Prevalence and plasmid D NA profiles of antibiotic resistant bacterial isolates from mobile phones of volunteer University students in Sagamu Nigeria.

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ABSTRACT

***Background*:** Mobile phones are devices that can receive and make calls over a radio frequency link while the user is moving within a telephone service area. They are indispensable devices for professional and social life. There is a potential for microbial colonization of this device from the users. This study evaluated the prevalence, resistance to conventional antibiotics and plasmid profiles of some selected bacterial isolates from the phones of volunteers' students.

Methods: A total number of one hundred (100) phone swab samples were collected and inoculated in peptone broth and incubated overnight. The inoculums were thereafter sub-cultured in different culture media for the isolation of various bacteria and their identity were confirmed using standard biochemical tests; catalase test, coagulase test, oxidase test, indole test, methyl red test, DNAse test, citrate utilization and haemolysis test. Antibiotic susceptibility using modified Kirby Bauer methods and plasmid profile analysis of some selected resistant isolates were determined.

Results: The prevalence of *Staphylococcus aureus* had the highest (62%) followed by *Escherichia coli (50%),Pseudomonas aeruginosa* (44%), *Streptococcus* spp (20%) and *coagulase negative staphylococci* (18%) respectively. The antibiogram of the biochemically characterized isolates showed varied patterns of antibiotics resistance and plasmid molecular weights profiles.

***Conclusion*:** The prevalence of pathogenic bacteria with remarkable resistance to a broad spectrum of antibiotics and with plasmids of varied molecular weights from the phones of the volunteers showed the potential of the phones as a possible agent of transmission of pathogenic infection.

Keywords: Mobile phone, antibiotic resistance, plasmid profiles, Sagamu campus.

1. INTRODUCTION

Mobile phones are devices that can receive and make calls over a radio frequency link while the user is moving within a telephone service area. It is used to transmit conversation across distances. It is the most indispensable accessory of professional and social life throughout the world [1].Modern mobile telephone services use cellular network architecture, and, therefore, are called cellular telephones or cell phones, in North America. The first handheld mobile phone was demonstrated by John F. Mitchell and Martin Cooper of Motorola in 1973, using a handset weighing 2 kilograms (4.4 lbs). In 1979, Nippon Telegraph and Telephone (NTT) launched the world's first cellular network [2].Super improvement was recorded in 2000s era-mobile phones has incorporated the potential of other services, such as text messaging, MMS (Multi-media Message Services), email, internet access,

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short-range wireless communications (infrared, Bluetooth), business applications, video games, and digital photography. Now, more people are using Smartphone than the old kind of mobile phone, which are called feature phones. Smartphone facilitates wider software and there have been some improvements in the modification of phones compared to the conventional functions [3].Mobile or cell phones are not as clean as we think they are. Each square inch of the mobile phone contains roughly 25,000 germs, making it one of the most microbial colonized devices we come in contact with on a daily basis .The average person checks their mobile phone approximately once every 30 minutes, this means our hands are perpetually on our device and we engage our hands in a lot of activities during the day and perhaps even when we visit the toilet. Research shows that 95% of people don't wash their hands properly and after shaking someone or touching things others have touched, there is a great germ adherence potential which may likely end on the mobile phone [4]. The more time we spend on our phones, the higher the phone temperature will become. Phone batteries have a temperature range of 37- 43°C or 98.6-109.4°F when active creating the ideal breeding ground for bacteria. With the advent of touch-screen phones, we use our microbe colonized hands, which if not well washed, increases the chances of infection. Also, we press the mobile phone against the mouth, cheeks and nose thereby adding up to the microbial load of the phone. There are some reports which indicate that the low emphasis on regular disinfection of hands and poor hand washing practices by health professional predispose their and other individuals' mobile phones to the colonization of bacteria. A study in the US revealed more than 80% of the common bacteria that make up our bacterial fingerprints end up on mobile phones bodies in entirety [5].Much of the disease-causing bacteria found on phone are transferred from person to person through touch, which means that once these bacteria get to the hands, one only have to then touch the eyes or nose for the bacteria to find an easy route into the body. Not everyone cleans or disinfects their phone, so the bacteria keeps multiplying. Mobile phones can be tagged mobile germ devices, with germs adhering to the hand, and the phone. Even if we wash our hands later, the germs are still on the mobile phone. Mobile phones have become veritable reservoirs of pathogens as they touches faces, ears, lips and hands of different users of different health conditions as observed by a researcher in a 2009 study of bacteria from personal calling devices [7]. A study by the University of Arizona found that the typical worker's desk, which tends to be the mobile phone's home for about 40 hours a week, has hundreds of times more bacteria per square inch than an office toilet seat. Other studies have found serious pathogens on Smartphone such as Streptococcus spp, Escherichia coli and methiciline resistant Staphylococcus aureus which is a type of bacteria that are resistant to several antibiotics [8].Epidemiological studies have confirmed that surfaces played a major role in the spread of infectious diseases. Many bacterial, fungal and viral pathogens can survive on the inanimate objects known as reservoir for a long time, and such pathogens could cause infections as a result of direct or indirect transmission [8]. In spite of the potential of microbial flora of mobile phone as agent of transmission of infection, not many studies in Nigeria have assessed the microbial burden of phones and its implication for public health. This study was therefore carried out to evaluate microbial pathogens associated with mobile phones of some volunteer students in Olabisi Onabanjo University campus in Sagamu Nigeria.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Reagents

Reagents used for this study include; Hydrogen peroxide (H_20_2), oxidase reagents, kovac's reagent, barium chloride, normal saline, sulphuric acid (H_2S0_4), Lugol's iodine, crystal violet, safranin, methylated spirit and 95% alcohol

2.1.2 Equipment

The equipment used for the study include; incubator, autoclave, electronic digital weighing balance, binocular microscope, laminar flow hood, water bath, graduated syringes, forcep, antibiotic disc dispenser and graduated glass wares.

2.1.3 Biological Materials

The biological materials and consumables used for this study include, nutrient agar, eosin methylene blue agar, cetrimide agar, mannitol salt agar, peptone powder, Mueller Hinton Agar and sheep blood agar base.(all the biological media were Oxoid made)

2.2 Methods

2.2.1 Study Design and selection criteria

This was a cross sectional study conducted between March and April 2021 amongst the students within the Faculty of Pharmacy and Basic Medical Sciences of Olabisi Onabanjo University Sagamu campus, with a total population of 3000 students.

2.2.2 Sample size
A total number of one hundred (100) cell phones, 50 each from pharmacy school and faculty of basic medical sciences were studied.

2.2.3 Sampling technique

Purposive sampling technique was used to select 100 cell phones to unravel the phenomenon that are associated with the subjects and phones collected.

2.2.4 Collection of Samples
Sterile swab sticks moistened in physiologic saline were used to swab the volunteers' cellphones mouthpiece, keypads, screen and the entire phone. The swab sticks were then dipped into the sterile peptone broth prepared in each test tube and incubated at 370Cfor 24 hours. The resulting culture was thereafter aseptically plated on different culture media; Eosin Methylene Blue, Cetrimide Nutrient Agar, Mannitol Salt Agar, Sheep Blood agar base supplemented with rabbit blood and incubated at optimum temperature required for 24 hours for the isolation of each different bacteria.

2.2.5 Biochemical identification of the isolates
The isolates obtained were Gram stained and the following biochemical tests were carried out to confirm their identities; catalase test, coagulase test, oxidase test, indole test, methyl red test, DNase test and citrate utilization and haemolysis test

2.2.5 Antibiotic susceptibility
The Kirby Bauer disc diffusion method was used in determining the antimicrobial susceptibility of the isolates. Four to five colonies of the overnight isolates were inoculated to nutrient broth and were incubated at incubated at 370C for 24 hours. The inoculums were standardized by diluting the broth cultures until turbidity matched the 0.5 McFarland standard. A sterile cotton swab was dipped into the suspension, impressed against the wall of test-tube to drain were used to inoculate 20mL of Mueller Hinton Agar on a 100-mm disposable Petri dishes. The air-dried plates were allowed to stand for 20 minutes and antibiotic discs were placed gently to make a contact on the agar with the aid of flamed forceps. Disc containing the following antibiotics were used; Gentamicin (GEN -10 µg), Ciprofloxacin (CIP -5µg), Ampicillin (AMP-10 µg), Meropenem (MEM-10 µg), Erythromycin (ERY-5 µg), Tetracycline (TET-30 µg), Cotrimoxazole (COT-25µg), Cefuroxime (CRX-10 µg), Cephalexin (CP-10 µg), Augmentin (AUG-30 µg), Ceftazidime (CPZ-10 µg) Vancomycin (VAN-30 µg) for Staphylococcus aureus and coagulase negative staphylococci while another brand of Gram negative spectrum antibiotics; Ciprofloxacin-(CPR-5µg), Ceftriaxone (CAZ-30 µg), Cefuroxime (CRX-30 µg), Gentamicin (GEN-10 µg), Ceftaxidime (CXM-30 µg), Ofloxacin (OFL-5 µg), Augmentin (AUG-30 µg), Nitrofurantoin (NIT-30) were used for Pseudomonas aeruginosa and Escherichia coli. The plates were incubated at 370C for 24 hours and the zones of growth inhibitions were measured in millimetres and interpreted with CLSI standard.

2.2.7 Plasmid DNA Extraction
The overnight (growing) nutrient broth culture of the bacterial cells in Eppendorf tubes were centrifuged at 13,000 rpm for 2 mins after which supernatants were discarded. The pellet was suspended in the remaining broth by vortexing at high speed. The suspended pellet was treated as follows; 300 µl of TENS (Tris 25 mM, EDTA 10 mM, NaOH 0.1 N, SDS 0.5 %) solution was added and mixed by inverting the tube gently until the solution became slimy.150 µl of Na2 acetate (3.0 M, pH 5.2) was added and vortex for 10 seconds. The mixture was centrifuged at 13,000 rpm for 5 mins. The supernatant was transferred from here into a sterile Eppendorf tube and 900 µl ice cold absolute ethanol was added. This was vortex mixed and centrifuged at 13,000 rpm for 10 minutes. After centrifugation, the supernatant was discarded and at this time the pellet turned white.1000 µl ice cold 70% ethanol was added to the white pellet and centrifuged at 13,000 rpm for 5 minutes. The supernatant was discarded and the pellet dried totally. The dried pellet was suspended in 40 µl of TE (Tris 10 mM, Na2EDTA 1 mM).

2.2.8 Gel electrophoresis of the suspended pellet
This was done as follows: 0.8 % agarose powder in ×0.5 TBE (Tris, Boric acid, EDTA) buffer was dissolved by boiling. This was allowed to cool to about 60oC before adding 10 µl of ethidium bromide (1 mg/mL). After gentle swirling, it was poured into electrophoresis tank and comb inserted. This was allowed to cool. After solidification (gelling) the comb was removed and the gel totally submerged in ×0.5 TBE. The sample (suspended pellet, 15 µL) was mixed with 2 µL of loading dye and carefully loaded into the wells created by the combs alongside 100 bp DNA ladder. This set up was connected to power pack and run at 100 V for 45 minutes. The gel was thereafter observed in gel photo documentation systems.

2.3 Statistical Analysis

Microsoft Excel (Window 8) was used to collate the data while descriptive statistics were used to analysed the frequency and interpret the data.

3.0 RESULTS

A total number of 100 mobile phones samples from volunteer students were studied for presence of bacterial contaminants. Both Gram positive and Gram negative bacteria were found in varied density (Figure 1).

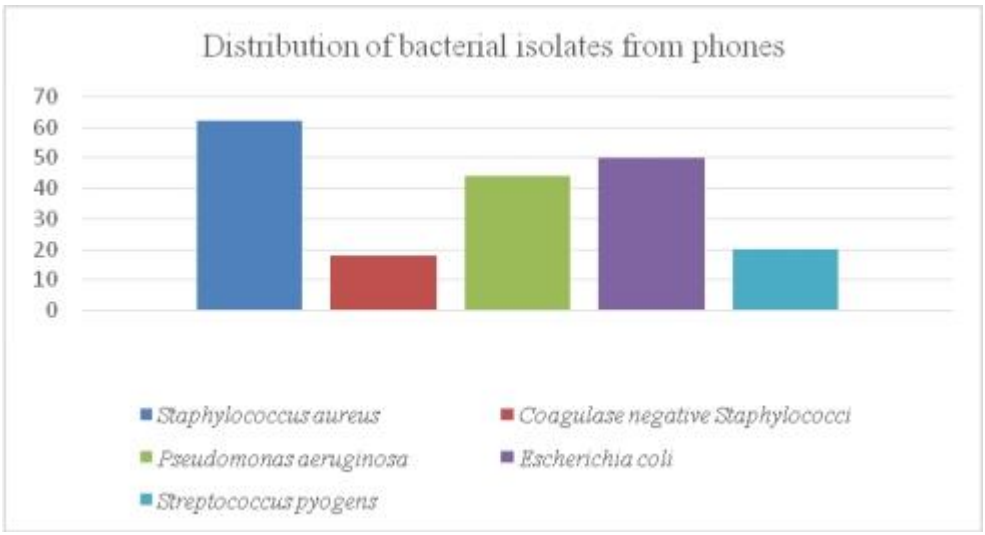

Figure 1: Distribution of isolates of bacteria from the phones of selected volunteers

The percentage of isolates of ***Staphylococcus aureus*** resistant to first and third generation β-lactams antibiotics was high., The isolates were 93.5% percent resistant to ampicillin, 100% resistant to Meropenem, cefuroxime, Cephalexin, Augmentin and Ceftaxidime. While the isolates were 22.5% resistant to gentamicin (Figure 2).

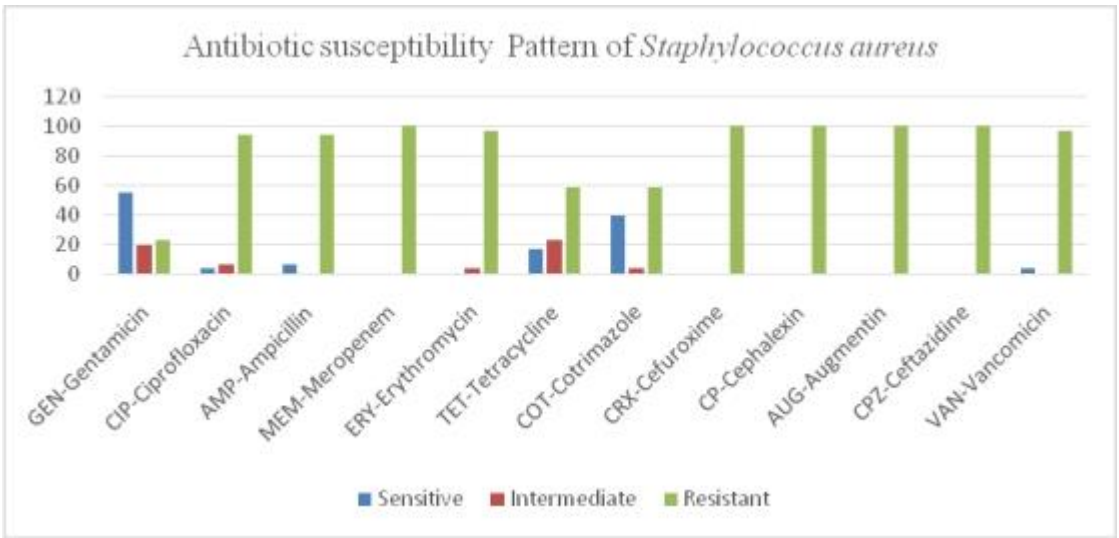

Figure 2: Antibiotic Susceptibility pattern of *Staphylococcus aureus* from volunteers' mobile phones

The percentage of coagulase negative *Staphylococci* isolates resistant to first and third generation β-lactams antibiotics was remarkable. The pattern of resistant was similar to *Staphylococcus aureus* with the exception of vancomycin which was 11.11% as shows in (Figure 3).

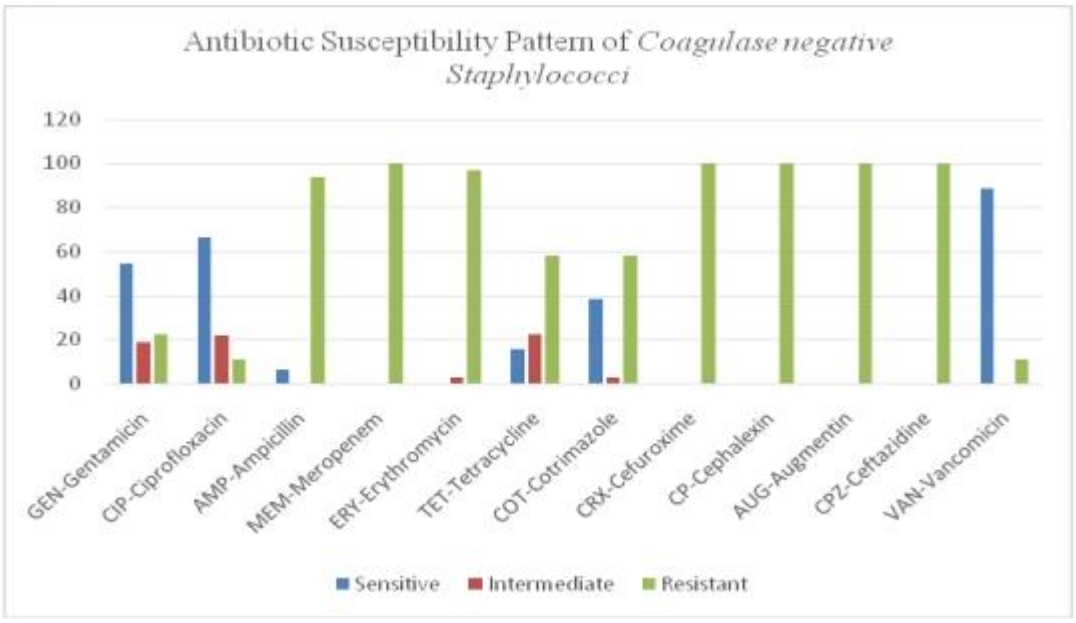

Figure 3: Susceptibility pattern of *coagulase negative staphylococci* from mobile phones of volunteers to antibiotics

The percentage of isolates of *Pseudomonas aeruginosa* resistant was 66.6% percent to ciprofloxacin and ceftriaxone, 100% to cefuroxime, Ceftaxidime and Augmentin, 40% to gentamicin- a broad spectrum antibiotics, 33.3% to Ofloxacin and 80% to nitrofurantoin (Figure 4).

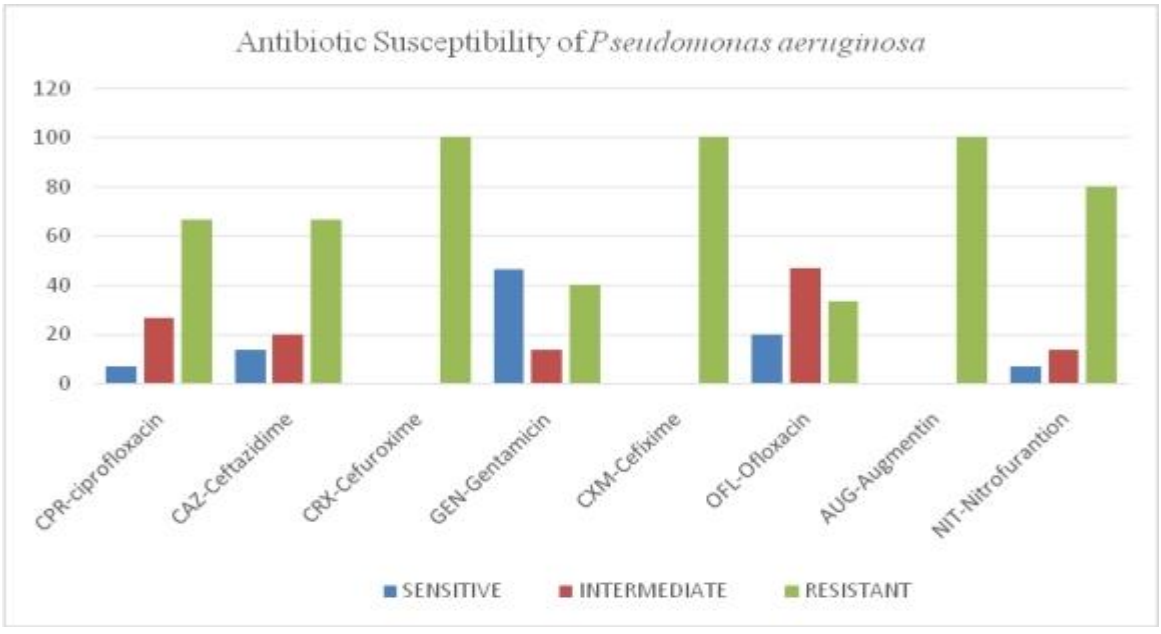

Figure 4: Antibiotic Susceptibility pattern of *Pseudomonas aeruginosa from* volunteers' mobile phones

The percentage of isolates of *Escherichia coli* resistant was 80% percent to ciprofloxacin, 100% to Ceftazidime, cefuroxime, cefixime and Augmentin, 40% to gentamicin- a broad spectrum antibiotics, 73.3% to Ofloxacin and 33.3% to nitrofurantoin (Figure 5).

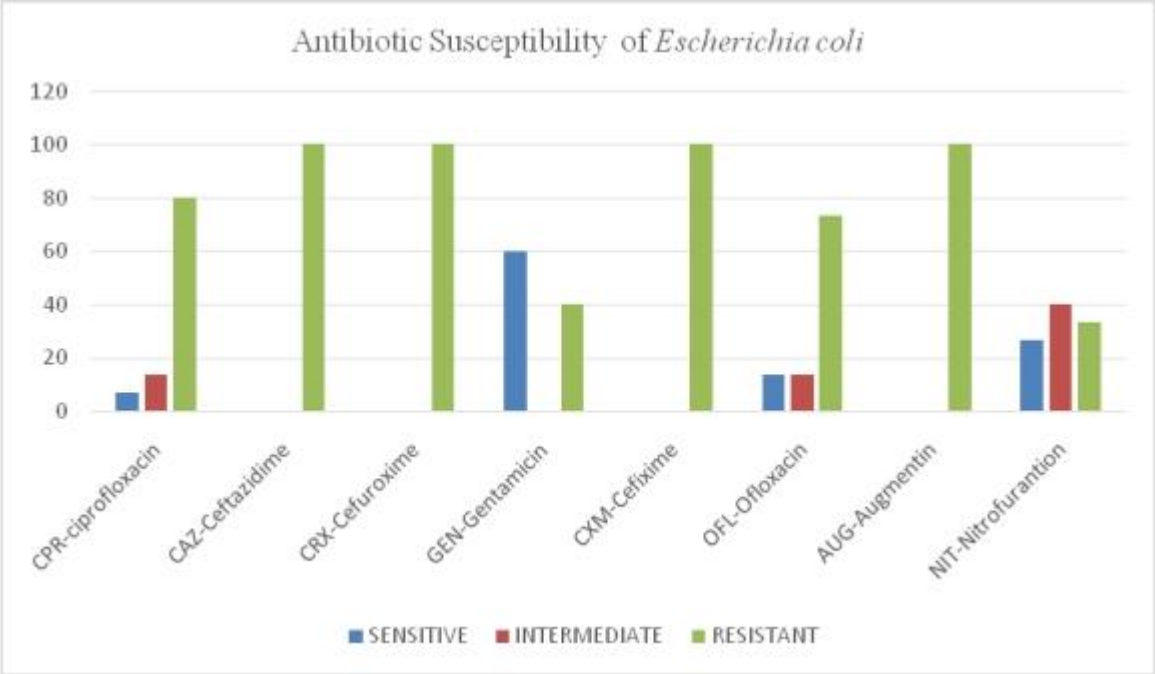

Figure 5: Susceptibility pattern of pattern of *Escherichia coli* from volunteers' mobile phones to antibiotics

Streptococcus spp were 100 % resistant to ciprofloxacin, ampicillin, tetracycline, cephalexin and Ceftazidime was alarming, but resistant to gentamicin was 10%, Meropenem 70%,erythromycin 50%, Cotrimoxazole 30%, cefuroxime 60%, Augmentin 90% and 10% to vancomycin (Figure 6).

3.1 Plasmid analysis of selected isolates

Fifteen (15) isolates of *Staphylococcus aureus* that are resistant to β-lactams antibiotic were selected for plasmid analysis and were found to contain plasmids of varied molecular weights (Figure 7).

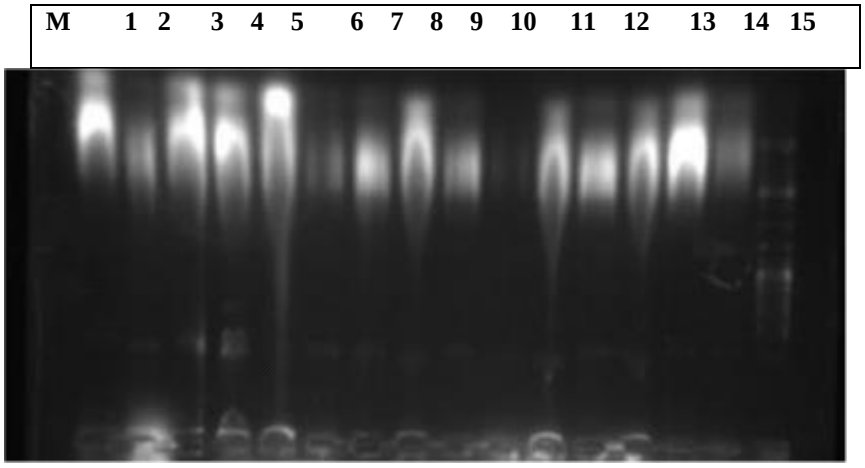

Figure 7: Plasmid analysis of selected isolates of *Staphylococcus aureus*

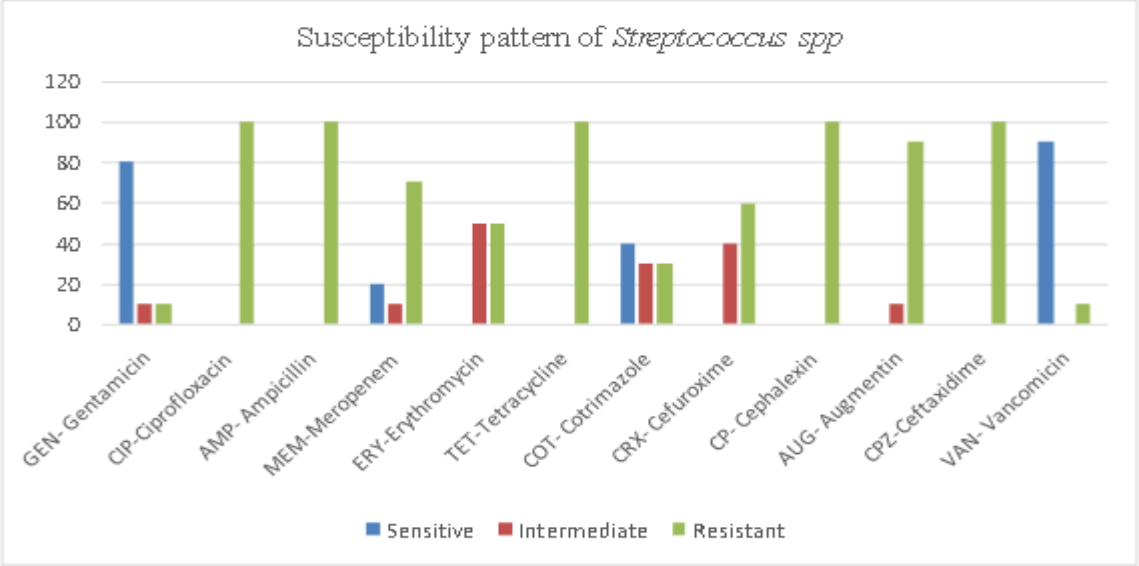

Figure 6: Susceptibility pattern of pattern of *Streptococcus spp.* from volunteers' mobile phones to antibiotics.

Table 1: Copies and Molecular Weights of the Plasmid of selected *Staphylococcus aureus*

Isolate Number	Plasmid Copies	Molecular Weight(Kb)
Sa 1	1	7.08
Sa 2	1	28.18
Sa 3	1	23.98
Sa 4	1	19.93
Sa 5	1	7.09
Sa 6	1	11.52
Sa 7	1	10.00
Sa 8	1	31.26
Sa 9	1	26.30
Sa 10	1	10.00
Sa 11	1	28.10
Sa 12	1	28.18
Sa 13	1	10.00
Sa 14	1	31.62
Sa 15	3	26.30
		28..22
		19.95

3.1 Plasmid analysis of selected isolates

Fifteen (14) isolates of *Pseudomonas aeruginosa* that were resistant to gentamicin, ciprofloxacin and nitrofurantoin antibiotics were selected for plasmid analysis and were found to contains no plasmids (Figure 8).

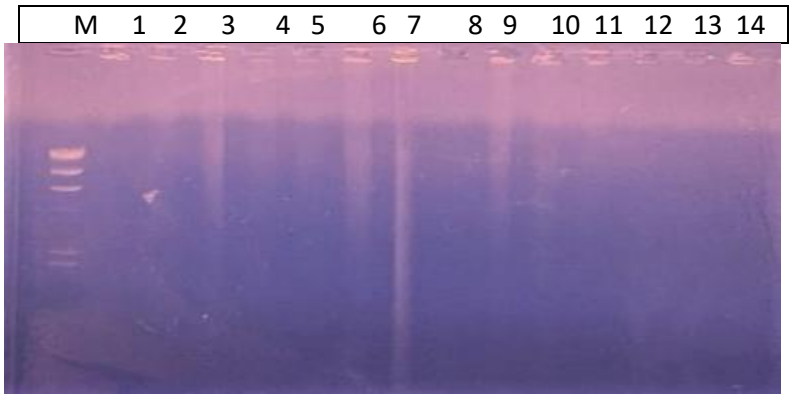

Figure 8: Plasmid DNA analysis of selected isolates of *Pseudomonas aeruginosa*

4. DISCUSSION

The distribution of isolates of bacterial obtained in this study showed variation in percentage prevalence. Staphylococcus aureus, *Escherichia coli* and Pseudomonas aeruginosa were found to be relatively higher while streptococcus spp and coagulase negative staphylococci were recorded to be lower. The incidence of bacteria recorded could be part of the microflora of the skin, mucous membranes, mouth of the users and their immediate environment [9]. The health implications of phones infested with pathogenic bacteria could be life threatening or a threat to public health. Staphylococcus aureus were found to be the most prevalent (62%), while coagulase negative Staphylococci (18%) were the least, this could be due to adaptability of the organism to the prevailing environmental milieu of the phones phone users that determined the survival rates of those bacteria and also, could be due to immune status of the volunteers. This corroborated the study of Detta *et al*., [10] on bacterial contamination of mobile phones of health care workers [10].The comparative percentage of isolates of Staphylococcus aureus resistant pattern especially to first and third generation beta-lactams antibiotic was alarming, the isolates was 93.5% percent resistant to ampicillin, 100% resistant to meropenem, cefuroxime, cephalexin augmentin and ceftaxidime - a third generation Cephalosporin while the isolates were 22.5% resistant to gentamicin. This resistance obtained could attributed to poor handlings of phones by the users and or indiscriminate use and misuse of antibiotics [12]. The percentage of isolates of coagulase negative Staphylococci resistant pattern to first and third generation beta-lactams antibiotic follows the same pattern of resistant obtained in Staphylococcus aureus with the exception of vancomycin which was 11.11% and this could be due to strain variations and inherent factors within the organisms, which agrees with the study of Khan *et al.*, [11] on clinically significant coagulase negative staphylococci and their antibiotic resistance pattern[13].The comparative percentage of isolates of Pseudomonas aeruginosa resistant was 66.6% percent to ciprofloxacin and ceftriaxone, 100% to cefuroxime, Ceftaxidime and Augmentin, 40% to gentamicin- a broad spectrum antibiotics, 33.3% to Ofloxacin and 80% to nitrofurantoin .This was in contrast with the study of Farida Anjum *et al*., [12] on susceptibility pattern of Pseudomonas aeruginosa against various antibiotics[14].The percentage of isolates of *Escherichia coli* resistant was 80% percent to ciprofloxacin, 100% to ceftazidime cefuroxime, cefixime and augmentin, 40% to gentamicin, 73.3% to Ofloxacin and 33.3% to nitrofurantoin. The prevalence pattern obtained from this study was in contrast with the percentage of Escherichia coli isolated by Famurewa and David [13] on mobile phone as a medium of transmission of bacteria pathogens which could also be due to immune status of the hosts and strains variation [14]. The comparative percentage of isolates of Streptococcus spp100 % resistant to ciprofloxacin, ampicillin, tetracycline, cephalexin and Ceftazidime was noteworthy, but resistant to gentamicin was 10%, Meropenem 70%,erythromycin 50%, Cotrimoxazole 30%, cefuroxime 60%, Augmentin 90% and 10% to vancomycin which corroborated the study of Stevens et al., (1994) on invasive group A streptococcal infections [15] Almost all isolates obtained in this study were highly resistant to the antibiotics tested which could worsen the issues of antibiotic resistance transfer and thereby, aggravate treatment if they eventually causes infection. Staphylococcus aureus were selected to represents Gram positive spectrum isolates in this study showed plasmids of varied molecular weights why Pseudomonas aeruginosa to represents Gram negative spectrum showed no plasmid. The plasmids pattern in Staphylococcus aureus obtained in this study with varied molecular weights suggests that the resistance to the antibiotics could be plasmid borne. The plasmids obtained in this study could be a threat to chemotherapy because of the capability to habour antibiotic resistant factors. Pseudomonas aeruginosa elicited no plasmid, but the resistance obtained could be chromosomal, which can also encode resistance in *Pseudomonas aeruginosa*[16].Handling of mobile phones with dirty hands and other environmental unhygienic related factors could contribute to microbial density and diversity found on the volunteers` mobile phones. This supports the previous findings of Rusin *et.al*. [17], on comparative surface to hand and fingertip to mouth transfer efficacy of bacteria. Since mobile phones are used in close proximity to sensitive parts of human body such as the face, ear lips and hands, this can lead to transmission of infections due to lack of personal hygiene and sanitation measures such as hand washing and phone cleaning, mobile phones slowly become pathogenic agents of microbial transfer [18]. It is therefore recommended to create public awareness on regular cleaning of phones, clean hand hygiene practices to reduce the bacterial and formites carriage and thereby reduce the possibility of transmission of infections from person to person. Further study is also recommended to assess the disinfectants that could be phone friendly for regularly cleansing of the phones when suspicious contacts had been touched.

5. CONCLUSION

The distribution of isolates of bacterial with clinical status obtained from the mobile phones of the volunteers in this study, suggest poor hygiene habit of some users. And since mobile phones has a role in the transmission of microorganisms, the users of mobile phone should be encourage to imbibe hygiene habit and regularly clean their mobile phones to reduce the risk of hand-to-hand, and hand-to-object transmission of bacterial pathogens.

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No conflict of interest

Contributions of Authors
O.L.Okunye - Conception, data collection design, analysis and manuscript preparation
PA Idowu - Data collection, analysis and manuscript preparation
BM Okanlawan - Data collection, analysis and manuscript preparation
AO Idowu - Data collection, design, manuscript preparation
OE Oyinloye - Data collection, analysis and manuscript preparation

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