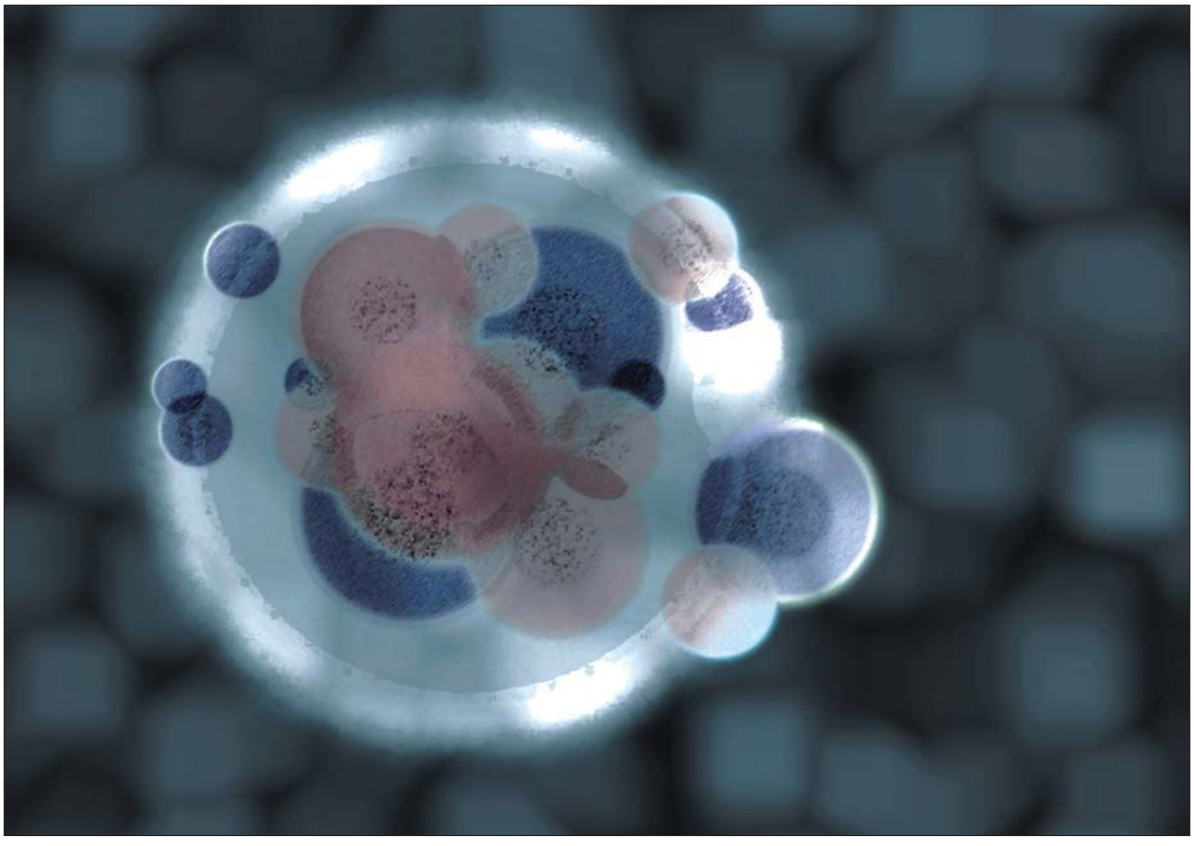

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Research Article
Antibiogram of Bacteria Species Isolated from Vegetables in Ado-Odo Ota, Nigeria

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Abstract

Background and Objective: There has been an unusual increase in the emergence of antimicrobial resistance to most of the commonly used antibiotics particularly in the developing countries. **Materials and Methods:** In this study, five bacteria species tentatively named NC1-5 were isolated from these vegetables: *Corchorus olitorius* (L.), *Celosia caracas*, *Vernonia amydalina* and *Abelmoschus esculentus* and the irrigation water in Ado-Odo Ota, Nigeria. The isolates were characterized culturally, morphologically and biochemically. The organisms were compared with standard reference organisms and were proposed to be species of *Escherichia coli*, *Chromobacterium violaceum*, *Micrococcus luteus*, *Shigella* and *Salmonella*. These bacteria species were tested for their susceptibility to standard Gram positive and Gram negative antibiotics by agar disk diffusion method. The tested antibiotics at different concentrations include: cotrimazole, cloxacillin, erythromycin, gentamycin, augmentin, streptomycin, tetracycline and chloramphenicol. **Results:** *Chromobacterium violaceum* exhibited 100% susceptibility to all antibiotics tested, while other bacteria species showed different susceptibility patterns ranging from susceptible, intermediate resistant and resistant patterns. **Conclusion:** From this study, vegetables can be an avenue for transferring antibiotic resistant genes in community settings. Hence it is necessary to monitor and understand the critical points in the control of antibiotic resistance among the developing countries.

Key words: Antiobiotics, vegetables, antibiotics resistance, bacteria species, irrigation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Vegetables include the leafy, fruit, seed or root vegetables that are fit for human consumption. These parts are consumed whole or in part, raw or cooked as a supplement to other food crops. Nowadays, there has been an increase in fresh vegetables consumption due to health benefits in eating natural, healthy and functional foods. Consequently, consumers are seeking for vegetables products that maintain its natural nutrition and other aesthetic qualities such as flavor, texture and color properties[1,2]. In the developing countries, vegetables are comparatively cheap and easy to grow. Thus, most rural women rely on these vegetable as source of income. Despite the numerous benefits of vegetables, it could be a medium for the spread of bacterial, parasitic, viral pathogens and antimicrobial resistance mechanisms[3]. As a consequence, the production and distribution of fresh vegetables could be a huge challenge to the food industry.

Water is one of the principal agents for the spread of various enteric diseases[4,5]. Some researchers[1,6,7] have stated that water from contaminated sources can propagate harmful microorganisms that can affect the safety of such vegetables. Since most of the populations that grow vegetables are from rural areas, majority of them cannot afford expensive technologies, such as use of chlorinated water to rinse their vegetables. Accordingly, they adopt locally-available and cheap technologies such as rinsing with ordinary water from rivers and shallow wells without recourse about the safety of the vegetables.

Food borne outbreaks associated with vegetables that are partially processed or consumed naturally have increased over the years[8-10]. This rise in the food-borne outbreaks from vegetables is due to the favorable conditions that the vegetables provide consequently encouraging the growth and survival of many types of microorganisms. Some of the favorable conditions include nutrient rich internal tissues-comprising polysaccharides (starch), pectin, hemicelluloses and cellulose. Enteropathogens such as *Escherichia coli* and *Salmonella* are among the greatest concerns with food-borne outbreaks. In 2007, these organisms were implicated in food-borne outbreaks in UK that resulted in the recalling of bagged lettuce[11]. Such recalls damage the consumer's confidence and hampers economically the income and corporate image of such food processing industry involved with the sale and distribution of such products. The enteropathogens *Escherichia coli* and *Salmonella* have been implicated in cases of diarrhea and typhoid fever following consumption of contaminated vegetables[12]. In most developing countries, street vending of fresh vegetables are on the increase and as such precautionary measures on the safety of the vegetables are not considered. Consequently, such vegetables could be a repository for various organisms that can severely affect the welfare of the consumers, shelf-life and nutritional worth of the vegetables.

In this study, selected vegetables popularly grown and consumed in the Ado-Odo Ota, Nigeria were assessed for epiphytic bacteria species associated with it and their antibiotics susceptibility patterns. The selected vegetables include: *Corchorus olitorius* (L.), *Vernonia amydalina* Delile, *Abelmoschus esculentus* and *Celosia caracas*. The selection of these vegetables were based on the huge culinary and medicinal benefits they offer the population when consumed raw or lightly cooked. For instance *Corchorus olitorius* when boiled or mashed as fresh leaves serves as rich sources of vitamins A, C and methionine[13,14]. Also, *Vernonia amydalina*. Delile is known for its antimalaria, antihelmenthic, antitumorigenic properties as well as antioxidant properties[15].

Recently, efforts have been devoted to prolonging the shelf life of vegetables, however, the determination of the antibiotic susceptibility patterns of the bacteria species associated with these vegetables have been scarcely reported. Therefore, the present study attempted to evaluate the presence of bacteria species associated with the selected local vegetables and their antibiogram to standard antibiotics.

MATERIALS AND METHODS

Study area: The Iju community in Ota, Ogun state, Nigeria is located at geographical coordinates of latitude 6.68333 and longitude 3.13333. The community has a major river called the Iju river, which is sourced by the community for various purposes. The river has tributaries, where other water bodies flow into it.

Sampling and sample preparation: In order to track the pathway between the source and the receptor (consumers), vegetables produced around the Iju river were selected. This is because the Iju river is used for the irrigation of most vegetable farms around Iju community. The vegetables sample selected included: *Abelmoschus esculentus*, *Vernonia amydalina*, *Celosia caracas* and *Corchorus olitorius* (L.). A total of 50 samples were sourced from various regions of the farms during this study. These include: *Celosia caracas* (12), *Abelmoschus esculentus* (10), *Corchorus olitorius* (L.) (18) and *Vernonia amydalina* (10). The vegetables were identified

by the farmers and further authenticated by Dr. Conrad Omohinmin, a certified botanist in the Department of Biological Sciences, Covenant University. All the vegetables samples were kept in sterile ziploc bags and stored at 4°C. In addition, water samples of the Iju river were collected. During the collection of the Iju river, the water lines were considered and collection done as described by Chinedu *et al.*[16]. For this, 2 L sterile plastic containers were used for the collection of irrigation water samples. The sterile containers were rinsed with distilled water prior to usage. The collection was done aseptically by careful immersion of the sample containers in the water body. The containers were then sealed with tight fitted corks avoiding air bubbles. In all cases, the collected samples were transported to the laboratory as soon as possible for further microbiological analyses as described by Cappuccino and Sherman[17,18].

Bacteriological analysis: Fifty grams of the vegetables samples were weighed and rinsed in 100 mL of sterile buffered peptone water under sterile conditions[19]. The wash water of the sterile buffered peptone water and the Iju river samples were diluted in sterile distilled water up to 10^{-5} and 0.1 mL aliquot from the corresponding samples inoculated using the spread plate technique on the different media plates: Nutrient agar, *Salmonella-Shigella* agar, plate count agar and MacConkey agar. All the media were prepared based on the manufacturer's instructions. The inoculated plates (triplicates) were incubated at 37°C for 24 h for nutrient agar, *Salmonella-Shigella* agar and plate count agar. The MacConkey agar was used for determination of total coliforms and was incubated at 30 and 44°C[17-19].

Purification and characterization of bacterial species: Isolates from the various media were plated out. In this, colonies were periodically transferred to the different media to obtain the pure cultures. These were tentatively named as NC1, NC2, NC3, NC4 and NC5. The isolates were classified by using the standard cultural, biochemical and morphological techniques and later compared with standard reference organisms[20,21]. The physiological tests carried out on the isolates include: Catalase, oxidase, colony motility, methyl red, voges proskauer, indole, nitrate reduction, gelatin hydrolysis, spore test, starch hydrolysis, coagulase, citrate and sugar utilization and Gram stain morphology.

Antibiotics susceptibility test: According to standard microbiological methods, the antibiotic susceptibility was determined by disk diffusion method[22,23]. In this, the bacterial suspensions (0.5 turbidity, McFarland standard, BioMerieux, Madrid, Spain) were seeded on Mueller-Hinton agar and the antibiotic-containing disks placed on the surface. For the Gram positive antibiotics (Abtex Biologicals Ltd., Liverpool, UK) assessed: Erythromycin 5 µg, cloxacillin 5 µg, cotrimoxazole 25 µg, augmentin 30 µg, tetracycline 30 µg, gentamicin 10 µg, chloramphenicol 10 µg and streptomycin 10 µg. The Gram negative antibiotics examined include: Ofloxacin 5 µg, augmentin 30 µg, gentamycin 10 µg, nalidixic acid 30 µg, tetracycline 25 µg, amoxicillin 25 µg, cotrimoxazole 25 µg and nirofurantoin 20 µg. All the plates were incubated at 37°C for 24 h. The antibiotic susceptibility assays were conducted at regular intervals of 24 h until there was a noticeable decline in the potency of the antibiotics to inhibit the growth of the test organisms. Zones of clearance round each test antibiotics were noted and the diameters of the zones were calculated. The zones of the inhibition were used to determine the clinical behavior of the isolates in terms of resistance, intermediate resistance and susceptible to the antibiotics[24].

RESULTS

The studied vegetables (*Corchorus olitorus*, *Abelmoschous esculentus*, *Celosia caracas*, *Veronina amygdalina*) and the Iju river samples were found to contain

Table 1: Notable bacteria species occurring in the samples

Probable organisms	*Celosia caracas*	*Vernonia amydalina*	*Corchorus olitorius*	*Abelmoschus esculentus*	Iju water
NC1					
Salmonella sp.	+	+	+	-	+
NC2					
Escherichia coli	-	-	+	-	-
NC3					
Chromobacterium violaceum	+	-	-	-	+
NC4					
Shigella	+	+	+	-	+
NC5					
Micrococcus luteus	+	+	-	+	_

+: Present and -: Absent

Table 2: Physiological characteristics of the isolated organisms

Probable organisms	*Samonella* spp. (NC1)	*Escherichia coli* (NC2)	*Chromobacterium violaceum* (NC3)	*Shigella* (NC4)	*Micrococcus luteus* (NC5)
Grams reaction	-	-	-	-	- (Gram variable)
	Cocci in chains	Cocci occur in chains		Short rods	Short rod
Colony morphology	Circular raised smooth butyrous	Yellow circular raised and viscous	Purple circular flat and butyrous	Cirular tiny raised butyrous	Yellow, circular, tiny, entire, smooth, raised and butyrous
Citrate test	+	-	-	-	+
Urease test	-	+	-	-	-
Oxidase test	-	-	+	+	
Indole test	-	+	+	-	
Starch utilization	-	-	+	+	+
Coagulase test	+	-	-	+	-
Catalase test	+	-	-	-	+
Methy red	-	+	+	+	-
Voges Proskauer	-	-	+	-	+
Motility	+	+	+	-	+
Glucose	+	+	-	+	+
Lactose	+	+	+	+	+

Salmonella spp., while *Escherichia coli* was obtained from the sterile buffered peptone water of *Corchorus olitorus* (Table 1). *Chromobacterium violaceum* occurred in the rinsed buffered peptone water for *Celosia caracas* and Iju water. *Shigella* species were found in the entire vegetable samples studied except for *Abelmoschous esculentus*. *Micrococcus luteus* was present on *Abelmoschous esculentus*, *Celosia caracas* and *Veronina amygdalina* rinsed buffered peptone water. Out of the five samples, *Salmonella* and *Shigella* species were detected in four of the samples while *Escherichia coli* occurred in only one sample. From the Table 2, the isolates NC1, 2, 3, 5 were all Gram negative organisms motile except for NC4. The NC1 and 5 were catalase positive. All the organisms were capable of fermenting lactose but NC3 showed inability to utilize glucose. Some notable medically important enteropathogen species that occurred in the samples were *Salmonella*, *E. coli* and *Shigella*.

Antibiotics susceptibility testing: In this assay, the recommendation of Clinical and Laboratory Standards Institute[25] was adopted. For this, the category Susceptible (S) implied that isolates are inhibited by the standard achievable concentrations of antimicrobial agent when applied at the right dosage, while the Intermediate (I) category implied that there was clinical efficacy at the sites, where the drugs are physiologically concentrated, which for instance could be antibiotics of classes of quinolones and β-lactams being present in the urine or where a higher than normal dosage of a drug is used (e.g., β-lactams). For the group designated Resistant (R) it implied, that the isolates are not inhibited by the normal concentrations of the agent when applied at normal dosage or where the zone of inhibition measured lie within the range, where specific microbial resistance mechanisms occurs.

DISCUSSION

Microorganisms often associated with vegetables are very large and diverse communities that maintain ecological balance in most agricultural systems[2]. Verraes *et al.*[26] stated that pathogenic bacteria species, which include but not limited to species of *Salmonella*, *Shigella*, *Escherichia coli*, *Escherichia coli* O157:H7, *Clostridium botulinum*, *Campylobacter*, *Listeria monocytogenes* and *Bacillus cereus* can contaminate vegetables. Remarkably; in this study, most of the samples examined showed the presence of fecal coliform such as *Salmonella*, *Shgiella* and *Escherichia coli* (*E. coli*). The contamination by these pathogenic microorganisms reveals the possibility of food borne diseases and illnesses when one consumes the raw or partially prepared vegetables[5,27]. Sources such as irrigation water, improper drainage and lack of sanitation workers hygiene have been identified as factors that could lead to vegetables contamination[19,27,28]. Consequently there is the possibility that some of these factors could provide possible link for the contamination of the surveyed vegetables[5]. As true with many developing countries, farmers locate their farms close to rivers and other water bodies for easy access to water for their crops and as such create potential risks of microbial pathogens contamination on the vegetables grown. Since there exists, an intricate involvement between sources of water and the overall vegetables quality, there is a possibility that high pathogenic organisms obtained may have come from the Iju river used for irrigation of the farms. Aside from irrigation purposes, it was discovered that the Iju community makes use of the Iju river for other purposes. Furthermore, during the direct interviews with the farmers they confirmed their rarity of use of fertilizers as they believed the water used for irrigation was rich enough for plant growth, therefore, a large portion of evidence as to the traceability of the source of contamination on these vegetables can be made to the Iju river. In addition, similar organisms isolated from the vegetables were also isolated from the water samples for instance *E. coli*, a Gram negative bacillus that is an opportunistic pathogen in the gastrointestinal tract was isolated. Some these pathogenic organisms could form biofilm matrix that enables to cope in difficult environment. For instance, Islam *et al.*[29] reported of the survivability and contamination of the vegetables (carrot and radish by *Salmonella typhimurium* inoculated in irrigation water. The *Salmonella* survived for 203 days post application. Solomon and Matthews[30] also reported of *E. coli* 0157:H7 occurring at harvest (30 days post-inoculation) of lettuce irrigated with *E. coli* 0157:H7 contaminated water. They found that lettuce farm irrigated with contaminated water between 7 and 14 days showed increase in their microbial populations. In this study authors observed the occurrence of different bacteria species on the vegetables. Hamilton *et al.*[31] using quantitative risk assessment models of reclaimed water for irrigation on the same farm, established risks that could vary between crops in the same farmland. The researcher and co-workers explained the possibility of different occurrence of bacteria species on vegetables even within the same farmland. This agrees to our findings in this study. Besides the developing world, the developed world still suffers from cases of food-borne illnesses from vegetables. This include but not limited to the iceberg lettuce imported from Spain in 2005 that caused outbreak of *S. typhimurium* in United

Kingdom and Finland and the *E. coli* 0157:H7 outbreak in Sweden in 2005[32]. Feroz *et al*.[33] stated that the occurrences of *E. coli* and *Shigella* species on vegetables not only poses as threat to the consumers but can also reduce the shelf life of such vegetables. From the results, majority of the organisms isolated were from the Gram negative group (*E. coli*, *Shigella*, *Salmonella* and *Chromobacterium violaceum*). Brocklehurst *et al*.[34], Carlin *et al*.[35] and Garg *et al*.[36] stated that 80-90% of mesophilic bacteria in the aerobic plate counts of vegetables were Gram negative rods. The high presence of these Gram negative groups could be due to general stress response, where the produce stress proteins that confer cross-resistance to the abiotic factors[37]. The cross-protection mechanisms enhance the survivability of this group of bacteria. Some of the isolated Gram negative species have been implicated in diseases such as multiple liver and lung abscesses and terminal septicemia, osteomyelitis, chronic granulomatosis, cellulitis and perorbital and ocular infections[38-41].

Immediacy is known to increase the likelihood of spreading resistant bacteria between these host species. Thus the occurrence of the different bacteria species on these vegetables and the direct link to human may cause the bacteria to transfer genetic cassettes among each other and to man consequently conferring resistance to several classes of drugs observed in this study. The antimicrobial resistance behaviors among isolates (Table 3 and Fig. 1 and 2) presents an indirect challenge to the public health, where they could serve as gene pool for horizontal gene spread of antibiotic resistance genes[26,42]. From Table 3, *C. violaceum* showed 100% susceptible to all antibiotics tested. *Shigella* spp. was susceptible to ofloxacin and amoxicillin, intermediate resistance with nalidixic acid and resistant to tetracycline, nitrofurantoin, gentamycin and augmentin. The *E. coli* was resistant to all test antibiotics except amoxicillin. *Salmonella* showed intermediate reaction to gentamycin, susceptible to tetracycline and resistant to cotrimazole, cloxacillin, erythromycin, augmentin, streptomycin and chloramphenicol. *Micrococcus luteus* was 100% susceptible to the antibiotics tested. According to Yah *et al*.[43], several antibiotics resistance to useful classes of antibiotics has generally increased among a number of Gram negative bacteria in particular the

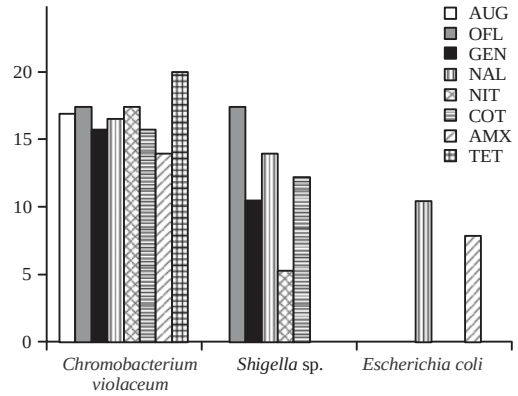

Fig. 1: Antibiotics susceptibility pattern of *C. violaceum*, *Shigella* sp. and *E. coli*

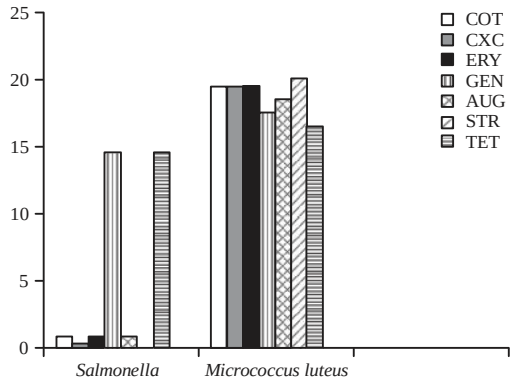

Fig. 2: Antibiotics susceptibility pattern of *Salmonella* sp. and *M. luteus*

Table 3: Zones of inhibition (mm) of antibiogram patterns of the bacteria isolates

		Salmonella			*Chromobacterium violaceum*			*Shigella*			*Micrococcus luteus*			*Escherichia coli*		
Class of antibiotics	Antibiotics	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Penicillin	Amoxicillin				14	-	-							-	7	-
	Cloxacillin	-	-	1							18	-	-			
	Augmentin	-	-	2	17	-	-				17	-	-	-	-	0
Aminoglycosides	Gentamycin	-	14	-	16	-	-	12	-	-	16	-	-	-	-	0
	Streptomycin										20	-	-			
Quinolones	Nalidixic acid				17	-	-	13	-	-				-	-	11
Trimethoprim/sulfonamides	Cotrimoxazole	-	-	1	16	-	-	12	-	-	18	-	-	-	-	0
Tetracyclines	Tetracycline	14	-	-	20	-	-				16	-	-	-	-	0
Fluoroquinolones	Ofloxacin				18	-	-	17	-	-				-	-	0
Macrolides	Erythromycin										0	-	-			
Nitrofuranes	Nitrofurantoin				18	-	-	-	-	4				-	-	0

R: Resistant, S: Susceptible/sensitive, I: Intermediate resistance

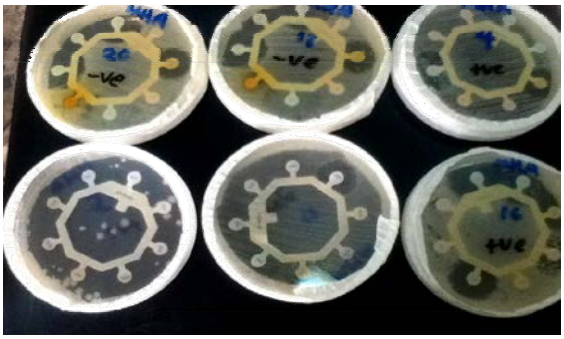

Plate 1: Antibiotic susceptibility patterns in bacteria lawn

enteropathogens. The implication of this is that when these vegetables are not properly prepared and consumed, it will certainly pose a significant challenge in man or animal, thus causing a wide range of infectious diseases and resistance to various antibiotics. In addition, these bacteria on these vegetables could acquire antibiotic resistance patterns from the various antibiotics that are applied during several agricultural processes[24,44]. Belguith *et al.*[45] established that antibiotic resistance in food-borne pathogens is a fact, though substantial qualitative and quantitative differences have been noted. The isolation of these bacteria species with multi resistant patterns indicates the need for continuous surveillance of antibiotic resistance from vegetables/food samples in the developing world with the view of effective rationalization of antibiotic in clinical system.

CONCLUSION

In conclusion, this study has revealed that although local vegetables could provide huge culinary and health benefits for mans sustenance and development, it could also harbor antimicrobial resistance genes that could be transferred to the consumers causing widespread resistance to the usual antibiotics. Given the significance of this study to the public health managers it is advocated that irrigation water supplies should be properly screened before use in other to eliminate/reduce minimally contaminations and antibiotic resistance gene transfers. In addition, safe wash water for the vegetables is advocated among the rural areas rather than the use of untreated and unsafe water that will negatively affect the community health.

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