Original Article

Contamination of Street Vended, Ready to Eat Vegetable Salad with Multidrug Resistant Bacteria

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Received: 4 April 2020 Accepted: 10 May 2021
doi: https://doi.org/10.3329/jemc.v11i3.66882

Abstract

Background: *Street vended, ready to eat vegetable salads are consumed raw, often without heat treatment. Consequently, understanding their role in the spread of antibiotic resistant bacteria cannot be overemphasized.* ***Objective***: *To study microbiological quality of street vended, vegetable salads sold in Minna metropolis in Nigeria.* ***Materials and Methods***: *Fourteen (14) vegetable salads were sampled randomly in duplicates from street venders within Minna metropolis between June 2019 to November 2019. These samples were examined for the presence of bacterial isolates using standard cultural methods. Antibiotic susceptibility testing of the bacterial isolates was achieved by the disk diffusion technique.* ***Results***: *Result analysis revealed* 1.06×10^7 *cfu/g mean total viable bacterial count. Similarly, the mean total coliform bacterial count was* 0.94×10^7 *cfu/g. The study revealed that all the sampled vegetable salads contained four or more bacteria. Staphylococcus aureus was the most frequent (19.5%), followed by Shigella and Klebsiella sp., (12.2%). However, E. coli, E. faecalis and Enterobacter sp. were less frequent (2.4%). The isolated Gram-negative bacteria exhibited resistance (≥ 73%) to amoxicillin/clavulanate, gentamicin, ofloxacin, cefuroxime and ampicillin. Furthermore, the isolated Gram-positive bacteria showed resistance to cefuroxime, ceftriaxone and amoxicillin/clavulanate in ≥ 40% cases. Multidrug resistant bacteria were 97.6% (40/41), of which 61.0% (25/41) was resistant to four or more antibiotics and have Multiple Antibiotic Resistance Index (MARI) ranging from 0.5 to 1.0.* ***Conclusion***: *The results of this study show that street vended, ready to eat vegetable salad facilitates the transmission of clinically relevant antibiotic resistant bacteria. Therefore, appropriate measure should be enforced to ascertain the wholesomeness of street vended, ready to eat vegetable salad as the spread of the bacteria contaminant to humans by ingestion cannot be dismissed.*

Key words: *Vegetable; Salad; Overemphasized; Coliform bacteria; Wholesomeness*

J Enam Med Col 2021; 11(3): 164−172

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Introduction

Salad mostly applies to food preparations made of a mixture of chopped or sliced vegetables[1], such as lettuce, cabbage, tomato, carrot, cucumber, onions and pepper.[2] The consumption rates of salad have greatly increased because of its medical and nutritional values consciousness.[3] Salad is normally consumed uncooked; therefore, the possibility of food infection or poisoning.[4–7]

The protective surface of salad vegetables are damaged by bruise during harvesting, transportation or food processing and are usually contaminated with spoilage and pathogenic microorganisms.[4] As a result, nutrients are released, that support the microbial growth particularly at non-refrigerated temperatures.[7–10] However, pathogens in salad do not result in spoilage,[5] even at relatively high populations. Therefore, in the absence of spoilage signs, salads are consumed because they are perceived as safe.

In the recent times, food-borne diseases and widespread resistance to antibiotic treatment is a very serious public health threat.[11,12] Thus, the microbial flora of uncooked foods such as vegetable salads is of great concern. The washing of vegetables in chlorinated water which may reduce bacterial loads is not common in the developing world.[13] Moreover, the unavailability of potable water for proper washing makes salad vegetables a significant contributor to the transmission of food borne diseases.[14]

Previous studies[10,15–17] conducted on the quality of vegetable salad in relation with the bacterial load and its public health significance have been reported in Nigeria. However, there seem to be dearth of information on the antibiotic resistance burden of food pathogens, especially for the street vended, ready to eat vegetable salad microbiota. This proves that a well-structured understanding of this is required.

Materials and Methods

Minna is a north-central urban center in Nigeria.[18] Vended, ready to eat vegetable salad samples were obtained in area dominated by diverse groups of people consisting mostly of civil servants, students, farmers, artisans, transporters and traders (Fig 1).

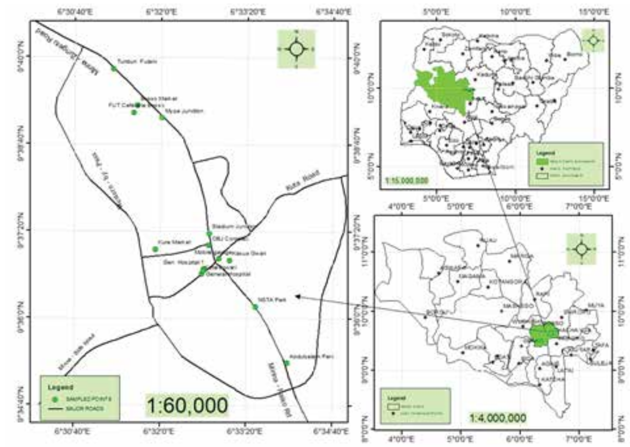

Fig 1. Study area and distribution of street venders of salad vegetable

Study design and period

A cross-sectional study was conducted from June 2019 to November 2019. Fourteen (14) street food vendors were included for data collection. A total of 28 vegetable salad samples (two samples from each selected site) were collected aseptically and analyzed.

Sample collection technique

Twenty eight (28) street vended vegetable salad were purchased randomly from venders within Minna metropolis. Each sample was collected separately in clean nylon bag pretreated with 70% ethanol. Properly labelled samples were transported to Centre for Genetic Engineering and Biotechnology (STEP B), Federal University of Technology, Minna for microbiological analysis.

Sample processing

From each sample, 5 g (with a representative portion of each vegetable that make up the sampled salad) was aseptically weighed and placed in electric blended blender jar. 45 mL of sterile water was added and blended for 5 minutes. 0.25 mL homogenized sample was transferred into 24.75 mL sterilized distilled water to prepare the inocula.[19]

Enumeration of Total Viable Bacterial Count and Total Coliform Count

Zero point one milliliter (0.1 mL) from each resultant inoculum was plated out on Nutrient agar and MacConkey agar using pour plate method. The resultant colonies after 24 hours of incubation at 37°C were counted using a colony counter and expressed as colony forming unit per milliliter (cfu/mL).[19]

Isolation and identification of the bacteria contaminants

Pure cultures of the bacterial contaminants were obtained by streak plate technique using Mannitol Salt Agar (MSA), Salmonella Shigella Agar (SSA) and Blood Agar (BA) plates (Cappuccino & Sherman, 2014). The emerged colonies were observed for their colonial morphology. Gram stained and pure cultures were stock in agar slant for further analysis. Each isolated pure culture was maintained at 4ºC for further analysis.

Identification of coliforms

The bacterial isolates were identified by Gram staining and standard biochemical tests (Triple Sugar Iron, Motility Indole Urease, Methyl Red, Voges-Proskaur, and Citrate utilization test).[19,20]

Antimicrobial susceptibility testing

Antibiotic susceptibility testing of the bacterial isolates was achieved by the disk diffusion technique using Clinical and Laboratory Standards Institute (CLSI) guidelines.[21] Eight (8) antibiotic discs (Rapid Labs, UK: LOT SK03/P), comprising of ceftazidime (CAZ) (30 µg), cefuroxime (CRX) (30), gentamicin (GEN) (10 µg), ceftriaxone (CTR) (30 µg), erythromycin (ERY) (5 µg), cloxacillin (CXC) (5 µg), ofloxacin (OFL) (5 µg) and amoxicillin/clavulanate (AUG) (30 µg), ciprofloxacin (CPR) (5 µg), nitofurantoin (NIT) (30 µg) and ampicillin (AMP) (10 µg) were used.

Pure bacterial cultures diluted to 0.5 McFarland standards were inoculated on Muller Hinton agar (MHA) using a sterile swab. The antibiotic discs were placed at 25 mm spacing apart (4 discs per agar plate) on the inoculated MHA plate. Sterile 6 mm Whatman filter paper No. 3 (Germany), impregnated with sterile distilled water was used as control. After 18 hours of incubation, the plates were examined, and the diameters of the zone of inhibition were measured. The inhibition zones were interpreted as susceptible, intermediate and resistant with the aid of CLSI interpretive standards chart.21

Multidrug resistant (MDR) bacterial isolates were defined by resistance to ≥1 agent in ≥3 antimicrobial classes.[21] Multiple Antibiotic Resistance Index (MARI) per isolate was defined as a/b, where "a" represents the number of antibiotics to which the isolate was resistant and "b" represents the number of antibiotics to which the isolate was exposed.[22]

Data analysis

Data obtained from this study were analyzed using statistical package for social sciences (SPSS) version 24. Cross tabulation was performed to determine the relationship between the rate of the resistant, intermediate and susceptible pattern of the isolated bacteria to the antibiotics. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to determine the significant differences between the bacterial load of vended ready to eat vegetable salads from Minna metropolis, Nigeria ($p > 0.05$).

Results

Bacterial counts in street vended, ready to eat vegetable salad

The mean total viable bacterial count (MTVBC) was 1.06×10^7 cfu/g which varied from 2.67×10^7 to 0.01×10^7 cfu/g. The mean total coliform bacterial count (MTCBC) was 0.94×10^7 cfu/g in which the value ranged from 2.35 to 0.01×10^7 cfu/g (Table I).

From 41 isolates identified, *Staphylococcus aureus* was the most frequent (19.5%), followed by *Shigella* and *Klebsiella* spp., (12.2%) and the least (2.4%) for *E. coli, E. faecalis* and *Enterobacter* spp. (Table II). The study revealed that all the sampled vegetable salads contained four or more bacterial contaminants.

Table I: Bacterial counts in street vended, ready to eat vegetable salad samples

Sample location	TVBC ($\times10^7$)	TCBC ($\times10^7$)
A	1.99^d	1.74^d
B	2.12^c	2.35^a
C	1.84^f	1.02^f
D	1.91^e	2.08^b
E	2.67^a	2.32^a
F	1.59^g	2.02^c
G	0.28^h	0.03^g
H	2.29^b	1.49^e
I	0.02^i	0.02^g
J	0.02^i	0.01^g
K9	0.02^i	0.02^g
L	0.02^i	0.02^g
M	0.02^i	0.02^g
N	0.01^i	0.01^g

Values are mean of three determinations. Means with dissimilar letter(s) (a,b,c,d) differ significantly, according to the Duncan's multiple range test (DMRT).

Key: TVBC (Total viable bacterial count) TCBC (Total coliform bacterial count)

Antibiotic susceptibility and resistance pattern of the bacterial isolates from ready to eat vegetable salad in Minna, Nigeria

The highest resistance (100%) exhibited by Gram-positive bacteria was against cloxacillin while the least (5.6%) was recorded against ofloxacin and gentamicin.

Table II: Frequency of bacterial isolates from ready to eat vegetable salad in Minna, Nigeria

Bacteria	Frequency	Percentage
E. coli	1	2.4
E. faecalis	1	2.4
Enterobacter spp.	1	2.4
Klebsiella spp.	5	12.2
M. leutus	3	7.3
Proteus spp.	3	7.3
Pseudomonas spp.	3	7.3
S. aureus	8	19.5
S. pyogenes	2	4.9
S. Typhi	4	9.8
Serratia spp.	1	2.4
Shigella spp.	5	12.2
Streptococcus spp.	4	9.8
Total	41	100

Similarly, Gram-positive bacterial isolates from ready to eat vegetable salad in Minna showed resistance to cefuroxime, ceftriaxone and amoxicillin/clavulanate in 40% cases (Table III).

The highest resistance (100%) shown by Gram-negative bacteria was against amoxicillin/clavulanate followed by 95.7% to ampicillin, and the least (4.3%) was recorded against ciprofloxacin. Similarly, Gram-negative bacteria isolates from ready to eat vegetable salad in Minna exhibited resistance to gentamicin, ofloxacin, cefuroxime and ampicillin in 73% cases (Table IV).

Table III: Antibiotic susceptibility and resistance pattern of the isolated Gram-positive bacteria

Antibiotics	µg	Number susceptible	Percentage	Number intermediate	Percentage	Number resistance	Percentage
CAZ	30	1	5.6	0	0	17	94.4
CRX	30	10	55.6	0	0	8	44.4
GEN	10	15	83.3	2	11.1	1	5.6
CTR	30	4	22.2	4	22.2	10	55.6
ERY	5	6	33.3	3	16.7	9	50
CXC	5	0	0	0	0	18	100
OFL	5	16	88.9	1	5.6	1	5.6
AUG	30	5	27.8	2	11.1	11	61.1

Table IV: Antibiotic susceptibility and resistance pattern of the isolated Gram-negative bacteria

Antibiotics	µg	Number susceptible	Percentage	Number intermediate	Percentage	Number resistance	Percentage
CAZ	30	15	65.2	2	8.7	6	26.1
CRX	30	2	8.7	1	4.3	20	87.0
GEN	10	3	13.0	3	13.0	17	73.0
CPR	5	20	87.0	2	8.7	1	4.3
OFL	5	3	13.0	3	13.0	17	73.0
AUG	30	0	0.0	0	0.0	23	100.0
NIT	300	7	30.4	5	21.7	11	47.8
AMP	10	1	4.3	0	0.0	22	95.7

Single and multidrug resistance pattern in bacterial isolates from ready to eat vegetable salad in Minna, Nigeria

MDR bacterial isolates accounted for 97.6% (40/41) of the bacteria from ready to eat vegetable salad in Minna, Nigeria while 61.0% (25/41) were resistant to four or more antibiotics and have MARI ranging from 0.5 to 1.0 (Table V).

Table V: Single and multidrug resistance pattern in bacteria isolates from ready to eat vegetable salad in Minna, Nigeria

Serial	Bacterial	RP	MARI
1	Shigella sp.	AUG	0.1
2	S. aureus	CAZ/CXC	0.3
3	S. aureus	CAZ/CXC	0.3
4	S. aureus	CAZ/CXC	0.3
5	M. leutus	CAZ/CTR/CXC	0.4
6	M. leutus	CTR/CXC/AUG	0.4
7	Streptococcus sp.	CAZ/CTR/CXC	0.4
8	S. aureus	CAZ/ERY/CXC	0.4
9	Streptococcus sp.	CAZ/CTR/CXC	0.4
10	S. pyogenes	CRX/CXC/AUG	0.4
11	Shigella sp.	CRX/AUG/AMP	0.4
12	S. typhi	CRX/AUG/AMP	0.4
13	Pseudomonas sp.	CRX/AUG/AMP	0.4
14	Klebsiella sp.	CRX/AUG/AMP	0.4
15	E. coli	AUG/NIT/AMP	0.4
16	Klebsiella sp.	CAZ/AUG/AMP	0.4
17	Proteus sp.	CRX/AUG/NIT/AMP	0.5
18	Proteus sp.	CAZ/CRX/AUG/AMP	0.5
19	Shigella sp.	CAZ/CRX/AUG/AMP	0.5
20	S. typhi	CAZ/CRX/AUG/AMP	0.5
21	Serratia sp.	CRX/AUG/NIT/AMP	0.5
22	Klebsiella sp.	CRX/AUG/NIT/AMP	0.5

Serial	Bacterial	RP	MARI
23	Klebsiella sp.	CAZ/CRX/AUG/AMP	0.5
24	Proteus sp.	CAZ/CRX/AUG/AMP	0.5
25	Klebsiella sp.	CRX/AUG/NIT/AMP	0.5
26	M. leutus	CAZ/ERY/CXC/AUG	0.5
27	E. faecalis	CAZ/ERY/CXC/AUG	0.5
28	S. pyogenes	CAZ/CRX/CTR/CXC/AUG	0.6
29	Enterobacter sp.	CAZ/CRX/AUG/NIT/AMP	0.6
30	Shigella sp.	CAZ/CRX/AUG/NIT/AMP	0.6
31	S. typhi	CAZ/CRX/AUG/NIT/AMP	0.6
32	Shigella sp.	CAZ/CRX/AUG/NIT/AMP	0.6
33	S. aureus	CAZ/CRX/ERY/CXC/AUG	0.6
34	S. aureus	CAZ/CRX/CTR/ERY/CXC/AUG	0.8
35	S. aureus	CAZ/CRX/CTR/ERY/CXC/AUG	0.8
36	Streptococcus sp.	CAZ/CRX/CTR/ERY/CXC/AUG	0.8
37	Streptococcus sp.	CAZ/CRX/CTR/ERY/CXC/AUG	0.8
38	S. typhi	CAZ/CRX/GEN/AUG/NIT/AMP	0.8
39	Pseudomonas sp.	CAZ/CRX/GEN/AUG/CPR/NIT/AMP	0.9
40	Pseudomonas sp.	CAZ/CRX/GEN/AUG/CPR/NIT/AMP	0.9
41	S. aureus	CAZ/CRX/GEN/CTR/ERY/CXC/OFL/AUG	1.0

Key: RP – Resistance Profile; MARI – Multi-antibiotic resistance index

Discussion

Street vended, ready to eat vegetable salads pose a risk to public health due to its potential to cause food-borne disease or poisoning.[23] The MTVBC (1.06×10^7 cfu/g) and MTCBC (0.94×10^7 cfu/g) as shown in Table I were higher when compared with previous studies on raw vegetable and raw-mixed vegetable salads carried out by Bala et al[10] in Nigeria and Pesewu et al[6] in Ghana respectively. In a similar related study, it has been established that the bacterial load is a function of time due to many factors which includes environmental condition, cultivation sources and issue relating to hygienic measures to avoid post-contamination of food.[5]

However, the high bacteria count of vended, vegetable salad indicates poor vender's personal hygiene, inappropriate washing, cutting and preparation processes.[2] Besides, the significant differences ($p>0.05$) in bacterial load of various street vended, vegetables salad can be linked to unrelated factors including resident microflora in the soil, inoculation of non-resident microflora via animal manures, sewage or irrigation water, as well as the transportation condition and handling by individual retailers.[24]

The presence of bacteria pathogen of public health significance in salad vegetables (Table II) mirrors its unwholesomeness for consumption[25-27] and the role of vended, ready to eat chopped vegetables in the continual spread of these pathogens[16,23]. Consistent with previous reports[6,10,23,28], *S. aureus* was the most frequently encountered bacterial isolates (19.5%). Staphylococcal food poisoning (SFP) is a major cause of morbidity and mortality worldwide therefore our finding underscores the role of street vended, salad vegetable as a major driver of *S. aureus.*[29] The ubiquitous nature of *S. aureus* in the environment such as soil and human skin may not be unconnected from this observation.[30]

High frequency of *Shigella* and *Klebsiella* spp. (12.2%) (Table II) may be due to fecal contamination through faeces, soil, irrigation water, and human contact.[6] The presence of these bacterial contaminants poses serious risk on the public health due to their ability to cause infections such as diarrhea, and gastroenteritis.[25,27]

The resistance (≥ 73%) to amoxicillin/clavulanate, gentamicin, ofloxacin, cefuroxime and ampicillin (Table III) exhibited by isolated Gram-negative bacteria can be credited to the incessant use of feces as manure in the cultivation of salad vegetables. Human and animal feces may contain antibiotic resistance gene that are horizontally transferred to soil bacteria which are ultimately introduced into vegetables during cultivation and harvesting processes: a progression that is improved by fertilizer application.[30,31] Similarly, 100% resistance to cloxacillin and ceftazidime activity by Gram-positive bacteria as revealed in Table V may be due to the widespread resistant gene(s) to these antibiotics in the study area. Furthermore, ≥ 40% resistance to cefuroxime, ceftriaxone and amoxicillin/clavulanate by Gram-positive bacteria isolates depicts the decline in activity of these agents and possible emergent of resistant strains (Table III).

It is evident from the results analysis that MDR bacterial isolates were 97.6% (40/41) (Table V). The prevalence rates of MDR bacteria in street vended, vegetable salad from this study is higher than those described by.[32-35] Therefore, this is a situation requiring serious attention as potential transmission of these MDR bacteria to the consumer is conceivable owing to the fact that these vegetable salads are consumed uncooked.

Anthropogenic pollutant such as antibiotic in sub-therapeutic doses, toxic or recalcitrant chemicals in the soil are known to increase antibiotic resistant genes selection in the environment.[36] Thus, high MARI obtained for most bacteria isolates pointed out the consequences of anthropogenic pollution in settings lacking adequate sewage management system like the sampling sites.

This study shows poor microbiological quality of street vended, vegetable salads sold in Minna metropolis. Street vended, vegetable salads are heavily contaminated with MDR bacteria strains of public health significance depicting lack of proper hygiene by venders and source of contamination. Therefore, appropriate strategies should be put in place to actualize the microbial level of street vended, vegetable salads.

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