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Antibiogram of bacteria isolated from palms of food handlers in Rivers State University

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Abstract
Multi-drug resistance has become a major public health threat, especially with the continuous emergence of resistant isolates causing delayed hospital stay. The study evaluated the antibiogram of bacterial isolates from the palms of food handlers at Rivers State University. The samples were collected by swabbing the hands of food handlers within the campus. The total heterotrophic bacteria (THB), coliform and staphylococcal loads were done using the spread plate count method and the isolates were identified using phenotypic and biochemical methods. The antibiogram was carried out using the Kirby-Bauer disc diffusion method. Plasmid profiling of antibiotic-resistant genes (oxa-48 and *QNRB*) was determined using gel electrophoresis. Results showed that the THB load of the food handlers' palms ranged from $2.9x10^3$ to $1.20x10^5$ CFU/cm^2. There was a significant difference (0.05) in the THB across the vendors. The staphylococcal counts of the food handlers' palms ranged from $p<0$ to $5.2x10^2$ CFU/cm^2 while the coliform counts ranged from 0 to $7.4x10^2$ CFU/cm^2. The percentage occurrence of the isolates was *Escherichia coli* 20(19.5%), *Staphylococcus* spp. 17(16.7%), *Acinetobacter* 3(2.9%), *Bacillus* 8(7.8%), *Campylobacter* 2(2.0%), *Corynebacterium* 7(6.9%), *Enterococcus* 3(2.9%); *Micrococcus* 4(3.9%), *Pneumococcus* 1(1.0%), *Proteus* 8(7.8%), *Pseudomonas* 7(6.9%) and *Klebsiella* spp. 14(13.7%). The antibiogram showed that the isolates exhibited multidrug resistance and high resistance was observed both in beta-lactam antibiotics and quinolones. Gentamycin was more effective in the control of both gram-negative and positive bacteria. Oxa-48 and *QNRB* genes were detected in most of the isolates which could have contributed to their resistance.

Keywords: Antibiogram, food handlers, bacteria, palms

Introduction
An antibiogram is a table that displays the antibiotic susceptibility pattern of specific microorganisms to different types of antimicrobial drugs (Wemedo and Robinson, 2018) [17]. According to the (WHO, 2011) [19], Antimicrobial resistance (AMR) has surfaced as a global problem facing public health and as a result of this, there is prolonged illness and a higher risk of death since diseases arising from these resistant microorganisms do not respond to the antimicrobial drugs. Furthermore, the diseases caused by multidrug-resistant microorganisms which are virtually non-treatable are the most alarming. The development of antibiotic resistance in many bacteria constitutes serious problems in the control of infectious diseases (WHO, 2011) [19]. The indiscriminate use of antibiotics is the most crucial factor in the resistance of bacteria to antibiotics. Resistance to antibiotics takes place when an antimicrobial drug is unable to completely inhibit the growth of bacteria and the resistant bacteria continue to multiply in the presence of therapeutic levels of the antibiotics (Zaman *et al.*, 2017) [21].

In developing countries like Nigeria in Western Africa, street vended food is very common. The consumption of such food products has increased tremendously, becoming a daily source of diet to millions of people in the country. Food is one of the most basic human needs and it is essential to sustain life (Agu, 2014) [1]. In as much as food is eaten primarily to sustain life, food should be nourishing, attractive and free from noxious substances such as poisonous chemicals, toxins and pathogenic microorganisms (McLauchlin and Little, 2007) [12]. Food if not carefully guarded against contaminations by the food vendors can do much harm to the consumer than the intended good. The safety of street food has become one of the major concerns of public health and a focus for governments and scientists to raise public awareness. Food contamination is caused by many factors including traditional food process methods, inappropriate holding temperatures, and poor personal hygiene of food handlers (Feglo and Sakyi, 2012) [6].

According to Monney *et al.,* (2013) [13] food vendors contaminate food by poor personal hygiene, cross-contaminating raw and processed food, as well as inadequate cooking and improper storage of food. Food is not supposed to cause harm to the consumer, but when it causes harm, a reverse of its intended good leads to food-borne diseases. The world Health Organisation (WHO) (2010) [20] stated that millions of people fall sick or die as a consequence of eating unsafe food. Although epidemiological data on the incidence of food-borne diseases are inadequate, and the outbreak is often not investigated; the recurrent incidences of food-borne illnesses with symptoms of gastrointestinal distress like diarrhoea, vomiting, abdominal cramps and nausea have remained a major cause of morbidity and mortality in Nigeria. According to Ihenkuronye (2012) [9], more than 200, 000 persons die of food contamination annually in Nigeria, and these deaths are caused by contaminated foods through improper processing, preservation and service. Assefa *et al.* (2015) [2] reported that food handlers are carriers of potential food pathogens like *Staphylococcus aureus, Klebsiella* spp, *Enterobacter* spp, *Citrobacter* spp, *marcescens, Pseudomonas aeruginosa, Providencia rettgeri* and *Salmonella* spp. Sharmila (2011) also reported that food vendors are carriers of food-borne pathogens like *Escherichia coli, Salmonella, Shigella, Campylobacter* and *Staphylococcus aureus* which they eventually transfer as food-borne hazards to the consumers. Recently, there is an upsurge in food vendors around the Rivers State University. Most of these vendors sell different types of foods including snacks to students as well as those who could assess them. In order to evaluate the microbiological standards of these street vended foods, this study was undertaken. Thus, this study evaluated the antibiogram of bacteria isolated from palms of food handlers in Rivers State University.

Materials and Methods

Study Area/Participants

The study area was restaurants within the Rivers State University where staff and students as well as other members of the university community feed. The school is one of the indigenous Universities of the State and it is located in Obio-Akpor Local Government Area, Rivers State, and Port Harcourt. Rivers State University.

Sample Collection

A total of twenty-two (22) swab samples from the hands of the participants were collected from all the participants from each restaurant using sterile swab sticks. The swab sticks were transported to the Microbiology laboratory, at Rivers State University for immediate analysis.

Enumeration and Isolation of Bacterial Isolates

The swab sticks were immersed into separate sterile 9 mL sterile normal saline. This served as stock. After which, 10-fold dilution was carried out of which 1 mL from the stock was withdrawn using a sterile 1mL pipette and transferred into another test tube containing 9mL sterile normal saline. This was done serially until a dilution of 10^{-4} was obtained. Aliquots of 10^{-3} was inoculated on Nutrient agar plates that have been pre-dried after preparation for the isolation of the total heterotrophic bacterial counts while 10^{-2} was inoculated on Eosin methylene blue agar and mannitol salt agar plates for the enumeration and isolation of the coliform and total staphylococcal counts, respectively. The plates were spread aseptically using sterile bent glass rod and incubated at 37 °C for 48 hours. After incubation, the colonies on the respective plates were counted and used for calculating the bacterial load while distinct colonies on the respective plates were picked using sterile wire loop and subcultured onto the surface of well labelled prepared nutrient agar plates. Plates were incubated at 37 °C for 48 hours. After incubation, the bacterium on plates void of contaminants were preserved refrigerated in nutrient agar slants while plates containing mixed cultures were subcultured repeatedly on the surface of nutrient agar plates until the bacterium was void of contaminants.

Identification of the Isolates

The ensuing colonies were identified based on cultural, morphological (appearance on plates and Gram staining) and biochemical techniques. The biochemical techniques employed included; sugar fermentation (lactose, mannitol, fructose, glucose, sucrose and lactose), methyl-red, Voges Proskauer, oxidase, triple sugar iron test, starch hydrolysis, methyl red test, and motility. The tests were carried out according to Cheesbrough (2006) [3].

Antibiotics Susceptibility Testing

Antibiotic sensitivity patterns of all the confirmed Gram's positive and negative isolates were performed by standard disk diffusion method according to Kirby-Bauer on Mueller-Hinton agar (Titan, Biotech Ltd, Indian) following the procedures recommended by CLSI (2013) [4]. From an overnight culture of the isolate, A 0.5 MacFarland turbidity standards bacterial culture was prepared in sterile saline, from which 0.1mL was inoculated by spreading onto Mueller Hinton agar, after which antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37 ^{o}C for 24h. Zone of inhibition was measured in millimeters. The gram negative discs contained the following antibiotics: Nitrofurantoin (30µg), Cefuroxime (30µg), Ceftriaxone (30µg), Ampiclox (10µg), Cefixime (5µg), Levofloxacin (5µg), Cefotaxime (25µg), Imipenem/ Cilistatin (30µg), Ofloxacin (5µg), Gentamicin (10µg), Nalidixic acid (30µg) and Augmentin (30µg) while the gram-positive were Amoxicillin (28µg), Bacitracin (10µg), Tetracycline (30µg), Erythromycin (15µg), Cefotaxime (30µg), Clindamycin (30µg), Chloramphenicol (30µg), Gentamicin (10µg), Levofloxacin (30µg), Carbanicillin (10µg), Ceforoxime (30µg) and Piperacillin (10µg).

Detection of Resistance Genes

The *QNRB* gene were detected using the primer F-5′ GATCGTGAAAGCCAGAAAGG-3′- R-5′ - ACGATGCCTGGTAGTTGTCC-3′, *QNRB* genes while the OXA-48 F-5′-GCTTGATCGCCCTCGATT-3′ and OXA-48R 3′-GATTTGCTCCGTGGCCGAAA-5′ primers were used for detection of Oxa-48 gene in the isolates. They were all amplified on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 µl for 35 cycles. The resultant product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV trans-illuminator for a 281 bp product size.

Results

Results of the bacterial load of the food handler's palms in Rivers State University are presented in Table 1. Results

showed that the total heterotrophic bacterial load of the food handler's palms ranged from $2.95x10^3$ to $1.20x10^5$ CFU/cm^2. The total heterotrophic bacterial load of the palms of vendor E was significantly (p<0.05) higher than the counts obtained from vendors F, H and N, respectively. Results also showed that the staphylococcal counts of the food handler's palms ranged from 0 to $5.2x10^2$ CFU/cm^2 while the coliform counts ranged from 0 to $7.4x10^2$ CFU/cm^2. Results further showed that most of the food handler's palms had no staphylococcal and coliform counts compared to other food handler's palms.

Results of the bacteria isolated from the palms of the food handlers showed that the 102 isolates belonged to twelve bacteria genera such as *Acinetobacter*, *Bacillus*, *Campylobacter*, *Corynebacterium*, *Enterobacter*, *Escherichia*, *Micrococcus*, *Klebsiella*, *Pneumococcus*, *Proteus*, *Pseudomonas* and *Staphylococcus.* The percentage occurrence highlighted that the most prevalent bacterial species on the palm of individuals handling food at Rivers State University was *Escherichia coli* 20 (19.5%) closely followed by *Staphylococcus* spp. 17 (16.7%). Others were *Acinetobacter* 3 (2.9%); *Bacillus* 8 (7.8%); *Campylobacter* 2 (2.0%), *Corynebacterium* 7 (6.9%), *Enterococcus* 3 (2.9%); *Micrococcus* 4 (3.9%), *Pneumococcus* 1 (1.0%), *Proteus* 8 (7.8%), *Pseudomonas* 7 (6.9%) and *Klebsiella* spp. (14 (13.7%).

Table 1: Mean total heterotrophic bacteria count of food handlers palm in Rivers State University

Sample Code	THB/cm^2	Staphylococci/cm^2	Coliform/cm^2
A	$3.65x10^4$	$4.25x10^2$	$7.4x10^2$
B	$5.65x10^4$	$1.95x10^2$	$3.0x10^2$
C	$1.70x10^4$	0	$4.6x10^2$
D	$5.75x10^4$	$3.1x10^2$	$2.4 x10^2$
E	$1.20x10^5$	$4.2x10^2$	0
F	$4.90x10^3$	$5.2x10^2$	$2.6 x10^2$
G	$1.98x10^4$	$2.35x10^2$	$1.4 x10^2$
H	$4.45x10^3$	0	$2.9 x10^2$
I	$8.65x10^3$	$5.2x10^2$	$3.65 x10^2$
J	$7.45x10^3$	$3.2x10^2$	0
K	$3.50x10^4$	0	$1.5 x10^2$
L	$1.46x10^4$	$4.6x10^2$	$2.9 x10^2$
M	$2.15x10^4$	0	$1.75 x10^2$
N	$2.95x10^3$	$9.0x10^1$	0
O	$5.00x10^3$	0	0
P	$1.18x10^4$	$2.15x10^2$	$1.25 x10^2$
Q	$6.50x10^3$	0	$2.5 x10^2$
S	$9.85x10^3$	$3.85x10^2$	$6.5 x10^1$
R	$5.70x10^3$	0	0
T	$1.13x10^4$	$3.4x10^2$	$1.4 x10^2$
U	$1.22x10^4$	$8.0x10^1$	0
V	$1.04x10^4$	$2.2x10^2$	$1.2 x10^2$

Legend: A-V represents the various vendors.

Results of the antibiotic susceptibility of the gram-negative and gram-positive bacterial isolates are presented in Tables 2 and 3, respectively. The antibiotics susceptibility pattern of the gram-negative bacterial isolates showed that 79% of *Klebsiella* isolates were resistant to Augmentin while 64% of the isolates were resistant to cefixime and ampiclox.

Some (50%) of the isolates were resistant to levofloxacin and norfloxacin while 36% were resistant to ofloxacin. Percentage resistant to gentamycin was 43% while 57% were resistant to cefotaxime. For *E. coli* isolates 90% was resistant to cefixime, 64% were resistant to ofloxacin and nalidixic acid while 10% was resistant to gentamycin. Results also showed that all the *Pseudomonas* isolates were susceptible to gentamycin while 86% were resistant to ceftriaxone and cefotaxime. A low percentage (14%) of the isolates were resistant to nalidixic acid and norfloxacin while 57% were resistant to levofloxacin. The *Proteus* isolates were 100% resistant to levofloxacin, 38% were resistant to gentamycin and ceftriaxone, 87% were resistant to ofloxacin while 50% were resistant to imipenem, cefixime, nalidixic acid, and ampiclox. The *Serratia* isolates exhibited 100% resistant to cefixime, 50% were resistant to gentamycin and ampiclox while 25% of the isolates were resistant to nalidixic acid and levofloxacin. *Campylobacter* isolates were 100% resistant to imipenem while 50% were resistant to levofloxacin and ampiclox, respectively. Thus, ofloxacin, nalidixic acid, gentamycin and norfloxacin completely (100%) inhibited the proliferation of *Campylobacter* isolates.

The antibiotics susceptibility pattern of the gram-positive bacterial isolates showed that 67% of *Enterococcus* isolates were resistant to erythromycin, carbenicillin and levofloxacin while 100% of the isolates were resistant to Piperacillin. The *Enterococcus* isolates were completely susceptible to tetracycline while 67% of the isolates were susceptible to ciprofloxacin and gentamycin. The *Micrococcus* isolates were 100% resistant to cefotaxime, erythromycin, and ciprofloxacin. Susceptibility to gentamycin which was the most potent against the isolates was 100% while 50% were susceptible to carbenicillin and cefuroxime, respectively. *Bacillus* species were completely resistant to cefotaxime while 62.5% were resistant to carbenicillin and cefuroxime, respectively. Resistance to erythromycin, ciprofloxacin and levofloxacin was 37.5%, respectively while 75% were resistant to tetracycline. For the *Staphylococcus* isolates, 58% were resistant to clindamycin, erythromycin and cefuroxime while 47.1% were resistant to gentamycin and ciprofloxacin. *Corynebacterium* sp were 100% resistant to cefotaxime 14.3%, and 28.6% were resistant to gentamycin and levofloxacin. Thus, gentamycin was more potent against *Corynebacterium* as 85.7% of the isolates were susceptible to the antibiotics.

Plasmid profiling of oxa-48 and *QNRB* resistant genes is presented in Plate 1 and 2, respectively. The results showed that *E. coli*, *E. coli* O157:H7, *Klebsiella pneumonia*, *Klebsiella pneumonia*, 37 and *Staphylococcus aureus* exhibited the gene while isolate 37 (*E. coli*) do not possess the gene.

On the other hand, the *QNRB* gene was detected in all the isolates except isolate 4 (*E. coli*).

Table 2: Antibiotic Susceptibility of the Gram's Negative Bacterial Isolates

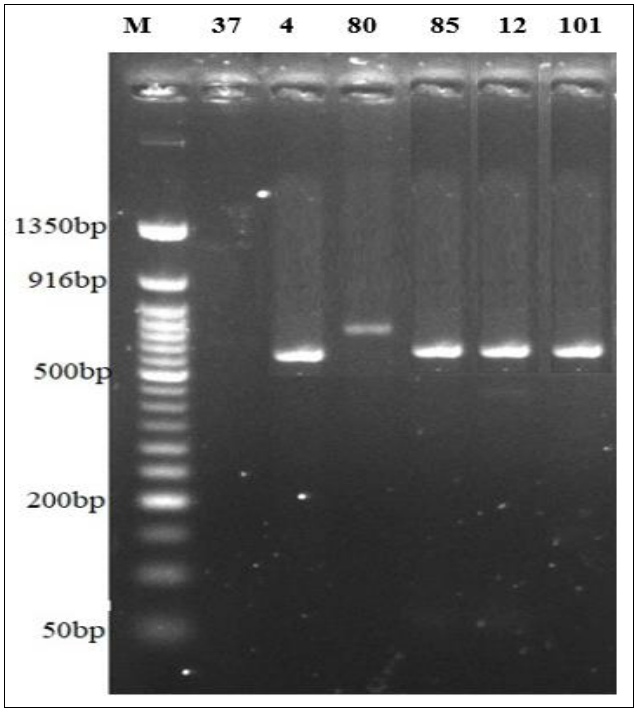
Antibiotics	*Klebsiella* (N=14) [N (%)]			*E. coli* (N=20) [N (%)]			*Pseudomonas* (N=7) [N (%)]			*Proteus* (N=8) [N (%)]			*Serratia* (N=8) [N (%)]			*Campylobacter* (N=2) [N (%)]		
	R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
IMP	7 (50)	1 (7)	6 (43)	12(60)	2(10)	6(40)	3 (43)	1(14)	3(43)	4(50)	2(25)	2(25)	4(50)	2(25)	2(25)	2(100)	0	0
ZEM	9(64)	4(29)	1(7)	18(90)	0	2(10)	3(43)	1(14)	3(43)	4(50)	2(25)	2(25)	8(100)	0	0	0	1(50%)	1(50)
CRO	6(43)	3(21)	5(36)	16(80)	3(15)	1(5)	6(86)	0	1(14)	3(38)	2(25)	3(38)	4(50)	3(38)	1(12)	0	2(100%)	0
OFX	5(36)	0(0)	9(64)	7(35)	0	13(65)	3(43)	0	4(57)	7(87)	0	1(13)	1(12)	0	7(88)	0	0	2(100)
CMX	8(57)	0	6(43)	13(65)	1 (5)	6 (30)	5(71)	0	2(29)	6(76)	1(12)	1(12)	1(12)	2(25)	5(62)	0	2(100%)	0
NA	5(36)	0	9(64)	6(30)	2(10)	12(60)	1(14)	1(14)	5(71)	4(50)	3(38)	1(12)	2(25)	1(12)	5(62)	0	0	2(100)
CTX	8 (57)	0	6(43)	15(75)	1(5)	4(20)	6(86)	0	1(14)	6(76)	0	2(25)	5(62)	1(12)	2(25)	2	0	0
AUG	11 (79)	1(7)	2 (14)	12(60)	2(10)	6(30)	4(57)	1(14)	2(29)	6(76)	1(12)	1(12)	3(38)	0	5(62)	0	1(50%)	1(50)
LBC	7(50)	2(14)	5(36)	7(35)	2(10)	11(55)	4(57)	1(14)	2(29)	8(100)	0	0	2(25)	0	6(75)	1(50)	0	1(50)
GN	6(43)	1(7%)	7(50)	2(10)	0	18(90)	0	0	7(100)	3(38)	0	5(62)	4(50)	0	4(50)	0	0	2(100)
NF	7(50)	1(7%)	6(43)	8(40)	2(10)	10(50)	1(14)	0	6(86)	5(62)	0	3(38)	1(12)	0	7(88)	0	0	2(100)
ACX	9(64)	1(7%)	4(29)	15(75)	0	5(25)	2(29)	1(14)	4(57)	4(50)	0	4(50)	4(50)	0	4(50)	1(50)	0	1(50)

Key: Nitrofurantoin(NF) 30µg; Cefuroxime(CXM) 30 µg; Ceftriaxone(CRO) 45 µg; Ampiclox (ACX) 10 µg; Cefixime(ZEM) 5 µg; Levofloxacin(LBC) 5 µg; Cefotaxime(CTX) 25 µg; Imipenem 10 µg; Ofloxacin 5 µg; Gentamicin 10 µg; Nalidixic acid 30 µg; Augmentin (AUG) µg.

Table 3: Antibiotic Susceptibility of the Gram's Positive Bacterial Isolates

	Enterococcus (N=3) [N (%)]			*Micrococcus* (N=4) [N (%)]			*Bacillus* (N=8) [N (%)]			*Staphylococcus* (N=17) [N (%)]			*Corynebacterium* (N=7) [N (%)]		
	R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
CD	1(33)	2 (67)	0	2(50.0)	0	2(50.0)	4(50)	0	4(50)	10(58.8)	2(11.8)	5(29.4)	6(85.7)	0	1(14.3)
CX	1(33)	1(33)	1(33)	4(100)	0	0	8(100)	0	0	16(94.1)	0	1(5.9)	7(100)	0	0
E	2(67)	0	1(33)	4(100)	0	0	3(37.5)	3(37.5)	2(25)	10(58.8)	4(23.5)	3(17.7)	6(85.7)	1(14.3)	0
TE	0	0	3(100)	2(50.0)	1(25)	1(25)	6(75)	1(12.5)	1(12.5)	9(52.9)	1(5.9)	7(41.2)	3(42.9)	3(42.9)	1(14.3)
BA	1(33)	1(33)	1(33)	0	1(25)	3(75)	2(25)	1(12.5)	5(62.5)	4(23.5)	1(5.9)	12(70.6)	6(85.7)	0	1(14.3)
AC	1(33)	1(33)	1(33)	1(25)	2(50.0)	1(25)	3(37.5)	2(25)	3(37.5)	9(52.9)	3(17.7)	5(29.4)	6(85.7)	0	1(14.3)
PR	3(100)	0	0	3(75)	0	1(25)	3(37.5)	2(25)	3(37.5)	13(76.5)	2(11.8)	2(11.8)	6(85.7)	1(14.3)	0
CP	0	1(33)	2(67)	4(100)	0	0	3(37.5)	1(12.5)	4(50)	8(47.1)	3(17.7)	6(35.3)	0	2(28.6)	5(71.4)
GN	1(33)	0	2(67)	0	0	4(100)	4(50)	0	4(50)	8(47.1)	2(11.8)	7(41.2)	1(14.3)	0	6(85.7)
CU	1(33)	1(33)	1(33)	2(50.0)	0	2(50.0)	5(62.5)	0	3(37.5)	10(58.8)	2(11.8)	5(29.4)	3(42.9)	0	4(57.1)
CR	2(67)	0	1(33)	1(25)	1(25)	2(50.0)	5(62.5)	2(25)	1(12.5)	13(76.5)	1(5.9)	3(17.7)	2(28.6)	1(14.3)	4(57.1)
LE	2(67)	0	1(33)	1(25)	0	3(75)	3(37.5)	2(25)	3(37.5)	6(35.3)	3(17.7)	8(47.1)	2(28.6)	0	5(71.4)

Key: Amoxicillin(AC) 28 µg; Bacitracin(BA) 10 µg; Tetracycline(TE) 30 µg; Erythromycin(E) 15 µg; Cefotaxime(CX) 30 µg; Clindamycin(CD) 2 µg; Chloramphenicol(CP) 30 µg; Gentamicin(GM) 10 µg; Levoflaxacin (LE) 5 µg; Carbanicillin (CR) 100 µg; Ceforoxime (CU) 30 µg; Piperacillin (PR) 100 µg, R = resistant; S = susceptibility; I = Intermediate



Key: 4 =*E. coli*, 80 = *E. coli* O157:H7, 85 = *Klebsiella pneumonia*, 12 = *Klebsiella pneumonia*, 37 = *E. coli* and 101 = *Staphylococcus aureus*

Plate 1: Gel image showing amplification of ESBL (OXA-48) at 550bp to 650bp. Isolates 4, 80, 85, 12 and 101 showed positive while isolate 37 was negative for the gene.

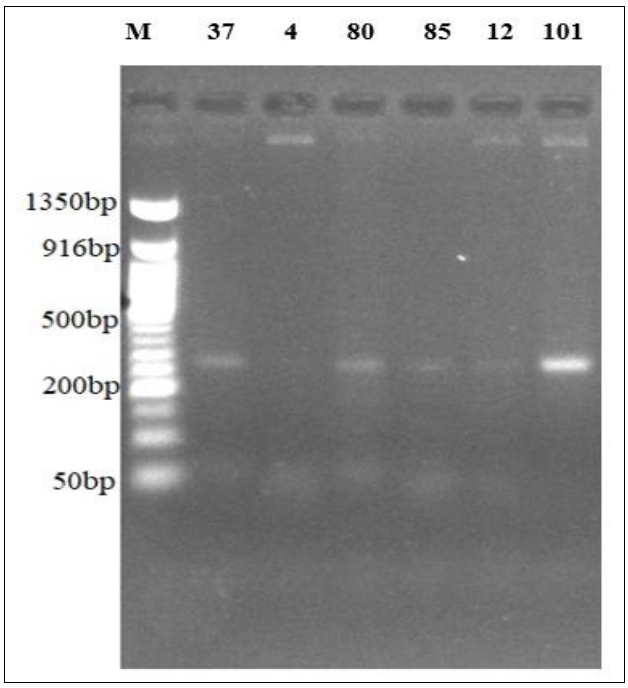


Plate 2: Gel Image showing the amplification of quinolones genes (QNRB) at about 250bp. All isolates showed positive amplification except for isolate 4 (*E. coli*).

Discussion

In assessing the total heterotrophic bacteria count of palm samples from food handlers at Rivers State University, the results showed viable counts ranging from the $2.25x10^3$ CFU/5cm^2 to $1.20x10^5$CFU/5cm^2. Based on standard guidelines, a total heterotrophic bacteria count below 100 CFU/ml is often considered acceptable for water and other related condiments used in food preparation (W.H.O., 2010) [20]. In this context, none of the twenty-two (22) hand swab samples were within this desirable range, suggesting poor hygiene practices. The staphylococcal count of palms of individuals handling food in Rivers State University however ranged from then $8.0x10^1$ CFU/5cm^2 to $5.2x10^2$ CFU/5cm^2. However, Samples C, H, M, Q and S had no *Staphylococcus* growth.

The coliform count of palms of food handlers in Rivers State University ranged from $6.5x10^1$ CFU/5cm^2 to $7.4x10^2$ CFU/5cm^2. Notwithstanding Samples E, J, N, O, N, S and U were negative for *Escherichia coli.* Results obtained for the total heterotrophic bacteria count are comparable to the report of Lambrechts *et al.*, (2014) [11] who reported a viable count ranging from 0 to $7.4x10^3$CFU/cm^2. Also, Woh *et al.*, (2017) [18] reported a similar range of mean aerobic bacteria counts among migrant food handlers in Peninsular Malaysia; in their study, they reported a total heterotrophic bacteria count of $3.7x10^3$CFU. They however recorded staphylococci count of $1.39x10^1$ and a mean *E. coli* count of $1.9x10^1$ CFU. Differences in microbial counts and diversity observed between our study and other studies compared could be attributed to variances in the composition of the food handler's population. Differences in personal hygiene as well as overall health; and different levels of adherence to recommended hand hygiene practices play a role in the differences observed in microbial population. Can result in disparity. Sampling methods and size including the swabbing technique and the sampled area as well as sampling frequency could also be reasons for differences in results. Also, variations in the laboratory techniques used for microbial analysis such as culture-based methods or molecular techniques can lead to differences in the sensitivity and specificity of detection.

A total of 102 bacterial isolates belonging to thirteen (13) genera were isolated from the 22 samples studied. Although most of the organisms isolated could be harmless commensals, some a potential pathogenic species. The presence of *E. coli* and *Staphylococcus* spp. in some samples pose risk of food contamination during processing and can be easily transferred to surfaces utensils and other food contact surfaces leading to cross contamination where pathogens could be spread from one surface to the other and increasing the risk of contamination of food both in the kitchen and when serving students already prepared meals. The presence of *E. coli*, *Klebsiella*, *Proteus* and other potential pathogens are indicators of the level of food handler hygiene practice ranging from inadequate hand washing to poor sanitation practices. These pathogens could potentially be transmitted to consumers through contaminated food which will result in gastrointestinal infection, food poisoning and other foodborne diseases associated with the isolated organisms.

The microorganisms isolated in this study are comparable to the 171 bacterial hand contaminants isolated by Assefa *et al.*, (2015) [2]. In their study, *S. aureus* 54(23.5%), *Klebsiella* spp. 37(16.1%), *E. coli* 25(10.9%), *Enterobacter* spp. 21(9.1%), *Citrobacter* spp. 10(4.3%), *Serratia marcescens* 6(2.6%), *Pseudomonas aeruginosa* 8(3.5%), *Proteus* spp. 5(2.2%), *Providencia rettegri* 3(1.3), and *Salmonella* spp. 2(0.9%) were isolated. Also, Jibrin *et al.*, (2016) isolated 8 different species of bacteria including *Escherichia coli* (20.3%), *Enterobacter* spp 15.4%, *Shigella* spp 14.7%, *Staphylococcus aureus* 14.7%, *Salmonella* spp 13.9%, *Klebsiella* spp 11.9%, *Streptococcus* spp 6.2%, and

Vibrio spp with occurrence of 2.8%. Similarly, *Staphylococcus aureus, S. epidermidis*, *Alcaligenes* spp. *Bacillus subtilis, Listeria* spp. *Enterobacter* sp, *Escherichia coli, Klebsiella aerogenes*, *Proteus vulgaris* and *Vibrio cholera* were isolated from the hand of food fast food handlers in Abeokwuta.

The antibiotics susceptibility of the gram-negative and gram-positive bacterial isolates showed that the resistance of the isolates to the different antibiotics varied with most of the isolates exhibiting multi-drug resistance with MAR index greater than 0.2. Gentamycin was one of the most potent antibiotics with broad spectrum extending to both gram-negative and gram-positive bacterial isolates. Since the antibiotics is not available in tablet, the inappropriate use of the drug could be controlled unlike those which are available in tablets that could be gotten over the counter. This agreed with previous study which had similar observations (Okafor *et al.*, 2023) [14]. More so, the present study agreed with Evbuomwan *et al.*, (2019) [5] who reported high susceptibility of *Corynebacterium* isolates to gentamycin in their study, although they reported 100% susceptibility of the isolates to gentamycin which is contradictory to the 85.7% susceptibility in the present study. The present study contradicts previous reports which showed that all *E. coli* isolates were 100% susceptible to Augmentin (Evbuomwan *et al.*, (2019) [5]. The high resistance of isolates including *Staphylococcus* sp to antibiotics could be due to the wide use of these drugs for the treatment of infections caused by these suspected pathogens, as wide consumption of antibiotics results to the emergence of antibiotics resistant species due to selective pressure (Kengne *et al.*, 2019) [10]. Furthermore, gentamycin as an aminoglycoside is known to inhibit protein synthesis in bacteria by binding to the 30S ribosomal subunit of the bacterial cells while ofloxacin, ciprofloxacin and levofloxacin are quinolone are known to interfere or inhibit nucleic acid synthesis (Robinson *et al.*, 2023) [16]. Thus, resistance to these antibiotics could partly be attributed to the presence of the *QNRB* gene and oxa-48 genes. The *QNRB* gene is known to enhance resistance to quinolone antibiotics while the oxa-48 gene confers resistance to penicillin antibiotics as well as the third-generation cephalosporin antibiotics (Gurung *et al.*, 2020; Pasom *et al.*, 2013) [8, 15].

Conclusion

In conclusion, the bacterial load on the palms of food handlers within the school campus was very high and could result to contamination of food by these microorganisms if proper hygiene is not emphasized. More so, the antibiotics susceptibility pattern has revealed that both the gram-positive and negative bacterial isolates exhibited multi-drug resistance with most of the isolates bearing the antibiotic-resistant genes (*QNRB* and oxa-48) known to confer resistance against quinolones and beta-lactam antibiotics.

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