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The Occurrence of Pathogenic Factors in *Escherichia coli* O157 Isolated from Mobile Phones of Ekiti State University Students Ekiti State, Nigeria.

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ABSTRACT:

Mobile phones have become an essential commodity used for communication, thereby making it an important accessory of human life. In fact, almost all the students in the university cannot do without a cell phone. In recent times, cell phones have been identified as one of the carriers of bacterial pathogens. The assessment of the different ways by which pathogens thrive on fomites and cause diseases in human being remains the aim of this study. Out of the one hundred and twenty seven (127) mobile phones screened only 21 (16.5%) were positive for *E. coli* while only 9 (7.1) were positive for *E. coli* O157. All the isolates were resistant to ampicillin; 8 (88.8%) were resistant to ofloxacin; 7 (77.8%) to Amoxycilin/ Clavulanate and cefuroxime; 5 (55.6%) to ceftadizime and 3(33.3%) to gentamycin and ciprofloxacin while all of the isolate were susceptible to nitrofurantoin. Out of nine (9) *E. coli* O157 isolates in this study, only three (3) (33.33%) were positive for haemolysis, four (4) (44.4%) isolates were positive for cell surface hydrophobicity (CSH) while only seven (77.8%) were positive for production of biofilm. The polymerase chain reaction revealed that seven isolates showed necessary amplification using biofilm producing (*Clf A*) gene P fimbrial adhesin *pap* gene primers with band of 650 bp and 655 bp, while only three isolates showed amplification using hemolysin (*hlyA*) gene with 725 bp. Mobile phones which make communication easy and accessible also have the potential to form good carriers of pathogenic microorganisms which can aid the transmission of diseases. If care is not taken, they could be vehicles for the transmission of biological weapons.

Keyword: Escherichia coli O157, lifestyle impact, bacterial infection,

INTRODUCTION

Since mobile phones have become an essential commodity used for communication and makes the world go round, it is therefore an important accessory of human life.

These mobile phones are handled frequently and the constant handling of the mobile phones by users especially by University students makes it a breeding place (fomite) for transmission of microorganisms [1]. Likewise, cell phones have been identified as one of the carriers of bacterial pathogens [2, 3, 1].

Different studies of bacterial contamination of mobile phones had been conducted in a teaching hospital in Turkey [4], and in New York where One-fifth of cellular telephones examined were found to harbor pathogenic microorganisms [5]. Several areas of the world most especially in Nigeria, Mobile phone usage has increased dramatically, and in environments such as hospitals, abattoirs, market places and places-of-convenience having a high percentage presence of bacteria.

Esherichia coli is a main component of the normal intestinal flora of humans and other mammals. A great diversity of commensal non-pathogenic *E. coli* strains belonging to many different serotypes can be isolated from the feces of healthy persons. They may contaminate any surfaces, surface and underground water, generally without any adverse effects on human health. *E. coli* from the normal intestinal flora are usually harmless to the host and represent opportunistic pathogens. Only in very rare cases can they become a threat to healthy persons.

In medicine *E. coli* is known as an important pathogen infecting worldwide millions of humans worldwide each year both in industrialized and in developing countries. Therefore, during the last few decades molecular biologists have started to work on the mechanisms of bacterial pathogenicity of *E. coli* [6, 7, 8, 9]. This study is aimed at examining the prevalence and occurrence of virulence factors in *E. coli* O157 isolated from mobile phones of Ekiti State University students.

MATERIALS AND METHODS

The study was conducted in Ekiti State University, Nigeria between February and March, 2015.

Sample Size

One hundred and twenty seven mobile phones of Ekiti State University students were randomly selected and screened for the presence of *E. coli* O157.

Sample Collection

All the mobile phones were aseptically swab using a sterile swab the swabs were transferred to test tubes containing 1 ml sterile saline and 0.1 ml of this was cultured on Eosin Methylene Blue Agar (Oxoid Ltd, Basingstoke, UK) and incubated for 24 hours at 37° C. After incubation the plates were examined, colonies with characteristic greenish metallic sheen on EMB agar was sub-cultured onto Sorbitol MacConkey Agar supplemented with cefixime and tellurite (CT- SMAC) (Oxoid Ltd, Basingstoke, UK) and incubated at 37° C for 24 hours. Whitish colonies on the plates were picked and sub-cultured on Nutrient agar slants.

The Gram reaction and biochemical tests were conducted on white (non-pigmented colonies) recovered from CT- SMAC. The isolates were tested to confirm if they were positive to both indole production and methyl red tests. Other tests carried out on the isolates were Voges-Proskauer (MR-VP), citrate utilization and catalase tests.

Serological characterization of *E. coli* O157

Two separate drops of normal saline (0.85% sodium chloride) was placed on a grease-free clean glass slide and thoroughly mixed with a loopful of 18 h culture of *Escherichia coli.* One drop of antisera was added to the bacterial suspension and to the control to the control (normal saline). The antiserum was mixed with the bacterial suspension. The slide was gently rocked for one minute and observes for agglutination under normal lighting conditions or using a low power objective. Clumping in only the antiserum test within 60 seconds was taking to be a positive result.

Antibiotic Sensitivity Test

The disc diffusion method was used for the susceptibility testing of the isolates to different antibiotics as described by Clinical and Laboratory Standard Institute [10]. Each of the isolates was grown at 37ºC in Mueller-Hinton broth (Oxoid) for 18 h and diluted to an optical density of 0.1 (0.5 McFarland Standard). The isolates was singly seeded on sterile Mueller-Hinton Agar (Oxoid) and the antibiotic discs gently place on the plate at equidistance and incubated at 37 ºC for 18 hours. The zone of clearance around each of the antibiotics was measure to the nearest millimeter and interpreted accordingly after incubation. The commercial antibiotics tested include: ampicillin (10μg), amoxicillin-clavulanate (30μg), ceftazidime (30μg), gentamicin (10μg), nitrofurantoin (300μg), ciprofloxacin (5μg), ofloxacin (5μg) and cefuroxime (30μg).

Virulence Factors in the Isolates

Test for Haemolysis

The *Escherichia coli* strains were streaked onto Mueller-Hinton agar supplemented with 5% human blood agar and incubated for 18h at 37°C. Colonies surrounded with a clear haemolytic zone was interpreted to be due to the production of haemolysin as described by [11].

Detection of Biofilm Production

Biofilm production among the O157 serotype of isolated *E. coli* was evaluated by streaking the isolates on nutrient agar supplemented with 0.08% Congo Red as described by [12]. The plates were incubated at 37°C in aerobic condition for 24 hours, followed by storage at room temperature for 48 hours. The result was interpreted according to their colony phenotypes. Isolates that developed black colonies with dry and smooth consistency with rough and round edges and shiny surface were considered a positive indication of biofilm production while colonies with no black pigmentation was considered to be non-biofilm formers.

Cell Surface Hydrophobicity (CSH)

Different molar concentrations of ammonium sulphate including 1 M, 1.4 M and 2 M were prepared. Forty microliter of 0.2 M phosphate-buffered saline PBS (pH 6.8) was dropped on a three sterile grease free slides while another forty microliter of 1M, 1.4M and 2M concentration of ammonium sulphate were added to the slides. Forty microliter of *E. coli* suspension (5×10^9 CFU/ml) was added with each of these slides. The clumps formed in different molar concentration of ammonium sulphate were observed microscopically under 100x and 400x magnification. Strains were considered hydrophobic if aggregation in a concentration of 1.4 M was seen [13].

Detection of Virulence Gene by Polymerase Chain Reaction

The presence of *ClfA*, *pap* and *hlyA* genes were detected in the isolates using the PCR method described by [14]. The following primers were used in this study: Biofilm producing, *ClfA* gene primer forward: 5'CCGGATCCGTAGCAGATGACC3'reverse: 5'GCTCTAGATCACTCATCAGGTTGTTCAGG3' [15], P fimbrial adhesin *pap* gene forward- 5'GCAACAGCAACGCTGGTTGCATCAT3' Reverse –5'AGAGAGAGCCACTCTTATACGGACA3' and Alpha hemolysin *(hlyA)* Foward- 5'AACAAGGATAAGCACTGTTCTGGCT3' Reverse-5'ACCATATAAGCGGTCATTCCCGTCA3' [16].

A 25μl of PCR amplification mixture contained deionized sterile water, 12.5μl Green Go *Taq* Master Mix pH 8 (Promega, USA) contained [(50unit/ml) of Go *Taq* DNA polymerase, (400Mm) of each dNTPs and (3mM) of $MgCl_2$], 1pmolfor specific primers (Alpha DNA, Canada). The PCR cycles for the genes were as follow initial denaturation at initial temperature-second (94-120), number of cycles (30), denaturation temperature 54 for 60 seconds, extension temperature of 72 for 60 seconds and the final extension of 72 for 600s using Gradient PCR (TechNet–500, USA). PCR products were electrophoresed on 1.5% agarose gels in TAE buffer (40mM Trisacetate pH 8.0; 2mM EDTA). Electrophoresis was performed on horizontal gel and the DNA samples were directly loaded into the gels. Samples were electrophoresed alongside a 100-bp DNA ladder (Sigma-Aldrich). Electrophoresis was performed at a constant voltage (100V) until the loading buffer fronts had moved to nearly the end of the gel. After electrophoresis, gels were stained in 0.5 mg/L ethidium bromide and visualized on a UV transilluminator. Photographs were taken by a digital camera.

RESULTS

In this study, 127 mobile phones were randomly in Ekiti State University, Nigeria. *Escherichia coli* were detected in only 21 of the samples while only 9 of the *E. coli* were O157 strains.

All the isolates exhibited 100% resistance to ampicillin, 8 (88.8%) were resistant to ofloxacin, 7 (77.8%) to Amoxycilin/ Clavulanate and cefuroxime, 5 (55.6%) to ceftadizime and 3(33.3%) to gentamycin and ciprofloxacin while all of the isolate were susceptible to nitrofurantoin (Table 1).

Multiple antibiotic resistances were exhibited by 2 (22.2%) of *E. coli* O157 isolates in this study showing multiple resistance to three different antibiotics with one resistance pattern (resistitype). Another 3 (33.3%) exhibited multiple resistance to four antibiotics showing three resistance patterns. One strains (11.1%) of *E. coli* O157 showed multiple resistance to five antibiotics with one pattern. Another set of 2 (22.2%) showed multiple resistance to six antibiotics with two different patterns. Only one organism (11.1%) showed multiple resistances to seven antibiotics and it occurs only in one pattern (Table 2).

Out of nine (9) *E. coli* O157 isolates in this study only three (33.33%) were positive for haemolysis, four (44.4%) isolates were positive for cell surface hydrophobicity (CSH) while only seven (77.8%) were positive for production of biofilm (Table 3).

The polymerase chain reaction revealed that seven isolates showed necessary amplification using biofilm producing (*Clf A*) gene and P fimbrial adhesin *pap* gene primers with band of 650 bp and 655 bp, while only three isolates showed amplification using hemolysin *(hlyA)* gene with 725 bp (Plates 1, 2 and 3).

Table 1: Antibiotics susceptibility pattern of *Escherichia coli* O157 isolates to antibiotics.

Isolates	Antibiotics								
	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP	MARI
FM 9	R	R	R	R	R	R	S	R	7(87.5%)
FM 27	R	R	S	S	R	R	S	R	5(62.5%)
FM 32	R	R	R	S	R	R	S	R	6(75%)
FM 51	S	S	S	S	R	R	S	R	3(37.5%)
FM 69	R	R	R	R	R	S	S	R	6(75%)
FM 81	S	R	S	S	R	R	S	R	4(50%)
FM 102	R	R	S	S	S	R	S	R	4(50%)
FM 103	S	S	S	S	R	R	S	R	3(37.5%)
FM 123	S	R	S	R	R	S	S	R	4(50%)
Resistant	5(55.6%)	7(77.8)	3(33.3%)	3(33.3%)	8(88.8%)	7(77.8)	0(0%)	9(100%)	
Susceptible	4(44.4%)	2(22.2)	6(66.7%)	6(66.7%)	1(11.11%)	2(22.2)	9(100%)	0(0%)	

Table 2: Phenotypic resistance pattern of *Escherichia coli* O157 organisms to antibiotics

No of antibiotics	Phenotypic pattern	No of organism
3	OFL/AUG/AMP	2
Total		2 (22.2)
4	CRX/OFL/AUG/AMP	1
	CAZ/CRX/AUG/AMP	1
	CRX/CPR/OFL/AMP	1
Total		3 (33.3)
5	CAZ/ CRX/OFL/AUG/AMP	
Total		1 (11.1)
6	CAZ/CRX/GEN/OFL/AUG/AMP	1
	CAZ/CRX/GEN/CIP/ OFL/AMP	1
Total		2(22.2)
7	CAZ/CRX/GEN/CIP/AUG/ OFL/AMP	1
Total		1 (11.1)

Figures in parenthesis are percentage values

Table 3: Virulence factors detected in *Escherichia coli* O157

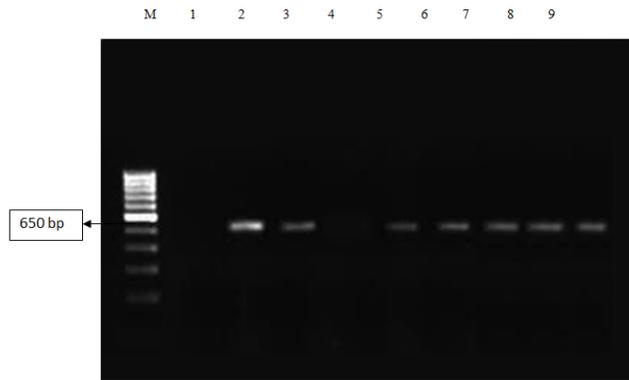| Test	*Escherichia coli* O157 (n=9)	
	Positive	Negative
Haemolysin	3(33.33)	6(66.7)
CSH	4(44.4)	5(55.5)
Biofilm	7(77.8)	2(22.2)

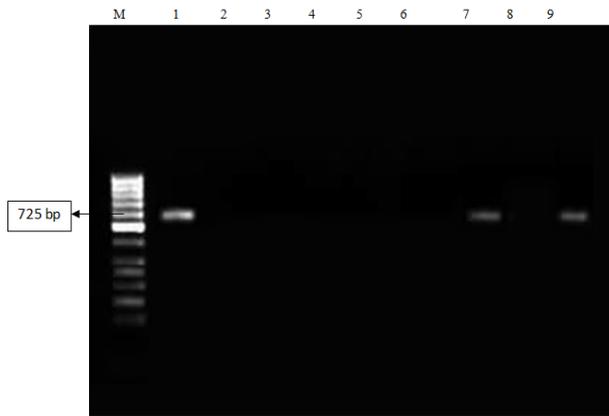Plate 1. PCR result for amplification of (*Clf A*) gene, Marker 1300 bp ladder

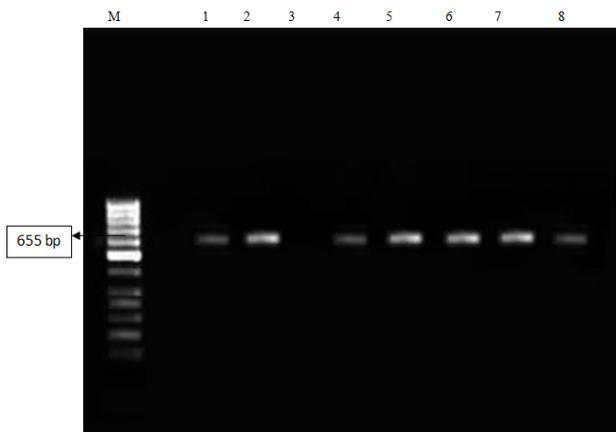Plate 3. PCR result for amplification of (*hly A*) gene, Marker 1300 bp ladder

Plate 2. PCR result for amplification of (*pap*) gene, Marker 1300 bp ladder

Discussion

Mobile phones have become an essential commodity used for communication and has become an important accessory of human life. The presence of *E coli* O157 indicates the possibility of the presence of faecal contamination on the mobile phones which can result in community-acquired infections and disease outbreaks. Mobile phones have become veritable reservoirs of pathogenic organisms as they touch faces, ears, lips and hands of different users of different health conditions [3].

Results from this study showed high levels of feacal contamination of mobile phones used by students in Ekiti State University, Nigeria. This correlates with the work by [5] who

found that One-fifth of the cellular telephones examined in New York harbor pathogenic microorganisms.

Depending on environmental conditions, pathogens may remain infectious on surfaces for weeks after being contaminated. In humid conditions, pathogens may actively colonize surfaces, transforming a passive reservoir into an active one. Furthermore, formation of biofilm by one bacterial agent can affect the survival of other pathogens on the same surface [17].

In general; the greater the concentration of the microbe, the longer it survives and survival can range from minutes to months. This is a cause for concern since these pathogenic isolates are capable of causing diseases in anyone who gets contaminated whilst using the mobile phone.

Hemolysin is a cytolytic enzyme secreted by many *E. coli* isolates from patients with extraintestinal infections. The results from this study suggest that hemolysin may contribute to virulence factors present in *E. coli* O157 isolates from this study in which 3 (33.3%) of the isolates were positive to haemolysis.

Escherichia coli to cause extraintestinal infections depend largely on several virulence factors which help to survive under adverse conditions [18]. Hydrophobicity is a recently described novel virulence mechanism by *Escherichia coli which* promotes their adherence to various surfaces like mucosal epithelial cells. Crystalline surface layers present on both Gram negative and Gram positive organisms play a role in this [19]

Biofilms are microbial communities of organism s adherent to each other and/or a target surface. Biofilm formation protects bacteria from hydrodynamic flow conditions, host defenses mechanisms and antibiotics. In the present study, biofilm production was observed in 7 (77.8%) of *Escherichia coli* O157 isolates in this study which correlate with the study of [20] Polymerase chain reaction (PCR) amplification was used to test for adhesion (*pap*) gene, biofilm producing (*Clf A*) and haemolysin (*hlyA*) gene as described previously.

The implication of the results in this study is that mobile phones which make communication easy and accessible also have the potential to form good carriers of pathogenic microorganisms which can also help in disease transmission of diseases. If care is not taken, they could be vehicles for the transmission of biological weapons.

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