Evaluation of some freshly sold salad vegetables and fruits for bacterial contaminants of clinical implication

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ABSTRACT

Background: Salads, a mixture of uncooked vegetables and fruits that can be partially boiled or eaten raw, are a good source of phytonutrients and antioxidants capable of immunizing the body against infection. Salads can, however, be contaminated by bacteria of clinical potential during pre and post harvest handlings.

Objectives: This study evaluated some selected salad vegetables and fruits from Sagamu-Iperu neighborhood markets, for bacterial contaminants of clinical implication and their resistance to conventional antibiotics of therapeutic values.

Methods: Exactly 20 gm each, of three vegetables selected for salads; carrot, cabbage and cucumber were macerated and suspended in 20 mL of sterile distilled water for 1 hour with intermittent vortexing. Ten-fold serial dilution of each salad sample was prepared, and selected dilution was spread plated on different selective, differential and general-purpose agar media. Total viable count was estimated on standard plate counts agar medium and MacConkey agar medium separately. Antibiogram of the isolates of bacteria obtained from selected and differential culture media prepared were determined by Kirby Bauer method with a little modification.

Results: Of the 81 salad vegetables and fruits samples examined, five (5) different bacterial genera and species in varied number; *Escherichia coli* (62), *Staphylococcus aureus* (31), *Pseudomonas aeruginosa* (26), *Klebsiella spp* (59) and *Shigella spp* (18) were obtained. A total viable count of bacterial load 4.9×10^8 cfu/g for cabbage was recorded as the highest while 1.3×10^9 cfu/g for carrot was found to be the lowest. *Klebsiella spp*. elicited susceptibility of 50 % to gentamicin, 67 % to ciprofloxacin, and 40 % to tetracycline, while other isolates exhibited varying degrees of antibiotic resistance pattern.

Conclusion: The isolation of bacteria of clinical importance capable of causing food borne illness from the samples examined, the aerobic count of bacteria observed capable of initiating rapid deterioration, coupled with the alarming resistance of the isolates to selected spectrum of antibiotics exposed indicates serious public health and economic challenges from possible therapeutic failures of such conventional antibiotics .

Keywords: salad vegetables, bacterial contaminants, clinical implication

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Évaluation de certaines salades de légumes et de fruits vendues fraiches en vue de détecter des contaminants bactériens ayant une incidence clinique

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RÉSUMÉ

Contexte: Les salades, mélange de légumes et de fruits crus pouvant être partiellement bouillis ou consommés crus, sont une bonne source de phytonutriments et d'antioxydants capables d'immuniser l'organisme contre les infections. Cependant, elles peuvent être contaminées par des bactéries potentiellement dangereuses lors des manipulations avant et après la récolte.

Objectifs: Cette étude a évalué certains légumes et fruits de salade sélectionnés sur les marchés du quartier de Sagamu- Iperu, afin de détecter les contaminants bactériens ayant des implications cliniques et leur résistance aux antibiotiques conventionnels ayant une valeur thérapeutique.

Méthodes: Exactement 20 g de chacun des trois légumes sélectionnés pour les salades (carotte, chou et concombre) ont été macérés et mis en suspension dans 20 ml d'eau distillée stérile pendant une heure, en agitant le vortex de façon intermittente. Une dilution en série dix fois supérieure de chaque échantillon de salade a été préparée et la dilution sélectionnée a été étalée sur différents milieux gélosés sélectifs, différentiels et à usage général. Le nombre total de bactéries viables a été estimé séparément sur des plaques standard en milieu gélosé et en milieu gélosé MacConkey. L'antibiogramme des isolats bactériens obtenus à partir des milieux de culture sélectionnés et différentiels préparés a été déterminé par la méthode de Kirby Bauer, avec une légère modification.

Résultats: Sur les 81 échantillons de salades de légumes et de fruits examinés, cinq (5) genres et espèces bactériens différents en nombre varié ont été obtenues: *Escherichia coli* (62), *Staphylococcus aureus* (31), *Pseudomonas aeruginosa* (26), *Klebsiella spp* (59) et *Shigella spp* (18). Le nombre total de bactéries viables était de $4,9 \times 10^8$ cfu/g pour le chou a été enregistré comme le plus élevé tandis que $1,3 \times 10^9$ cfu/g pour la carotte s'est avéré être le plus faible. Klebsiella spp. a suscité une sensibilité de 50 % à la gentamicine, de 67 % à la ciprofloxacine et de 40 % à la tétracycline, tandis que d'autres isolats présentaient des degrés variables de résistance aux antibiotiques.

Conclusion: L'isolement de bactéries d'importance clinique capables de provoquer des maladies d'origine alimentaire à partir des échantillons examinés, le nombre de bactéries aérobies observées capables de déclencher une détérioration rapide, associé à la résistance alarmante des isolats à un spectre sélectionné d'antibiotiques exposés, indiquent de graves défis de santé publique et économiques liés à d'éventuels échecs thérapeutiques de ces antibiotiques conventionnels.

Mots clés: salades de légumes, contaminants bactériens, implications cliniques

INTRODUCTION

Salad, a provisional term commonly applied to a mixture of uncooked vegetables and fruits that can be eaten raw or partially boiled. It has the potential of promoting good health and fortifying immunity when regularly consumed. Salad can harbor a wide spectrum of enteric pathogens capable of causing human diseases. Salad vegetables include cabbage, carrot, broccoli, cucumber, green onions, lettuce, endive, escaroles, mushrooms, peas, bell peppers, radishes, spinach and tomatoes. Most leafy vegetables that can be eaten raw are classified as salad. The five basic types of salad are green salads, bound, vegetables, fruit and combination made up of the base, the body, the garnish and the dressings.[1]

Though salads are categorized as low calories, they are a good source of beta carotene, fiber and vitamins. Some groups of salad are classified as detoxifiers and blood purifiers while some have been found to stabilize blood pressure, urinary abnormalities and cardiovascular related conditions. Fruits like avocado, orange and grapefruits can be added to green salads, and when composed of fruit mixture and dressings, are eaten as desserts. Salads are sold in almost every neighborhood market and are readily available from street hawkers and farm produce outlets.

They can be contaminated by pathogenic bacteria associated with vegetation. Also, unhealthy handlers, nomadic cow dungs, sewage sludge, pre-and post-harvest handlings can be another source of salad contamination. The use of untreated water for irrigation, wastewater supplies, equipment and locally sourced manures are potential sources of salad contamination. The inherent contaminant microbial pathogens that salad could harbour include, *Salmonella, Staphylococcus aureus,* Shigella, *Klebsiella, Escherichia coli, Listeria spp,* Clostridia, *Pseudomonas aeruginosa,* and bacteria of faecal-oral origin.[2]

The pathogenic microbial contaminants, if consumed with salads, have the potential of causing infection of clinical implication in immunocompromised human entity when the salads are not properly decontaminated.[3]

In the local markets within the vicinity of this project execution, consumers prefer a well washed fruits and vegetable salads. It has been discovered that some retailers of these salads, mostly immigrated northerners, usually soak the salads to be sold in parozone (a brand of sodium hypochlorite disinfectant) and detergent overnight to keep it clean and appealing to the buyers, not having the knowledge that the disinfectant can percolate into the products overnight, and cause another health challenges of unimaginable magnitudes.[4]

This study evaluated some selected salads vegetables and fruits from neighborhood markets within the axis of Sagamu-Iperu located within the latitude 6.832201N0 and longitude 3.631913E0 geographical coordinates in Ogun State, for bacteria of clinical implication, enumerated the total viable counts and determined the antibiogram of some of the culprit isolates to some selected antibiotics of therapeutic potentials.

METHODS

Materials

Samples of carrot (vegetables), cucumber (fruits) and cabbage(vegetables), bacteriological media; MacConkey agar media, mannitol salt agar, Plate count agar media, eosin methylene blue, vortex mixer, glassware for bacteriological culture works.

Study area

The salad vegetable samples used in this very study were obtained from Iperu, Awolowo and Sabo markets in Sagamu located within the latitude 6.832201N0 and longitude 3.631913E0 in Ogun State Southwest Nigeria.

Collection of samples

A total of nine (9) samples per each of three varieties, each of salad vegetables and fruits; cabbage (vegetables), carrot (vegetables) and cucumber (fruits), were sourced from community retailers and were transfered in sterile waterproof nylon to the laboratory for immediate microbial analysis.

Bacteriology

Exactly 20 gm each, of 3 vegetable types selected for salads; carrot, cabbage and cucumber were macerated and suspended in 20 mL of sterile distilled water for 1 hour with intermittent vortexing. The samples were diluted serially in 10 folds by inoculating 1 mL of the samples on to 9 mL of sterile distilled water. And from the (10-1 dilution) stock, a repeated dilution of 10-2 to 10-10 were made and aliquots of 0.1 mL from selected dilution factor 10-3 were spread plated on melted and cooled standard plate count agar medium, and other differential and selective agar medium available, the preparation was incubated at 37^{o}C for 24 - 48 hours and

thereafter biochemical characterization, which include catalase test and coagulase test for S*taphylococcus aureus,* indole test and methyl red test for *Escherichia coli,* oxidase test and citrate test for *Pseudomonas aeruginosa,* citrate test and Voges-Proskauer test for *Klebsiella spp*, urea agar implant, triple sugar iron for *Shigella spp* and other relevant biochemical identification tests on the isolates obtained were carried out.

Total viable count

Total viable bacterial count was carried out on the food samples to determine the microbial load of the samples as described by American Public Health association. Exactly 20 gm of each salad sample were sliced and rinsed in 200 mL of sterile distilled water. The samples were diluted serially in 10 folds by inoculating 1 ml of the sample on to 9 ml of sterile distilled water and from the (10-1 dilution) and this stock, a repeated dilution of 10-2 to 10-10 dilutions of the resultant homogenates were made and aliquots of 0.1 mL from selected dilution factor 10-3 were spread plated on standard plate count agar for the enumeration of aerobic viable bacteria. The plates were thereafter incubated at 37^{o}C for 24-48 hours. The colonies on standard plate count agar were counted and expressed as colony forming units per gram (cfu/g) of samples. All counts were done in duplicate using the Quebec colony counter.

Antimicrobial susceptibility test

The antimicrobial susceptibility pattern of the isolates obtained from the samples was determined on Mueller Hinton agar using the method of Kirby Bauer (1968) with a little modification. A volume of 20 ml of Mueller Hinton agar was prepared in universal bottles and allowed to cool to about 45 ^{o}C, 0.1 ml of 10-2 dilution of an overnight broth culture of each test bacterium was aseptically transferred into each bottle of agar and gently rolled between the palm and poured into the petri-dishes. The plates were then allowed to set. With strict aseptic precautions, the antibiotic discs were placed on the surface of seeded agar plates such that the centers were 25 mm apart as designed by the manufacturers and not less than 10 mm from the petri dish edge. The plates were allowed for 15 minutes after the application of the antibiotic disc and incubated at 37^{o}C for 18-24 hrs. The plates were examined for zones of growth inhibition, measured in millimeter (mm) and interpreted as recommended by Clinical and Laboratory Standards Institute, (CLSI, 2020). The spectrum of selected antibiotics tested includes: Gentamicin (GEN -10 µg), Ciprofloxacin (CIP -5 µg), Ampicillin (AMP-10 µg), Meropenem (MEM-10 µg), Erythromycin (ERY-5 µg), Tetracycline (TET-30 µg), Cotrimoxazole (COT-25 µg), Cefuroxime (CRX-10 µg), Cephalexin (CP-10 µg), Augmentin (AUG-30 µg), Ceftazidime (CPZ-10 µg) Vancomycin (VAN-30 µg) and others

RESULTS

A total of 81 salad vegetable samples (9 samples per each type for 3 trips, for 3 different salad vegetable samples) were propagated on different selective and differential media respectively. The culture yield per gram from each sample varied from market to market of the total sum of the isolates of bacteria obtained as shown in Table 1 below.

Table 1: The culture yields (n) of bacteria from the salad samples studied

Sample site	Sample studied	Isolates obtained and average of thee total culture yield									
		E. coli	yield	*S. aureus*	yield	*P. aeruginosa*	yield	*Klebsiella Sp.*	yield	*Shigella Sp.*	yield
Iperu Market	C R1	+	8	+	2	+	5	+	7	+	5
Iperu Market	CR2	+	5	+	6	+	3	+	6	+	1
Iperu Market	CB3	+	10	+	5	+	2	+	7	+	2
Awolowo Market	CC4	+	9	+	1	+	0	+	7	+	3
Awolowo Market	CR5	+	10	+	2	+	4	+	8	+	0
Awolowo Market	CB6	+	7	+	0	+	6	+	7	+	4
Sabo Market	CC7	+	8	+	6	+	4	+	7	+	2
Sabo Market	CR8	+	8	+	4	+	0	+	5	+	0
Sabo Market	CB9	+	7	+	5	+	2	+	5	+	1
Total yield			62		31		26		59		18

Key: CC: Cucumber CB: Cabbage CR: Carrot Yield (n) : number of isolates of bacteria from the samples culture

The total viable counts from the salads samples studied elicited a wide variation in numeration ranging from 4.9×108 recorded for cabbage as the highest and 1.3 ×109 for carrot

Table 2: Total viable counts from the salads samples evaluated.

Sample code	Date of sampling	Sampling site	Salad types	Total viable counts cfu/g (PCA)	Total viable counts cfu/g (MCA)
CC1	10/06/2024	Iperu market	Cucumber	$3.4 \times 10^7 \pm 2.3 \times 10^8$	---
CR2	10/06/2024	Iperu market	Carrot	$2.2 \times 10^7 \pm 1.12 \times 10^8$	---
CB3	10/06/2024	Iperu market	Cabbage	$2.9 \times 10^7 \pm 2.3 \times 10^8$	$1.1 \times 10^7 \pm 2.2 \times 10^8$
CC4	5/06/2024	Awolowo market	Cucumber	$1.4 \times 10^8 \pm 1.9 \times 10^9$	$4.1 \times 10^6 \pm 1.3 \times 10^6$
CR5	5/06/2024	Awolowo market	Carrot	$1.6 \times 10^8 \pm 5.9 \times 10^9$	$3.1 \times 10^6 \pm 0$
CB6	5/06/2024	Awolowo market	Cabbage	$1.7 \times 10^8 \pm 3.5 \times 10^9$	$3.1 \times 10^6 \pm 0$
CC7	2/06/2024	Sabo market	Cucumber	$2.3 \times 10^8 \pm 6.2 \times 10^8$	---
CR8	2/05/2024	Sabo market	Carrot	$1.3 \times 10^9 \pm 2.9 \times 10^{10}$	$1.1 \times 10^7 \pm 0$
CB9	2/06/2024	Sabo market	Cabbage	$4.9 \times 10^8 \pm 1.5 \times 10^9$	$1.1 \times 10^7 \pm 0$

Key: CC: Cucumber CR: Carrot CB: Cabbage PCA: Plate Count Agar MCA: MacConkey Agar Count, ±: SD

Antibiogram study of selected representative isolates elicited varied patterns of antibiotics resistance as shown in Figure1 to Figure 5 below

Antibiogram of *Staphylococcus aureus* from representative cucumber (CB3) samples

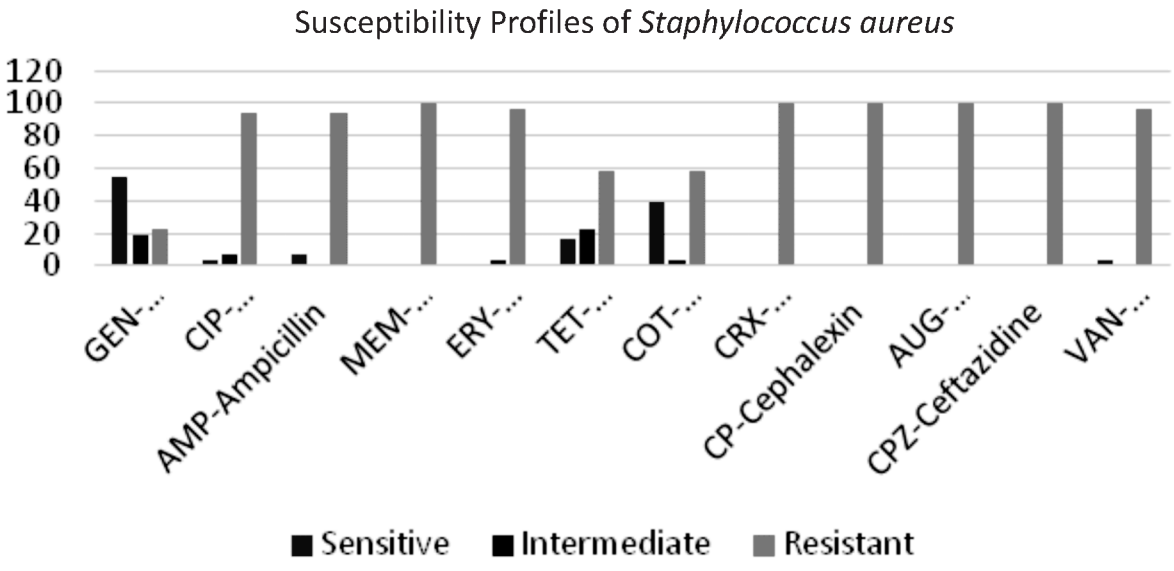

Figure 1: Percentage susceptibility profiles of *Staphylococcus aureus* to selected antibiotics

Gentamicin (GEN -10 µg), Ciprofloxacin (CIP -5µg), Ampicillin (AMP-10 µg), Meropenem (MEM-10 µg), Erythromycin (ERY-5 µg), Tetracycline (TET-30 µg), Cotrimoxazole (COT-25µg), Cefuroxime (CRX-10 µg), Cephalexin (CP-10 µg), Augmentin (AUG-30 µg), Ceftazidime (CPZ-10 µg) Vancomycin (VAN-30 µg).

Antibiogram of *Escherichia coli from* representative carrot (CR5) samples

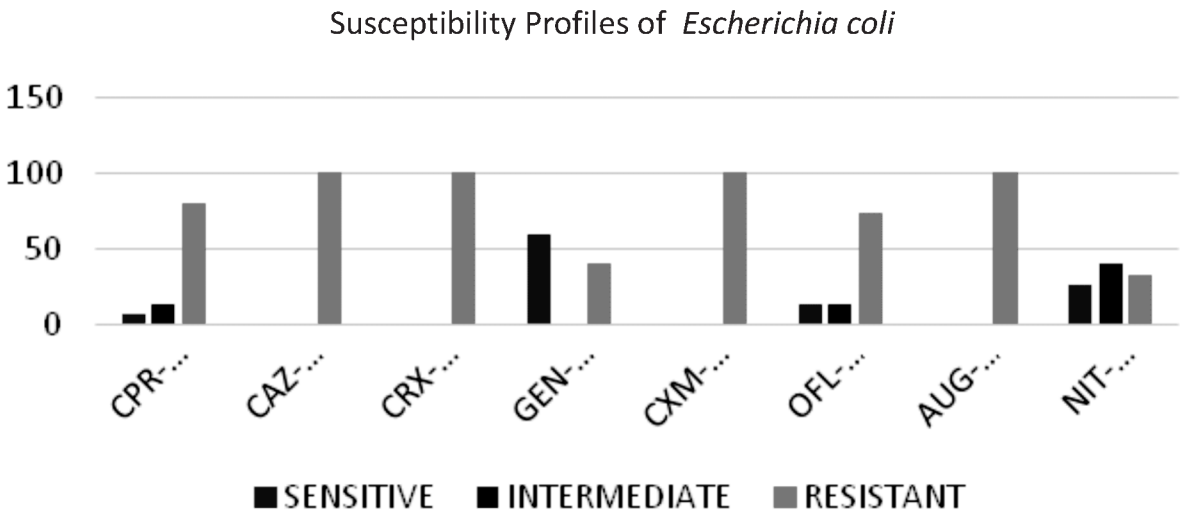

Figure 2: Percentage susceptibility profiles of *Escherichia coli* to selected antibiotics.

Ciprofloxacin-(CPR-5µg), Ceftriaxone (CAZ-30 µg), Cefuroxime (CRX-30 µg), Gentamycin (GEN-10 µg), Ceftaxidime (CXM-30 µg), Ofloxacin (OFL-5 µg), Augmentin (AUG-30 µg), Nitrofurantoin (NIT-)

Antibiogram of *Pseudomonas aeruginosa* from representative cabbage (CB6) samples

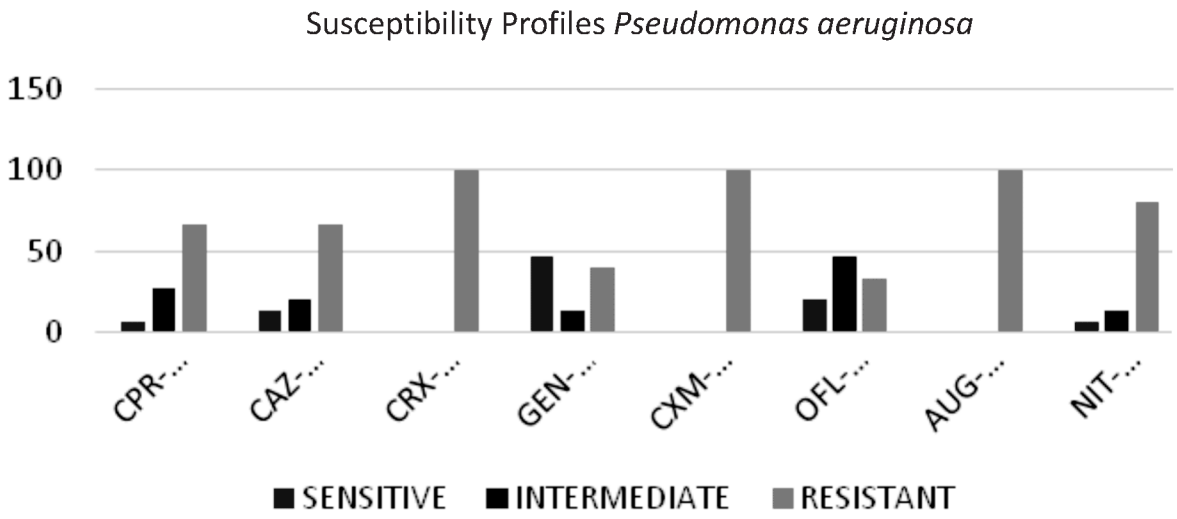

Figure 3: Percentage susceptibility profiles of *Pseudomonas aeruginosa* to selected antibiotics.

Ciprofloxacin-(CPR-5µg), Ceftriaxone (CAZ-30 µg), Cefuroxime (CRX-30 µg), Gentamicin (GEN-10 µg), Ceftaxidime (CXM-30 µg), Ofloxacin (OFL-5 µg), Augmentin (AUG-30 µg), Nitrofurantoin (NIT-)

Antibiogram of *Klebsiella spp.* from representative cucumber(CB9) samples

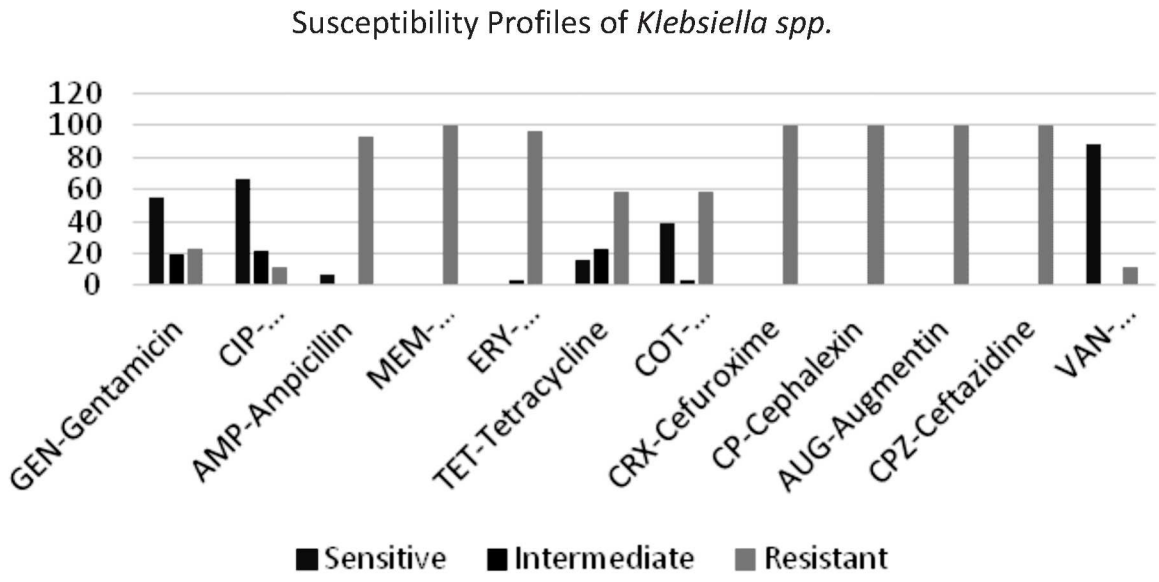

Figure 4: Percentage susceptibility profiles of *Klebsiella spp.* to selected antibiotics.

Gentamicin (GEN -10 µg), Ciprofloxacin (CIP -5µg), Ampicilin (AMP-10 µg), Meropenem (MEM-10 µg), Erythromycin (ERY-5 µg), Tetracycline (TET-30 µg), Cotrimoxazole (COT-25 µg), Cefuroxime (CRX-10 µg), Cephalaxin (CP-10 µg), Augmentin (AUG-30 µg), Ceftazidime (CPZ-10 µg) Vancomycin (VAN-30 µg).

Antibiogram of *Shigella spp* from representative cabbage (CB6) samples

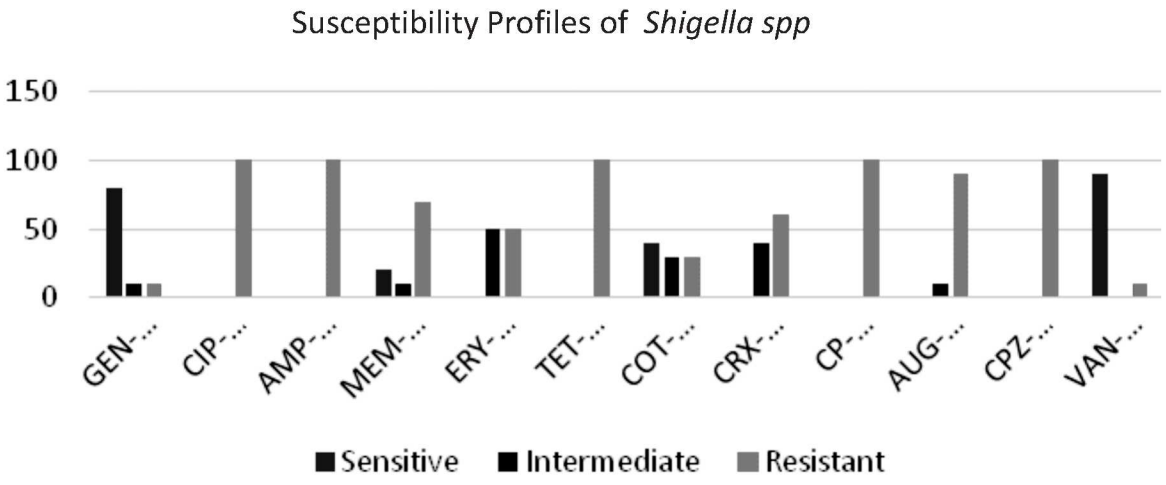

Figure 5: Percentage susceptibility profiles of *Shigella spp.* to selected antibiotics.

Gentamicin (GEN -10 µg), Ciprofloxacin (CIP -5µg), Ampicillin (AMP-10 µg), Meropenem (MEM-10 µg), Erythromycin (ERY-5 µg), Tetracycline (TET-30 µg), Cotrimoxazole (COT-25 µg), Cefuroxime (CRX-10 µg), Cephalexin (CP-10 µg), Augmentin (AUG-30 µg), Ceftazidime (CPZ-10 µg), Vancomycin (VAN-30 µg).

Samples of the salad vegetables evaluated

Figure 6: Carrot, Cucumber and Cabbage

DISCUSSION

Salad fruits or vegetables, depending on the type can be eaten raw or cooked. Salads are a good source of vitamins and minerals but can also constitute hazards to the health of the consumers when laden with microbial pathogens. Each of the salad samples; cucumber, carrot and cabbage investigated carried mostly enteric bacterial pathogens, and other related microbes.[5] The culture yield of *Escherichia coli* (62) an enteric pathogen was found to be the highest. This could be due to the abundant availability of *Escerichia coli* in the soil due to defecation of warm blooded animals and other environmental sanitary violations. *Klebsiella spp* also had a considerable yield (59). *Klebsiella spp* are easily abundant and survive in vegetation. *Staphylococcus aureus* had (31 culture yield), while *Pseudomonas aeruginosa* and *Shigella spp* had (26) and (18) culture yields respectively. Their yields were found to be comparatively less to the *Escherichia coli* and *Klebsiella spp* in this study. This could be attributed to the health status of the handlers, the pre - and post - harvest contaminants and human contacts.[6] The differences in carriage rates of salad samples studied could also be traced to the soil microbial pathogens, poor preservation and storage methods which corroborate the study of Denis *et.al.*, (2016) on prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada.[7]

The total viable counts from the salad samples studied elicited a wide variation in numeration, ranging from 4.9× 108 recorded for cabbage as the highest and 1.3 × 109 for carrots .The variation in the total viable counts obtained in this study could be attributed to the quality of the samples, inherent microbial loads, environmental contamination within the markets, poor storage, and unsterile market displaying receptacles. This corroborates the study of Feng (2016) on enumeration of *Esherichia coli* and the coliform bacteria.[8]

Different spectrum of antibiotics was tested on the isolates obtained in this study and different susceptibility patterns for each isolate were exhibited. *Staphylococcus aureus* elicited the highest resistance to the antibiotics exposed as shown in Figure 1. The isolates were found to be 100 % resistant to meropenem, cefuroxime, augmentin, ceftazidime and 90 % resistant to vancomycin, erythromycin, ampicillin and ciprofloxacin but were susceptible to gentamicin (50 %), cotrimoxazole (30 %) and tetracycline (10 %). The percentage of resistance exhibited in this study could be chromosomal, plasmid mediated or any other inherent genetic factors. This agrees with the study of Adekanle *et.al.*, (2015) on a study of microbial analysis of fresh fruit and vegetables in Sagamu markets South-West, Nigeria.[9]

Escherichia coli was 100 % resistant to ceftazidine, cefuroxime, cefixime and augmentin while it was observed to be 60 % and 30 % resistant to gentamicin and nitrofurantoin respectively as shown in Figure 2. The presence of *Escherichia coli* could be due to microbial sludge or enteric bacteria associated with animal

manure. This corroborates the study of Benjamin *et.al*., (2013) on the occurrence of generic *Escherichia coli, E. coli* O157 and *Salmonella spp.* in water and sediment from leafy green produce farms and streams on the central California coast.[10]

Pseudomonas aeruginosa was also 100 % resistant to cefuroxime, cefixime and augmentin. While variation in resistance to ciprofloxacin, ceftazidime and nitrofurantoin were observed, 46.6 % and 20 % susceptibility were recorded for gentamicin and ofloxacin as shown in Figure 3. The presence of *Pseudomonas aeruginosa* could be attributed to the sewage dripping, irrigation and water sources for post harvest cleansing. The resistant patterns observed in this study were in contrast with the study of Farida Anjum *et al*., (2010) on susceptibility pattern of *Pseudomonas aeruginosa* against various antibiotics.[11]

Klebsiella spp. elicited susceptibility profiles of 50 % to gentamicin, 67 % to ciprofloxacin, 40 % to tetracycline, and 88.8 % to vancomycin as shown in Figure 4, while 100 % resistance were recorded for ceftazidime, augmentin, cefuroxime and cephalexin. The presence of *Klebsiella spp.* could be attributed to ecological climate and vegetation around the salad source. This study supports the findings of Vincent (2022) on prevalence of parasites of medical importance on fruits and vegetables sold in markets of Makurdi and Otukpo, Benue State Nigeria.[12]

Shigella spp. was 90 %, 40 % and 80 % susceptible to vancomycin, cotrimoxazole and gentamicin respectively and were found to be 100 % resistant to ciprofloxacin, ampicillin, tetracycline , Cephalexin, Ceftazidime and 90% resistant to augmentin as shown in Figure 5. *Shigella spp* presence could be attributed to the pre harvest and post harvest handling and preservation of the salad source. This observation corroborates with the findings of Abdulahi and Daphey *et.al*., (2023) on parasites associated with open markets vegetables: implication for public health interventions in Ethiope East, southern Nigeria.[13]

CONCLUSION

Eat your food as a medicine so as not to eat your medicine as a food says ancient British proverb, but when the food to be eaten as a medicine now becomes a 'metabolic poison', conscious action must be taken. The preponderance of resistant isolates of clinical implication obtained from the salad samples to selected spectrum of antibiotics studied is alarming. These organisms are potential pathogens with the ability to transfer inherent resistant factors horizontally amongst their family into an immune-compromised entity. It is therefore recommended that food hygiene professionals and specialists in food safety that understand the microbial ecology of foods and epidemiology of food borne diseases should be deplored to the markets where all these farm produce are been sold to enforce food safety rules, sensitize and create awareness to the consumers. Regular washing of salad fruits and vegetables is herewith advocated to reduce or prevent microbes being consumed with the desired salads, vegetables or fruits.

Declaration of conflicting interests:

The authors declare no conflicts of interest concerning the publication of this article.

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The authors declare that there are no conflicts of interest.

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