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The Antibiotic Sensitivity Pattern of *Esherichia coli* isolated from Street Vendored Vegetable Salad in Umuahia, Nigeria

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The antibiotic sensitivity pattern of *Escherichia coli* isolated from vegetable salad samples sold in Umuahia, Nigeria was investigated. A total of 30 different *E. coli* isolates from 30 different vendors were used for the antibiotic sensitivity test which was carried out by the Kirby-Bauer disc diffusion method. Eight different antibiotics, Ofloxacin (OFL), Augmentin (AUG), Tetracycline (TET), Amoxycillin (AMX), Co-trimoxazole (COT), Nitrofurantoin (NIT), Gentamicin (GEN) and Nalidixic acid (NAL) were used in this study. All *E. coli* isolates were sensitive to Ofloxacin (100%). The percentage sensitivity recorded in the case of Gentamicin, Nalidixic acid, Nitrofurantoin, Co-trimoxazole and tetracycline were 96.7%, 90%, 80%, 50% and 6.7%, respectively. All the isolates were resistant to Augmentin and Amoxycillin. This study suggests the need for better personal, environmental and food hygiene of street vendors as well as judicious application of antibiotics for the safety of the public.

Key Words: *E.coli*, vegetable salad, antibiotic sensitivity, Umuahia, Nigeria.

In Nigeria, antibiotics are among the most abused drugs. This is evidenced by the fact that in the course of any ailment, especially common cold, cough and gastrointestinal disorder, it is very common to hear such phrases like "Have you taken an antibiotic?" This shows that either not so much has been done in creating awareness and /or controlling or even regulating the use of antibiotics in the system or the impact created in line with this is minimal, not forgetting the survival instinct of the microorganisms anyway.

According to Willey *et al*. (2008) the causes of antibiotic resistance include: inappropriate prescribing practices, unregulated sale of antibiotics, failure to complete course of antibiotics, use of suboptimal antibiotic dosages, and use of antibiotics as animal growth enhancers. The treatment of serious community infections based on clinical symptoms rather than following laboratory confirmation of the exact organism and its drug sensitivities adds to the list. (Pitout and Laupland, 2008).

Escherichia coli, one of the common microbial flora of the gastrointestinal tract of human beings and animals is an important food-borne disease organism (Levine, 1987, Brooks *et al.*, 2007). It is the most common cause of food and water-borne diarrhea worldwide, especially in developing countries, causing 800,000 deaths out of 650 million cases per year primarily in children under the age of five (Turner *et al.*, 2006). Also, it has been documented as the most important pathogen associated with urinary tract infections (UTIs) in many countries (Samra *et al.*, 2005; Zinnah *et al*., 2008).

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Antibiotics used in the treatment of *E. coli* infections include Penicillins, Cephalosporins, Aminoglycosides, Tetracyclines, Sulphonamides and Sulphonamide- containing compounds, Quinolones, and Nitrofurantoin (Cheesbrough, 2006; Willey *et al.*, 2008).

The problem of antibiotic resistance in *E. coli* is of great concern to medical experts mostly because; antibiotic resistance can be transmitted within and across its genera through R plasmids, genetic elements (transposons and integrons) and by spontaneous mutation of *E. coli* chromosome, although this is rare (Goldstein *et al*., 2001; Davies, 1994; Willey *et al.*, 2008)

As has happened with other bacteria, such as Methicillin Resistant *Staphylococcus aureus* (MRSA), cases where strains of *E. coli* develop resistance to commonly used antibacterial drugs have been recorded. The multi-drug resistant *E. coli* and other bacteria within the same group are able to produce enzymes called Extended-Spectrum Beta-Lactamases (ESBLs) that stop certain antibiotics from working, among which are some of the most widely used in hospitals. There is a particularly virulent form of these bacteria that produce a distinct group of ESBLs (CTX-M enzymes). These bacteria are resistant to the groups of antibiotics that are commonly used to treat most infections (penicillins and cephalosporins) and also to certain higher classes of antibiotics normally reserved for more severe infections (e.g. fluoroquinolones, co-trimoxazole and gentamicin). (Kumar *et al*., 2006; Sinha *et al*., 2008).

Pitout and Laupland, (2008), reported that ESBL-producing bacteria are being found in the community although infections caused by these bacteria in the community are rare. They speculate that in the near future, clinicians will be regularly confronted with the hospital types of bacteria causing infections in patients in the community, just like community-acquired MRSA.

The growing concern as healthy non-hospitalized people have become infected with the antibiotic-resistant form of *E. coli*, the reported cases of intra- and inter- household transmission (Lo *et al.*, 2010) and the listing of food chain as possible cause of high rate of occurrence of CTX-M ESBL producing Enterobacteriaceae (Tadahiro *et al*,. 2010) motivated the choice of food sample for this work since the presence of *E. coli* in food generally indicates direct or indirect faecal contamination (Anigo *et al.*, 2007).

This work is therefore aimed at determining the prevalence and antibiotic sensitivity pattern of the *E. coli* isolates from street vendored vegetable salads in Umuahia, Abia State, Nigeria.

Table 1: *Escherichia coli* Count in each Vegetable salad sample

Sample	Colony Forming Units (CFU/g)	Sample	Colony Forming Units (CFU/g)
1	3.4×10^5	16	6.6×10^5
2	4.6×10^5	17	5.4×10^5
3	3.7×10^5	18	4.7×10^5
4	5.4×10^5	19	9.1×10^5
5	6.1×10^5	20	5.4×10^5
6	5.7×10^5	21	1.50×10^6
7	6.0×10^5	22	1.83×10^6
8	7.3×10^5	23	8.5×10^5
9	6.3×10^5	24	5.0×10^5
10	8.6×10^5	25	9.7×10^5
11	6.8×10^5	26	7.0×10^5
12	6.5×10^5	27	1.47×10^6
13	2.7×10^5	28	8.0×10^5
14	6.4×10^5	29	5.2×10^5
15	6.4×10^5	30	7.6×10^5

MATERIAL AND METHODS

A total of 30 vegetable salad samples were each collected from different street vendors in Umuahia. The samples were already packaged but were put in clean polythene bags and immediately brought to the Department of Microbiology laboratory, Michael Okpara University of Agriculture Umudike for analyses.

Isolation, enumeration and identification

The samples were homogenized, serially diluted to 10^4 and cultured on MacConkey agar using the pour plate method. These were incubated aerobically at 37°C for 24h. After incubation, *E. coli* was identified on the basis of colony characteristics and biochemical tests which included Gram's stain, motility, carbohydrate fermentation (glucose, sucrose, and lactose), indole and citrate utilization tests. Enumeration of the *E. coli* isolates was done using a colony counter (Gallencamp).

Presumptive identification

Cultures that were Gram negative rod, motile, lactose, glucose and sucrose fermenter with gas production, indole positive and citrate negative were identified as *E. coli*

Antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion method as described by Bauer *et al.*, (1966) and modified by the National Committee for Clinical Laboratory

Table 2. The antimicrobial sensitivity pattern of the *E. coli* isolates

SAMPLE	OFL	AUG	TET	AMX	COT	NIT	GEN	NAL
1	26 S	Nil R	8 R	Nil R	Nil R	Nil R	17 S	Nil R
2	30 S	Nil R	Nil R	Nil R	10 R	Nil R	Nil R	20 S
3	35 S	Nil R	16 I	Nil R	10 R	27 S	25 S	24 S
4	30 S	Nil R	18 I	Nil R	10 R	22 S	22 S	23 S
5	27 S	Nil R	18 I	Nil R	24 S	23 S	21 S	24 S
6	26 S	Nil R	16 I	7 R	11 I	21 S	23 S	24 S
7	26 S	Nil R	16 I	Nil R	14 I	16 I	17 S	16 I
8	29 S	Nil R	12 R	Nil R	Nil R	7 R	18 S	18 I
9	30 S	Nil R	6 R	Nil R	5 R	22 S	20 S	19 S
10	30 S	Nil R	13 R	Nil R	8 R	19 S	21 S	20 S
11	33 S	Nil R	10 R	5 R	5 R	20 S	20 S	21 S
12	35 S	Nil R	6 R	4 R	Nil R	24 S	22 S	21 S
13	33 S	Nil R	15 I	5 R	18 S	22 S	24 S	20 S
14	32 S	Nil R	14 R	5 R	23 S	24 S	23 S	19 S
15	31 S	Nil R	4 R	Nil R	4 R	23 S	22 S	22 S
16	26 S	Nil R	6 R	Nil R	19 S	22 S	20 S	19 S
17	27 S	Nil R	17 I	5 R	24 S	25 S	22 S	23 S
18	26 S	Nil R	14 R	Nil R	19 S	24 S	20 S	23 S
19	32 S	Nil R	16 I	Nil R	23 S	24 S	24 S	23 S
20	35 S	Nil R	19 S	Nil R	27 S	25 S	26 S	25 S
21	35 S	Nil R	6 R	Nil R	Nil R	25 S	23 S	21 S
22	32 S	Nil R	10 R	Nil R	6 R	24 S	21 S	20 S
23	35 S	Nil R	9 R	Nil R	23 S	5 R	23 S	24 S
24	37 S	Nil R	9 R	Nil R	28 S	24 S	22 S	23 S
25	33 S	Nil R	15 I	Nil R	20 S	24 S	23 S	23 S
26	35 S	Nil R	20 S	Nil R	15 I	25 S	26 S	25S
27	34 S	Nil R	8 R	Nil R	25 S	11 R	25 S	24 S
28	34 S	Nil R	16 I	Nil R	23 S	20 S	21 S	21 S
29	35 S	Nil R	17 I	Nil R	25 S	24 S	23 S	20 S
30	35 S	Nil R	6 R	Nil R	23 S	20 S	22 S	21 S

Key:
OFL =Ofloxacin
NIT = Nitrofurantoin
R = Resistance
COT = Co-Trimoxazole
I = Intermediate
AMX = Amoxycillin
S = Sensitive
TET = Tetracycline
NAL = Nalidixic acid
AUG =Augmentin
GEN = Gentamicin

Standards (NCCLS) was used. A multidisc containing eight different commercially prepared antibiotics discs (Abtek Biologicals Ltd., UK) in the following concentration: Ofloxacin (30µg), Augmentin (30µg), Tetracycline (30µg), Amoxycillin (25µg), Cotrimoxazole (25µg), Nitrofurantoin (30µg), Gentamicin (10µg) and Nalidixic acid (30µg) were placed on the surface of Mueller-Hinton agar that had been streaked uniformly with a pure bacterial suspension. After incubation for 18-24h, inhibition of growth was seen as clear zones around the different discs and depending on the width of the zone in relation to the antibiotic was interpreted as intermediate or sensitive.

RESULTS

Escherichia coli was isolated from all the samples analyzed. The count ranged from 2.70×10^5 to 1.83×10^6cfu/g (Table 1).

Tables 2 and 3 show the antimicrobial sensitivity pattern of the *E. coli* isol ates and the percentage Antibiotic sensitivity/Resistance Pattern of the *E. coli* isolates. *E. coli* showed the highest sensitivity of 30 (100%) to Ofloxacin (30µg), followed by Gentamicin (10µg), 29 (96.7%), and lastly by Tetracycline(30µ g) 2(6.7%), while the highest resistance was recorded against Augmentin (30µg) and Amoxycillin (25µg) with 30(100%) respectively. Intermediate action was recorded mostly against Tetracycline (30µg) 11(36.7%) followed by Co-trimoxazole (25µg) 3(10%).

Table 3. The Percentage Antibiotic sensitivity/Resistance Pattern of the *E. coli* isolates

Antibiotics	Number and Percentage (%) of isolates		
	Sensitive (S)	Intermediate (I)	Resistant (R)
Ofloxacin (30 µg)	30 (100%)	0 (0%)	0 (0%)
Augmentin (30 µg)	0 (0%)	0 (0%)	30 (100%)
Tetracycline (30 µg)	2 (6.7%)	11(36.7%)	17 (56.7%)
Amoxycillin (25 µg)	0 (0%)	0 (0%)	30 (100%)
Co-Trimoxazole (25 µg)	15 (50%)	3 (10%)	12 (40%)
Nitrofurantoin (30 µg)	24 (80%)	1.33%)	5 (16.7%)
Gentamicin (10 µg)	29 (96.7%)	0 (0%)	1 (3.3%)
Nalidixic acid (30 µg)	27 (90%)	2 (6.7%)	1 (3.3%)

DISCUSSION

The isolation of *Escherichia coli* in all the thirty vegetable salad samples analyzed implies that the vegetables used in the preparation of the salads or the vegetable salads themselves were faecally contaminated, directly or indirectly (Table 1). This can be attributed to poor environmental and food hygiene as well as poor personal hygiene of the street vendors who sold such foods (Dawson and Canet, 1991).

The *E. coli* load of each of the samples exceeded $100g^{-1}$(Table 1). Such levels of contamination are highly unacceptable and indicate that not just *E. coli* might be present but other food borne pathogens as well (Sagoo *et al.*, 2003; Anigo *et al*., 2007; Froder *et al*., 2007). Thus, such vegetable salads are highly unsafe for consumption as they may be sources of food borne diseases especially, *E. coli* infection.

Table 2 showed that the *E. coli* isolates obtained from each of the vegetable salad samples showed resistance to at least two antibiotics especially Augmentin and Amoxycillin. This implies that the *E. coli* isolates had acquired multiple antibiotic resistances which could render them resistant to the other antibiotics with time, if care is not taken (Willey *et al.*, 2008; Pitout and Laupland, 2008). Also the ingestion of these resistant strains by humans and animals may confer resistance to the normal flora of the gastrointestinal tract.

All the E. coli isolates were sensitive to Ofloxacin. (Tables 2 and 3) This is in agreement with the works of Oluyege *et al.*, (2009) and Kebira *et al.*, (2009) which showed that all *E. coli* isolates were resistant to Ofloxacin. Most of the E. coli

isolates were sensitive to Gentamicin, Nalidixic acid and Nitrofurantoin (96.7%, 90% and 80% respectively). This agrees with the works of Kebira *et al.* 2009) which revealed 93%, 91% and 77% *E. coli* sensitivity to Gentamicin, Nalidixic acid and Nitrofurantoin respectively, and Akond *et al. (*2009) which revealed 100% *E. coli* sensitivity to Gentamicin. The low resistance of the organism to these antibiotics might be as a result of its cost, route of administration and their not being easily gotten from chemist shops like some of the most abused drugs.

The resistance of *E. coli* isolates to Augmentin and Amoxycillin was in agreement with the work of Oluyege *et al.* (2009) which showed 95.7% and 100% *E. coli* resistance to Augmentin and Amoxycillin respectively. This may be as a result of excessive and inappropriate use of these antibiotics and confirms the *E. coli* isolates as ESBL producers. Hence, they are not the drugs of choice in the treatment of *E. coli* infections.

The result showed that over 50% of the *E. coli* isolates were resistant to Tetracycline. (Table 3) This is agreement with the work of Akond *et al.* (2009) which revealed 52% *E. coli* resistance to it, and Zinnah *et al.* (2008), which had 70% *E. coli* resistance to it. Also, fifty (50) % of the isolates were resistant to Co-Trimoxazole. This disagrees with the work of Oluyege *et al.*, (2009) which showed 30.4% *E. coli* resistance to it. This may be due to the abuse of this antibiotic as it can be gotten easily from any chemist shop (Patent medicine dealers) and also the fact that it is cheap. However, this result is of public health concern since the antibiotics concerned are used in the treatment of more severe infections.

A number of *E. coli* isolates showed intermediate reaction to Tetracycline (36.7%), Co-Trimoxazole (10%), Nalidixic acid (6.7%) and Nitrofurantoin (3.3%). This may be an intermediate phases for the conversion of *E. coli* isolates from sensitive to resistant form.

This study confirms the isolates as ESBL producers although they are still sensitive to some of the higher antibiotics (Nalixidic acid, Gentamicin and Nitrofurantoin). Urgent action is therefore needed in the fight against antibiotic resistance, else it will continue to pose a problem not only to our medical and health experts, but to the world at large.

CONCLUSION

The level of contamination of the analyzed samples is alarming considering the fact that its presence is an indicator of the presence of more pathogenic organisms. The sensitivity/ resistance pattern also is a serious indicator that all is not well and that something must be done fast either in the way of hygiene or prevention of abuse of the antibiotics since this is one of the factors that confers resistance to the microorganisms.

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