

Antibiogram of pre-harvest *Escherichia coli* 0157:H7 on cabbage irrigated with wastewater

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Abstract

This research investigated the pre-harvest prevalence of *E. coli* 0157:H7 on cabbage (*Brassica oleracea* var. *capitata*) grown with contaminated irrigations' water, of Sharada canal in Kano Nigeria and determined the level of antibiotic's susceptibility phenotype. *E. coli* 0157:H7 was isolated by enrichment on Trypticase Soy Broth (TSB), and transferred onto Sorbitol MacConkey agar plates, supplemented with 0.5% sodium thioglycolate, cefexime and potassium telurite (CT-SMAC) and Eosin Methylene Blue (EMB) repetitively. Non Sorbitol fermented colonies were biochemically screened on Microbact 24E, Latex agglutination and tested on standard antibiotic. A total of 11(22.00%) *E. coli* 0157:H7 were observed with highest resistance of 54.55% on nalidixic acid, followed by gentimycin and ceporex with 36.36% each. A number of strains showed significant positive correlations, in susceptibility response to drugs like ceporex and streptomycin ($r=0.624$, $P<0.05$), pefloxacin and ofloxacin ($r=0.624$, $P<0.05$), gentimicin and pefloxacin ($r=0.624$, $P<0.05$), and cotrimothaxole and pefloxacin ($r=0.624$, $P<0.05$) respectively. Similarly eight patterns of multidrug resistance were observed, 27.27% of the isolates showed resistance of 2 to 5 antibiotics. The prevalence of *E. coli* 0157:H7 in cabbage coupled with the high multi-drugs resistance, is a subject of public health concerned and therefore call for active surveillance mechanism by government health agencies, for detection of possible *E. coli* 0157:H7 disease outbreak in the State in routine services.

Keywords

Pre-Harvest, Cabbage, Susceptibility, Multi-Drug Resistance, Public Health

1. Introduction

Epidemiological data indicated that the number of documented *Escherichia coli* 0157:H7 outbreaks associated with the consumption of raw fruits, vegetables, and unpasteurized fruit juices has increased in recent years (CDC, 2001 and Codex, 2003). Outbreaks with identified etiology were predominantly of bacterial origin, primarily *Salmonella*, and *E. coli* 0157:H7 infection has been associated with lettuce, sprouts, and apple juice, and other enterotoxigenic bacteria (Buck *et al.*, 2003). However, researches are fewer, in developing countries, which link directly the transmission of *E. coli* 0157:H7 from contamination sources to vegetables.

Reports were concerned with possible sources of vegetables contaminations, for example Chigor *et al.*, (2010) only report on the occurrences of *E. coli* 0157:H7 in irrigation wastewater in Zaria, Kaduna Nigeria, while others reports on post harvest prevalence of *E. coli* 0157:H7 in Nigeria, concentrated on the vegetables sold at market places. For example, Odu and Okomuda, (2013) reported 79.6% prevalence of *Escherichia coli* in cabbage sold at Port Harcourt, Nigeria and speculated that the pathogens in the vegetables may have been a direct reflection of sanitary quality of the cultivation water, harvesting, transportation,

storage, and processing of the plant produce. With the occurrence of these pathogenic *E. coli* O157:H7 strain in the environment, the possibility of development of antibiotic resistance phenotype is most likely, especially due increase phenomenon of increasing incidence of antimicrobial resistance (AMR) bacteria in humans, animals and the environment that has continue to be a challenge to public health, in both human and veterinary medicine, which needs increased monitoring, Willis, (2000). Uses of herbicides and insecticides have in recent years, increased the level and distribution of AMR phenotypes, Chang *et al.* (2013) and resistance genes. Similarly, indiscriminate uses of antimicrobial drugs in the treatment of infectious diseases, and as animal feed supplements have lead to rapid development of antibiotics resistance bacteria. Recently, methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* O157:H7, *Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa* were considered some of the most virulent microorganisms for the human population (Hafith *et al.*, 2001).

This research therefore, aimed to investigate the pre-harvest prevalence of *E. coli* O157:H7 on cabbage irrigated with wastewater from Sharada canal, and determined the level of antibiotic' susceptibility phenotype among *E. coli* O157:H7 strains. Sharada canal collects wastewater from Sharada Industrial Layout (Phases I and II), Kano municipal, Kumbotso Local Government Areas and some parts of Tarauni Local Government areas. A part from domestic waste, sludge collected by gully is being emptied in to the canal. The wastewater from this canal serves as source of irrigation water for vegetables farms in Kano city and its environs.

2. Methodology

2.1. Samples Collection

Fifty (50) samples of cabbage (*Brassica oleracea* var. *capitata*) were collected, from irrigation farms along the Canal, at three different points, the points which were determined according to the degree of irrigation activities relative to the farms' sizes, volumes of the cultivars produced and position of the farm along the canal (16 samples at initial point of utilization, 17 samples at mid-point and 17 samples at upstream/ terminal point of utilization). Samples were transported in a sterile polyethylene bag, to laboratory and processed.

2.2. Identification of *E. coli* O157:H7

One gram (1 g) each of samples was added to nine milliliter (9 ml) of distilled water and mix well to form a sample homogenate. The homogenates were serially diluted 10^4 from which 1 ml each was inoculated in 9 ml Trypticase Soy Broth (TSB) (Lab M, UK) incubated at 37°C for 4 hrs Sata *et al.*, (2003) for enrichment. Enriched samples were cultured on Sorbitol MacConkey agar (US Biological) plates (SMAC) supplemented with 0.5% sodium thioglycolate,

cefexime and potassium telurite (CT-SMAC) and Eosin Methylene Blue (EMB) respectively, incubated at 37°C for 24 hrs, Wallace *at al.*, (1997). Non Sorbitol fermented (colorless) colonies on CT-SMAC were Grams' stained, and subjected for biochemical identified on Microbact 24E gram negative identification system and screened by *E. coli* O157:H7 Latex agglutination Kit (Oxoid) (Koneman *et al.*, 1994; Wallace *at al.*, 1997 and Collee *et al.*, 2007).

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility profile of *E. coli* O157:H7 was determined by disc diffusion KirbyAuer techniques (Cheesbrough, 2005) on standard antibiotic discs (G-VE, Polytes Laboratories, Enugu, Nigeria) using the following antibiotics: Ampicillin (30 μg), Ofloxacin (10 μg), Streptomycin (30 μg), Ceporex (10 μg), Cotrimoxazole (30 μg), Gentamicin (10 μg), Nalidixic acid (30 μg), Augumentin (30 μg), Ciprofloxacin (10 μg) and Pepflacin (10 μg). The results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria (2007).

3. Result

The non Sorbitol fermented colonies observed in cabbage from Sharada canal irrigation farms were 17 (34.00). Of these 65% were biochemically and serologically positive for *E. coli* O157:H7. In other words, 11(22.00%) of the 50 cabbage samples were positive for *E. coli* O157:H7. The percentage prevalence or occurrence were not normal along the sampling points, but rather positively skewed distributed, there was 45.5% and 54.5% in the first and second sampling points respectively, while on strains was detected in the last point of collection.

Strains isolated have demonstrated high antibiotic resistance phenotypes $n = 11$, (Table 1). A high resistance of 54.55% to Nalidixic acid was observed, followed by gentimycin and ceporex with 36.36% each. Amoxicillin clavulanate and ciprofloxacin were only 9.09% among the entire antibiotic tested. Similarly, significant positive correlations were also observed in susceptibility response to antibiotics, with ceporex and streptomycin ($r = .624$, $P < 0.05$), pefloxacin and ofloxacin ($r = .624$, $P < 0.05$), gentimycin and ofloxacin ($r = .607$, $P < 0.05$), gentimycin and pefloxacin ($r = .624$, $P < 0.05$), and cotrimothaxole and pefloxacin and cotrimothaxole and gentimycin , $r = .624$ and $.607$, $P < 0.05$ respectively. The level of antibiotic shared variability of susceptibility on strains, ranged from 37.00% to 39.00% (Table 2).

On the other hand, eight (8) different patterns of multidrug resistance were equally observed, with varied number and types of antibiotic involved, ranged from 2 to 5 (Table 3). There were 6 (54.55%) of isolates resistant to nalidixic acid and 4 (36.36%) to gentimycin. Twenty seven percent (27.27%) of the isolates showed resistance to 2 and 5 antibiotic.

Table 1. Antibiotic resistance profile of *Escherichia coli* O157:H7 from cabbage along Sharada canal wastewater irrigation farms.

Sample code	S	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
SH 4	S	S	S	R	R	R	R	S	S	R
SH 5	R	R	R	S	R	S	S	S	S	S
SH 7	S	S	S	R	R	S	S	S	R	S
SH 11	S	R	S	S	S	S	S	S	S	S
SH 14	S	S	S	S	S	S	R	S	S	R
SH 18	S	S	S	S	R	S	S	S	S	S
SH 20	S	R	S	S	S	S	S	S	S	S
SH 24	S	S	R	S	R	S	S	S	R	S
SH 25	S	S	R	S	S	S	S	R	R	R
SH 26	R	S	R	R	S	S	R	S	R	S
SH 29	S	S	S	R	R	R	R	S	S	R
Resistance %	18.18	27.27	36.36	36.33	54.55	18.18	36.36	9.09	9.09	27.27

Key: SH = sharada irrigation farms samples, PN = Ampicilin CEP = Ceporex, OFX = Ofloxacin, NA = Nalidixic acid, PEF = Pefloxacin, CN = Gentamycin, AU= Amoxicillin-clavulanate, CPX = Ciprofloxacin, SXT = Co-trimotxazole.

Table 2. Correlations of cabbage samples collected along Sharada irrigation farms

Antibiotics	Statistics	S	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
S	R	1									
PN	R	.241	1								
CEP	R	.624*	-.039	1							
OFX	R	.134	-.463	-.179	1						
NA	R	-.043	-.261	-.069	.311	1					
PEF	R	-.222	-.289	-.356	.624*	.430	1				
CN	R	.134	-.463	-.179	.607*	-.069	.624*	1			
AU	R	-.149	-.194	.418	-.239	-.346	-.149	-.239	1		
CPX	R	.134	-.463	.607*	.214	-.069	-.356	-.179	.418	1	
SXT	R	-.356	-.463	-.179	.214	-.069	.624*	.607*	.418	-.179	1

Keys: * = Significant at 0.05 level, S = Streptomycin, PN = Ampicilin CEP = Ceporex, OFX = Ofloxacin, NA = Nalidixic acid, PEF = Pefloxacin, CN = Gentamycin, AU= Amoxicillin-clavulanate, CPX = Ciprofloxacin, SXT = Co-trimotxazole.

Table 3. Antibiotic resistance pattern of *E. coli* O157:H7 from cabbage irrigated with wastewater from Sharada canals in Kano City, Nigeria.

S.N	Number of Antibiotics	Resistance Pattern	Number of strain/s	Source of Isolate
1	1	NA	1 (9.09)	Sharada
2	1	PN	1 (9.09)	Sharada
4	1	PN	1 (9.09)	Sharada
6	2	CN, SXT	1 (9.09)	Sharada
7	2	NA, CEP	1 (9.09)	Sharada
8	2	CEP, AU	1 (9.09)	Sharada
10	3	NA, OFX, CPX	1 (9.09)	Sharada
11	4	NA, CEP, S, PN	1 (9.09)	Sharada
17	5	NA, OFX, PEF, CN, SXT	1 (9.09)	Sharada
18	5	CEP, S, OFX, CN, CPX	1 (9.09)	Sharada
19	5	NA, OFX, PEF, CN, SXT	1 (9.09)	Sharada

Keys: S = Streptomycin, PN = Ampicilin CEP = Ceporex, OFX = Ofloxacin, NA = Nalidixic acid, PEF = Pefloxacin, CN = Gentamycin, AU= Amoxicillin-clavulanate, CPX = Ciprofloxacin, SXT = Co-trimotxazole.

4. Discussion

The bacterial prevalence observed in this work was high, by implication cabbages grown with wastewater from this canal poses public health hazard of potential disease transmission, even when aseptically handled and processed after harvest. The speculations in Kano state by health agency, of vegetables leveled as major vehicle for diarrhea outbreaks, could be true, based on this finding and the contribution of the pre-harvest contamination sources could therefore be considered enormous, going by recorded high prevalence of *E. coli* O157:H7 in this study. Similar result was reported by Oliveira *et al.*, (2011) in Chang *et al.* (2013) of *E. coli*

O157:H7 present in 53.1% of minimally processed leafy vegetable samples in Brazil which. Chang and his colleagues concluded, that *E. coli* O157:H7 contamination of organic foods usually originated from environment, human or animal sources. Thus environmental sources of contamination play a great role in the transmission of *E. coli* O157:H7 to vegetables. In line with this, Enabulele and Uraih (2009) did not isolate *E. coli* O157:H7 in cabbage sampled from Benin City, Nigeria, although their samples were not collected directly from farms as in the case of this research and got Zero prevalence. The Zero post-harvest prevalence further demonstrated the likely source by which vegetables become contaminated. Probably, it was not irrigated with contaminated water or soil. Temperature and other

environmental factors are important factor that affects the growth and distribution of *E. coli* O157:H7 at pre-harvest stage (Steinmuller *et al.*, 2006). At pre-harvest activities fresh produce are more likely to be contaminated through irrigation water containing untreated sewage and improperly composted manure and in some cases water, birds and flies were used as vectors of transmission (Dahiru *et al.*, 2013). The positively skewed distribution of *E. coli* O157:H7 noticed along and between samples point could probably have been as a result of some bactericidal chemical substances released from industrial waste or other abiotic factor such as temperature and salinity, especially in the last segment of sampling point, as pointed by Richard *et al.*, (2004) that the survival of fecal indicator bacteria like *Escherichia coli*, in ambient environments is strongly influenced by abiotic (e.g., salinity, sunlight, and temperature) and biotic (predation and competition) factors. High algal growth observed during sampling at 1st and 2nd sampling point in the wastewater surface could have also probably influence the occurrence of this bacteria. In previous studies algal-derived surface films and slimes were found to enhance growth of the bacteria by creating turbulence-free microenvironments (Epstein Jenkinson, 1993).

Although EHEC (*E. coli* O157:H7) strains are generally susceptible to a variety of antibiotics. Our result revealed high rate of resistance, especially to quinalones drugs by *E. coli* O157:H7 strains and this is subject of high clinical and public health importance. It could probably be due to excessive usage of the drugs (for both human and veterinary purposes), inappropriate prescription or substandard drugs administration. Low *et al.* (2002) attributed the problem of drug resistance, with rapid increase in the routine use of these drugs, thereby facilitating the increase resistance level of pathogenic microorganisms, to even newer drugs. Similar to this work, Umolu *et al.*, (2006) reported antibiotic resistant *E. coli* in Lagos – Nigeria, to augmentin 94.2%, amoxicillin 88.4%, cotrimoxazole 51.2%, gentamicin 48.8% and nalidixic acid. However Schroeder *et al.*, (2002) demonstrated lower resistance to flouroquinolone drugs in *E. coli* O157:H7 isolated from humans, cattle, swine, and food sources. The implication of this result is that outbreak related to *E. coli* O157:H7 especially in Kano state, administration of these drugs may not be effective in the treatment of patients involved. Further researches are needed to find out effective drugs of choice for this pathogen, especially in the area of study.

Multiple antibiotic resistances observed in this work was quite high, and similar to Yang *et al.*, (2004) in the characterization of multiple antibiotic resistant *E. coli* isolated from disease chickens and swine in China, who reported majority of isolates resistant to sulfamethoxazole (84%), ampicillin (79%), streptomycin (77%) and trimethoprim-sulfamethoxazole (76%), and all *E. coli* isolated were, regardless of their origin, resistant to the quinolone antibiotics.

The high resistance and multiple drugs resistance profile recorded in this work could probably be due to extensive use

of the drugs, for the treatment of bacterial infections. Similarly, the prohibitive cost of newer and potent drugs could necessitate the poor (low-income earners) to use of substandard, less potent or ineffective drugs, thereby promoting the development of multiple resistance phenotypes. These substandard drugs may be prone to poor storage condition, (which are patronized due to prohibiting cost of newer and effective drugs), which affect the potency of drugs and translate negatively to the efficacy of drugs on microorganisms. Drug like amoxi-clavulanate was reported to be volatile and unstable when exposed to high temperatures and high pH (Khan *et al* 2013), can therefore quickly be rendered less potent

Rasheed *et al.*, (2014) noted that about 32.30% of STEC elicited eight different patterns of antibiotic resistance ranging from one to eight antibiotics that are widely used. The indiscriminate use of antibiotics in agriculture and medicine were observed, to be a major selective force in the high incidence of antibiotic resistance among gram's negative bacteria. This could also probably explain why high multi-drugs resistance was observed in this result. Rasheed and his colleagues indicated that the bacterium which contains both types of Shiga toxin genes (*stx1+stx2*) were resistant to most of the antibiotics tested. Bacteriophages contain antibiotic resistance genes and are potentially excellent vehicles for the propagation of bacterial resistance genes in the environment (Colomer-Lluch M *et al.*, 2011).

5. Conclusion

Contamination of vegetables with *E. coli* O157:H7 can pose possible health risks to the consumers. Results of the current study can be used as baseline data in risk estimation for the prediction of human illness associated with vegetables and provide useful information for the microbial risk assessment of *E. coli* O157:H7 and control of epidemic.

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