International Journal of Research and Review
Volume 11; Issue: 6; June 2024
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Microbiological Analysis and Antimicrobial Susceptibility Pattern of *Salmonella typhi* and *Escherichia coli* Isolated from Raw Vegetables Sold in Some Selected Markets in Damaturu, Potiskum and Bade L.G.A, Yobe State, Nigeria

Sheriff Wakil[1], Rabi Yakubu Bello[2], Saheed Yunusa[3]

[1,2,3]Department of Microbiology, Yobe State University, Damaturu, Nigeria

Corresponding author: Sheriff Wakil

DOI: https://doi.org/10.52403/ijrr.20240602

ABSTRACT

The contamination of vegetables by pathogenic microorganisms is a global challenge to food safety and human health. This study was carried out to isolate *Salmonella typhi* and *Escherichia coli* on three different raw vegetables (tomato, lettuce and carrot) randomly sampled from central markets (Damaturu, Potiskum and Bade) in Yobe State, Northeast, Nigeria. A total of two hundred and seventy (270) raw vegetable samples; (30) tomatoes, (30) lettuce and (30) carrots from each market were collected in clean-sterilized sampling bag and taken to Microbiology Laboratory, Department of Microbiology, Yobe State University Damaturu, Nigeria, for the isolation of *S. typhi* and *E. coli* using standard laboratory protocol. One hundred and thirty-six (136) positive isolates; Sixty-seven (67) *S. typhi* and sixty-nine (69) *E. coli* were obtained from raw vegetables. Damaturu market *S. typhi* were high in lettuce 47.1%, tomato 41.7% and carrot 37.5% while the *E. coli* has highest percentage on carrot 62.5%, tomato 58.3% and lettuce 52.9%. In Potiskum market, *S. typhi* occur higher on lettuce 57.7%, carrot 56.3% and tomato 42.9% while *E. coli* has high percentage on tomato 57.1%, carrot 43.7% and lettuce 42.3%. In Gashua (Bade) market, *S. typhi* has high occurrence on lettuce 54.5%, tomato 45.5% and carrot 40.0% while *E. coli* has highest occurrence in carrot 60.0%, tomato 54.5% and lettuce 45.5% respectively. The antibiotic sensitivity test of the resistant isolates (*S. typhi* and *E. coli*) shows one hundred and thirty-six (136) were isolated, thirty-seven (37) resistant isolates in Damaturu, fifty-six (56) in Potiskum and forty-five (45) in Gashua markets. The resistant genes were observed using an agarose gel electrophoresis of amplified 16SrRNA gene for *S. typhi* at 500bp and 586bp for *E. coli* were detected. The high presence of the resistant isolates in raw vegetables indicates contamination of faecal origin probability during harvesting, processing, selling and/or distribution under inadequate hygienic conditions. Surveillance and enlightenment were recommended to the general public on the health risk of consuming unhygienic raw vegetables.

Keywords: *Salmonella spp., Escherichia coli*, Enterobacteriaceae, Raw vegetables,

Antibiotic resistance, Food safety, public health.

INTRODUCTION

The vegetables include the tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*), 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 (Nwinyi and Nduchukwuka, 2016). In the developing countries, vegetables are comparatively cheap and easy to grow. Thus, most rural women rely on this 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 (Abadias *et al.*, 2008). As a consequence, the production and distribution of fresh vegetables could be a huge challenge to the food industry.

The water is one of the principal agents for the spread of various enteric diseases (Ahmed *et al*., 2014). Some researchers 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 (Chigor, Sibanda and Okoh, 2013). Food borne outbreaks associated with vegetables that are partially processed or consumed naturally have increased over the years (Altekruse and Swerdlow, 1996.). This rise in the food-borne outbreaks from vegetables is due to the favourable conditions that the vegetables provide consequently encouraging the growth and survival of many types of microorganisms. Some of the favourable conditions include nutrient rich internal tissues-comprising polysaccharides, pectin, hemicelluloses and cellulose. Entero-pathogens such as *Salmonella spp*. and *Escherichia coli* 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, 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 entero-pathogens *Salmonella spp*. and *Escherichia coli* have been implicated in cases of typhoid fever and diarrhoea following consumption of contaminated raw vegetables. In most developing countries, street vending of fresh raw vegetables is 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 (Beuchat, 2002.). The Food and Agriculture Organization and the World Health Organization strongly recommend more than 400 g/day intake of fruits and vegetables in diets to promote good health (FAO/WHO, 2014). Despite the health benefits derived from consuming fresh vegetables, the risk of microbiological contaminations in raw vegetables is of public concern due to the possibility of vegetable contaminations along the food chain, beginning from the vegetable farm up to the consumer's (Kuan *et al*., 2017); this concern is compounded by the fact that these vegetables are mostly eaten fresh (not cooked) and

washing may not guarantee decontamination, so that any resident microorganism easily enter the alimentary canal. Thus, these public concerns are justifiably informed based on reported cases of numerous foodborne disease outbreaks caused by consumption of fresh vegetables contaminated by microorganisms like Listeria monocytogenes, *Escherichia coli* O157:H7, and *Salmonella spp.* (CDC, 2011 and 2012; Maffei *et al*., 2013; Beuchat, 2017). Various estimates of incidences of foodborne disease outbreaks have resulted in illnesses, hospitalizations, deaths, and even food recalls in some countries, particularly United States of America (Scallan *et al*., 2011; WHO, 2015; CDC, 2016). Microbiological contamination of raw vegetables can occur directly or indirectly through (a) contact with soil, dust, water, and (b) punctures and open cuts of tissues of vegetables; thus, contaminations of vegetables may occur internally or externally during cultivation, harvest, packaging, storage, transporting and marketing (Solomon *et al*., 2002; Bernstein, 2007; Eni *et al*., 2010; Giusti *et al*., 2010). Contaminated vegetables rot more quickly and become unwholesome due to microbiological activities of resident microorganisms. Vegetable spoilage by microorganisms is possible, because vegetables serve as suitable substrates that present the microorganisms with a plethora of valuable nutrients essential for microbial growth. This is a concern for consumers and public health and safety practitioners. Vegetables, whether sold as part of street-vended foods, as part of the menu at eateries, or used domestically for family meals, have understandably come under strict scrutiny by the Nigerian vegetable consumers. This is particularly so since there is widespread belief and acknowledgement among most consumers that vegetables sold on the Nigerian markets are largely produced and handled under unhygienic conditions and practices, i.e., application of manure to soils during cultivation of vegetables, irrigation of vegetables farms with heavily contaminated water, use of unclean water to wash vegetables, dressing vegetables on the bare floor and concrete slabs on the farm and at the grocery, and insufficient pre- and post-harvest inspections of vegetables (Wachtel *et al* ., 2002a & 2002b).

In Nigeria, pipe-borne water is the most commonly used water source to irrigate vegetable farms, but due to the high cost and unreliable water supply, most vegetable farmers have re-sorted to the use of water from streams, wells and storm drains as alternate water sources to irrigate vegetable farms. Unfortunately, these alternative water sources are heavily polluted, especially with pathogenic and toxigenic microorganisms (Keraita *et al*., 2008; Donkor *et al*., 2010; Adentunde *et al*., 2015).

Salmonella typhi and *Escherichia coli* are Gram-negative, rod-shaped bacteria under Enterobacteriaceae family. *Salmonella typhi* is the causative agent of typhoid fever, paratyphoid fever, and food poisoning worldwide (Furchtgott *et al*., 2011). *Escherichia coli* is considered as a part of normal enteric microflora, and most of them are opportunistic pathogens for animal and human; however, this bacterium may cause serious diarrhea and other systemic diseases in healthy humans and animals (Levine, 1984).

In this study, selected raw vegetables; tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*) popularly grown and consumed in the Damaturu, Potiskum and Bade (Gashua) Yobe State, Nigeria were assessed for epiphytic bacteria species (*Salmonella typhi* and *Escherichia coli*) associated with it, their antibiotics susceptibility patterns and molecular detection of the resistant genes of the isolates. The selected vegetables include: (tomato, lettuce and carrot). The selection of these raw vegetables was based on the huge culinary and medicinal benefits they offer the population when consumed raw or lightly cooked.

Therefore, the present study attempted to evaluate the presence of bacteria species (*Salmonella typhi* and *Escherichia coli*) associated with the selected raw vegetables.

MATERIAL AND METHODS

Study area

The study was carried out in Damaturu, Potiskum and Bade (Gashua) Local Government Area, Yobe State- Nigeria. The sampling was carried out between the month of January- February, 2024. The Yobe State is located in the North-Eastern geopolitical zones of Nigeria. The state borders four states: Bauchi, Borno, Gombe, and Jigawa. Yobe State shares borders with Borno State to the east for about 421 km, Gombe State to the south for 140 km (in the vicinity of Gongola River), Bauchi State for 188 km (117 miles) and Jigawa State for 193 km (120 miles) to the west and the Republic of Niger to the north for about 352 km. It borders to the north the Diffa and Zinder Regions of Niger. Because the state lies mainly in the dry savanna belt, conditions are hot and dry for most of the year, except in the southern part of the state which has more annual rainfall (NPC, 2006).

Sample collection

Two hundred and seventy (270) were collected thirty (30) of each raw vegetables tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*) was randomly collected from vegetable sellers in the study areas using sterile sampling bags and stored at in a cool container. The samples were labelled accordingly and transported immediately to Microbiology Laboratory, Yobe State University for further microbiological analysis.

Samples enrichments

Ten (10)g of tomato, lettuce and carrot samples each were weighed separately and transferred into selenite-f-broth medium and allowed to stand for 60minutes at room temperature, then incubated at 37^{o}C for 24hours (Cheesbrough, 2006).

Isolation and identification of *S. typhi* and *E. coli*

The enriched tomato, lettuce and carrot samples was inoculated aseptically onto Deoxycholate citrate agar for *Salmonella typhi* and Eosin Methylene blue agar for *Escherichia coli* isolations and were incubated at 37^{o}C for 24hours. The *S. typhi* appeared colourless with transparent black centre while the *E. coli* appeared with greenish metallic sheen colour. The colonies were further identify using gram staining and biochemical techniques (citrate, methyl red, triple sugar iron and indole tests) (CLSI, 2016).

Antimicrobial susceptibility testing

According to standard microbiological methods, the antibiotic susceptibility was determined by disk diffusion method (22). In this, the bacterial suspensions (0.5 turbidity, McFarland standard) were seeded on Mueller-Hinton agar and the antibiotic-containing disks chloramphenicol 10 µg, ciprofloxacin 10 µg, amoxicillin 10 µg, gentamicin 10 µg, streptomycin 10 µg, ampicillin 10 µg, augmentin 10 µg, cefazolin 10 µg, ofloxacin 10 µg, pefloxacin 10 µg were placed on the surface. All the plates were incubated at 37^{o}C for 24 hours. The zones of clearance round each test antibiotics were noted and the diameters of the zones were measured. The zones of the inhibition were used to determine the antibiotic susceptibility pattern of the isolates in terms of resistance, intermediate resistance and susceptible to the antibiotics (CLSI, 2016).

DNA extraction and molecular identification

The DNA templates of each of the confirmed pure *Salmonella typhi* and *Escherichia coli* isolates were generated by dispensing most of the pure colonies of overnight growth of the isolates onto 100-µL1X Tris- EDTA buffer, vortex mixed and boiled at 100°C for 10 minutes. Then it was transferred immediately

to the freezer (-20°C) for 10minutes, maintained at room temperature, vortex mixed again and centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant containing DNA templates of the isolates were separate, stored at 4°C, and used as DNA template for PCR amplification (Zhang *et al*., 2019).

16SrRNA gene amplification

PCR reaction (25 µL scale) contained master mixture (12.5 µL), forward and reverse primers (1 µL each), DNA template (4 µL), and nucleus free water (6.5 µL). The *16SrRNA* gene from *S. typhi* was amplified using the thermocycler (Eppendorf, Germany). The reaction conditions included an initial denaturation at 94°C for 5 min, followed by 33 cycles of reaction of denaturation at 94°C for 30 Sec, annealing of primers at 65°C for 30 Sec, elongation at 72°C for 45 Sec, and finally an extension at 72°C for 7 min. Similarly, the *16SrRNA* gene of *E. coli* was amplified using an initial denaturation at 95°C for 3 min, followed by 30 cycles of reaction of denaturation at 94°C for 45 Sec, annealing of primers at 58°C for 45 Sec, elongation at 72°C for 1 min, and a final extension at 72°C for 3 min. All the PCR amplicons were visualized using gel electrophoresis on 2% agar after staining with ethidium bromide under UV transilluminator (UV Solo, Germany) (Saidu, Ishaleku, and Sheriff., 2023).

RESULTS AND DISCUSSION

The studied raw vegetables tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*) samples were found to contain *Salmonella typhi* and *Escherichia coli*. The *S. typhi* were detected in the vegetables on Deoxycholate citrate agar, all of the positive isolates appeared colourless with transparent black centre while the *E. coli* were detected on Eosin Methylene blue agar, all of the positive isolates appeared with greenish metallic sheen colour. Gram stain revealed the presence of Gram-negative, pink coloured, short rod bacteria, arranged in single and paired. The bacteria were motile seen by hanging drop slide technique. The were confirmed by conventional biochemical tests. All *E. coli* isolates fermented the five basic sugars (dextrose, sucrose, lactose, maltose and mannitol) producing both acid and gas. On the other hand, *S. typhi* could not ferment Sucrose and Lactose. All of the test isolates *S. typhi* were indole negative, positive for Methyl-Red (M-R) test and negative for V-P test. *E. coli* isolates were catalase positive, V-P test negative, M-R positive, citrate negative and Indole test positive. The isolated *E. coli* was found to be motile using hanging drop slide technique. The results were summarized using a graphic chart based on percentages show in Fig. 1

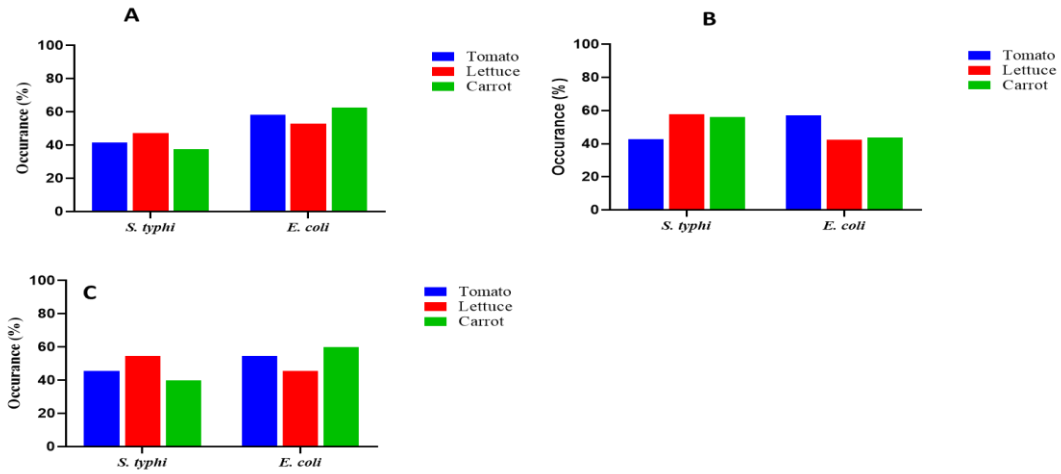

Figure 1: Some bacterial isolates (*S. typhi* and *E. coli*) in raw vegetable samples collected from A) Damaturu B) Potiskum and C) Gashu'a central markets, Yobe State, Nigeria.

Antibiotics susceptibility testing: In this study, the recommendation of Clinical and Laboratory Standards Institute 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 vegetable samples 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 antibiotics when applied at normal dosage or where the zone of inhibition measured lie within the range, where specific microbial resistance mechanisms occur (CLSI, 2016). Therefore, in this study resistant isolates were considered. The table 1 show how the number of the resistant isolates on the raw vegetables collected from the three central markets across the Yobe State-Nigeria.

Table 1: Antibiotic Sensitivity Testing Pattern of *Salmonella typhi* and *Escherichia coli* isolated in raw vegetable samples collected at Damaturu, Potiskum and Bade central market

Antibiotics (10µg)	DCM						PCM						BCM					
	S. typhi (R)			*E. coli* (R)			*S. typhi* (R)			*E. coli* (R)			*S. typhi* (R)			*E. coli* (R)		
	T	L	C	T	L	C	T	L	C	T	L	C	T	L	C	T	L	C
CH	2	-	1	2	1	-	-	-	-	2	-	1	-	-	-	2	3	-
CPX	1	5	2	3	4	-	2	3	1	-	4	-	1	-	-	-	-	-
AM	1	2	-	-	-	-	1	4	2	3	-	2	2	4	2	3	4	2
CN	-	-	-	-	1	-	-	-	3	-	2	1	-	1	-	-	1	-
S	-	-	-	-	2	1	2	-	-	-	-	3	-	-	1	1	-	3
AMC	-	-	-	-	1	-	-	2	-	-	1	-	1	3	1	-	-	1
AU	1	1	-	1	-	2	-	2	2	2	3	1	-	-	-	-	2	-
CZ	-	-	-	-	-	1	1	3	-	-	1	-	1	2	-	-	-	-
OFX	-	-	-	1	-	1	-	1	1	-	-	-	-	1	-	-	-	-
PEF	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-	-

CH= Chloramphericol, CPX= Ciprofloxacin, AM= Amoxicillin, CN= Gentamicin, S= Streptomycin, AMC= Ampicillin, AU= Augmentin, CZ= Cefazolin, OFX= Ofloxacin, PEF= Pefloxacin. µg= microgram, R= Resistant, DCM= Damaturu central market, PCM= Potiskum central market, BCM= Bade central market, T= Tomato, L= Lettuce, C= Carrot, µg= microgram.

Table 2: Primer used for PCR analysis

Targeted genes	Base pair size (bp)	Primers (5´-3´)	Reference
16SrRNA (*S. typhi*)	500	ACTGGCGTTATCCCTTTCTCTGGTG ATGTTGTCCTGCCCCTGGTAAGAGA	Serena *et al*., 2010
16SrRNA (*E. coli*)	586	GACCTCGGTTTAGTTCACAGA CACACGCTGACGCTGACCA	Hassan *et al*., 2014

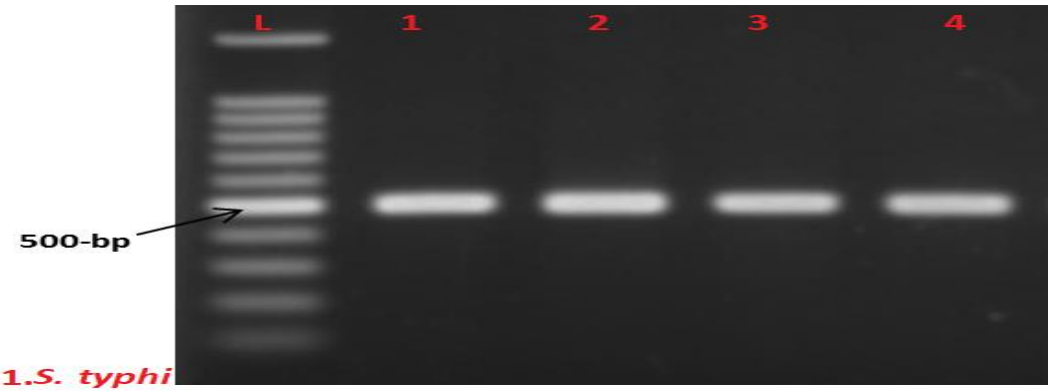

Figure 2: PCR image of *S. typhi*, Lane L: 1000-bp DNA ladder, Lane 1 positive control, Lane 2-4 isolated *S. typhi* at 500-bp.

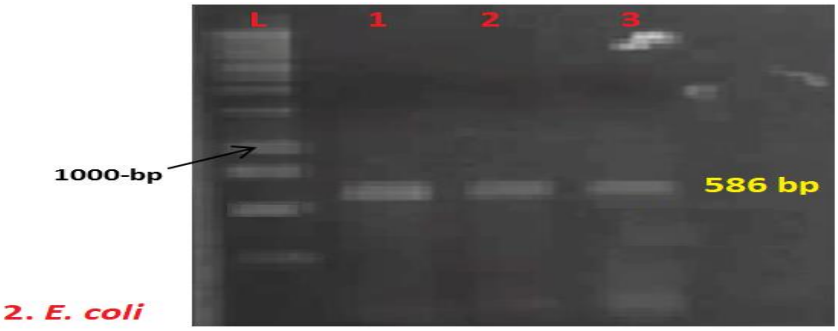

Figure 3: PCR image of *E. coli*, Lane L: 1000-bp DNA ladder, Lane 1 positive control, Lane 2-3 isolated *E. coli* at 586-bp.

Figure 4: Shows *S. typhi* on Deoxycholate citrate agar appeared colourless with transparent black centre.

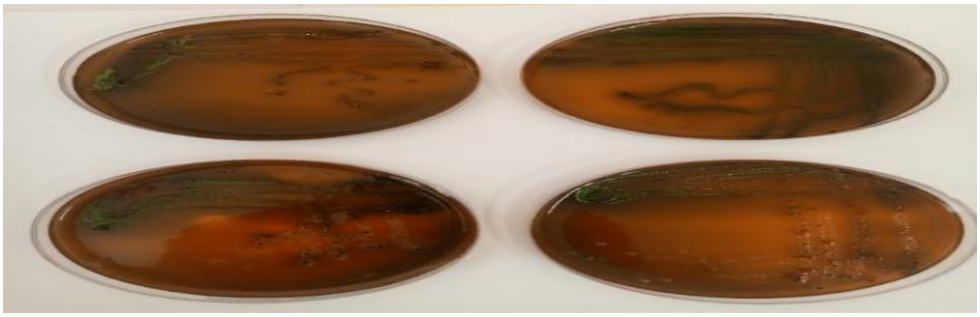

Figure 5: Shows *E. coli* on Eosin Methylene blue agar appeared with greenish metallic sheen colour.

Figure 6: Show images of some biochemical tests on *S. typhi* and *E. coli*

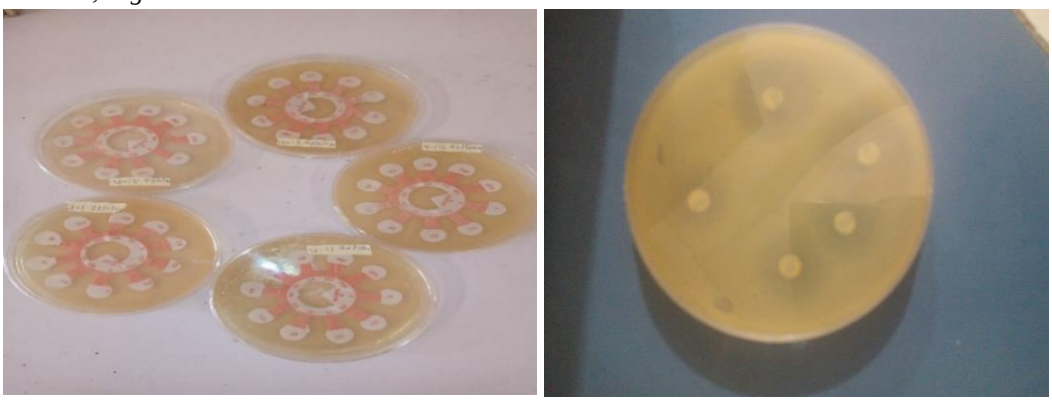

Figure 7: Image of some antimicrobial susceptibility pattern tests on *S. typhi* and *E. coli*

a.  **b.**  **c.** 

Figure 8: Images of raw vegetables collected from the Damaturu, Potiskum and Bade (Gahu'a) markets in Yobe State-Nigeria. a. Tomato (*Solanum lycopersicum*), b. Lettuce (*Lactuca sativa*) and c. Carrot (*Daucus carota*)

DISCUSSION

Microorganisms often associated with vegetables are very large and diverse communities that maintain ecological balance in most agricultural systems (Smetanska *et al*., 2013). Verraes *et al*., 2013 stated that pathogenic bacteria species, which include but not limited to species of *Salmonella, Shigella, Escherichia coli, Escherichia coli, Clostridium botulinum, Campylobacter, Listeria monocytogenes* and *Bacillus cereus* can contaminate vegetables.

The main goal/aims of this study was to; isolate *S. typhi* and *E. coli*, characterization of the isolates based on gram stain and biochemical tests, antimicrobial resistant pattern of the positive isolates and molecular detection of the isolates associated with raw vegetables sold in Damaturu, Potiskum and Gashua (Bade) central markets in Yobe State-Nigeria.

S. typhi and *E. coli* are members of the family *Enterobacteriaceae* are gram-negative, non-spore forming rods. Some of them are human and animal pathogens which produce intestinal infection and food poisoning. The genera of importance in raw vegetables disease include *Salmonella* and *Escherichia* (Goodbum and Wallace, 2013). Salmonellosis is a major public health concern and continues to have a serious economic importance in the raw vegetables in all countries. Broilers meat, raw vegetables and raw poultry products are considered to be a reservoir of infection to human where *Salmonella* and *E. coli* food poising in human is often associated with the consumption of raw vegetable (Jay *et al*., 2014 and Olsen *et al*., 2000). Brandl 2006, reported outbreaks of food borne illnesses between developing and developed countries. This can be explained due to habits of eaten raw vegetables. There is challenge associated with reporting of food borne diseases, however, over 1.8 million peoples, mostly children reported incidence in 2005 in developing countries (WHO, 2007). The main cause of diarrheal diseases cases was due to contaminations of raw vegetables, food and

water. The occurrence of the disease is no respecter of country's status, for instance, in US, about 250-350 million individuals reported acute gastroenteritis each year, and significant number (30%) is due to foodborne pathogens especially *S. typhi* and *E. coli* (Lynch *et al*., 2002). Outbreaks of infectious diseases due to food contamination are common and it associated with health threat and economic loss. For instance, over $152 was added to health care system of US due to impact of food borne infections annually (Scharff, 2010).

This study was conducted to isolate the *S. typhi* and *E. coli* in raw vegetables in some markets in Yobe State. One hundred and thirty-six (136) positive isolates were obtained in this study, Fig. 1 reveals that in Damaturu market *S. typhi* were high in lettuce 47.1%, tomato 41.7% and carrot 37.5% while the *E. coli* has highest percentage on carrot 62.5%, tomato 58.3% and lettuce 52.9%. In Potiskum market, *S. typhi* occur higher on lettuce 57.7%, carrot 56.3% and tomato 42.9% while *E. coli* has high percentage on tomato 57.1%, carrot 43.7% and lettuce 42.3%. In Gashua (Bade) market, *S. typhi* has high occurrence on lettuce 54.5%, tomato 45.5% and carrot 40.0% while *E. coli* has highest occurrence in carrot 60.0%, tomato 54.5% and lettuce 45.5% respectively. This finding was similar with previous study which shows the positive isolates of *S. typhi* that indicates possible contaminations of raw vegetables or poor handling of the vegetables from farm to consumers (Sheriff, Adam and Abdulrahman, 2021). The contamination by these pathogenic microorganisms reveals the possibility of food borne diseases and illnesses when one consumes the raw or partially prepared vegetables (Khan *et al*., 2014). Sources such as irrigation water, improper drainage and lack of sanitation workers hygiene have been identified as factors that could lead to vegetables contamination (Halablab, Sheet and Holail, 2011). Consequently, there is the possibility that some of these factors could provide possible link for the contamination of the surveyed vegetables. 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 (5). Table 1, shows the antibiotic sensitivity test of the resistant isolates (*S. typhi* and *E. coli*) in raw vegetable samples collected from three (3) different markets in Yobe State. A total of one hundred and thirty-six (136) were isolated, thirty-seven (37) resistant isolates in Damaturu, fifty-six (56) in Potiskum and forty-five (45) in Gashua markets. Table 2, shows the primers used for the PCR analysis to detect the resistant genes of *S. typhi* and *E. coli.* Fig. 2, show an agarose gel electrophoresis of amplified 16SrRNA gene for *S. typhi* at 500bp. Fig. 3, show an agarose gel electrophoresis of amplified 16SrRNA gene for *E. coli* at 586bp.

CONCLUSION

The high presence of *S. typhi* and *E. coli* in raw vegetables indicates that these vegetables could be contaminated with faecal probably during harvesting, processing, selling and/or distribution under inadequate hygiene conditions. Therefore, thorough surveillance and enlighten the seller, harvesters is needed in order to ensure the safety and quality of these raw vegetables. The contamination of *S. typhi* and *E. coli* in raw vegetables was a result of poor food handling. All raw vegetables consumers of young and old are exposed to these potential health hazards. Proper temperature control and shorter storage period of fresh vegetables are encouraged.

Acknowledgement: We acknowledge the Chief Technologist; Isa Muhammad at Microbiology Laboratory Unit, Yobe State University, Damaturu-Nigeria for helping in samples preparation, culture media, isolation and identifications of the resistant isolates, and

Scientist; Idriss Baba Machina at Molecular Laboratory Unit, Nigerian Institute for Trypanosomiasis Research Kaduna State, Nigeria, for helping in conducting DNA extraction, detection of the resistant gene. Dr. Musa Adamu of Biological Sciences Department, University of Maiduguri, Borno State, Nigeria for helping us in statistical analysis.

Author's contributions: Sheriff Wakil, Rabi Yakubu Bello and Saheed Yunusa conducts the research and all authors read and approved the final manuscript.

Funding: This study was sponsor by Tertiary Education Trust Fund (TET-Fund) through the Institutional Based Research Grant, Yobe State University, Damaturu-Nigeria.

Competing interest: The authors declare that they have no competing interest.

Ethical Approval: Not required

REFERENCES

1. Abadias, M., J. Usall, M. Anguera, C. Solsona and I. Vinas, 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables and sprouts from retail establishments. Int. J. Food Microbiol., 123: 121-129.
2. Adetunde, L. A., Sackey, I., D ombirl, D. and Mari-ama, Z. W. (2015): Potential links between irrigation water microbial quality and fresh vegetables quality in Upper East Region of Ghana subsistence farming. Annual Research and Review in Biology 6 (6), 347 – 354.
3. Ahmed, T., N.J. Urmi, M.S. Munna, K.K. Das, M. Acharjee, M.M. Rahman and R. Noor, 2014. Assessment of microbiological proliferation and in vitro demonstration of the antimicrobial activity of the commonly available salad vegetables within Dhaka metropolis, Bangladesh. Am. J. Agric. For., 2: 55-60.
4. Altekruse, S.F. and D.L. Swerdlow, 1996. The changing epidemiology of foodborne diseases. Am. J. Med. Sci., 311: 23-29.
5. Bernstein, N., Sela S. and Neder L. Avon, S. (2017) Assessment of contamination potential of lettuce by *Salmonella enterica* Newport added to the plant growing medium. Journal of Food Protection 70, 1717 – 1722.
6. Beuchat, L. R. (2002) Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes and Infection 4, 413 – 423.
7. Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect., 4: 413-423.
8. CDC. 2010. *Salmonella*. Available at: *http://www.cdc.gov/* Center for Disease Control and Prevention (2013). *"Attribution of Foodborne Illness, 1998–2008"*. Estimates of Foodborne Illness in the United States. 19 (3).
9. Center for Disease Control and Prevention 2014. Pathogens causing US foodborne illness, hospitalizations, and death, 2000-2008. Downloaded from *http://www.cdc.gov/foodborneburden/PDFs/pathogens compete list 01-12 pdf* on 30/1/15
10. Center for disease control and prevention 2014. Pathogens causing US foodborne illness, hospitalizations, and death, 2008-2009. Downloaded from https://www.cdc.gov/foodborneburden/PDFs/pathogens complete list 01-12pdf on 30/1/15
11. Center for disease control and prevention 2014. Reports of selected Salmonella outbreaks in Malawi. Clinical Infectious Diseases 46(7): 963-969.
12. Center for food Safety. (2014) Microbiological Guidelines for food: For ready-to-eat food in general and specific food items. Revised Edition; August 2014. p.7 – 8.
13. Centers for Disease Control and Prevention 2014. Reports of selected *Salmonella* outbreaks in Malawi. Clinical Infectious Diseases 46(7): 963- 969.
14. Centers for disease control and Prevention. (2011) Multistate Outbreak of Human *Salmonella enteritidis* Infections Linked to Alfalfa Sprouts and Spicy Sprout (Final Update: 6th July, 2011; cited 10th April, 2019). Available from: http://www.cdc.gov/salmonella/2011/alfalfa-spicy-sprouts-7-6-2011.html.
15. Centers for disease control and Prevention. (2012). Multistate Outbreak of *E. coli* Infections Linked to Romaine Lettuce (Final Update: 23rd March, 2012; cited 10th April,

2019). Available from: http://www.cdc.gov/ecoli/2011/romaine-lettuce-3-23-12.html.

16. Cheesbrough, M. (2006). Microbiological Tests: Gram stain and Biochemical Tests to Identify Bacteria. District Laboratory Practice in Tropical Countries. 2nd ed. Cambridge: Cambridge University Press. p. 62 – 70.
17. Chigor, V.N., T. Sibanda and A.I. Okoh, 2013. Studies on the bacteriological qualities of the Buffalo River and three source water dams along its course in the Eastern Cape Province of South Africa. Environ. Sci. Pollut. Res., 20: 4125-4136.
18. CLSI 2016. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 26th Edition, M100S. Wayne, Pennsylvania 19087 USA: Clinical and Laboratory Standards Institute. 2016.
19. CLSI., 2011. Performance standards for antimicrobial susceptibility testing; twenty first informational supplement. CLSI Document M100-S21, Clinical and Laboratory Standards Institute, Wayne, PA., USA.
20. Coyle, E.F.; Plamer, S.R.; Ribeiro, C.D.; Jones, H.I.; Howard, A.J.; Ward, L. and Rowe, B. (1988*). Salmonella enteritidis* phage type 4 infection: Association with hen's eggs. Lancet, 86: 1295-1297.
21. Donkor, E. S., L Anyo, R., Kayang, B. B., Quaye, J. & Edoh, D. A (2010). Internalization of microbes in vegetables: Microbial load of Ghanaian vegetables and the relationship with different water sources of irrigation. Pakistan Journal of Biological Sciences 13 (17), 857 – 861
22. Droby S. Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Horticulture, 2006; 709: 45–51.
23. Eni, A. O., Oluwawemitan, I. A. & Solomon, A. U. (2010) Microbial quality of fruits and vegetables sold in Sango, Ota, Nigeria. African Journal of Food Science 4, 291 – 296.
24. Food and Safety Authority of Ireland Scientific Criteria to ensure safe food. Institute of Medicine
25. Furchtgott L, Wingreen NS, Huang KC. Mechanisms for maintaining cell shape in rod-shaped Gram-negative bacteria. Molecular Microbiology. 2010; 81(2):340–353. https://doi.org/10.1111/j.1365-2958.2011.07616.x
26. Goodbum C. and C.A Wallace, 'Positive microbiological efficacy of decontamination methodologies for fresh produce: A review food control' vol. 32, no. 2, pp. 418-427, 2023.
27. Halablab, M.A., I.H. Sheet and H.M. Holail, 2011. Microbiological quality of raw vegetables grown in Bekaa Valley, Lebanon. Am. J. Food Technol., 6: 129-139.
28. Hassan J, Parvej MS, Rahman MB, Khan MSR, Rahman MT, Kamal T, Nazir KHMNH. Prevalence and characterization of *Escherichia coli* from rectal swab of apparently healthy cattle in Mymensingh, Bangladesh. Microbes and Health. 2014; 3:12–14. https://doi.org/10.3329/mh.v3i1.19775
29. Ifeanyichukwu I, Chika E, Ogonna A, Chidinma I, Monique A, Ikechukwu M, Stanley E, Emmanuel N, Ngozi A, Agabus N. Prevalence and antibiogram of *Salmonella* species isolated from poultry products in Ebonyi State, Nigeria. Journal of Advanced Veterinary and Animal Research. 2016; 3(4):353–359. https://doi.org/10.5455/javar.2016.c-172
30. Jay, M.J. (2005). Modern Food Microbiology 4th Edition. Chapman and Hall, New York.p.187.
31. Jay, S., Grau, F. H., Smith, K., Lightfoot, D., Murray, C. and Davey, G. R. 2014. *Salmonella* and *E. coli* in hocking, Foodborne microorganisms of public health significance, p. 169-229. Australia: Australian Institutes of Food Science and Technology Inc.
32. Kemal J. (2014). A review on the public health importance of bovine Salmonellosis. Journal of veterinary science technology,5: 175.doi:1041722/2157-7579.1000175.
33. Keraita, B., Drechsel, P. & Konradsen, F. (2008). Using on farm sedimentation ponds to improve
34. Khan, F., Y.N. Jolly, G.M. Islam, S. Akhter and J. Kabir, 2014. Contamination status and health risk assessment of trace elements in foodstuffs collected from the Buriganga River embankments, Dhaka, Bangladesh. Int. J. Food Contamination., Vol. 1.
35. Kuan, C. H., Rukayadi, Y., Ahmad, S. H., Che, J., Thung, T., Premarathne, K., Chang, WS.,

Loo, Y., Tan, CW., Ramzi, O., Fadzil, S., Kuan, C. S., Yeo, S.K., Nishibuchi, M., & Radu, S. (2017) Comparison of the microbiological quality and safety between conventional and organic vegetables sold in Malaysia. Frontiers in Microbiology 8, 1 – 10.

36. Levine MM. *Escherichia coli* infections. Germander (ed), Bacterial vaccines, Academic Press, Inc, New York. 1984; p. 187–235.
37. Lynch, M., J. Painter, R. Woodruff, and C. Braden. 2006. Surveillance for foodborne diseases outbreaks united states, 1998-2002.
38. Maffei, D. F., Silveira, N. F. A. & Catanozi, M. P. L. M. (2013). Microbiological quality of organic and conventional vegetables sold in Brazil. Food Control 29, 226 – 230.
39. National Research Council. The National Academies Press, Washington, 2001. microbial quality of irrigation water in urban vegetable farming in Ghana. Water Science and Technology 57 (4), 519 – 525.
40. NPC (2006). Population census data, Yobe State, Nigeria. Federal Republic of Nigeria official gazette, National Provisional total census. Printed and published in 2007 by the Federal Govt. Printer, Lagos, Nigeria, Number 21, Volume 94 pp: 175-198.
41. Nwachukwu E, Chukwu CM. Effect of chemical treatments on the microbial load of fruits and vegetables. International Journal of Applied Microbiology and Biotechnology Research. 2013; 1:16–19.
42. Olsen, S. J.; Machinnon, L.C; Goulding, J.S.; Been, N.H. and Stusker., L. (2000). Surveillance for foodborne diseases outbreaks in USA (1993-1997). 49: 1-62.
43. Saidu, I. A., Ishaleku, D. and Sheriff, W. (2023). Antibiotics Susceptibilities and Plasmid Profiles of *Salmonella typhi* from Patients Attending Yobe State Specialist Hospital, Damaturu, Yobe State, Nigeria. Sahel Journal of Life Sciences FUDMA, 1(1):45-52. DOI: https://doi.org/10.33003/sajols-2023-0101-006
44. Scallan, E., Hoeskstra, R. M., Angulo, F. J., Tauxe, R. V. & Hoekstra, R. M. (2011). Foodborne illness acquired in the United States major pathogens. Emerging Infectious Diseases 17(1), 7 – 15.
45. Scharf, R. L. 2010. Health-related costs from foodborne illness in the US. The produce safety project at George Town University. Available at: www.producesafetyproject.org. accessed 20th march, 2010.
46. Serena S, Valerio I, Maria B, Giovanni DN, Valentina T, Monica PC, Catia L, Giulia M, Salvatore C, Maria PC. A distinctive 'microbial signature' in celiac paediatric patients. BMC Microbiology. 2010; 10:175. https://doi.org/10.1186/1471-2180-10-175
47. Sheriff Wakil, Adam, M., Dadile, M.A., 2021. Isolation and Identification of *Salmonella* species from raw vegetables sold in some markets in Maiduguri metropolis. Baobab: A Journal of Research in Science and Technology, Vol.2, No.2, pp. 97-112.
48. Smetanska, I., D. Hunaefi, V. Gustavo and S. Barbosa-Ca'novas, 2013. Nonthermal Technologies to Extend the Shelf Life of Fresh-Cut Fruits and Vegetables. In: Advances in Food Process Engineering Research and Applications, Yanniotis,S., P. Taoukis, N.G. Stoforos and V.T. Karathanos (Eds.). Springer Science and Business Media, New York, ISBN: 9781461479062, pp: 375.
49. Solomon, E. B., Yaron, S. & Mathews, K. R. (2002). Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Applied and Environmental Microbiology 68, 397 – 400.
50. Verraes, C., S. Van Boxstael, E. Van Meervenne, E. Van Coillie and P. Butaye et al., 2013. Antimicrobial resistance in the food chain: A review. Int. J. Environ. Res. Public Health, 10: 2643-2669.
51. Wachte L, M. R., Whitehand, L. C. & Mandrell, R. E. (2002a). Association of *Escherichia coli* with preharvest leaf lettuce upon exposure to contaminated irrigation water. Journal of Food Protection 65, 18 – 25.
52. Wachte L, M. R., Whitehand, L. C. & Mandrell, R. E. (2002b) Prevalence of *Escherichia coli* associated with cabbage, lettuce, carrot and tomato crop inadvertently irrigated with partially treated sewage wastewater. Journal of Food Protection 65, 471 – 475.
53. World Health Organization (2015). WHO estimates of the global burden of foodborne

diseases. A report by the Foodborne Disease Burden Epidemiology Reference Group 2007-2015. Geneva, Switzerland.

54. World Health Organization WHO (2014). Global Salmonella surveillance and laboratory support project. Fourth edition, April, 2003.Pp. 1-18
55. World Health Organization, 2014. Global *Salmonella* and *E. coli* surveillance and laboratory support project. Fourth edition, April, 2003. Pp. 1-18.
56. Yusuf IZ, Oyaweye OM, Yongabi KA, Pemu AT. Bacteriological Quality Assessment of Salad Vegetables sold in Bauchi Metropolis. Nigeria Journal of Microbiology, 2004;18:316-320.
57. Zhang, S.,Zhu, X., Wu, Q., Zhang, J., Xu, X. and Li, H. (2019). Prevalence and characterization of *Escherichia coli* O157 and O157:H7 in retail fresh raw meat in South China. Annal of Microbiology. 65 (4): 1993-1999.
58. Zhao, B.; Villena, R.; Sudler, E.; Yeh, S.; Zhao, D.G.; White, D. Wagner and J. Meng. (2001). Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington. Applied Environment Microbiology, 67: 5431–5436.

How to cite this article: Sheriff Wakil, Rabi Yakubu Bello, Saheed Yunusa. Microbiological analysis and antimicrobial susceptibility pattern of *Salmonella typhi* and *Escherichia coli* isolated from raw vegetables sold in some selected markets in Damaturu, Potiskum and Bade L.G.A, Yobe State, Nigeria. *International Journal of Research and Review*. 2024; 11(6): 5-17. DOI: *https://doi.org/10.52403/ijrr.20240602*

******