Multiple Antibiotic Resistant Index and Plasmid of *Escherichia coli* in Beef in Ekpoma

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ABSTRACT: *Escherichia coli (E. coli),* a member of the family Enterobacteriacease, has been known to cause infection in man and animals. Sixty samples of beef were collected randomly from Ekpoma market, in Edo State. *E. coli* were isolated from 40/60 (66.7%) of samples. All isolates were sensitive to ciprofloxacin, 78% were resistant to tetracycline, 73% resistant to cefuroxime, 43% resistant to cotrimoxazole, 35% to nalidixic acid. All isolates were 100% resistant to chloramphenicol and ampicillin. Nine different resistant patterns were observed. Multiple antibiotics resistance was observed among isolates. Seven of the nine resistant patterns observed were screened for plasmid, it was observed that they haboured one or more plasmid that was of sizes 23.13 kb and 4.361 kb. This study point to the fact that farmers should exercise caution in the use of antibiotics in farms. [The Journal of American Science. 2006;2(3):16-18].

Keywords: Multiple Antibiotic Resistant Index; infection; plasmid

INTRODUCTION

Meat is defined as the flesh of animals, which are suitable for use as food (Forest *et al*., 1975). Meat also include the parts of livestock muscles that is skeletal or is found in the tongue diaphragm, heart, oesophagus with or without the accompanying of over laying fat and the portion of the bone, skin sinew, nerves and blood tissue that normally accompany the muscle tissue and are not separated from it in the process of dressing (Billy, 1996).

The quality of meat is affected by the physiology of the animal and handling of animal before slaughtering. The environmental conditions also play a vital role in determining the appearance of fresh meat (Ikeme, 1990). The main source of spoilage bacteria with which fresh meat become contaminated is the hide of cattle or the skin of hogs. It has been shown that cattle hide and hogs, skin may contain millions of bacteria (anaerobes and aerobes) per square centimeter of surface in the area where the stick knife is inserted.

Contamination caused by hides, skin and intestinal tract of animals get into meat during bleeding, handling, and processing of animal meat (like slaughtering, scalding, eviscerating and washing). Meat is an ideal culture medium for many organisms because it is high in moisture and rich in vitamin and nitrogen compound. Some organisms involved in meat contamination are *Acetobacter species, Acinetobacter species , Citrobacter species, Proteus species, Salmonella species, Escherichia species, Shigella species, Staphylococcus species,* and *Streptococcus species* (Alonge, 1982).

The wide spread of antibiotic by farmers have resulted in increasing antibiotic resistance. The aims of this paper are to determine the prevalence of multi-drug resistant *Escherichia coli (E. coli)* isolates from raw beef and to determine the plasmid profile of the isolates.

MATERIALS AND METHODS

Sixty beef samples were randomly purchased from different sites in Ekpoma market, Ekpoma, Edo State. Specimen comprises of stomach, liver, heart muscles and lungs. They were transported in sterile cellophane to the laboratory.

One gram of the meat were weighed and suspended in 9 ml of sterile nutrient broth. 10-fold dilution was made. 0.1 ml of each dilution was plated on Nutrient agar and MacConkey agar and incubated aerobically at 37^0C for 24 h. *E. coli* was identified with conventional biochemical tests (Forbes *et al*, 1998).

Susceptibility of the organisms to antibiotics were tested by the disk diffusion method on Brain Heart Infusion medium according to Bauer *et al* (1966). Antibiotics used include Nitrofurantion, Cefuroxime, Norfloxacin, Cotrimozaxole, Gentamycin, Tetracycline,

Ciprofloxacin, Nalidxic acid, Chloramphenicol and Ampicillin.

The method of Birboin and Doly (1979) was used to screen for plasmid. The DNA was electrophoresed on 0.8% agarose gel, stained with ethidium bromide, visualized by UV trans-illumination. Molecular weights were calculated based on molecular weight standard.

RESULTS

Out of the sixty samples from different parts of beef, *E coli* were isolated from 40 samples (66.7%). Fifteen from stomach, eight from muscle and liver, five from heart and four from lungs (Table 1).

All isolates were resistant to ampicillin and chloramphenicol, 90% resistance to nitrofurantion, 78% resistance to tetracycline, 73% to cefuroxime, 43% resistance to cotrimozole, 35% resistance to nalidixic acid, 33% resistance to norfloxacin, 28% resistance to gentamycin, while all isolates were sensitive to ciprofloxacin (Table 2).

Multiple resistances were observed all through the study. Nine different resistance patterns were observed (Table 3).

These different resistance patterns isolated were screened for plasmid, they were found to harbour one or more plasmid of sizes 23.1 and 4.36 kb (Table 4).

Table 1. Isolation of *E. coli* from various beef samples

Meat	Number examined	Number positive
Muscles	12	8+ve
Liver	14	8+ve
Heart	10	5+ve
Lungs	07	4+ve
Stomach	17	15+ve

Table 2. Percentage Antibiotics Resistance of *E. coli* isolates from beef

Antibiotics	No. of Resistant Strain	Percentage Resistance
Nitrofurantion	36	90
Cefuroxime	29	73
Norfloxacin	13	33
Cotrimozaxole	17	43
Gentamycin	11	28
Tetracycline	31	78
Ciprofloxacin	0	0
Nalidixic	14	35
Chloramphenicol	40	100
Ampicillin	40	100

Table 3. Antibiotic Resistance Patterns of *E. coli* from beef

Pattern of Resistance	Frequency of Occurrence	Percentage
Cf,NB,Gn,Te,Na,C,Am	4	10
N,Cf,Co,Gn,Te,C,Am	4	10
N,Cf,Na,C,Am	4	10
N,Te,C,Am	2	5
N,Cf,Te,C,Am	8	20
N,Cf,NB,Co,Gn,Te,C,Am	3	7.5
N,Cf,C,Am	5	12.5
N,Cf,NB,Co,Te,Na,C,Am	6	15
N,Co,Te,C,Am	4	10

Key: Cf = Cefuroxime, NB = Norfloxacin, Gn = Gentamycin, Te = Tetracycline, Na = Nalidixic acid, C = Chloramphenicol, Am = Ampicillin, N = Nitrofurantion

Table 4. Plasmid containing *E.coli* isolates and their Molecular Weight in Kilobase

Resistance Pattern	Number of Plasmid	Plasmid size (kb)
N,Cf,NB,Co,Gn,Te,C,Am	1	23.1
Cf,NB,Gn,Te,Na,C,Am	1	23.1
N,Cf,C,Am	2	23.1, 4.36
N,Co,Te,C,Am	1	4.36
N,Cf,NB,Co,Te,N,C,Am	2	23.1, 4.36
N,Cf,Co,Gn,Te,C,Am	1	23.1
N,Cf,Te,C,Am	1	23.1

DISCUSSION

The use of antibiotic in food animal product has been controversial from the standpoint of health workers and food consumers. Although the drugs afford the food animal industry the ability to generate affordable products, the resulting increases in antibiotics resistance bacteria associated with their use is both well documented and problematic (Langlios and Dawson, 1999).

From this study, it was observed that *E. coli* isolates were resistant to commonly used antibiotics in clinical medicine. Multiple antibiotic resistance was observed in this study. There has been increasing concern of the possible development of resistance to antimicrobial agents in the *Enterbacteriaceae*, especially *E. coli*, as a result of the use of such agent in animal feed (Willis, 2000). This resistance is quite high and it could be as a result of the widespread use of such agent in animal feeds in Nigeria. It could also be from drinking water. Calomiris *et al*. (1984) isolated multi resistance bacteria from drinking water. Another possible source of the resistance isolates may be from the hands, clothing of butcher/sellers and buyers; and knives or water used in washing the beef.

Plasmid profile analysis of the isolates revealed that most of the strains that were resistant to 4 antibiotics harboured plasmid.

The plasmid isolated were 23.1 kb and 4.36 kb. This was similar to what was observed by Smith *et al*. (2003). They isolated 3 plasmids from cow, which were 23.13, 4.361 and 0.564 kb.

Beef is commonly consumed in Nigeria and from this study; it would be observed that eating beef that is not properly cooked could be a source of multi drug resistant bacteria.

The isolates were highly resistant to commonly used antibiotic. Farmers should be advice on the dangers of using antibiotic in feeds. Since multi drug resistance can be transferred from animals to human and also taking into account the limited choice of antimicrobial agents for treatment and the possibility of transfer of resistance to other enteric organism.

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REFERENCES

1. Alonge, O.O. (1982). Bacteria causing beef spoilage in a meat shop in Ibadan. *Nig. J. Microbiol. 22*:168-172.
2. Bauer, A.W.; Kirby, W.M.; Sherris, J.C. and Turck, M. (1966). Antibiotic susceptility testing by a standard single disc method. *Am. J. Clin. Pathol. 45*:493-496.
3. Billy TJ (1996). What is meat?
3. http/www.fsis.usda.gov/OPPDE/FSISDirective/FSISDir7/60-2.
4. Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res. 7:* 1513- 1523.
5. Calomiris, J.; Armstrong, L. and Seidler, J. (1994). Association of metal tolerance with multiple antibiotics resistance of bacteria isolated from drinking water. *Appl. Environ. Microbiol. 47:*1238- 1242.
6. Forbes. B.A. (1998). Enterobacteriaceae. Bail and Scott's Diagnostic Microbiology, Baltimore, Mosby.Pp. 509-526.
7. Forest, J.C.; Aberle, E.D.; Hedrick, B.B.; Judge, M.D. and Markel, R.A. (1995). *Principle of Meat Science*. W.H. Freeman and Co. San Francisco. Pp.500.
8. Ikeme, M. (1990). *Meat Science and Technology.* African Feb Publishers ltd. Pp. 370.
9. Langlois B.E. and Dawson, K.A. (1999). Antimicrobial resistance of Gram-negative enteric bacteria from pigs in a non-antimicrobial exposed herb before and after transportation. *J. Food Protect. 62*: 797- 799.
10. Smith, S.; Aboaba, O.O.; Odeigha, P.; Shodipo, K.; Adeyeye, T.A.; Ibrahim, A.; Adebiyi, T.; Onibokun, H. and Odunukwe, N.N. (2003). Plasmid profile of *Escherichia coli* 0157:H7 from apparently healthy animals. *Afr. J. Biotechnol. 2 (9):* 322- 324.
11. Willis, C. (2000). Antibiotics in the food chain: their impact on the consumer. *Rev. Med. Microbiol. 11:* 153-160.