for 10 min. Clear zones around the bacterial colonies indicated amylase production [23,24].

3. RESULTS

The results of the morphological and biochemical characteristics of bacterial isolates from ATM

Keypads, computer keyboards and mice swab samples are shown in Table 1. A total of 62 bacterial isolates, comprising 52 Gram positive and 10 Gram negative bacteria, in the genera *Staphylococcus, Enterococcus, Streptococcus, Escherichia*, *Bacillus* and *Pseudomonas,* were isolated from computer mice swab samples obtained from personal computers, business centres and cyber cafés (Table 2). The Gram positive bacteria harboured on the surfaces of the computer mice from the business centres were *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus* spp., *Streptococcus* spp. and *Bacillus* spp, while the Gram negative obtained were *Escherichia coli* and *Pseudomonas aureginosa* (Table 2). The most common bacterial isolate on the surfaces of computer mice from cyber cafés was *S. aureus* 58.3% (n = 7), followed by *Bacillus* spp 41.7% (n = 5), *S. epidermidis* 33.3% (n= 4), *Streptococcus* spp 33.3 % (n= 4), *Enterococcus* spp 16.7% (n= 2) and *E. coli* 16.7% (n= 2), while *P. aureginosa* had the lowest occurrence with 8.3% (n= 1). *S. aureus* was the predominant bacterial isolate from computer keyboards and ATM keypads, while *Bacillus* spp. had the lowest percentage of occurrence (Table 3). Of the 12 ATM keypads swab samples, 8 /12 (66.7%) had *S. aureus,* 4/12 (33.3%) had *S. epidermidis,* 3/12 (25.0%) had *Enterococcus* spp, 3/12 (25.0%) had *Streptococcus* spp, 5/12 (41.7%) had *E. coli*, while *P. aureginosa* and *Bacillus* spp had 2/12 (16.7%) each (Table 3). The percentages of occurrences of bacterial isolates from computer keyboards in business centres and cyber cafés are shown in Table 3.

All the 36 (100%) computer keyboards and 12 (100%) ATM keypads swab samples examined showed bacterial growth. A total of 23 (47.9%) computer keyboard swab samples had single bacterial growth, while 25 (52.1%) had mixed bacterial flora. The highest and lowest numbers of mixed bacterial flora were obtained from ATM keypads and computer keyboards of personal computers, respectively. Of the 12 computer mice from cybercafés, 3/12 (25.0%) showed growth of single bacterial isolate, while 9 /12 (75.0%) had mixed bacterial growth (Table 4). The occurrences of single and mixed bacterial flora on computer keyboards and mice swab samples obtained from business centres are similarly shown in Table 4.

The antibiotic susceptibility patterns of *S aureus*, *S. epidermidis* and *Enterococcus* spp isolated from ATM keypads, computer mice and keyboards are shown in Figs. 4, 5 and 6. More than 70% of *S. aureus*, *S. epidermidis* and *Enterococcus* spp were sensitive to ofloxacin and ciprofloxacin. ≥ 52% of *Streptococcus* spp were resistant to streptomycin (Fig. 7), between 39.1% and 44.4% *E. coli* and *P. aureginosa* were resistant to ceftriaxone and ampicillin (Figs. 8 and 9), while ≥ 68% of *Bacillus* spp. were sensitive to ceftriaxone, gentamycin, nalidixic acid and ciprofloxacin (Fig. 10).

Of the 146 bacteria isolated from ATM keypads, computer keyboards and mice swab samples, 61 (41.8%), 46 (31.5%) and 61 (41.8%) produced DNase, TNase and amylase, respectively (Table 5). *S. aureus* was the highest

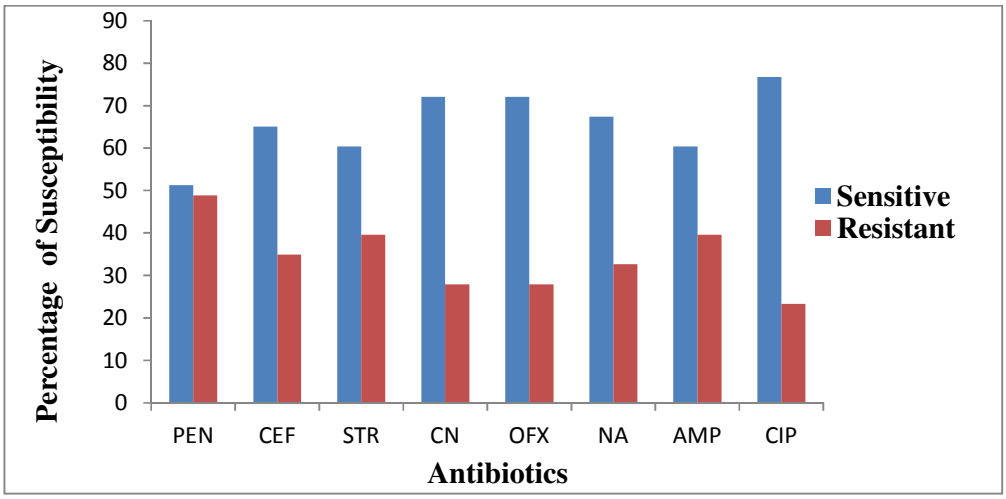

Fig. 4. Antibiotic susceptibility pattern of *S. aureus* isolated from ATM keypads, computer keyboards and mice

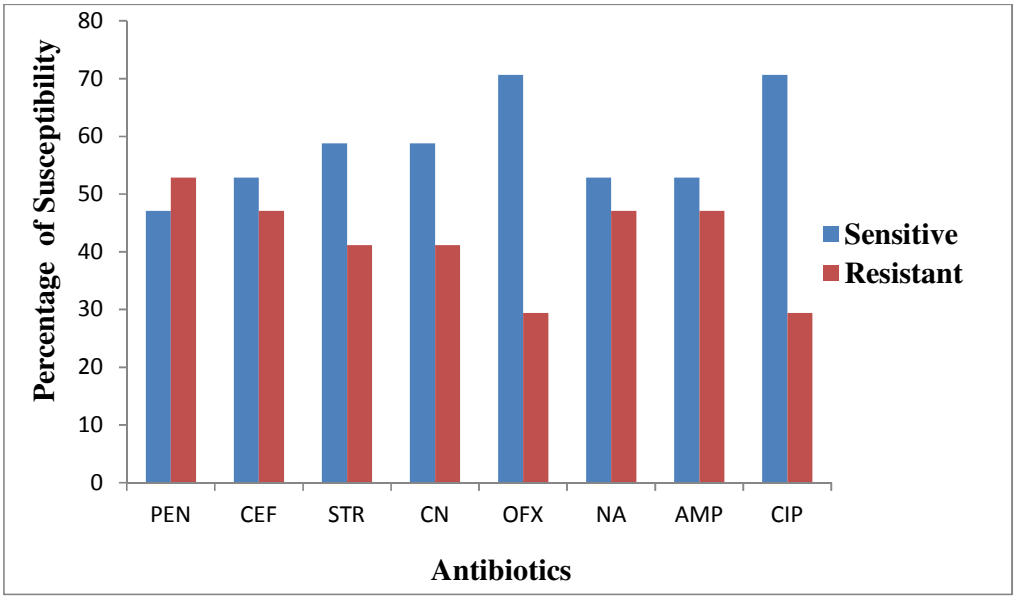

Fig. 5. Antibiotic susceptibility pattern of *S. epidermidis* isolated from ATM keypads, computer keyboards and mice

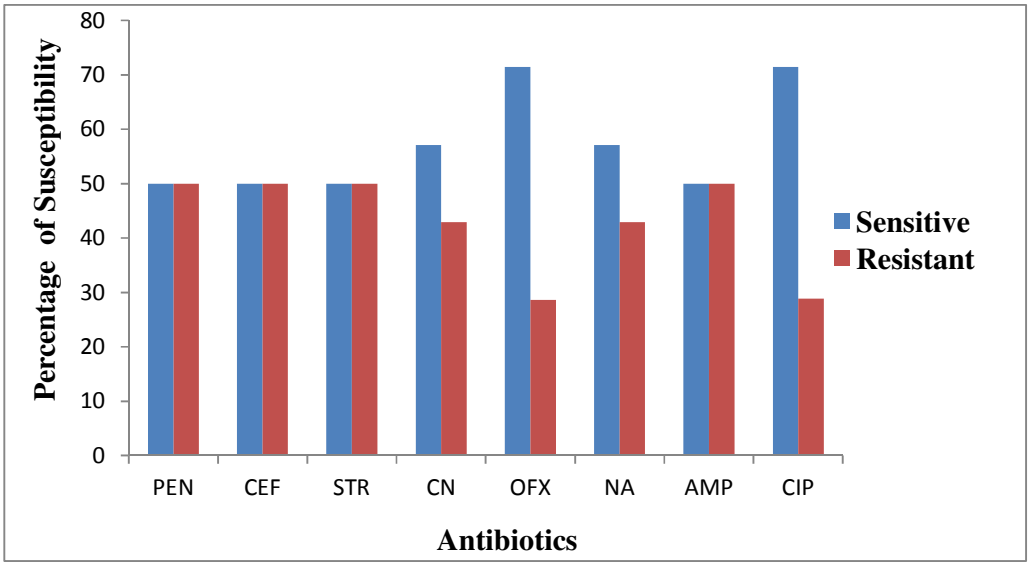

Fig. 6. Antibiotic susceptibility pattern of *Enterococcus* spp isolated from ATM keypads, computer keyboards and mice

DNase and TNase producers, while none of the *P. aureginosa* isolated had the capability to produce DNase. The virulence markers detected in the *E. coli* were DNase, TNase, lipase, amylase and haemolysin. Of the 73 / 146 (50.0%) lipase producing bacteria isolated, the widest clear zone was observed on the tributyrin agar plate containing *S. aureus* SA-C2. The results also showed that ≥ 60.3% and ≥ 32.9% of the bacterial isolates had the capabilities to produce haemolysins and proteases (caseinase and gelatinase), respectively. None of the *S. epidermidis* and *E. coli* produced caseinase and gelatinase. Thirty–two encapsulated bacterial isolates in the families *Staphylococcaceae*, *Enterococcaceae, Streptococcaceae and Pseudomonadaceae* were isolated from these fo mites (Table 5).

Table 1. Morphological and biochemical characteristics of bacterial isolates from ATM keypads, computer keyboards and mice

Bacterial spp	Gram's staining	Spore staining	Catalase test	Citrate test	Oxidase test	Coagulase test	Indole test	Urease test	Glucose test	Lactose test	Sucrose test	Mannitol test	Motility test	Methyl red	Vogues Proskauer	Hydrogen sulphide
Streptococcus pyogenes	+cocci	-	-	+	-	-	-	-	+	-	+	-	-	+	-	+
Staphylococcus aureus	+cocci	-	+	+	-	+	-	+	+	+	+	+	-	+	-	+
S. epidermidis	+cocci	-	+	+	-	-	-	+	+	+	+	+	-	+	-	-
Pseudomonas aeruginosa	-rod	-	+	+	+	-	-	-	-	+	+	+	+	-	+	-
Escherichia coli	-rod	-	+	-	-	-	+	-	+	+	+	+	+	-	-	-
Enterococcus faecalis	+cocci	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+
Bacillus subtilis	+rod	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-

Keys: - : Negative; +: Positive

Table 2. Percentage of occurrence of bacterial isolates from computer mice

Bacterial isolates	Personal computers (N = 12)	Business centres (N=12)	Cyber cafés (N=12)	Total
	No (%) of occurrence	No (%) of occurrence	No (%) of occurrence	No (%)
S. aureus	5(41.7)	7 (58.3)	7 (58.3)	19 (30.6)
S. epidermidis	3(25.0)	1 (8.3)	4 (33.3)	8 (12.9)
Enterococcus spp	0(0.0)	1 (8.3)	2 (16.7)	3 (4.8)
Streptococcus spp	2(16.7)	2 (16.7)	4 (33.3)	8 (12.9)
E. coli	2(16.7)	3 (25.0)	2 (16.7)	7 (11.2)
P. aureginosa	0(0.0)	2 (16.7)	1 (8.3)	3 (4.8)
Bacillus spp	5(41.7)	4 (33.3)	5 (41.7)	14 (22.6)

Table 3. Percentage of occurrence of bacterial isolates from computer keyboards and ATM keypads

Bacterial isolates	Personal computers (N = 12)	ATM (N=12)	Business centres (N=12)	Cyber cafés (N=12)	Total
	No (%) of occurrence	No (%) of occurrence	No (%) of occurrence	No (%) of occurrence	No (%)
S. aureus	5(41.7)	8(66.7)	5(41.7)	6(50.0)	24(28.6)
S. epidermidis	2(16.7)	4(33.3)	2(16.7)	1(8.3)	9(10.7)
Enterococcus spp	2(16.7)	3(25.0)	2(16.7)	4(33.3)	11(13.1)
Streptococcus spp	2(16.7)	3(25.0)	5(41.7)	3(25.0)	13(27.4)
E. coli	3(25.0)	5(41.7)	3(25.0)	5(41.7)	16(19.0)
P. aureginosa	1(8.3)	2(16.7)	1(8.3)	2(16.7)	6(7.1)
Bacillus spp	0(0.0)	2(16.7)	1(8.3)	2(16.7)	5(6.0)

Table 4. Occurence of single and mixed bacterial contamination of ATM keypads, computer keyboards and mice

Source	No of samples collected	No (%) of samples with one isolate	No (%) of samples with two isolates	No (%) of samples with three isolates	Total No (%) of isolates
Computer keyboards					
Personal computers	12	10 (83.3)	1 (8.3)	1 (8.3)	15 (17.9)
Cyber cafés	12	4 (33.3)	5 (41.7)	3 (25.0)	23 (27.4)
Business centres	12	7 (58.3)	3 (25.0)	2 (16.7)	19 (22.6)
ATM keypads	12	2 (16.7)	5 (58.3)	5 (58.3)	27 (32.1)
Total	48	23 (47.9)	14 (29.2)	11 (22.9)	84 (100)
Computer mice					
Personal computers	12	8 (66.7)	3 (25.0)	1 (8.3)	17 (27.4)
Cyber cafés	12	3 (25.0)	5 (41.7)	4 (33.3)	25 (40.3)
Business centres	12	6 (50.0)	4 (33.3)	2 (16.7)	20 (32.3)
Total	36	17 (47.2)	12 (33.3)	7 (19.4)	62 (100)

The results of antibacterial activities of the varied concentrations of disinfectants on the bacteria isolated from the surfaces of ATM keypads, computer mice and keyboards are presented in Tables 6 and 7. Of the three disinfectants examined, Savlon showed the highest antibacterial activities on both Gram positive and Gram negative bacterial isolates. None of the disinfectants evaluated in this study inhibited the growth of *Streptococcus* spp SS-A9, *Bacillus* spp BS-P7 and *E. coli* EC-C8 (Tables 6 and 7). Among the Gram-positive bacteria, the highest and lowest inhibitory zones were observed in the plates containing *Enterococcus* spp EN-P10 and *Staphylococcus aureus* SA-A6 with the corresponding (mean ± SD) of 13.2 ± 2.0 mm

and 6.4 ± 1.2 mm, respectively (Table 6). The results obtained showed that Gram negative bacteria were more susceptible to the growth inhibition of the disinfectants than the Gram positive bacteria (Tables 6 and 7). The discs containing 20% disinfectants showed more antibacterial activities than those containing 10% concentrations. Statistical differences at $P < 0.05$ in the inhibitory antibacterial activities of the different concentrations of the disinfectants were equally observed.

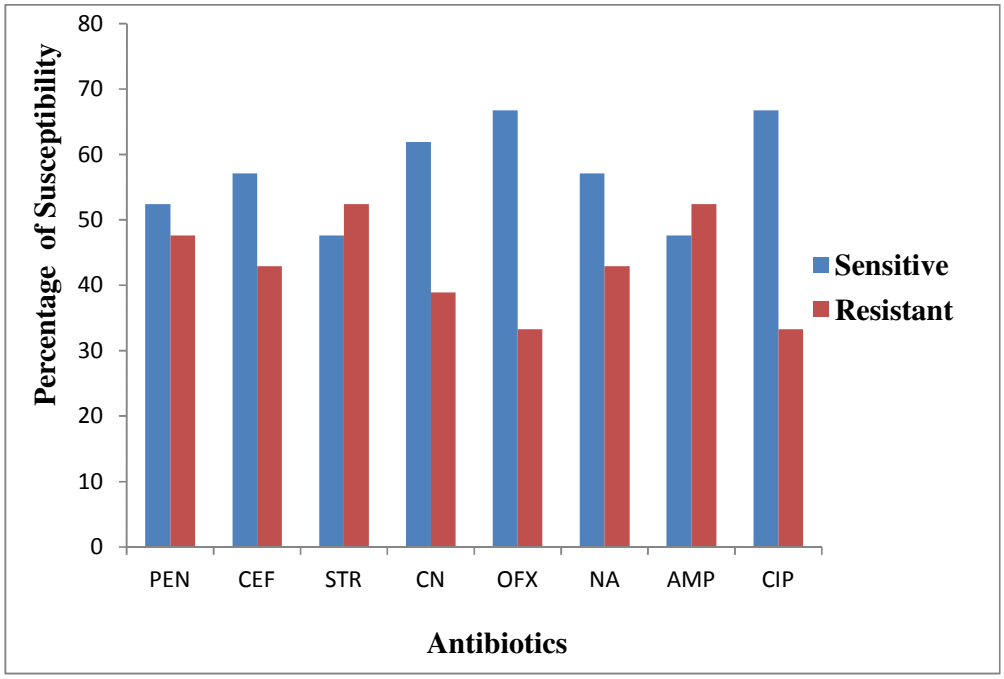

Fig. 7. Antibiotic susceptibility pattern of *Streptococcus* spp isolated from ATM keypads, computer keyboards and mice

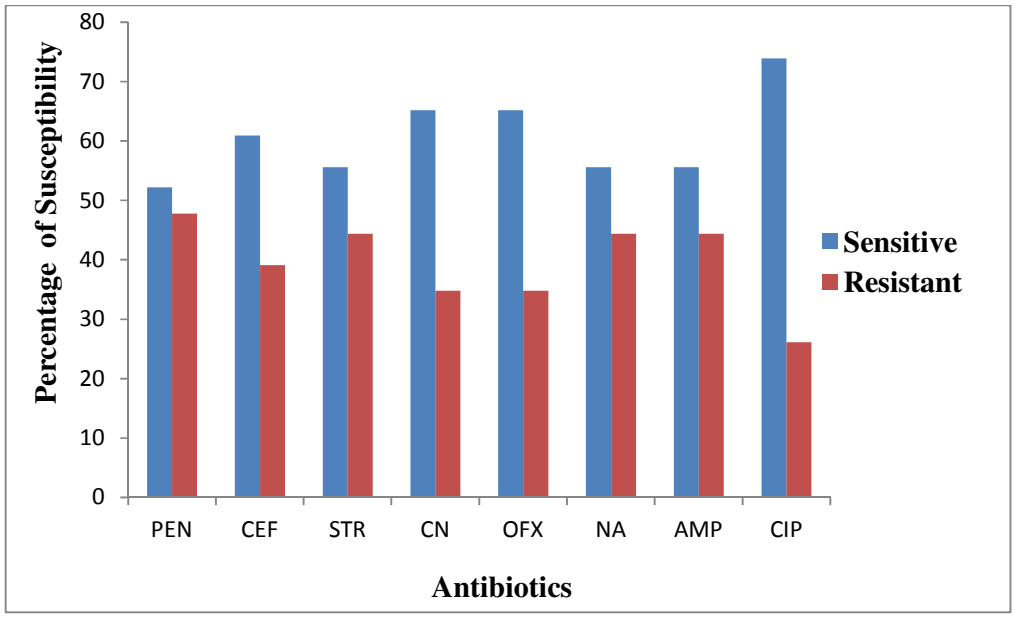

Fig. 8. Antibiotic susceptibility pattern of *E. coli* isolated from ATM keypads, computer keyboards and mice

Table 5. Virulence Markers in bacterial isolates from ATM keypads, computer keyboards and mice

Bacterial isolates	No of isolates	Virulence markers							
		DNase no (%)	TNase no (%)	Lipase no (%)	Amylase no (%)	Caseinase no (%)	Gelatinase no (%)	Haemolysin no (%)	Capsule no (%)
S. aureus	43	32 (74.4)	20 (46.5)	24 (55.8)	22 (51.2)	17 (39.5)	17 (39.5)	32 (74.4)	14 (32.6)
S. epidermidis	17	5 (29.4)	5 (29.4)	12 (70.1)	0 (0.0)	0 (0.0)	0 (0.0)	13 (76.5)	0 (0.0)
Enterococcus spp	14	5 (35.7)	3 (21.4)	6 (42.9)	5 (35.7)	8 (57.1)	8 (57.1)	10 (71.4)	5 (35.7)
Streptococcus spp	21	8 (38.1)	3 (14.3)	10 (47.6)	8 (38.1)	11 (52.4)	10 (47.6)	15 (71.4)	7 (33.3)
E. coli	23	4 (17.4)	3 (13.0)	4 (17.4)	9 (39.1)	0 (0.0)	0 (0.0)	10 (43.5)	0 (0.0)
P. aureginosa	9	0 (0.0)	4 (44.4)	6 (66.7)	4 (44.4)	3 (33.3)	4 (44.4)	2 (22.2)	6 (66.7)
Bacillus spp	19	7 (36.8)	8 (42.1)	11 (57.9)	13 (68.4)	9 (47.4)	9 (47.4)	6 (31.6)	0 (0.0)
Total	146	61 (41.8)	46 (31.5)	73 (50.0)	61 (41.8)	48 (32.9)	48 (32.9)	88 (60.3)	32 (21.9)

Table 6. Susceptibilities of gram positive bacterial isolates from ATM keypads, computer keyboard and mice to disinfectants

Bacterial isolates	Isolates codes	Dettol		Savlon		Hydrogen peroxide	
		10%	20%	10%	20%	10%	20%
	SA-P4	NZ	8.9±1.0[a]	NZ	9.5±1.0[b]	NZ	NZ
S. aureus	SA-A6	6.4±1.2[a]	8.0±1.0[a]	8.4±1.0[a]	10.6±2.[b]	6.9±1.0[a]	6.9±2.5[a]
	SA-B1	6.6±2.0[a]	9.1±0.5[a]	8.2±1.5[a]	9.0±0.5[a]	NZ	7.2±1.0[a]
	SA-C2	7.4±1.4[a]	8.9±1.0[a]	7.0±1.5[a]	8.7±1.3[b]	NZ	7.0±1.5[a]
	SE-P1	7.0±1.0[a]	7.5±1.5[a]	8.6±2.5[a]	10.2±1.0[b]	7.0±1.5[a]	9.6±1.2[b]
S. epidermidis	SE-A9	7.9±1.5[a]	9.8±1.5[b]	8.0±0.5[a]	9.6±1.6[b]	6.8±1.5[a]	8.1±0.5[a]
	SE-B12	7.5±1.0[a]	8.4±1.5[a]	8.8±1.3[a]	11.0±1.0[b]	NZ	7.6±1.5[a]
	SE-C5	6.8±1.0[a]	8.2±2.0[a]	9.3±0.5[a]	12.0±2.0[b]	NZ	8.3±0.7[a]
	EN-P10	NZ	NZ	9.3±0.6[a]	13.2±2.0[b]	NZ	NZ
Enterococcus spp	EN-A10	7.2±1.3[a]	8.9±1.5[a]	8.6±1.5[a]	9.0±0.5[a]	NZ	7.6±1.0[a]
	EN-B3	8.0±0.7[a]	9.3±1.0[a]	10.6±1.0[b]	11.0±0.5[b]	7.1±2.0[a]	7.9±0.8[a]
	EN-C2	7.7±1.2[a]	8.5±1.0[a]	8.9±1.2[b]	9.0±1.4[b]	7.5±1.0[a]	9.0±0.5[a]
	SS-P4	8.1±1.0[a]	8.8±1.5[b]	9.0±0.5[a]	9.6±1.0[b]	6.8±1.5[a]	8.1±1.5[a]
Streptococcus	SS-A9	NZ	NZ	NZ	NZ	NZ	NZ
spp	SS-B5	8.6±1.5[a]	10.1±1.0[b]	8.0±2.5[a]	10.6±1.6[b]	8.1±1.0[a]	8.9±1.6[a]
	SS-C2	NZ	7.3±0.5[a]	NZ	NZ	NZ	NZ
	BS-P7	NZ	NZ	NZ	NZ	NZ	NZ
Bacillus spp	BS-A12	7.0±0.5[a]	9.7±1.0[b]	7.7±2.0[a]	10.1±1.0[b]	NZ	NZ
	BS-B10	NZ	9.0±0.5[a]	NZ	8.3±1.0[a]	NZ	NZ
	BS-C6	7.9±0.5[a]	9.4±1.5[a]	7.5±1.5[a]	8.7±1.5[a]	NZ	NZ

Keys: NZ: No zone of inhibition; values in parenthesis are percentages; each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test (P <0.05).

Table 7. Susceptibilities of gram negative bacterial isolates from ATM keypads, computer keyboards and mice to disinfectants

Bacterial isolates	Isolates codes	Dettol		Savlon		Hydrogen peroxide	
		10%	20%	10%	20%	10%	20%
	EC-P6	NZ	9.4±1.0[a]	NZ	10.0±1.0[b]	NZ	NZ
	EC-A6	6.8±1.4[a]	8.5±0.5[a]	8.9±1.0[a]	11.1±2.5[b]	7.4±1.0[a]	7.4±2.0[a]
E. coli	EC-B7	7.1±2.0[a]	9.6±0.5[b]	8.7±1.5[a]	9.5±0.5[b]	NZ	7.7±1.0[a]
	EC-C8	NZ	NZ	NZ	NZ	NZ	NZ
	EC-A8	7.0±1.0[a]	9.4±1.5[a]	7.5±1.5[a]	9.2±1.0[a]	NZ	7.5±1.5[a]
	PA-P12	7.5±1.0[a]	8.1±1.2[a]	8.9±2.5[a]	10.7±1.5[b]	7.5±0.5[a]	10.1±1.0[b]
P. aureginosa	PA-A3	8.4±2.5[a]	10.3±1.0[b]	8.5±0.5[a]	10.1±1.0[b]	7.3±1.5[a]	8.6±2.5[a]
	PA-P3	8.0±1.0[a]	8.9±1.0[a]	9.3±1.3[a]	11.5±1.0[b]	NZ	8.1±1.5[a]
	PA-B2	7.3±1.3[a]	8.7±2.0[a]	9.8±0.5[b]	14.0±0.5[b]	NZ	8.8±0.7[a]
	PA-C1	8.8±0.5[b]	9.4±0.6[a]	8.6±1.5[a]	10.8±1.4[b]	9.1±1.2[a]	11.5±1.5[b]

Keys: NZ: No zone of inhibition; values in parenthesis are percentages; each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test (P <0.05)

4. DISCUSSION

Studies have indicated that computer keyboards and mice can become contaminated with bacteria [26,27]. *S. aureus, S. epidermidis, Enterococcus* spp., *Streptococcus* spp., *E. coli, P. aureginosa* and *Bacillus* spp were isolated from computer keyboards of cyber cafés, business centres, personal computers and ATM keypads in this study. Of particular interest was the isolation of 23.1% *E. coli* and 13.1% *E. faecalis* from keyboards, which was an indicative of faecal contamination. Isolation of *S. aureus* and *E. coli* in this study corroborates

the earlier investigations of bacterial contamination of computer keyboards and mice by [28,29]. The occurrence of *S. aureus* from the computer keyboards in this study is in agreement with the results of [7,26]. *S. aureus* was the most prevalent bacterial isolates from the computer keyboards from individual, business centres, cyber cafes and ATM keypads. The high occurrence of *S. aureus* may be attributed to fact that one's palm is usually moist due to varying degrees of perspiration, which contains sodium chloride that sustains the growth of this halophilic *S. aureus* [30,31]. *Bacillus* spp had the lowest percentage of occurrence in the computer keyboards in our study and this is contrary to [32] who reported *Bacillus* spp as the predominant bacteria associated with computer keyboards. The isolation of *Bacillus* spp, a common soil bacterium, was evidence of environmental contamination.

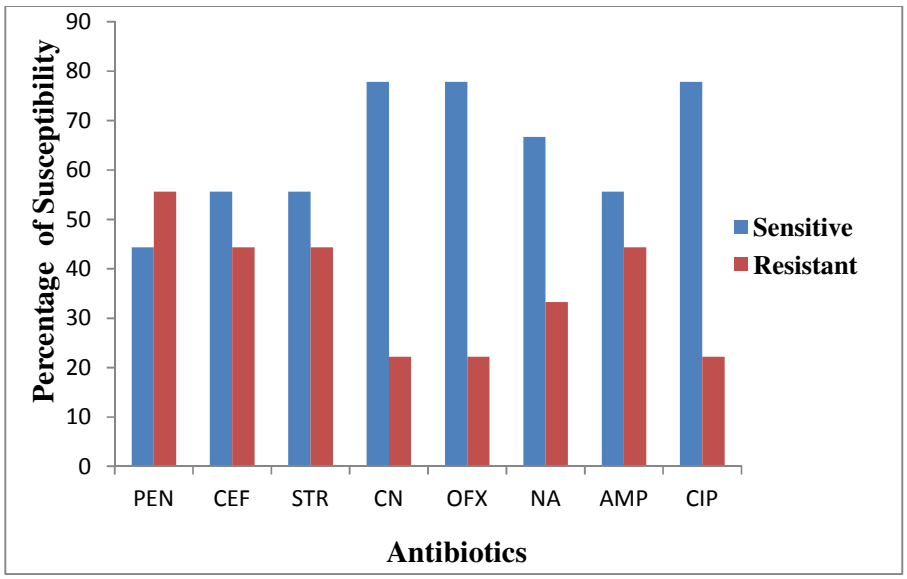

Fig. 9. Antibiotic susceptibility pattern of *P. aeruginosa* isolated from ATM keypads, computer keyboards and mice

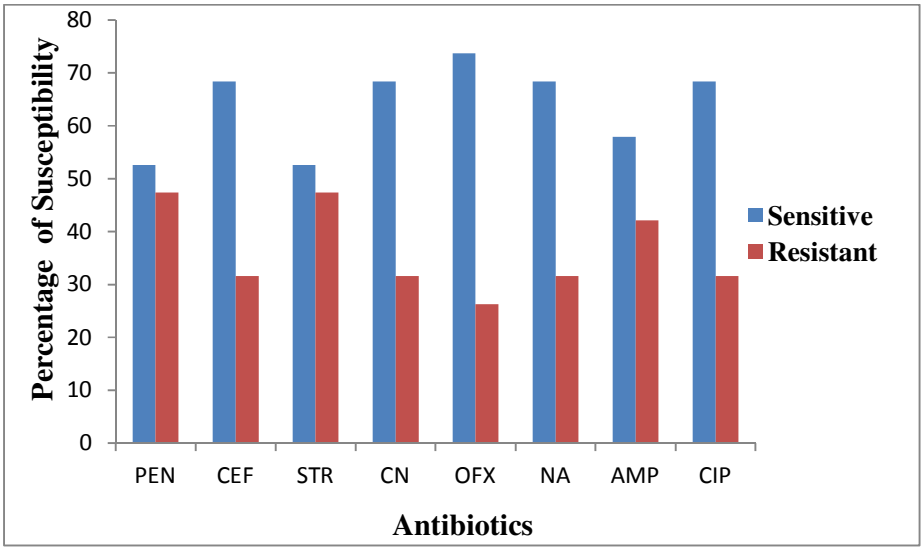

Fig. 10. Antibiotic susceptibility pattern of *Bacillus* spp isolated from ATM keypads, computer keyboards and mice

Keys: PEN: Penicillin; CEF: Ceftriaxone; STR: Streptomycin; CN: Gentamycin OFX: Ofloxacin; NA: Nalidixic Acid; AMP: Ampicillin; CIP: Ciprofloxacin

It was reported that computer keyboards harboured more bacterial contaminants than mice due to their large surface area and our study confirmed it [6]. Of the 36 computer keyboards and 12 ATM keypads examined in our study, 25 (52.1%) were colonized by mixed bacteria. The colonization of computer keyboards and mice by mixed bacterial isolates agree with the previous results of [33]. In this study, highest bacterial isolates were obtained from ATMs keypads and this may be attributed to their open location, exposure to wind and rain and also high number of multiple users.

In this study, 32 (74.4%) *S. aureus* and 8 (38.1%) *Streptococcus* spp produced DNase. The production of DNase by greater than 70% *S. aureus* isolated agrees with the previous results of [11,21]. Isolation of amylase producing *Bacillus* spp in our study corroborates the results obtained by [34] who isolated species of *Bacillus* that had capability to produce amylase. The results of our study are also in conformity with [14,35] who obtained amylase producing *Streptococcus* spp. The possession of virulence markers such as lipase, caseinase, gelatinase and haemolysin observed in the bacteria isolated from the ATM keypads, computer keyboards and mice confirmed the reports of [36,37] that computer harboured pathogenic bacteria. This study also showed that the bacterial isolates from ATM keypads, computer keyboards and computer mice were moderately sensitive to practically all the antibiotics used and these results are in disagreement with [12] who reported between 80% to 100% antibiotic resistant bacterial isolates from computer keyboards and computer mice.

Dettol and Savlon are widely used for various purposes including disinfection of skin, objects and equipment, as well as environmental surfaces [38,39]. The antimicrobial properties of disinfectants on some pathogenic bacteria have also been reported [40,41,42]. All the *P. aureginosa, Enterococcus* spp and *S. epidermidis* isolated were sensitive to Savlon. The sensitivity of *P. aureginosa* to Savlon in this research corroborates the earlier reports of [41,43]. The mechanism of action of disinfectant is by production of destructive chemicals that attacks membrane lipids, DNA and other essential cell components of various pathogenic bacteria [38,44]. Microorganisms are continuously acquiring resistance to new disinfectants; as a result, no single disinfectant will be appropriate for all pathogens [45]. The *Streptococcus* spp SS-P9, *Bacillus* spp BS-P7 and *E. coli* EC-C8 were resistant to different concentrations of Dettol, Savlon and H_2O_2. [46] reported that Gram-negative bacteria were generally more resistant to disinfectants than other bacteria, but in this study, there was no remarkable difference in the susceptibility of the Gram-positive and Gram-negative bacteria isolated from the computer keyboards, mice and ATM keypads to disinfectants. [39] reported that all the bacterial isolates tested were sensitive to 5% H_2O_2 and this is in contradiction to our results as > 50% of the bacteria were resistant to the 10% H_2O_2.

The inanimate objects play a role in the transmission of human pathogens either directly by surface to mouth contact or indirectly by contamination of fingers and subsequent hand to mouth contact, thus, hands must be properly washed with detergents whenever the ATMs and computers are used. In addition, cleaning of the ATM and computer with disinfectants on a regular basis should be adopted.

5. CONCLUSION

This study has further established that automated teller machines (ATM), Computer keyboards and mice harbour multidrug resistant pathogenic bacteria that may be transferred to / among the users.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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