Original Research Article

The Incidence of Extended Spectrum Beta-Lactamase (ESBL)-Producing Bacteria in Salad Vegetables in Ondo City, Nigeria

Abstract

Aim: This study was carried out to determine the occurrence of extended spectrum beta-lactamase (ESBL) producing bacteria in salad vegetables in Ondo City, Nigeria.

Study Design: An experimental study design with randomized sampling

Place and Duration of the Study: The research was carried out in the Department of Biological Sciences of Wesley University, Ondo State, Nigeria.

Methodology: Samples of cucumber, carrot, green pea, green beans, sweet corn and cabbage were analysed on appropriate agar medium. Pure isolates were identified by biochemical tests and confirmation was done by the use of API 20 E and API 20 NE in accordance with standard procedures. ESBLs screening was carried out using the double disk synergy test. Data were statistically analyzed using MedCalc statistical software (version 17.2).

Results: Total viable bacterial counts (TVBCs) ranged from 1.1×10^3 to 7.1×10^5 cfu/ml; total coliform counts (TCC) ranged from 1.2×10^2 to 3.9×10^3 cfu/ml while total faecal counts (TFC) ranged from 0 to 2.9×10^2 cfu/ml. There were statistical differences in mean TVBCs of the samples ($P \leq 0.05$). The mean TCCs of cabbage, carrot and cucumber showed no statistical significance; green beans, green pea and sweet corn also showed no statistical significance ($P \geq 0.05$). One hundred and sixty (166) isolates obtained from the samples were identified as *Bacillus cereus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Serratia marcesens* and *Staphylococcus saprophyticus*. At least one member of all bacterial species, except *S. saprophyticus*, produced ESBL.

Conclusion: This study revealed that salad vegetables could be a vehicle for the spread of extended-spectrum beta-lactamase-producing bacteria which translates to a threat to public health around the world as salads are loved and consumed by all categories of people globally.

Keywords: Extended-spectrum Beta-lactamase, Bacterial resistance, Salad vegetables and Foodborne pathogen.

Introduction

The beginning of food safety is not usually at the grocery store or in the kitchen. It starts on the farm. Concerns about the safety of food, plants' and animals' welfares, as well as traceability are more preferred to the food products being supplied in plenitude. Vegetables are considered as the major reservoirs of opportunistic and emerging pathogens due to its diverse microbiome and they are also strongly influenced by biogeographic aspects of farming and food processing practices [1].

Fresh vegetables are considered as the essential components of healthy diet of people and the consumption of vegetables in the form of salads has increased in many parts of the world, including Africa. In contrast to the potential health benefits of fresh vegetables, a concern about the safety and the quality of vegetables has also raised due to outbreaks of infectious diseases reported from by Center for Disease Control and Prevention (CDC), US Food and Drug Administration (FDA), World Health Organization (WHO) and Center for Science in the Public Interest (CSPI). These changes are mainly due to change in the ecology of human pathogens to persist in non-host environments. Fresh vegetables, generally, are known to harbour huge bacterial populations [2], which could be of plant endophytes, plant pathogenic and human pathogenic in nature. The most important features of plant host colonization is by the adaptation of pathogens to the host defence response, physiology, immunity, native microflora, physical barriers, mobility and temperature.

Since the fresh vegetables in the form of salads are consumed raw, the pathogens present in it lead to widespread disease outbreaks. The microbes, non-pathogenic in nature, associated with plants as a commensal may lead to allergies due to change in the interaction strategies of microbes with the endophytic bacterial community and the plant host [3].

Extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated and the genes encoding these enzymes could be transferred easily among different bacteria [4]. ESBLs hydrolyze oxyimino-cephalosporins and transmit resistance to bacteria against the penicillins, cephalosporins (first to third generations), and aztreonam. These are repressed by β-lactamase inhibitors. ESBLs are classified as β-lactamases containing three main families: TEM (named after the patient Temoneria), SHV (sulfhydryl reagent variable), and CTX-M (active on cefotaxime, first isolated in Munich) [5,6]. Most of these plasmids not only contain DNA encoding ESBLs but also carry genes conferring resistance to several non-β-lactam antibiotics [7].

The presence of ESBL in bacterial isolate has been documented as a very serious problem and a significant risk to quick survival of patients in the hospital, high economic burden, loss of hours in life's activities and huge treatment failure [8]. The phenotypic methods are currently the gold standard in determination of susceptibility or resistance of bacterial isolates. The most widely used methods to screen ESBL are E-test, or double-disk synergy test (DDST) [9].

There are several reported outbreaks related to salad vegetables from the past decades to the present but none has reported salad vegetables as an important source of ESBLs-producing bacteria which is germane in controlling the spread multi-drug resistant pathogens, and thus, reduce the morbidity and mortality rates associated with foodborne pathogens. Reports of Fody *et al*. [8] in Niamey, Niger; Andrew *et al*. [10] in southwestern, Uganda; Nepal *et al*. [11] in Kathmandu, Nepal; Jose *et al*. [12] in Meppadi, Wayanad; Mashwal *et al*. [13] in Saudi Arabia and many others have only described the prevalence of ESBLs in clinical samples but studies on food products such as salad vegetables as potential vehicle of ESBLs-producing bacteria is scanty, and none has been reported in southwestern, Nigeria. This study was, therefore, carried out to investigate ESBLs production from bacteria isolated from salad vegetables in Ondo city, Nigeria.

Materials and Methods

Collection of Samples

The salad vegetables were obtained from different stalls in Ondo-West LGA by random sampling. Three samples each of the salad vegetables were collected prior to preparation. These include cucumber, carrot, green pea, green beans, sweet corn and cabbage from three different eateries. The samples were collected in sterilized (by irradiation) polyethylene bags and taken to the laboratory for analysis.

Microbiological analysis and identification of isolates

Twenty five grams (25g) of each sample was blended and homogenized with 225 ml buffer peptone water. Serial dilutions were prepared up to 10^{-6} following the standard. A volume of 0.1 ml from each sample suspension was spread onto nutrient agar and incubated at 37 ^{o}C for 24 hours for enumerating total viable bacteria. For enumerating coliforms, 0.1 ml of suspension was spread over MacConkey agar for each samples and incubated at 37 ^{o}C for 18-24 hours. A 0.1 ml of suspension was spread onto Mannitol salt agar for the estimation of *Staphylococcus aureus* and the plates were incubated at 37 ^{o}C for 24 hours. Pure cultures of the isolates were obtained by subsequent sub-culturing. Pure isolates were carefully examined macroscopically for cultural characteristic such as extent of growth, colour, shape, pigmentation and consistency. Gram's staining, spore staining, motility testing and standard biochemical tests were performed to characterize the isolates in accordance with standard procedures. API 20 E and API 20 NE were used to confirm the identity of the isolates according to manufacturer's instructions.

Standardization of Inoculum

The isolates of organisms were cultured on nutrient agar (Oxoid, England) plates and incubated for 24 hours at 37 ^{o}C to obtain confluent growth for sensitivity testing. Few colonies were taken from the nutrient agar plates and dispensed in sterile normal saline to turbidity equivalent to 0.5 McFarland standard. A 0.6 ml proportion of 1% Barium Chloride was mixed with 99.4 ml of Sulphuric acid to obtain a Barium Sulphate solution used for sensitivity [14].

Screening of Bacteria for ESBLs Production

The sensitivity of standard inocula of isolates to ceftriaxone (CTR 30µg, Oxoid UK) and ceftazidime (CAZ 30µg, Oxoid, UK) discs was determined on Mueller Hinton Agar (Oxoid, UK) using Kirby-Bauer (1966) method. The test organism of appropriate inoculums size was emulsified on the surface of MHA (Oxoid, England) using sterile cotton swab (220210 BD SWUBE, India). Then, aseptic application of the ceftriaxone (CTR 30 µg) and ceftazidime (CAZ 30 µg) was carried out on the surface of the inoculated Mueller Hinton Agar (MHA) 20mm from each disc and 15 mm from the edge of the plate using sterile forceps. After 30 minute of disc application, the plates were incubated at 37^{o}C for 24 hours at inverted position [15,16]. After an overnight incubation, the diameters for inhibition zones were measured in millimetre using a meter rule [17].

Confirmation of ESBLs-Producing Bacteria

The isolates were subcultured on nutrient agar by streak late method and incubated at 35^{o}C for 18-24 hours so as to obtain confluent growth. Improved procedure of Jarlier *et al*. [18] was employed for screening of isolates for ESBLs production on Mueller-Hinton Agar (MHA) using standard inocula from nutrient agar (NA) plates. The isolates were further inoculated using sterile swab stick onto the surface of MHA. The discs containing two 3rd generation

cephalosporins (ceftriaxone 30µg and ceftazidime 30µg) both placed at 20mm distance apart centre to centre from an augmentin (amoxycillin/clavulanic acid, 30µg, CT0223B Oxoid, UK) disc placed at the centre. The plates were then incubated at 35 ^{o}C for 18-24 hours after which the plates were read [19,20].

Statistical Analysis

Data were collated and statistically analyzed using MedCalc statistical software version 17.2 (a statistical software package designed for the biomedical sciences). Simple means, percentages and frequencies from different locations were computed and compared using one-way Analysis of Variance (ANOVA) and independent t- test.

Results

Table 1 showed the bacteriological quality of salad vegetables sold in Ondo city, Nigeria. The quality was determined in terms of total viable bacterial count (TVBC), total coliform count (TCC) and total fungal count (FCC). Three replicate samples of each of the vegetables (cucumber, carrot, green pea, green beans, sweet corn and lettuce), making eighteen samples in all, were analyzed. TVBCs in samples ranged from 1.1×10^3 to 7.1×10^5 cfu/ml as occurred in samples GP2 and Cu1, respectively; TCC ranged from 1.2×10^2 to 3.9×10^3 cfu/ml as encountered in samples GP3 and Cab1, respectively while FCC ranged from 0 to 2.9×10^2 cfu/ml. The mean TVBC was highest in cucumber (5.3×10^5 cfu/ml) and lowest in green pea with 1.5×10^3 cfu/ml; mean TCC was highest in cabbage and carrot samples with 2.8×10^3 cfu/ml while green pea had the lowest with 1.3×10^2 cfu/ml; however, mean FCC ranged from 0 to 2.1×10^2 cfu/ml. Faecal coliforms were not encountered in green beans and green pea samples. There were significant differences in TVBC counts of salad vegetable samples analysed in this study. Data and mean counts with same superscripts along same column in Table 1 showed no statistically significance. There were statistical differences in mean TVBCs of the samples ($P \leq 0.05$). The mean TCCs of cabbage, carrot and cucumber showed no statistical significance ($P \geq 0.05$); green beans, green pea and sweet corn also showed no statistical significance ($P \geq 0.05$) while there was a statistical difference between the former and latter groups. In the same vein, the mean FCC of cabbage was statistically different from other samples.

Table 2 showed the morphological and biochemical characteristics of bacteria isolated from salad vegetables sold in Ondo city, Nigeria. One hundred and sixty-six (166) isolates were obtained from the various samples and these were characterized into nine (9) genera but ten (10) species. These isolates were identified as *Bacillus cereus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumonia*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Serratia marcesens* and *Staphylococcus saprophyticus*.

The distribution of bacteria in vegetable salad samples in Ondo city, Nigeria was shown in Table 3. All samples except GP3 contributed to the bacterial diversity recorded in this study. The bacteria occurred randomly in the different samples and, at least, one bacterium was encountered in all samples except GP3 in which none was found.

Figure 1 showed the percentage occurrence of bacteria isolated from salad vegetables in Ondo city, Nigeria. *S. aureus* had the highest percentage occurrence of 19.28%, followed by *P. aeruginosa* (13.86%), *K. pneumoniae* (12.65%), *M. marcesens* (10.24%), *B. cereus* (9.64%), *P. mirabilis* (9.64%), *M. morganii* (7.83%), *S. saprophyticus* (7.23%), *E. coli* (5.42%) while *C. freundii* had the lowest occurrence of 4.22%.

The zones of inhibition to screen for potential ESBL-producers based on CLSI breakpoint using ceftriaxone and ceftazidime antibiotics was shown in Table 4. Strains of test organisms that were resistant to any of the cephalosporins as illustrated in Table 4 were suspected to be ESBL producers, and were further subjected to double discs synergy test (DDST) to phenotypically confirm if they are ESBL-producers as shown in Table 5.

It was, however, confirmed that 4 out of 12 *B. cereus* isolates, 1 of 7 *C. freundii*, 4 of 10 *E. coli*, 2 of 10 *K. pneumoniae*, 2 of 6 *M. morganii*, 3 of 17 *P. aeruginosa*, 1 of 9 *P. mirabilis*, 3 of 22 *S. aureus* and 1 of 11 *Serratia marcecens* isolates were ESBL producers. None of the eight *S. saprophyticus* produced ESBL. This indicated that 17% of *B. cereus* isolates, 8% *C. freundii*, 21% *E. coli*, 10% *K. pneumoniae*, 17% *M. morganii*, 9% *P. aeruginosa*, 6% *P. mirabilis*, 7% *S. aureus* and 5% *Serratia marcecens* isolates were ESBL-producing isolates in the salad vegetables within the study area (Figure 2).

Table 1: Bacteriological quality of vegetable salads sold in Ondo West LGA, Ondo State.

Ingredients	Sample code	Total viable bacterial count (TVBC) (cfu/ml)	Total coliform count (TCC) (cfu/ml)	Faecal coliform count (FCC) (cfu/ml)
Cabbage	Cab1	5.9×10^{5b}	3.9×10^{3a}	2.9×10^{2a}
	Cab2	2.7×10^{5a}	2.6×10^{3b}	2.1×10^{2}b
	Cab3	3.3×10^{5a}	2.0×10^{3b}	1.2×10^{2c}
	Mean	**4.0×10^{5a}**	**2.8×10^{3b}**	**2.1×10^{2b}**
Carrot	Car1	4.2×10^{5b}	3.5×10^{3a}	1.6×10^{2a}
	Car2	2.1×10^{5a}	2.7×10^{3b}	1.3×10^{2a}
	Car3	3.7×10^{5b}	2.2×10^{3b}	1.5×10^{2a}
	Mean	**3.3×10^{5b}**	**2.8×10^{3b}**	**1.5×10^{2a}**
Cucumber	Cu1	7.1×10^{5b}	3.2×10^{3a}	1.8×10^{2a}
	Cu2	3.8×10^{5a}	1.2×10^{3c}	1.1×10^{2a}
	Cu3	4.9×10^{5c}	2.7×10^{3b}	1.4×10^{2a}
	Mean	**5.3×10^{5c}**	**2.4×10^{3b}**	**1.4×10^{2a}**
Green beans	GB1	3.2×10^{3a}	1.8×10^{2a}	0
	GB2	3.1×10^{3a}	1.3×10^{2a}	0
	GB3	2.2×10^{3b}	1.5×10^{2a}	0
	Mean	**2.8×10^{3d}**	**1.5×10^{2a}**	0
Green pea	GP1	1.5×10^{3a}	1.3×10^{2a}	0
	GP2	1.1×10^{3a}	1.4×10^{2} a	0
	GP3	1.8×10^{3a}	1.2×10^{2a}	0
	Mean	**1.5×10^{3e}**	**1.3×10^{2a}**	**0**
Sweet corn	SC1	3.8×10^{3a}	1.4×10^{2a}	1.0×10^{2}
	SC2	6.2×10^{3c}	1.2×10^{2a}	1.0×10^{2}
	SC3	3.2×10^{3a}	1.6×10^{2a}	0
	Mean	**4.4×10^{3a}**	**1.4×10^{2a}**	**1.0×10^{2a}**

Data and mean counts with same superscripts along same column are not statistically significant at 95% level of confidence i.e. $P \leq 0.05$.

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184 Table 2: Morphological and biochemical characteristics of bacteria isolated from vegetable salads sold in Ondo city, Nigeria

Gram Reaction	Cellular morphology	Catalase	Oxidase	Indole	Motility	Methyl-Red	Voges Proskauer	Urease activity	Citrate Utilization	Starch Hydrolysis	Gelatin Hydrolysis	Casein Hydrolysis	Spore test	NO_3 Reduction	Glucose	Sucrose	Arabinose	Maltose	Mannitol	Xylose	Galactose	Sorbitol	Inositol	Raffinose	Frauction	Number of isolates showing characteristics	Most Probable Identity
+ve	R	+	+	-	+	-	+		+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	16	*Bacillus cereus*
+ve	R	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+	+	7	*Citrobacter freundii*
-ve	R	-	+	-	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	+	9	*Escherichia coli*
-ve	R	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	21	*K. pneumoniae*
-ve	R	+	-	+	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	13	*Morganella morganii*
-ve	R	+	+	-	+	-	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	23	*P. aeruginosa*
-ve	R	+	-	-	+	+	-	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	16	*Proteus mirabilis*
+ve	C	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	-	-	-	+	32	*S. aureus*
-ve	R	+	+	-	+	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	17	*Serratia marcesens*
+ve	C	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	-	-	-	+	12	*S. saprophyticus*

185 Keys: Cb = Coccobacilli; R = Rods; C = Cocci; + = Positive reaction; - = Negative reaction; ND = Not determined

186

187 Table 3: Distribution of bacteria in vegetable salad samples sold in Ondo State, Nigeria

Ingredients	Sample code	*Bacillus* sp.	*Citrobacter freundii*	*E. coli*	*K. pneumoniae*	*M. morganii*	*P. aeruginosa*	*Proteus mirabilis*	*S. aureus*	*Serratia. marcesens*	*S. saprophyticus*
Cabbage	Cab1	+	+	+	+	-	-	+	+	+	+
	Cab2	-	+	-	-	+	+	-	+	+	-
	Cab3	+	-	-	+	-	+	-	+	+	-
Carrot	Car1	-	+	+	+	+	+	+	+	-	+
	Car2	+	-	-	-	-	+	-	+	+	+
	Car3	+	-	+	+	-	+	-	+	+	-
Cucumber	Cu1	-	+	-	+	-	+	+	+	-	+
	Cu2	-	-	-	-	+	+	-	+	+	+
	Cu3	+	-	-	-	-	+	-	+	+	+
Green beans	GB1	+	-	-	-	-	-	-	+	-	+
	GB2	-	-	-	+	-	+	-	-	-	-
	GB3	-	-	-	-	-	+	-	-	-	-
Green pea	GP1	-	-	-	-	-	-	-	-	+	-
	GP2	-	-	-	-	-	-	-	+	-	-
	GP3	-	-	-	-	-	-	-	-	-	-
Sweet corn	SC1	+	-	-	-	-	-	-	+	-	-
	SC2	-	-	+	-	-	-	-	+		-
	SC3	-	-	-	-	-	+	-	+	-	-

188 Keys: + = present; - = absent.

189

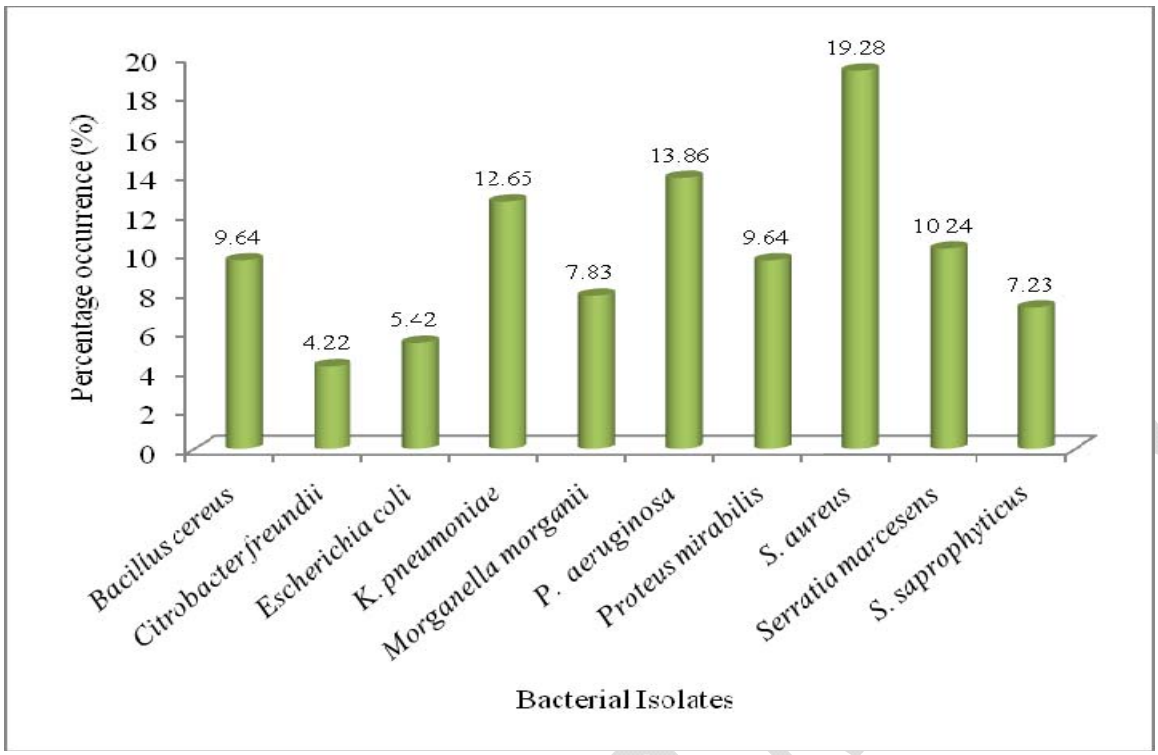

Figure 1: Percentage occurrence of bacteria isolated from vegetable salads in Ondo city, Nigeria

203 Table 4: Zones of inhibition to screen for potential ESBL-producers based on CLSI breakpoint using ceftriaxone and ceftazidime
204 antibiotics

Isolates	Ceftazidime (CAZ) ≤ 22 mm	Ceftriaxone (CTR) ≤ 25 mm	No. screened	No. of potential ESBL producer	Isolates	Ceftazidime (CAZ) ≤ 22 mm	Ceftriaxone (CTR) ≤ 25 mm	No. screened	No. of potential ESBL producer
B. cereus	21	20	12	6		15	24		
	17	20				18	16		
	27	32			*K. pneumoniae*	17	20	10	6
	19	22				21	22		
	20	23				13	21		
	25	29				25	26		
	17	21				24	27		
	23	21				23	30		
	21	23				26	25		
	23	27				19	22		
	23	31				21	23		
	25	25				16	21		
C. freundii	19	21	7	3	*M. morganii*	20	21	6	2
	23	27				23	27		
	30	26				25	28		
	25	27				23	19		
	14	20				28	26		
	21	15				21	18		
	23	30			*S. saprophyticus*	27	26	8	3
E. coli	20	17	10	7		32	27		
	16	21				27	30		
	23	26				19	25		
	19	16				15	27		
	24	28				23	26		
	18	22				20	23		
	23	25				25	30		
	20	21							

Isolates	Ceftazidime (CAZ) ≤ 22 mm	Ceftriaxone (CTR) ≤ 25 mm	No. screened	No. of potential ESBL producer	Isolates	Ceftazidime (CAZ) ≤ 22 mm	Ceftriaxone (CTR) ≤ 25 mm	No. screened	No. of potential ESBL producer
P. aeruginosa	21	19	17	5		23	27		
	25	27				31	28		
	23	31				25	26		
	25	26				24	26		
	21	23				21	23		
	19	22				24	27		
	23	29				30	33		
	27	27				19	21		
	24	25				26	24		
	25	29				23	33		
	19	21				27	25		
	27	25				15	21		
	30	27				18	26		
	26	28				26	31		
	21	19				23	33		
	25	27				27	21		
	27	26				33	32		
Pr. mirabilis	25	31	9	4		21	21		
	25	29			*S. marcesens*	23	15	11	4
	15	18				33	18		
	21	23				25	32		
	23	31				21	27		
	21	25				26	25		
	21	22				31	29		
	24	28				33	22		
	25	29				21	20		
S. aureus	21	23	22	8		32	25		
	24	26				21	23		
	10	23				15	20		
	17	20							

Table 5: Double Disks Synergy Test (DDST) for confirmation of ESBL-producers from salad vegetables in Ondo city, Nigeria

Isolates	Number of potential ESBLs producers	Number confirmed as ESBLs producers
Bacillus cereus	6	4
Citrobacter freundii	3	1
Escherichia coli	7	4
K. pneumoniae	6	2
Morganella morganii	2	2
P. aeruginosa	5	3
Proteus mirabilis	4	1
S. aureus	8	3
Serratia marcesens	4	1
S. saprophyticus	3	0
TOTAL	48	

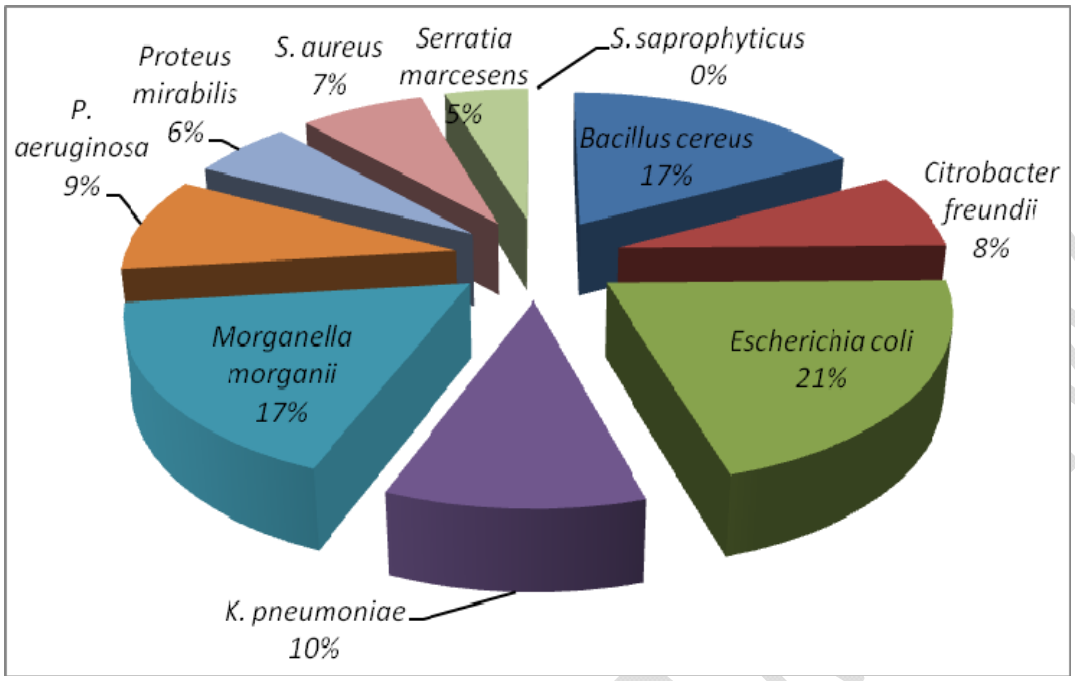

Figure 2: Percentage ESBL-producing species of bacteria associated with salad vegetables in Ondo city, Nigeria.

CHAPTER FIVE

5.0 Discussion and Conclusion

Concerns over the safety and quality of salad vegetables have risen, in spite of potential beneficial aspects. The occurrence of fresh produce-associated outbreaks highlighted our deficiencies in understanding the ecology of enteric pathogens outside human and animal host [21].

The high microbial loads recorded from cucumber samples in this study could be attributed contaminated soil environment from where the sample were obtained, poor storage method and lack of good hygiene practices of handlers. The high microbial loads in cabbages could be due to their surface structures which have folds that provide more surface area that harbors microorganisms. The low level of microbial loads in green pea and green beans could be as a result of the fact that green peas were protected in a pod and, thus, preventing them from direct contamination. Green beans could be as a result of their smooth surface and their protective manner of display, even at point of sale [22].

In this part of the country, domestic sewage, industrial and municipal waste water is used for irrigating vegetable crops. This could be responsible for the presence of human pathogenic bacterial load observed in salad vegetables, in addition to contamination through human and farm animal waste in agricultural lands, postharvest handling, transport, storage and poor hygiene conditions prevailing in market places [23].

Most of the bacteria isolated from this study are contaminants from soil, irrigation water, and the environment during transportation, washing rinsing water or handling. The high microbial contamination observed might be a reflection of storage conditions and how long these produce were kept before they were obtained for preparation of salads. The total viable bacterial counts obtained in this study were lower than those reported by Kaneko *et al*. [24]. Cantwell and Suslow [25] reported that high loads of microorganisms in ready-to-eat could be due to the presence of cut surfaces which allowed increased nutrient availability and, thus, favour the microbiota associated with the fresh product.

In this study, *S. aureus* had the highest percentage occurrence of 19.28%, followed by *P. aeruginosa* (13.86%), *K. pneumoniae* (12.65%), *M. marcesens* (10.24%), *B. cereus* (9.64%), *P. mirabilis* (9.64%), *M. morganii* (7.83%), *S. saprophyticus* (7.23%), *E. coli* (5.42%) while *C. freundii* had the lowest occurrence of 4.22%. These bacteria could adapt and persist in plant environment and increase the chance of transmitting to humans via consumption of plants or plant-derived products. Nithya and Babu [23] reported the existence of *Stenotrophomonas rhizophila*, *Arthrobacter mysorens*, *Xanthomonas axonopodis,* and *Aeromonas hydrophila* that were not encountered in this study. The findings of this study also differ from the results of Kemajou *et al*. [26] who reported *Escherichia coli* as the predominant (29.3%) and which was followed by *Staphylococcus aureus* (22.9%). *Enterobacter aerogenes* and *Salmonella* species were not also encountered in this study as reported by the authors.

Pseudomonas and *Bacillus* species are part of the natural flora and are among the most common vegetable spoilage bacteria, though some *Bacillus* sp are capable of causing food borne illness. *Enterobacter* sp*., E. coli, S. aureus* and *Klebsiella* sp. found in this sample is indicative of the

fact that some of the samples were actually contaminated with matters that originated from faeces, soil, sewage and poor quality water. The presence of *S. aureus* may also be due to poor hygiene practices as it is a normal flora of man and its carriage in nasal passage of food handlers or infected workers could aid easy distribution of the organism. Bello *et al*. [27] previously reported that improper handling and inadequate hygiene might lead to the contamination of food and, thus, serious health effects on consumers. Most strains of *Staphylococcus aureus* produce heat-stable enterotoxins and, thus, are antibiotic-resistant and pathogenic in nature [28].

The bacterial community normally could vary due to morphological and chemical differences among vegetable genera as some microorganisms attach preferentially to cut edges, and are able to internalize the leaf tissue [29]. Pathogens that have internalized are known to be more resistant against sanitation agents and washing by physical means [30].

It is worthy to mention that pathogens with a human reservoir and a low infectious dose can be readily transferred onto salad vegetables and fruit by infected food handlers. The source of these pathogens is most likely to be of animal origin, but prepared salads can also become cross-contaminated through poor handling or storage practices. Therefore the application of good basic food hygiene would greatly reduce the risk of transmission via infected food handlers or cross-contamination.

This highlights the significance of international surveillance systems, which can be vital mechanisms in recognizing and investigating epidemics. This becomes a matter of particular importance when the potential for disseminating multi-drug resistant strains of pathogens is taken into consideration. This requires international collaboration to address food safety issue. Microbiological food surveillance studies contribute to a greater understanding of the microbiological and food hygiene problems associated with food, and of how food safety may be improved. The results from studies such as this could be applied to monitor trends, assess risks in food safety and judge the effectiveness of regulation. Information from food studies can also form part of the science base for the development of food policy and informing microbiological risk assessments. Such studies, therefore, help to establish sound evidence on which advice could be based.

In addition, the occurrence of antibiotic-resistant microorganisms in salad vegetables may contribute to horizontal spreading of resistances between different isolates, species and genera. The occurrence of resistant genes on transferable elements facilitates distribution of resistance, and the widespread use of antibiotics allows direct selection or co-selection of resistances. Hospitals are prime areas of antibiotic resistance development [31]. The use of huge amounts of antibiotics in plant agriculture and commercial animal husbandry could lead to a selection of resistant bacteria; applying manure from animal farming to agricultural fields or the use of contaminated water for irrigation could also contribute to spread of resistant bacteria to plants. Bacteria serving as a reservoir for resistance determinants may have great influence on resistance gene transfer in natural habitats, such as vegetal surfaces or human colon. Therefore, the presence of antibiotic-resistant bacteria in fresh salad vegetables constitutes an additional concern for consumer safety [32].

This study showed that 17% of *B. cereus*, 8% *C. freundii*, 21% *E. coli*, 10% *K. pneumoniae*, 17% *M. morganii*, 9% *P. aeruginosa*, 6% *P. mirabilis*, 7% *S. aureus* and 5% *Serratia marcecens* isolates were ESBL-producing isolates in the salad vegetables. The prevalence of ESBLs-

producing bacteria in this study was similar to that recorded in other developing countries such as Khartoum Teaching Hospital, Sudan (45.1%) [33], and in Lebanon (15.4%) [34]. *K. pneumoniae* and *E. coli* are the most common species producing ESBLs in this study. This was supported by a similar observation previously reported by Ogefere *et al*. [35] and Yadav and Chauhan [36]. However, *B. cereus,* which was not previously reported, has been indicted in this study. Alarmingly, most of these bacterial isolates are common culprits of nosocomial infections [34,37]. Significantly, they are the main cause of UTI and septicaemia, in agreement with observation of Ogefere *et al*. [35] and Yadav and Chauhan [36].

This study revealed that salad vegetables could be a vehicle for the spread of extended-spectrum beta-lactamase-producing bacteria which translates to a threat to public health around the world as salads are loved and consumed by all categories of people globally. There is need to educate the vendors and consumers on good sanitary practices during processing, display and sale of vegetables and also dangers associated with misuse of antibiotics.

Conflict of Interest

Authors have no conflicts of interest to disclose.

Disclaimer: - This manuscript was presented in a Conference.

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