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Full Length Research Paper

Incidence and antibiotic susceptibility profile of bacteria isolated from mobile phones of food handlers in Birnin Kebbi, Kebbi State, Nigeria

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This research was aimed to determine the incidence and antibiotic susceptibility profile of bacteria isolated from mobile phones of food handlers in Birnin Kebbi, Kebbi state Nigeria, using aseptic swab technique. Hundred swab samples from mobile phones of food handlers were collected. Bacteria Isolated were enumerated using pour plate method and overall mean aerobic mesophilic bacterial counts of 2.70x10^{6}CFU/ml was obtained. Bacterial isolates (n=111) belonging to three genera; *S. aureus* (n=88) *Escherichia coli* (n=19) *and Salmonella spp* (n=4) were identified. Antibiotic susceptibility profile of the isolates indicated that the highest sensitivity of 40mm against *S. aureus* was ciprofloxacin, 30mm against *E. coli* and *Salmonella* spp has 26mm. However, *Salmonella spp* was resistance to nitrofloxacin and chloramphenicol with 0.0mm. The highest zone of inhibition was recorded against ciprofloxacin with 30 and the least resistant drug was chloramphenicol with 0.0mm respectively. The findings are of public health concern since mobile phone may serve as a vehicle for transmission of various bacterial diseases. Since using mobile phones become day to day activities, food vendors and other common people should be sensitized to adhere to infection control measures, such as hand washing, to avoid possible cross-contamination between mobile phones and food items.

Keywords: Antibiotic susceptibility, *S. aureus*, Mobile phones, zone of inhibition, food handlers.

INTRODUCTION

The global system for mobile telecommunication (GSM) was established in 1982 in Europe with a view to providing and improving communication network. Today, mobile phones have become one of the most indispensable accessories of professional and social life. Although they are usually stored in bags or pockets, mobile phones are handled frequently and held close to the face (Kawo and Musa, 2013). Mobile phones are increasingly being used by people in day to day life, and these phones are usually in contact with various surfaces and are thus likely to get contaminated with various organisms (Tambe and Pai, 2012). Several pathogenic microbes including *Salmonella* spp and *S. aureus* have been isolated in different countries from mobile phones by many researchers (Ekrakene and Igeleke, 2007; Akinyemi *et al*., 2009, Al-Abdalall, 2010). The presence of pathogenic microbes on mobile phones is an indication that it has played a great role in spreading the infectious agents in the community and cause disease outbreaks (Akinyemi *et al*., 2009).

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Street foods are ready-to-eat foods and beverages prepared and or sold by vendors, especially on streets and other public places. Types of vending sites encompass stalls, a variety of push-carts, roadside stands, hawkers with head-loads and other arrangements depending upon the ingenuity of the individual, resources available, type of food sold and the availability of other facilities. Street vendors are common in both developing and industrialized countries with a considerable expansion in the former. Evidently, in large cities of developing countries, various food items of animal and plant origin are commonly vended at areas with busy economic activities and heavy movement of people (Chuiv *et.al.,* 2002) These areas include transportation centres, large construction sites, schools, factories, hospitals, churches, checkpoints and other similar business centres. Both males and females are involved actively in the activities of street vending (Ashen *et. al.,* 1995).

Research has shown that mobile phones could be a health hazard with ten thousands of microbes living on each square inch of phones. The normal microbiota is harmless and may be beneficial in their normal location in the host and in the absence of coincident abnormalities. However, they can produce disease condition if introduced into foreign locations or compromised host (Ekrakene and Igeleke, 2007). The normal microbial of the skin include among others; Staphylococci which are also found regularly on clothes, bed linen and other human environments (Roth and Jenner, 1998). *Staphylococcus epidermidis* is also a member of the normal flora of the human skin, respiratory and gastrointestinal tracts.

Staphylococcus aureus remains one of the major economical human and animal diseases in Nigeria, it results in economic losses to producers by increasing the cost of production through replacement cost of drugs services for treatment and control of the affected humans and animals (Nielsen 2009; Amosun *et al.,* 2010); despite its importance very little attention is given to it (Salihu *et al.,* 2011). Among several bacterial pathogens that can cause boils, sepsis and abscess, *S. aureus* is probably the most common agent because it causes chronic and deep infections in human and animals such as cattle herd that is extremely difficult to be cured (Smith *et al.,* 2001 and Gruet *et al.,* 2001; Anayo *et al.,* 2013).

Phenotypic, genotypic and biochemical characteristics knowledge of *Staphylococcus aureus* infection in both humans and animals in Nigeria are scanty (Shittu *et al.,* 2011), therefore there is a need for more intensive research work in this area especially the study area (Kebbi State), (Mamman 2005). Despite the fact that mobile phones make most human activities easier; they may pose a number of serious public health problems. There is no published data on the growth potential of microbes isolated from mobile phones in Birnin Kebbi.

The handling of the phone by different users exposes it to an array of microorganisms, and makes it a good carrier for microbes, especially those associated with the skin resulting in the spread of different microorganisms from the users to another user (Austin *et al*., 1999; Al-Abdalall, 2010; Butz *et al*., 1993).

The constant handling of mobile phones by users makes it a breeding place for the transmission of microorganisms as well as hospital-associated infections (Yusha'u *et al*., 2010). Growing evidence have indicated that contaminated fomites or surfaces (cell phones inclusive) play a key role in the spread of bacterial infections (Kawo *et al.,* 2012; Kawo *et al.,* 2009; 2012).

The subscription of mobile phone technology has highly increased worldwide. It is estimated that in Nigeria, over 33 million people have their own mobile phones including adults and children (National Communication Commission, 2015).

For the fact that cell phones have been identified as one of the carriers of bacterial pathogens (Akinyemi *et al*., 2009; Yusha'u *et al*., 2010; Shamebo *et al*., 2015), therefore, becomes imperative to carry out this investigation with a view to recommending ways of tackling the menace. This study was aimed at isolating and determining the antibiotic susceptibility profile of bacteria associated with mobile phones of food handlers in Birnin Kebbi, Kebbi state Nigeria.

MATERIALS AND METHODS

The study was conducted in Birnin Kebbi, the capital of Kebbi state, Nigeria. A hundred samples were collected from mobile phones aseptically using sterile commercially purchased swab cotton wool stick moistened with 0.8 normal saline solutions by rolling it over the exposed surface of the mobile phone. Each cotton swab sample was transferred into10ml of prepared buffered peptone water, kept in an ice box and transported to veterinary public health and preventive medicine laboratory of the Faculty of Veterinary Medicine of Usmanu Danfodiyo University, Sokoto Nigeria for Microbiological Analysis. The sample processing was done after three hours of sample collection following standard microbiology methods (Cheesbrough, 2006).

Aerobic, mesophilic bacterial count was determined using 10-fold serial dilution technique as described by (Chesebrough, 2006). Briefly, from the stock culture in the peptone water medium, 1.0 ml of the sample was aseptically pipetted into a sterile test tube containing 9.0 ml of peptone water and the contents were mixed thoroughly. A millilitre of the mixture from the second tube (10^2) and continuously to tube Ten (10). Then 0.1ml was aseptically pipetted and transferred into correspondingly-labeled Petri dishes in duplicates. This was followed by pouring of prepared, cooled but molten nutrient agar (Oxoid) medium onto the plates. The contents were gently

swirled and allowed to solidify at room temperature and incubated at 37°C for 24 hours. The plates were counted and the mean count obtained was recorded and expressed in colony forming unit per milliliter (CFU/ml) of the sample analyzed.
The streaks plate streaking was used to obtain isolated bacterial colonies that are required for observing colonial morphology, antibiotic sensitivity testing and for biochemical identification (Quinn *et al.,* 2004). The quadrant streak method, using the whole plate was employed for this study. Cells from clearly defined colony were picked up using a sterile wire loop and streaked by rubbing the wire loop horizontally across the surface of the agar, creating visible “streak”. After streaking area one, the loop will be sterilized and then rub across the edges of the area one to make area two, this was repeated for area three and four (Quinn *et al.,* 2004).
The coliforms were enumerated, using multiple tube fermentation technique (Most Probable Number) method. This technique is normally in three stages, viz: Presumptive test; confirmed test and completed test.
The presumptive test was carried out by means of five to three sets of lactose broth tubes. Respective sets were inoculated with volumes (10ml, 1.0ml and 0.1ml) volume of water samples to give an estimate of the Most Probable Number (MPN) of coliforms in the water. The tubes were then incubated at 37°C for 24 to 48 hours. The confirmation of gas in any of the tubes within 24 to 48 hrs is positive for the presumptive test. The probable number of coliforms per 100ml of water sample was determined by McCrady’s probability Table (Chang *et. al*., 2006).
Cultures from positive tubes in the presumptive test were streaked onto Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. The typical colonies (Small, dark, almost black centre with greenish metallic sheen and large, pinkish mucoid, dark centre colonies) indicated a positive confirmed test.
The typical colonies from EMB agar plates in the confirmed test were selected and inoculated into lactose broth and nutrient agar slant and incubated at 35°C for 24 to 48 hours. Fermentation of lactose broth and demonstration of gram-negative, non-sporulating bacilli on the agar slants were considered to be a positive test and that demonstrated the presence of some members of the coliform group in the volume of samples examined.

Isolation and Identification of *E coli, Staphylococcus aureus* and *Salmonella* spp

One ml of each mobile phone swab samples were transferred aseptically into 9 ml of buffered peptone water (BPW), and vortex mixed thoroughly for five minutes. The homogenates were serially diluted from 10^{-1} to 10^{-6} and a volume of 0.1 ml aliquot of appropriate dilution was spread-plated on pre-solidified plates of Eosin methylene Blue agar EMB). The plates were incubated at 37°C for 24hrs.
Golden yellow colonies from the mannitol salt agar plates were aseptically picked and transferred into 5 ml nutrient broth and incubated at 37°C for 24 hrs for further purification. Then, a loopful of culture from the nutrient broth was streaked on nutrient agar medium and incubated at 37°C for 24 hrs. Finally, the distinct colonies were characterized using the established microbiological methods (Acco *et al*., 2003). Gram-positive cocci with cluster arrangement under the microscope were subjected to preliminary biochemical tests (coagulase, catalase and MSA.). Blood agar was used (apart from isolation and cultivation of any type of bacteria) in determining the type of haemolysis produced by *Staphylococcus aureus* isolate.
A loopful of suspension from the growth culture was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Oxoid) and incubated at 37°C for 18 hrs. Characteristic black centered red colonies from the selective media were picked, further purified and biochemically tested in (Triple iron sugar iron, oxidase, indole agar, Simmons Citrate agar, Unease agar and media) based on the standard method (Johnson and Case, 2007; Nesa *et al*. 2011).
To test the presence of S*almonella species*, in the sampled food handlers and Health care workers, 1ml cotton swab sample of each were aseptically transferred into a test tube containing 9 ml of buffer peptone water, homogenized for 5 minutes and then incubated at 37°C for 24 hrs for recovery of the organism. Following the buffer peptone water primary enrichment, the secondary enrichment broth namely selenite F broth (Oxoid®) was used due to its selective property inhibit the growth of non-targeted microorganisms, Allowing Gram-negative bacteria and coliforms. However, it permits rapid multiplication of *Salmonella*. After the enrichment in peptone water, 1 ml of the culture from the peptone water was transferred into 10ml of selenite F broth (Oxoid®) and was incubated at 43°C for 48 hrs. Solid media such as Xylose Lysine Deoxycholate Agar (XLD) (Oxoid®) was used for plating purpose. A loopful of suspension from selenite F broth (Oxoid®) tube was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Oxoid®) and incubated at 37°C for 18 hrs. Characteristic black centered red colonies from the selective media were picked, further purified and biochemically tested according to Johnson and Case*,* 2007.

Antibiotic susceptibility testing

Antimicrobial susceptibility profiles of the bacteria isolated from mobile phones were performed using the disk diffusion method in Muller Hinton Agar (Oxoid®). Briefly, a standardized suspension of the bacterial isolates was prepared and the turbidity of the inoculums was matched

Table 1. Mean aerobic mesophilic bacterial counts (CFU/ml) of mobile phones of food handlers in Birnin–Kebbi, Kebbi State, Nigeria.

Sample SITE	Months				
	January	**February**	**March**	**April**	**Mean**
A	2.20 x 10^1	1.80 x 10^1	1.40 x 10^1	1.60 x 10^1	1.75 x 10^1
B	4.20 x 10^1	2.00 x 10^1	6.00 x 10^1	1.80 x 10^1	3.5 x 10^1
C	2.20 x 10^1	1.00 x 10^1	1.20 x 10^1	2.20 x 10^1	1.65 x 10^1
D	9.20 x 10^1	1.40 x 10^1	1.00 x 10^1	1.60 x 10^1	3.30 x 10^1
E	1.86 x 10^1	1.60 x 10^1	1.08 x 10^1	3.20 x 10^1	2.18 x 10^1
F	1.40 x 10^1	1.20 x 10^1	1.00 x 10^1	8.00 x 10^1	2.90 x 10^1
G	1.60 x 10^1	1.00 x 10^1	8.00 x 10^1	1.20 x 10^1	2.95 x 10^1
H	1.20 x 10^1	1.40 x 10^1	1.20 x 10^1	1.00 x 10^1	1.20 x 10^1
I	1.60 x 10^1	1.00 x 10^1	8.00 x 10^1	8.00 x 10^1	4.65 x 10^1
J	1.00 x 10^1	1.00 x 10^1	1.20 x 10^1	8.00 x 10^1	2.80 x 10^1
K	8.00 x 10^1	1.00 x 10^1	1.20 x 10^1	1.00 x 10^1	2.80 x 10^1
Mean	3.13 x10^1	1.31 x 10^1	2.84 x 10^1	3.42 x 10^1	2.70x10^1

Table 2. Coliform and faecal coliform count (MPN/ml) of mobile phones of food handlers in Birnin Kebbi, Kebbi State Nigeria (figures in parentheses are faecal coliform counts).

Sample	Months				
	January	**February**	**March**	**April**	**Mean**
A	1.80X10^1(7.00X10^1)	2.20X10^1(2.30X10^1)	1.20X10^1(1.40X10^1)	1.81X10^1(1.00X10^1)	1.75 2.92X10^1)
B	2.50X10^1(2.20X10^1)	1.81X10^1(1.00X10^1)	1.60X10^1(2.10X10^1)	1.40X10^1(2.00X10^1)	1.85 (1.82 x 10^1)
C	2.20X10^1(2.30X10^1)	2.40X10^1(2.20X10^1)	1.40X10^1(2.00X10^1)	1.81X10^1(1.00X10^1)	1.95 (1.88 x 10^1)
D	4.10X10^2(2.50X10^1)	1.20X10^1(1.40X10^1)	2.50X10^1(1.50X10^1)	1.60X10^1(2.10X10^1)	1.35 (1.88 x 10^1)
E	2.50X10^1(1.50X10^1)	3.50X10^1(2.50X10^1)	3.50X10^1(2.50X10^1)	1.60X10^1(2.10X10^1)	2.78 (2.15 x 10^1)
F	5.30X10^2(4.60X10^1)	2.80X10^1(2.60X10^1)	2.50X10^1(1.50X10^1)	1.60X10^1(2.10X10^1)	3.05 (2.7 x 10^1)
G	2.40X10^1(2.20X10^1)	4.50X10^1(4.20X10^1)	1.20X10^1(1.40X10^1)	1.81X10^1(1.00X10^1)	2.47 (2.20 x 10^1)
H	1.10X10^1(1.20X10^1)	2.10X10^1(1.40X10^1)	1.00X10^1(1.80X10^1)	1.10X10^1(1.20X10^1)	1.32 (5.60 x 10^1)
I	3.20X10^1(3.40X10^1)	2.20X10^1(2.30X10^1)	1.20X10^1(1.40X10^1)	1.81X10^1(1.00X10^1)	8.41 (2.02 x 10^1)
J	1.00X10^1(2.00X10^1)	1.20X10^1(1.50X10^1)	2.20X10^1(2.30X10^1)	1.20X10^1(1.40X10^1)	1.40 (1.80 x 10^1)
K	1.30X10^1(1.20X10^1)	1.00X10^1(2.00X10^1)	1.40X10^1(2.00X10^1)	1.30X10^1(1.20X10^1)	1.25 (1.60 x 10^1)
Mean	2.49 X10^1(2.74 X10^1)	2.26 X10^1(2.13 X10^1)	1.79 X$10^{1(}$1.81 X10^1)	1.54 X10^1(1.46 X10^1)	2.51(2.41 X10^1)

Table 3. Morphology and biochemical characteristics of bacteria isolated from mobile phones of food handlers in Birnin Kebbi, Kebbi State, Nigeria.

Gram Reaction	**Cat**	**Coa**	**Ind**	**MSA**	**Butt**	**Lac**	**Slope**	**H_2s**	**Gas**	**Urea**	**Cit**	**Oxi**	**Mot.**	**Haem**	**Mr**	**Vp**	**Organism Isolated**
+cocci	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	Beta	NA	NA	*S. aureus*
-Rods	NA	NA	+	NA	y	y	y	NA	NA	NA	NA	NA	NA	Beta	-	-	*E. coli*
-Rods NA	NA	NA	NA	y	r	r	NA	NA	NA	NA	NA	Non Hemolytic	NA	NA	+	+	*Salmonella* species

KEY: NA: Not Applicable

Test Isolated	**AMX (30u)**	**CRX (10u)**	**ERY (10u)**	**AUG (30u)**	**GEN (10u)**	**AMP (30u)**	**CTR (30u)**	**NIT (20u)**	**CPX (10u)**	**CH (30u)**	**CAZ**
E. coli	**16**	**26**	**0**	**18**	**20**	**16**	**0**	**20**	**30**	**18**	**16**
S. aureus	**16**	**24**	**20**	**18**	**20**	**16**	**26**	**0**	**40**	**0**	**20**
Salmonella	**16**	**0**	**0**	**22**	**18**	**16**	**0**	**20**	**26**	**16**	**0**

Key

CH	-	Chloramphencol	CPX	-	Ciprofloxacin
Gen	-	Gentamycin	AMX	-	Amoxillin
CAZ	-	Cefluzidime	ERY	-	Erythromycin
AMP	-	Amphicillin	OFL	-	Ofloxacin
CAZ	-	Ceftazidime	CRX	-	Cefuroxime
CTR	-	Ceftriaxone	AUG	-	Augmentin
MM-	-	Millimeter	NIT	-	Nitroxacin

with the turbidity standard 0.5 McFarland (Bauer *et al.*, 1966). A cotton swab was dipped into the suspension and the swab was pressed against the side of the bottle to remove excess fluid. The inoculated swab was then spread across the surface of Mueller Hinton agar and allowed to soak for five minutes after which sterile forceps were used to carefully remove the disc from its pack and gently pressed onto the agar surface. The plates were finally incubated at 37°C for24 hours. After the incubation period, the diameter of zone of inhibition (clearance) was measured using millimeter rule from the

center of the disc to the edge of the circumference of the clearance zone and recorded to the nearest millimeter. The results were recorded and interpreted on the basis of the Clinical Standards laboratory Institute guidelines (CSLI, 2005).
The antibiotics use for this research include, chloramphenicol (30µg),ciprofloxacin(5µg), cefuroxime (30µg), erythromycin (15µg), gentamycin (10µg),augmentin(30µg), amphicillin(10µg), Nitrocillin(10µg),Cefluzime (30µg), (10µg) and ceflriaxone (30µg)(10µg), amoxicillin (30µg) NCCL, 2007.

RESULTS

In this research, a total of 100 samples from mobile phones of food handlers in Birnin Kebbi, Kebbi State, were cultured, and phynotypically & biochemically chracterised. Antibiotic sensitivity profile of the isolates (*E. coli, S. aureus and Salmonella spp.*) were evaluated. Overall aerobic mesophilic bacterial counts of $2.70x10^6$CFU/ml was obtained from mobile phones of food handlers (Table 1). Morphological and Biochemical Identification (Table 3) of bacterial isolates shows the recovery of 111 isolates comprising of three (3) genera of *E. coli* (n=19) *S. aureus* (n=88) and *Salmonella* species (n=4). It is observed that *S. aureus* was isolated from sample collected from Unguwar Sarakuna 6, Badariya/army Barracks 6, Tudunwada 4, Sabon Tasha 4, Bayan Sofon Tasha 6, Bayan Kara 5, Go slow/Zabarmawa 8, Aleiro Quarters 8, Gessefale 1.20, GRA 20 and Rafin Atiku 2 while *E. coli* and *Salmonella spp* occur only 2 each.
The results of antibiotic susceptibility profile of the isolates as shown in (Table 4) indicated that the highest sensitivity of 40mm against *S. aureus* was ciprofloxacin followed by 30mm against *E. coli* and 26mm against S*almonella spp.* Augmentin is the second antibiotic the isolated are susceptible to with 18mm on *E. coli* and *S. aureus* and 22mm against *salmonella spp*. However, the *salmonella spp* are resistant to ceforoxime, erythromycin, and ceftriaxone with 00mm. The highest zone of inhibition was recorded against ciprofloxacin with 40mm and the least resistant drug was chloramphenicol, and ceftriaxone respectively. Generally the bacterial isolates (*E. coli, S. aureus* and *Salmonella spp*) were susceptible to ciprofloxacin, Ampicillin, amoxicillin, augmentin Erythromycin, and gentamycin as shown in (Table 4).

DISCUSSION

The results of this study shows the aerobic mesophilic bacterial counts of $2.70x10^6$CFU/ml. The high number of bacteria isolated from mobile phone of food handlers might be as a results of poor handling, among other factors, might account for the high level of the bacterial load or count in the phones of food handlers (Mohamed *et al.,* 2006; Akinyemi *et al*., 2009).
Brady *et al.,* (2006) had documented that the combination of constant handling and heat generation provides a favorable breeding ground for microbes that are normally found on our skin, environment and clothes. The results obtained in my study are contrary to the finding of Yusha'u *et al.,* (2010) who reported a gross contamination rate of both commercial and personal mobile phones in some location in Kano metropolis.
In the study, *Staphylococcus aureus* was the most frequent bacterial isolated with 46 from food handler of all the mobile phone's studied. This corresponds with the findings of David, 2009 and Altaee *et al.* 2013 in which S. aureus was the most frequently encountered bacterial isolated from 32.9 of the samples evaluated.

CONCLUSION

The research or study shows that the mobile phones examined from food handlers in Birnin Kebbi metropolis were loaded with numbers of bacteria such as *salmonella, E. coli and S. aureus,* their number or presence may cause foodborne disease such as diarrhea, typhoid fever and food poisoning. Moreover, the results are of health implications of using other people's phone as they could be loaded with bacteria capable of causing various diseases and infections. As the using mobile phones become a day to day activity, Ready to eat food vendors and other common people should be aware to avoid possible cross-contamination between mobile phones and Food items.

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