INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

Molecular Identification and Antimicrobial Sensitivity Profile of Bacteria Isolated from Food Handlers in Akure Nigeria

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Abstract:
Hand washing and hand swab samples culminating to a total of 477 samples were collected from different food vending locations in Akure. Bacteria were isolated and identified, after which the sensitivity patterns of the isolates to standard antibiotics and plant extracts were determined using disc diffusion agar and well diffusion methods, respectively. The bacterial isolates include Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Micrococcus luteus, Proteus mirabilis, Proteusvulgaris, Pseudomonas aeruginosa, Salmonella enteritica, Salmonella typhi, Serratia marcescens, Shigella sonnei, Staphylococcus epidermidis, Staphylococcus aureus and, Streptococcus pyogenes. Staphylococcus aureus was observed to be the most prominent bacteria harboured by the food handlers. The isolated bacteria were subjected to 16s rRNA polymerase chain reaction, DNA gene extraction and sequence blasting using the amplified length of 1500 base pair. The sequence obtained was blasted in National Centre for Biotechnology Information (NCBI) database. Based on the 16s rRNA sequences, the following bacteria were confirmed; Escherichia coli str. K12 substr. DH10B, Klebsiella pneumoniae strain J1, Proteus mirabilis strain HI4320, Pseudomonas fluorescens SBW25, Salmonella enterica subsp.enterica serovar Typhi str. CT18, Salmonella enterica subsp. enterica serovar Infantis, Shigella flexneri 2a str. 2457T and Staphylococcus cohnii subsp. cohnii strain 532 Contig16. Phylogeny was employed in the phylogenetic tree construction due to its robustness, reliability and less-laboriousness. The result of the sequence was aligned and the phylogenetic tree constructed shows the evolutionary relationship between eight bacterial species. Two clusters were generated; Salmonella enterica subsp. enterica serovar Typhi, Salmoenella typhi LN854584.1 Staphylococcus cohnii, and Staphylococcus aureus are in cluster 1, Proteusmirabilis NC0100554.1, Bacillus thuringiensis KY652120.1 and Bacillus subtilis MG231261.1 is incluster 2. However, bacteria in the same cluster were observed to have different phenotypic characteristics. The antibacterial efficacy of the selected medicinal plant extracts (Ocimum gratissimum and Annona muricata) were copared to that of standard antibiotics (Ciprofloxacin, Nitrofurantoin, Ampliclox, and Chloramphenicol) using three different solvents (methanol, water and petroleum ether) at 50 mg/ml, 100 mg/ml, 200mg/ml and 300mg/ml, concentrations. Bioactive characterization of the selected plants revealed the presence ofvarious active components such as Thymol, Eugenol, Caryophyllene, Gamma-terpinene, 8-methyl-4-vanillyl-7-nonenamide, N-4-Hydroxy-3-methoxybenyl 8methyldec 6 enamide, DL-6, 8-tio acidamide and N-4-Hydroxy-3-methoxyphenyl methyl nonanamide. Phytochemical analysis of the plants affirmed the presence of Tannins, Alkaloids, Flavonoids, Anthraquinone and Saponins in O. gratissimum and A. muricata. It can be deduced from this study that food handlers play a major role in the transfer of bacterial contaminants to the edible food. Also, the metabolites present in O. gratissimum and A. muricata render these plants cost effective alternative source of therapy to food borne illnesses.

Keywords: *Antimicrobial sensitivity, molecular identification, ocimum gratissimum, annona muricata, food handlers, antibiotics*

1. Introduction

Food handler refers to any person involved in food processing from its raw form to the edible form. The human skin surface harbours large numbers of bacteria that can be readily dislodged and transferred to surfaces upon touching, hence the importance of proper hand hygiene by health care practitioners and food handlers (Jarvis, 2017). When an individual swallows bacteria that cause food poisoning, there is a delay (incubation period) before symptoms begin. This is because most bacteria that cause food poisoning need time to multiply in the intestine. The length of the incubation period depends on the type of bacteria and how many are swallowed. It could be hours or days. The bacteria stick to the lining of the intestine and destroy those cells, either by sheer weight of numbers or by the toxins (poisons) they produce (Liu, 2017). Sometimes these toxins are absorbed and cause damage elsewhere in the body. Some bacteria produce toxins when they grow in food. Because the toxins themselves are harmful, the bacteria don't need to multiply in the intestine to make someone ill, so the symptoms come on very quickly (Weisblum and Davies, 2015). Since the bacteria enter the body

through the digestive system, symptoms will generally be in the form of nausea, vomiting, abdominal cramps and diarrhoea. In some cases, food poisoning can cause very serious illness or even death (Liu, 2017).

Traditional methods of bacterial identification rely on phenotypic identification of the causative organism using gram staining, culture and biochemical methods. However, these methods of bacterial identification suffer from two major drawbacks.

First, they can be used only for organisms that can be cultivated in vitro. Secondly, some of the strains exhibit unique biochemical characteristicsthat do not fit into patterns that have been used as a characteristic of any known genus and species (Ashelford *et al.,* 2015).Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in clinical laboratories and research laboratories for a variety of applications. In the past decade or so, molecular techniques have proven beneficial in overcoming some limitations of traditional phenotypic procedures for the detection and characterization of bacterial phenotypes.

2. Materials and Methods

2.1. Collection of Samples

A total of four hundred and seventy-seven (477) samples were collected between the hours of 8am-9am within October and December 2017 using sterile swab sticks already moistened with normal saline at the cotton tips so as to ensure easy adherence of microbes to it. The sticks were used to swab the palms of food handlers in various locations within Akure metropolis. Hand washing technique was also employed in collection of the samples. Distilled water was used to rinse the hands of food handlers into sterile containers. All the samples collected were placed in an ice bag.

2.2. Sterilization of Materials Used

All the glassware (Petri dishes, beakers, conical flasks) used were washed thoroughly with detergent, rinsed with tap water and then oven dried at 160°C - 170°C for 2 hours. Forceps and inoculating loops were flamed to red-hot and then dipped in 70% ethanol. Laboratory benches and inoculating chambers were thoroughly disinfected with cotton wool previously soaked in 70% ethanol before and after investigations.

2.3. Culture Media Preparation

Growth media used for this study were prepared according to manufacturer's specifications. Dehydrated Nutrient agar (2.8g), EMB (3.8g), MacConkey agar (5g), Salmonella Shigella agar (6g), Mannitol salt agar (11.1g), Simmon citrate agar (2.4g) and Muellar Hinton agar (3.8g) were separately dissolved in 100ml of distilled water, in a 250 ml capacity conical flask. Complete dissolution was achieved by placing on hot plate at 50^{o}C for 45 mins. Thereafter, the flask was corked with cotton plug and then wrapped with Aluminium foil. The medium was later sterilized in an autoclave at 121^{o}C for 15mins.

2.4. Isolation and Identification of Bacterial Isolates

Samples were transported to the laboratory and analyzed within 1 hour of collection. Serial dilution of the swab sticks and the water samples was employed in transferring the bacteria to the agar plates so as to reduce the microbial population. Samples were inoculated onto nutrient, chocolate, blood, and MacConkey plates. All plates were incubated aerobically at 37°C for 24 to 48 hr except the chocolate agar plates that were incubated in a candle jar. Emergent bacterial colonies were identified by standard bacteriological techniques (Cheesbrough, 2010). The pure isolates were stored on slants and kept at 4^{o}C for further use (Fawole and Oso, 2007).

2.5. Cultural Identification

Cultural characteristics of the distinguished bacteria colonies such as colour, shape, pigmentation and opacity were observed and noted after 24 hours of incubation. Microscopic characterization was done using Gram staining procedure, biochemical tests were done according to the methods of Olutiola *et al*. (2011); Fawole and Oso (2007); Cheesbrough (2010) and identification of bacterial isolates was carried out using the method of Cowan and Steel (2016).

2.6. Biochemical Characterization of Bacterial Isolates

The standard methods of Fawole and Oso (2007) and Cheesebrough (2010) were used for the biochemical characterization of bacterial isolates.

2.7. Molecular Identification of Bacterial Isolates

This test was carried out according to the method of Heikens *et al*. (2015).

2.8. Antibiotic Sensitivity Test

The antibiotic sensitivity test was carried out according to Asoso *et al*., (2016).

2.9. Preparation of Plant Extracts

The leaves of the *Ocimum gratissimum* (efinrin) and *Annona muricata* (sour sop) plants was harvested and identified at the Crop, Soil and Pest Management Department (CSP) Department of the Federal University of Technology, Akure. Then they were properly rinsed with tap water, air-dried at room temperature and then pulverized to powdered form. A 400g portion each of the powdered sample was soaked separately for 72 hr in three solvents namely: 70%

methanol, distilled water and petroleum ether. Each solution was first sieved using muslin cloth and then filtered with No 1 Whatman (Asoso *et al.,* 2016). The filtrate was evaporated in a rotary evaporator (Buchi Rotavapor R-200 Heidolph, V-assembly, manufactured by Lyman C. Craig) to concentrate the crude extracts. The 100% stock concentration of the extracts was obtained and stored at 4°C in a corked universal bottle. It was reconstituted with 30% Tween-20 to obtain 50 mg/ml, 100 mg/ml, 200 mg/ml and 300 mg/ml of each of the plant extracts. Prior to sensitivity test, the various concentrations of the extracts were filtered using a millipore membrane filter of 0.45µm pore size to ensure the sterility of the crude extracts.

2.10. Phytochemical Screening

The phytochemical analysis was carried out according to the standard methods of analysis by analytical methods Committee of Royal Society of Chemistry, (2002).

2.11. Data Analysis

The experiment was carried out in triplicates and the data obtained was subjected to statistical analysis using SPSS version 22, Treatment means were compared using Duncan's New Multiple Range test and significant differences were evaluated at $p \leq 0.05$.

3. Results and Discussions

All the bacterial isolates showed different biochemical reactions and were characterized morphologically based on microscopy into cocci and bacilli. The isolates were identified as *Alcaligenes faecalis, Bacillus cereus, Bacillus subtilis, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Micrococcus luteus, Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa, Salmonella typhi,Serratia marcescens, Shigella sonnei, Staphylococcus aureus, S. epidermidis,* and*, Streptococcus pyogenes*. After the presumptive identification, *S aureus* was observed to be the most prominent having eighty-five (85) isolates while *Serratia marcescens, Shigella sonnei,* recorded the least number of isolates two (2) isolates.

The length of amplified products was 1500 base pair. The sequence obtained was blasted in National Centre for Biotechnology Information (NCBI) database. Based on the 16s rRNA sequences, the following bacterium were confirmed; *Escherichia coli* str. K12 substr. DH10B, *Klebsiella pneumoniae* strain J1, *Proteus mirabilis* strain HI4320, *Pseudomonas fluorescens* SBW25, *Salmonella enterica* subsp.*enterica* serovar Typhi str. CT18, *Salmonella enterica* subsp. enterica serovar Infantis, *Shigella flexneri* 2a str. 2457T and *Staphylococcus cohnii* subsp. cohnii strain 532 Contig16. The result also revealed a difference in cultural identification of *Staphylococcus cohnii* subsp. cohnii strain 532 Contig16, *Salmonella enterica* subsp. enterica serovar Infantis and *Pseudomonas fluorescens* SBW25.

The antibiotic sensitivity profile of bacteria isolated from food handlers in Akure metropolis is shown in Table 1.0. Ciprofloxacin was the most potent antibiotic agent on the isolates. The zone of inhibition ranged from 9.25±0.31 to 11.57±0.30 mm. This antibacterial agent exerted its highest effect on *Staphylococcus aureus* while the least effect was on *Enterobacter aerogenes*. *Staphylococcus aureus* was also observed to be the most susceptible bacterium to Nitrofurantoin with an inhibitory zone of 10.46±0.23 mm while *Proteus mirabilis* was the least susceptible with a zone of 8.60±0.27 mm. Ampiclox demonstrated its highest antimicrobial effect on *Streptococcus pyogenes* (10.46±0.26 mm), while *Bacillus thuringiensis* was least susceptible to Ampiclox with an inhibitory zone of 8.26±0.26 mm. Chloramphenicol appeared to be the antibacterial agent with the least efficacy, the zone of inhibition ranged from7.52±0.28 mm (*Serattia marcescens*) to 9.57±0.28 mm (*S. aureus*).

Tables 2 to 7 represent the antibiotic sensitivity patterns of the bacterial isolates to methanol, water and petroleum ether extracts of *O. gratissimum* and *A. muricata* at the concentrations of 50 mg/ml, 100mg/ml, 200mg/ml and 300mg/ml. The zone of inhibition of methanolic extract of *O. gratissimum* extracts ranged from 5.32±0.37mm to 20.56±0.70 mm. Its water extracts recorded inhibitory range 3.67±0.10 mm to 18.42±0.28 mm. Petroleum ether extracts demonstrated the least efficacy with inhibition zones ranging from 1.80±0.34 mm to 9.13±0.35 mm. On the other hand, water extracts of *A. muricata* was the least effective with zones of inhibition ranging from 3.02±0.10 mm to 12.99±0.98 mm.

	A	B	C	D
Escherichia coli	9.42 ± 0.04^{c}	8.50 ± 0.03^{b}	8.43 ± 0.32^{b}	9.91 ± 0.38^{d}
Klebsiella oxytoca	9.50 ± 0.01^{d}	8.70 ± 0.04^{c}	8.43 ± 0.04^{b}	10.04 ± 0.01^{e}
Klebsiella pneumonia	10.37 ± 0.20^{d}	9.30 ± 0.52^{c}	8.53 ± 0.27^{b}	10.47 ± 0.24^{d}
Proteus vulgaris	8.53 ± 0.27^{b}	8.18 ± 0.16^{c}	8.57 ± 0.28^{b}	9.26 ± 0.26^{c}
Shigella flexneri	8.63 ± 0.32^{c}	9.47 ± 0.26^{c}	8.30 ± 0.84^{b}	10.29 ± 0.25^{c}
Enterobacter aerogenes	9.25 ± 0.31^{b}	8.60 ± 0.30^{b}	9.28 ± 0.31^{b}	9.25 ± 0.31^{c}
Salmonella typhi	9.50 ± 0.26^{c}	9.22 ± 0.22^{c}	8.53 ± 0.28^{b}	9.65 ± 0.30^{c}
Shigella sonnei	9.66 ± 0.28^{c}	9.09 ± 0.16^{bc}	8.66 ± 0.33^{b}	10.33 ± 0.24^{d}
Citrobacter freundii	9.53 ± 0.24^{c}	8.43 ± 0.26^{b}	8.25 ± 0.31^{b}	9.69 ± 0.30^{d}
Streptococcus pyogenes	10.21 ± 0.24^{d}	9.33 ± 0.28^{c}	7.80 ± 0.15^{b}	10.65 ± 0.24^{c}
Serrattia marcescens	9.57 ± 0.28^{c}	9.09 ± 0.55^{c}	7.52 ± 0.28^{b}	10.67 ± 0.39^{c}
Pseudomonas aeruginosa	9.29 ± 0.28^{c}	8.30 ± 0.31^{b}	8.40 ± 0.31^{b}	10.32 ± 0.33^{d}
Proteus mirabilis	8.60 ± 0.27^{b}	8.30 ± 0.31^{b}	8.20 ± 0.30^{b}	9.33 ± 0.30^{c}

	A	B	C	D
Staphylococcus epidermidis	9.53±0.29c	9.83±0.17c	7.57±0.28b	9.40±0.30c
Staphylococcus aureus	10.46±0.23c	8.75±0.23b	9.57±0.28c	11.57±0.30c
Bacillus subtilis	9.42±0.20c	9.33±0.29b	8.69±0.25b	9.50±0.21c
Bacillus aureus	9.46±0.24c	8.75±0.23c	7.57±0.28b	9.57±0.30d
Micrococcus luteus	9.29±0.20c	8.40±0.27b	8.37±0.29b	10.43±0.26d
Bacillus thuringiensis	9.47±0.26c	8.26±0.26b	7.66±0.288b	10.60±0.31d
Alcaligenes faecalis	10.29±0.31c	8.33±0.30b	8.26±0.32b	10.23±0.34c
Staphylococcus cohnii	9.47±0.27c	8.46±0.26b	7.90±0.15b	10.53±0.26d

Table 1: Antibiotic Sensitivity Patterns of Bacterial Isolates
Data Are Presented as Mean ± S.E (N=3). Values with the Same Superscript Letter(S) Along the Same Rows Are Not Significantly Different (P<0.05).
KEYS: A= Nitrofurantoin (5mg/Ml) B= Ampiclox (5mg/Ml) C= Chloramphenicol (5mg/Ml) D= Ciprofloxacin (5mg/Ml)

	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml	Positive control	Tween 20
Escherichia coli	4.60±0.18 a	6.06±0.98a	10.60±0.43b	15.15±0.39c	9.91±0.38d	0
Klebsiella oxytoca	3.23±0.17 a	5.32±0.37a	10.73±0.54 b	14.3±0.34 c	10.04±0.01e	0
Klebsiella pneumonia	6.52±0.10 a	8.25±0.60 a	12.86±0.48 b	17.08±0.36 c	10.47±0.24d	0
Proteus vulgaris	4.52±0.15 a	6.45±0.11 a	10.49±0.56 b	16.26±0.45 c	9.26±0.26c	0
Shigella flexneri	3.16±0.22 a	5.91±0.11 a	9.58±0.40 b	16.11±0.48 c	10.29±0.25c	0
Enterobacter aerogenes	6.14±0.28 a	8.41±0.32 a	11.71±0.36 b	16.48±0.51 c	9.25±0.31c	0
Salmonella typhi	5.91±0.35 a	7.39±0.53 a	10.99±0.45 b	16.77±0.46 c	9.65±0.30c	0
Shigella sonnei	4.24±0.30 a	6.42±0.02 a	11.00±0.57 b	15.35±0.35 c	10.33±0.24d	0
Citrobacter freundii	3.24±0.12 a	5.42±0.21 a	9.90±0.58 b	14.28±0.60 c	9.69±0.30d	0
Streptococcus pyogenes	5.52±0.29 a	7.25±0.28 a	11.11±0.59 b	16.07±0.39 c	10.65±0.24c	0
Serrattia marcescens	6.04±0.52 a	8.63±0.25 a	13.23±0.46 b	18.18±0.52 c	10.67±0.39c	0
Pseudomonas aeruginosa	6.20±0.66 a	8.62±0.31 a	12.98±0.33 b	17.99±0.26 c	10.32±0.33d	0
Proteus mirabilis	6.66±0.37 a	8.62±0.21 a	12.21±0.23 b	17.52±0.36 c	9.33±0.30c	0
Staphylococcus epidermidis	5.55±0.92 a	8.58±0.29 a	14.34±0.47 b	20.56±0.70 c	9.40±0.30c	0
Bacillus subtilis	4.88±0.64 a	7.38±0.27 a	11.66±0.33 b	17.25±0.59 c	9.50±0.21c	0
Staphylococcus aureus	7.87±0.21 a	10.56±0.29 a	15.31±0.44 b	20.51±0.39 c	11.50±0.21c	0
Micrococcus luteus	7.77±0.33a	9.13±0.57 a	13.11±0.31 b	20.24±0.34 c	10.43±0.26d	0
Bacillus thuringiensis	6.01±0.86a	8.10±0.52 a	14.32±0.37 b	18.49±0.29 c	10.60±0.31d	0
Alcaligenes faecalis	4.07±0.42a	6.60±0.35 a	10.74±0.42 b	14.07±0.46 c	10.23±0.34c	0
Staphylococcus cohnii	5.09±0.38a	7.35±0.20 a	13.49±0.38 b	18.44±0.58 c	10.53±0.26d	0
Bacillus aureus	4.19±0.19a	6.59±0.30 a	13.06±0.29 b	18.04±0.58 c	9.57±0.30c	0

Table 2: Sensitivity Patterns of Bacterial Isolates to Methanol Extracts of Ocimum Gratissimum

	50 mg/ml	100mg/ml	200mg/ml	300mg/ml	Positive control	Tween 20
Escherichia coli	2.13±0.11b	4.99±0.10 a	9.48±0.43c	16.63±0.44c	9.91±0.38d	0
Klebsiella oxytoca	3.34±0.10a	6.78±0.10 a	10.24±0.39c	16.37±0.71c	10.04±0.01e	0
Klebsiella pneumonia	2.19±0.32b	4.11±0.12 a	9.04±0.47c	14.83±0.45c	10.47±0.24d	0
Proteus vulgaris	3.11±0.10b	6.11±0.10 a	10.01±0.35c	14.44±0.32c	9.26±0.26c	0
Shigella flexneri	2.00±0.12a	3.90±0.10 b	8.50±0.33 a	15.33±0.48c	10.29±0.25c	0
Enterobacter aerogenes	5.62±0.78a	7.00±0.15 b	10.88±0.58b	17.53±0.66b	9.25±0.31c	0
Salmonella typhi	3.31±0.53a	6.00±0.12 a	11.90±0.52b	15.40±0.59c	9.65±0.30c	0

	50 mg/ml	100mg/ml	200mg/ml	300mg/ml	Positive control	Tween 20
Shigella sonnei	5.09±0.13[a]	7.10±0.12[b]	11.32±0.7[c]	16.89±0.89[a]	9.20±0.27[c]	0
Citrobacter freundii	3.21±0.41[b]	5.20±0.10[a]	10.60±0.63[b]	17.80±0.29[c]	10.33±0.24[d]	0
Streptococcus pyogenes	4.00±0.10[b]	7.31±0.10[a]	11.40±0.81[b]	15.5±0.47[b]	9.69±0.30[d]	0
Serrattia marcescens	3.21±0.11[b]	6.10±0.10[a]	10.49±0.65[b]	15.65±0.43[c]	10.65±0.24[c]	0
Pseudomonas aeruginosa	2.22±0.31[b]	4.30±0.10[a]	9.00±0.58[c]	15.90±0.29[b]	10.67±0.39[c]	0
Proteus mirabilis	1.05±0.21[b]	3.67±0.10[a]	8.11±0.55[c]	14.99±0.27[c]	10.32±0.33[d]	0
Staphylococcus epidermidis	5.02±0.21[b]	7.30±0.10[a]	11.41±0.32[c]	16.54±0.30[c]	9.33±0.30[c]	0
Bacillus subtilis	4.00±0.23[b]	6.02±0.10[a]	12.4±0.60[c]	18.42±0.28[b]	9.40±0.30[c]	0
Staphylococcus aureus	5.02±0.87[b]	7.06±0.10[a]	11.67±0.78[b]	16.01±0.23[c]	11.57±0.30[c]	0
Micrococcus luteus	2.21±0.12[b]	4.60±0.10[a]	8.20±0.39[c]	15.94±0.54[c]	9.50±0.21[c]	0
Bacillus thuringiensis	5.19±0.21[b]	7.23±0.10[a]	11.43±0.55[c]	16.41±0.45[c]	9.57±0.30[d]	0
Alcaligenes faecalis	2.98±0.10[b]	5.50±0.10[a]	9.49±0.80[b]	14.06±0.28[c]	10.43±0.26[d]	0
Staphylococcus cohnii	3.33±0.37[b]	5.62±0.10[a]	10.17±0.60[c]	16.14±0.53[b]	10.6±0.31[d]	0
Bacillus aureus	4.21±0.63[b]	6.51±0.10[a]	12.42±0.68[b]	16.48±0.44[b]	10.23±0.34[c]	0

Table 3: In-Vitro Sensitivity Profile of Bacterial Isolates Treated with Water Extracts of Ocimum Gratissimum
KEY: Positive Control= Ciprofloxacin (5mg/Ml)

	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml	Positive control	Tween 20
Escherichia coli	1.06±0.14[a]	3.08±0.12[a]	4.85±0.27[b]	8.13±0.19[c]	9.91±0.38[d]	0
Klebsiella oxytoca	1.09±0.21[a]	3.42±0.30[a]	5.50±.0.26[b]	7.01±0. .34[c]	10.04±0.01[e]	0
Klebsiella pneumonia	1.00±0.19[a]	1.80±0.34[a]	4.05±0. 13[b]	7.07±0.98[c]	10.47±0.24[d]	0
Proteus vulgaris	1.12±0.13[a]	3.37±0.35[a]	5.36±0.35[b]	8.22±0.12[c]	9.26±0.26[c]	0
Shigella flexneri	1.81±0.14[a]	3.08±0.17[a]	4.94±0.24[b]	7.06±0.41[c]	10.29±0.25[c]	0
Enterobacter aerogenes	1.11±0.12[a]	2.83±0.17[a]	5.15±0.34[b]	7.99±0.29[c]	9.25±0.31[c]	0
Salmonella typhi	1.21±0.21[a]	3.50±0.26[a]	5.34±0.21[b]	7.13±0.15[c]	9.65±0.30[c]	0
Shigella sonnei	1.18±0.87[a]	2.83±0.17[a]	4.97±0.38[b]	7.47±0.29[c]	9.20±0.27[c]	0
Citrobacter freundii	1.17±0.41[a]	2.90±0.50[a]	5.13±0.75[b]	7.17±0.27[c]	10.33±0.24[d]	0
Streptococcus pyogenes	1.90±0.19[a]	2.07±0.17[a]	4.67±0.27[b]	6.21±0.15[c]	9.69±0.30[d]	0
Serrattia marcescens	1.03±0.12[a]	3.22±0.14[a]	5.29±0.18[b]	7.28±0.19[c]	10.65±0.24[c]	0
Pseudomonas aeruginosa	1.21±0.04[a]	3.50±0.01[a]	5.42±0.24[b]	7.40±0.26[c]	10.67±0.39[c]	0
Proteus mirabilis	1.32±0.09[a]	2.40±0.22[a]	4.22±0.23[b]	5.99±0.16[c]	10.32±0.33[d]	0
Staphylococcus epidermidis	1.32±0.21[a]	3.57±0.30[a]	7.12±0.16[b]	8.53±0.58[c]	9.33±0.30[c]	0
Staphylococcus aureus	2.31±0.18[a]	4.02±0.40[a]	7.52±0.26[b]	9.13±0.35[c]	11.40±0.30[c]	0
Bacillus subtilis	1.15±0.21[a]	3.12±0.18[a]	5.10±0.14[b]	6.6±0.21[c]	9.57±0.30[c]	0
Bacillus aureus	1.32±0.27[a]	3.70±0.37[a]	5.05±0.15[b]	5.99±0.37[b]	9.50±0.21[c]	0
Micrococcus luteus	2.01±0.31[a]	4.23±0.66[a]	6.15±0.25[b]	6.31±0.29[b]	9.57±0.30[d]	0
Bacillus thuringiensis	1.00±0.16[a]	2.08±0.18[a]	5.19±0.39[b]	6.33±0.21[c]	10.43±0.26[d]	0
Alcaligenes faecalis	1.09±0.15[a]	2.14±0.14[a]	4.33±0.32[b]	6.38±0.30[c]	10.6±0.31[d]	0
Staphylococcus cohnii	1.56±0.12[a]	3.29±0.19[a]	5.23±0.30[b]	5.24±0.30[b]	10.23±0.34[c]	0

Table 4: Effects of Petroleum Ether Extracts of Ocimum Gratissimum on the Survival of Bacterial Isolates
KEY: Positive Control= Ciprofloxacin (5mg/Ml)

	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml	Positive control	Tween 20
Escherichia coli	3.03±0.35^{b}	6.69±0.23^{a}	9.84±0.34^{c}	15.36±0.27^{c}	9.91±0.38^{d}	0
Klebsiella oxytoca	4.02±0.14^{b}	7.18±0.11^{a}	10.54±0.48^{c}	14.73±0.76^{c}	10.04±0.01^{e}	0
Klebsiella pneumonia	3.21±0.28^{b}	6.11±0.12^{a}	10.04±0.63^{c}	16.03±0.54^{c}	10.47±0.24^{d}	0
Proteus vulgaris	2.16±0.23^{b}	5.11±0.20^{a}	11.01±0.15^{c}	15.64±0.22^{c}	9.26±0.26^{c}	0
Shigella flexneri	5.53±0.16^{a}	8.03±0.13^{b}	10.50±0.23^{a}	15.73±0.88^{c}	10.29±0.25^{c}	0
Enterobacter aerogenes	5.22±0.12^{a}	7.00±0.25^{b}	10.28±0.18^{b}	17.59±0.26^{b}	9.25±0.31^{c}	0
Salmonella typhi	4.01±0.31^{b}	7.49±0.17^{a}	11.82±0.22^{b}	16.60±0.89^{c}	9.65±0.30^{c}	0
Shigella sonnei	3.87±0.16^{a}	7.11±0.18^{b}	10.32±0.16^{c}	16.49±0.29^{a}	9.20±0.27^{c}	0
Citrobacter freundii	2.99±0.17^{b}	6.20±0.10^{a}	9.66±0.63^{b}	15.80±0.22^{c}	10.33±0.24^{d}	0
Streptococcus pyogenes	3.91±0.12^{a}	7.30±0.14^{a}	11.40±0.87^{b}	16.50±0.47^{b}	9.69±0.30^{d}	0
Serrattia marcescens	3.28±0.11^{a}	6.82±0.10^{a}	10.49±0.45^{b}	15.60±0.33^{c}	10.65±0.24^{c}	0
Pseudomonas aeruginosa	3.46±0.21^{a}	6.66±0.10^{a}	9.38±0.38^{c}	14.90±0.29^{b}	10.67±0.39^{c}	0
Proteus mirabilis	3.69±0.10^{a}	7.67±0.26^{a}	10.11±0.75^{c}	15.99±0.28^{c}	10.32±0.33^{d}	0
Staphylococcus epidermidis	4.62±0.12^{a}	8.30±0.17^{a}	12.41±0.31^{bc}	17.54±0.49^{c}	9.33±0.30^{c}	0
Bacillus subtilis	3.42±0.10^{a}	7.02±0.10^{a}	13.40±0.40^{c}	15.82±0.78^{b}	9.40±0.30^{c}	0
Staphylococcus aureus	3.32±0.10^{a}	7.06±0.10^{a}	11.67±0.78^{b}	16.01±0.23^{c}	11.57±0.30^{c}	0
Micrococcus luteus	3.01±0.12^{a}	6.28±0.10^{a}	10.22±0.36^{b}	14.99±0.42^{a}	9.50±0.21^{c}	0
Bacillus thuringiensis	3.21±0.18^{a}	7.26±0.13^{a}	12.43±0.51^{b}	16.81±0.45^{c}	9.57±0.30^{d}	0
Alcaligenes faecalis	4.01±0.11^{a}	6.99±0.11^{a}	11.29±0.70^{b}	15.06±0.48^{c}	10.43±0.26^{d}	0
Staphylococcus cohnii	4.20±0.12^{a}	8.69±0.14^{a}	11.18±0.60^{b}	16.14±0.53^{b}	10.6±0.31^{d}	0
Bacillus aureus	3.39±0.19^{a}	6.51±0.10^{a}	12.42±0.68^{b}	16.48±0.44^{b}	10.23±0.34^{c}	0

Table 5: In-Vitro Sensitivity Patterns of Bacterial Isolates Treated with Methanol Extracts of Annona Muricata
KEY: Positive Control= Ciprofloxacin (5mg/Ml)

	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml	Positive Control	Tween 20
Escherichia coli	2.23±0.19^{a}	4.59±0.13^{a}	7.42±0.94^{c}	12.36±0.88^{b}	9.91±0.38^{d}	0
Klebsiella oxytoca	1.98±0.12^{a}	3.98±0.20^{a}	7.04±0.38^{c}	11.13±0.28^{c}	10.04±0.01^{e}	0
Klebsiella pneumonia	1.32±0.17^{a}	3.45±0.28^{a}	6.73±0.28^{c}	10.22±031^{c}	10.47±0.24^{d}	0
Proteus vulgaris	2.01±0.15^{a}	4.13±0.20^{a}	9.09±0.83^{c}	12.32±0.24^{c}	9.26±0.26^{c}	0
Shigella flexneri	1.09±0.15^{a}	3.06±0.13^{b}	7.76±0.96^{a}	11.40±0.88^{c}	10.29±0.25^{c}	0
Enterobacter aerogenes	2.50±0.12^{a}	5.00±0.25^{b}	9.28±0.19^{b}	14.59±0.60^{c}	9.25±0.31^{c}	0
Salmonella typhi	2.23±0.13^{a}	4.49±0.17^{a}	9.82±0.22^{b}	13.60±0.89^{c}	9.65±0.30^{c}	0
Shigella sonnei	2.02±0.12^{a}	4.11±0.18^{b}	10.39±0.16^{c}	12.59±0.29^{a}	9.20±0.27^{c}	0
Citrobacter freundii	1.09±0.17^{a}	3.24±0.14^{a}	5.99±0.43^{C}	9.80±0.72^{c}	10.33±0.24^{d}	0
Streptococcus pyogenes	1.21±0.19^{a}	3.30±0.14^{a}	8.60±0.87^{b}	11.53±0.27^{c}	9.69±0.30^{d}	0
Serrattia marcescens	1.23±0.10^{a}	3.33±0.10^{a}	7.49±0.45^{b}	11.60±0.33^{c}	10.65±0.24^{c}	0
Pseudomonas aeruginosa	1.45±0.18^{a}	3.66±0.10^{a}	6.38±0.38^{c}	10.90±0.29^{b}	10.67±0.39^{c}	0
Proteus mirabilis	2.22±0.29^{a}	4.19±0.26^{a}	8.11±0.75^{c}	12.99±0.98^{c}	10.32±0.33^{d}	0

	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml	Positive Control	Tween 20
Staphylococcus epidermidis	2.78±0.12[a]	5.50±0.19[a]	9.41±0.31[b]	15.54±0.49[c]	9.33±0.30[c]	0
Bacillus subtilis	1.90±0.15[a]	3.02±0.10[a]	7.40±0.40[c]	10.22±0.38[b]	9.40±0.30[c]	0
Staphylococcus aureus	2.91±0.19[a]	4.02±0.13[a]	8.67±0.48[b]	11.01±0.93[c]	10.57±0.30[c]	0
Micrococcus luteus	2.22±0.11[a]	4.18±0.10[a]	8.22±0.26[b]	11.99±0.42[a]	9.50±0.21[c]	0
Bacillus thuringiensis	1.32±0.12[a]	3.06±0.13[a]	7.43±0.51[c]	12.81±0.45[c]	9.57±0.30[d]	0
Alcaligenes faecalis	1.45±0.20[a]	3.99±0.11[a]	8.29±0.70[b]	10.06±0.31[c]	10.43±0.26[d]	0
Staphylococcus cohnii	2.33±0.21[a]	4.92±0.11[a]	9.18±0.73[b]	12.14±0.53[b]	10.6±0.31[d]	0
Bacillus aureus	2.81±0.24[a]	4.18±0.27[a]	8.42±0.28[b]	11.59±0.44[b]	10.23±0.34[c]	0

Table 6: Sensitivity Profile of Bacterial Isolates to Water Extracts of Annona Muricata
KEY: Positive Control= Ciprofloxacin (5mg/Ml)

	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml	Positive control	Tween 20
Escherichia coli	2.23±0.18[b]	5.69±0.26[a]	8.99±0.75[b]	14.29±0.27[c]	9.91±0.38[d]	0
Klebsiella oxytoca	2.91±0.18[a]	5.18±0.11[a]	8.22±0.48[b]	13.73±0.56[c]	10.04±0.01[e]	0
Klebsiella pneumonia	3.33±0.19[a]	6.71±0.12[a]	10.14±0.63[c]	16.03±0.54[c]	10.47±0.24[d]	0
Proteus vulgaris	2.91±0.16[ab]	5.11±0.14[a]	11.11±0.44[c]	17.64±0.62[b]	9.26±0.26[c]	0
Shigella flexneri	5.06±0.19[a]	8.03±0.13[b]	10.50±0.23[a]	15.73±0.88[c]	10.29±0.25[c]	0
Enterobacter aerogenes	3.83±0.18[a]	6.10±0.45[b]	12.30±0.24[b]	16.91±0.26[b]	9.25±0.31[c]	0
Salmonella typhi	2.71±0.19[a]	5.44±0.33[a]	10.62±0.12[c]	16.90±0.23[c]	9.65±0.30[c]	0
Shigella sonnei	3.91±0.15[b]	6.11±0.48[a]	12.32±0.66[c]	16.59±0.22[a]	9.20±0.27[c]	0
Citrobacter freundii	3.01±0.17[a]	6.20±0.10[a]	9.66±0.63[b]	15.80±0.82[c]	10.33±0.24[d]	0
Streptococcus pyogenes	2.41±0.13[b]	5.30±0.10[a]	10.40±0.17[b]	14.51±0.74[b]	9.69±0.30[d]	0
Serrattia marcescens	2.09±0.19[a]	4.02±0.10[a]	8.39±0.88[c]	13.61±0.33[c]	10.65±0.24[c]	0
Pseudomonas aeruginosa	2.18±0.13[a]	5.66±0.10[a]	10.38±0.34[c]	14.62±0.24[b]	10.67±0.39[c]	0
Proteus mirabilis	3.20±0.18[a]	6.97±0.26[a]	11.11±0.84[c]	15.99±0.28[c]	10.32±0.33[d]	0
Staphylococcus epidermidis	4.72±0.17[b]	7.70±0.14[a]	12.45±0.31[bc]	16.84±0.42[c]	9.33±0.30[c]	0
Bacillus subtilis	3.00±0.12[b]	5.72±0.13[a]	10.44±0.40[b]	16.10±0.72[b]	9.40±0.30[c]	0
Staphylococcus aureus	4.21±0.16[a]	7.11±0.10[a]	13.07±0.78[b]	18.01±0.23[b]	11.57±0.30[c]	0
Micrococcus luteus	3.82±0.14[a]	6.66±0.17[a]	11.92±0.36[b]	14.99±0.42[a]	9.50±0.21[c]	0
Bacillus thuringiensis	4.21±0.19[a]	7.26±0.13[a]	12.43±0.51[b]	15.88±0.85[c]	9.57±0.30[d]	0
Alcaligenes faecalis	3.24±0.19[a]	6.52±0.11[a]	10.29±0.40[b]	15.06±0.48[c]	10.43±0.26[d]	0
Staphylococcus cohnii	4.21±0.17[b]	7.77±0.39[a]	12.28±0.60[c]	17.24±0.35[b]	10.6±0.31[d]	0
Bacillus aureus	3.38±0.13[a]	7.51±0.20[a]	11.12±0.55[b]	16.48±0.51[c]	10.23±0.34[c]	0

Table 7: Sensitivity Patterns of the Bacterial Isolates Exposed to Petroleum Ether Extracts of Annona Muricata
KEY: Positive Control= Ciprofloxacin (5mg/Ml

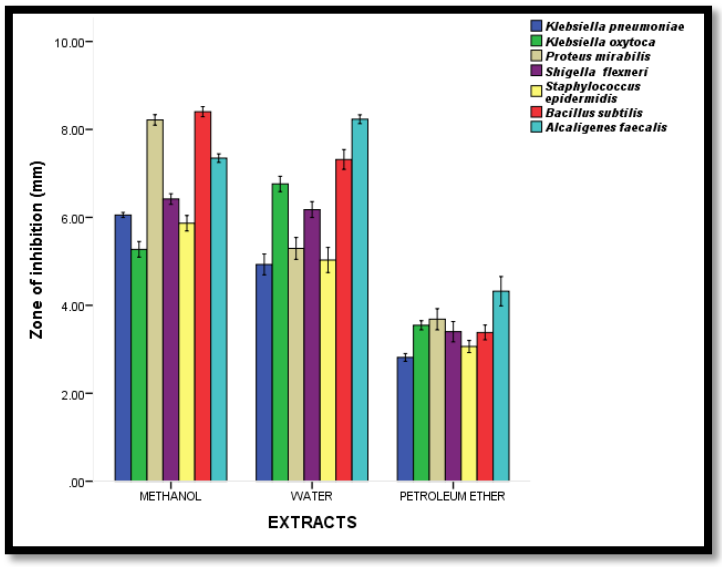

Figure 1: Sensitivity Patterns of Bacterial Isolates against Ocimum Gratissimum Extracts

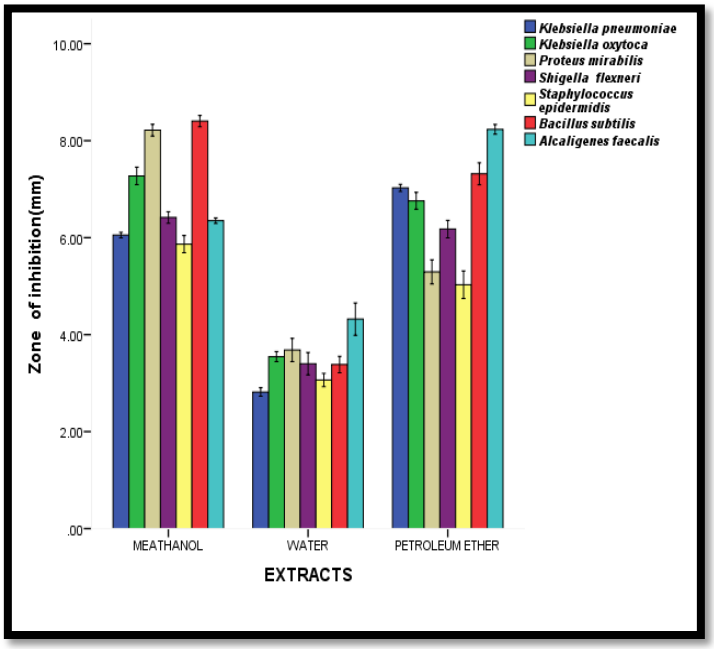

Figure 2: Sensitivity Patterns of Bacterial Isolates against Annona Muricata Extracts

S/N	Cultural and Biochemical Identities	16s rRNA Sequence Identification	Max Identity	Accession Number
1	*Proteus mirabilis*	*Proteus mirabilis* strain HI4320	97%	NC010554.1
2	*Salmonella typhi*	*Salmonella enterica* subsp.enterica serovar Typhi str. CT18	100%	NC003198.1
3	*Pseudomonas aeruginosa*	*Pseudomonas fluorescens* SBW25	92%	NC012660.1
4	*Staphylococcus aureus*	*Staphylococcus cohnii* subsp. cohnii strain 532 Contig16	99%	NZLATV01000012.1
5	*Salmonella enterica*	*Salmonella enterica* subsp. enterica serovar Infantis	80%	NZ LN649235.1
6	*Escherichia coli*	*Escherichia coli* str. K12 substr. DH10B	100%	NC010473.1
7	*Shigella* flexneri	*Shigella flexneri* 2a str. 2457T	100%	NC004741.1

S/N	Cultural and biochemical identities	16s rRNA sequence identification	Max Identity	Accession number
8	*Klebsiella pneumoniae*	*Klebsiella pneumoniae* strain J1	99%	NZ CP013711.1
9	*Citrobacter freundii*	Citrobacterfreundii partial strain R4-2	95%	LN854584.1
10	*Bacillus subtilis*	*Bacillus subtilis* strain DBS-LAZ-03/17	90%	MG23126.1
11	*Bacillus thuringensis*	*Bacillus thuringiensis* strain AN11	92%	KY652120.1
12	*Alcaligenes faecalis*	*Alcaligenes faecalis* strain UG2-MRL	83%	KC762741.1

Table 8: Molecular Identity of Bacterial Isolates from Food Handlers in Akure

Phytochemicals	Methanol Extract	Water Extract	Petroleum ether Extract
Monosaccharides	+	+	-
Pentoses	+	+	-
Alkaloids	+	+	-
Steroids	+	++	-
Tannins	++	+	+
Ketoses	+	+	+
Arginine	+	+	+
Cysteine	+	+	+
Phenolic amino acids	+	+	+
Anthraquinones	-	-	-
Nitrogen and Halides (Cl^-)	+	+	+
Sulphur and Sulphate ion	+	+	-
Aromatic amino acid	+	+	+
Flavonoids	+	+	+
Saponins	++	+	+
Terpenoids	+	-	+
Phlobatannins	+	-	+
Glycoside	+	-	+
Cardiac compounds	+	+	+

Table 9: Qualitative Phytochemical Properties of Ocimum Gratissimum and Annona Muricata Leaves

Phytochemicals	Ocimum gratissimum			Annona muricata		
	Methanol Extract	Water Extract	Petroleum ether Extract	Methanol Extract	Water Extract	Petroleum Ether Extract
Arginine	4.75±0.27^c	3.81±0.00^a	1.24±0.06^a	ND	ND	ND
Steroids	2.73±0.14^a	3.33±0.07^b	0	ND	ND	ND
Sulphate ions	2.85±0.28^b	2.42±0.12^c	0	ND	ND	ND
Alkaloids	3.99±0.32^b	4.16±0.19^a	0	3.89±0.82^b	0.78±0.33^c	4.56±0.49^b
Amino acids	2.97±0.16^b	1.38±0.02^a	1.43±0.35^b	ND	ND	ND
Tannins	4.42±0.27^a	2.03±0.34^b	1.00±0.21^a	4.44±0.27^c	2.40±0.17^b	3.43±0.09^b
Saponins	4.02±0.08^a	3.45±0.26^a	1.01±0.36^a	3.03±0.24^b	0	0
Flavonoids	3.67±0.11^c	3.00±0.12^b	1.26±0.22^c	1.05±0.29^b	0	1.89±0.16^c
Polyphenol	1.02±0.32^b	ND	ND	1.97±0.14^b	0.26±0.07^a	1.43±0.35^b

Table 10: Quantitative Phytochemical Properties of Ocimum Gratissimum and Annona Muricata Leaves

4 Conclusion and Recommendations

It can be deduced from this study that food handlers play a major role in the transfer of bacterial contaminants to the edible food that we purchase in our daily activities, although some of these foods may naturally habour these pathogens. Most of the bacteria present were susceptible to commercial antibiotics and extracts from selected medicinal plants. In addition, this research was developed to determine the most rampant bacterial pathogen that human can encounter when contaminated food is ingested. *Staphylococcus aureus* happens to be the most prominent bacteria found on the hands of food handlers and can cause notable food borne infections if not taken care of. Other bacteria like *Bacillus spp, Citrobacter freundii, Escherichia coli, Klebsiella spp, Salmonella spp, Pseudomonas spp,* should be taken cognisant of as they were isolated from the food handlers in the study. The use of PCR for the identification of the isolated bacteria was

found to be more effective and accurate compared to the normal conventional method. The results of antimicrobial activity of *Annona muricata and Ocimum gratissimum* indicate the antimicrobial potential of the leaf extract which may be a source of new bioactive compounds for drug development and also suggests that the test plants could be promising in the treatment of food borne diseases.

The availability and accessibility to plant makes the use of *A. muricata and O. gratissimum* a cost-effective alternative medicine to the commercial antibiotics to which most organisms are now developing resistance. Further purification of the extract and identification of the active component is necessary to enhance greater antimicrobial potency.

Every microorganism has a set of nucleotide arrays which appears in series of sequenced data and are specific to each organism, thus making the identification accurate.

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