ISOLATION, IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY OF *Escherichia coli* ISOLATED FROM RAW MEAT FROM MODAKE AND ILE-IFE, OSUN STATE. NIGERIA

ABSTRACT

The study reported isolation, identification and antibiotic susceptibility of Escherichia coli isolated from raw meat from Modakeke and Ile-ife, Osun State, Nigeria, with the view to determining the antibiogram profiling of the bacterial isolates.

In this study, five samples of fresh meat were collected from different abattoirs in Ile-Ife and Modakeke, Osun State. Isolates of *Escherichia coli* were isolated, identified morphologically based on their growth on nutrient agar and subjected to antibiotic susceptibility test on Mueller Hinton agar. The mean microbial load from the meat samples ranged from 8.85 x 10^2cfu/ml to 5.77 x 10^4cfu/ml. A total of 69 *E. coli* isolates were recovered from the meat sampled. All the isolates appeared cream, translucent, entire, convex, circular, smooth and glistering. The isolates were identified as Gram negative rods, non-motile, lactose fermenters, positive for indole test and negative for citrate utilization test. All the *E. coli* isolates were resistant to augmentin, ceftriazone, nitrofurantoin and gentamycin. 98.55% of *E. coli* isolated was resistant to amoxillin and the least resistant was recorded in ofloxacin (8.70%). However, 91.30% of the *E. coli* isolates was sensitive to ofloxacin, 81.16% to ciprofloxacin and 36.23% to pefloxacin while none was sensitive to augmentin, ceftriazone, nitrofurantoin and gentamycin. A total of 19 different multiple antibiotic resistance patterns were observed among the isolates. Thirty isolates (43.48%) showed multiple antibiotic resistance to 5 and 10 different antibiotic types each.

The study concluded that occurrence of *E. coli* infection is high in the study area with high level of multiple antibiotic resistance.

Key words: Meat, *E.coli*, Gram negative rods, antibiotics and multiple antibiotic resistance

1.0 INTRODUCTION

Meat is significant in human nutritional needs. Meat is not only rich in protein but also has complete and balanced essential amino acids (Soepranianondo *et al*., 2019). The damage rate of meat depends on the number of initial microbes. The beef meat will be damaged more quickly if it has a higher number of initial microbes (Soepranianondo *et al*., 2019). Meat, like any food, can also transmit certain diseases, but complete cooking and avoiding recontamination reduces this possibility. Minced beef can be contaminated during slaughter with disease-causing *Escherichia coli* originating from the intestinal tract or hide if proper precautions (such as steam pasteurization or organic acid treatment) are not taken (Gayathri *et al*., 2015).

Typically, the meat of healthy animals is sterile; however, contamination may occur during the various stages of slaughter, preparation, and transportation (Ercolin *et al*., 2006). A variety of

microbes can contaminate meat, although different species may become dominant depending on factors that include pH, oxygen, water availability, and storage temperatures (Ercolin *et al*., 2006; Weigand *et al*., 2007). Aside from spoilage, infection of meat can be pathogenic to the consumer. This study is aimed at isolating *E. coli* isolates from raw meat (beef), characterising them and determining their antibiograms.

2.0 Methodology:

2.1 Sample collection

The raw meat samples were collected directly from different abattoirs located in Ile-Ife and Modakeke. The samples were collected in small clean bowls.

2.2 Method of isolation

Pour plate method was used for the isolation as well as serial dilution. Small pieces were cut from the different samples of meat and each piece was inserted in test tubes containing 10ml of freshly prepared nutrient broth. The serial dilution was then carried out. Different Petri dishes were labelled according to the dilution factor of each meat sample.

EMB agar was poured into the plates containing the inoculums and the plates were left to set. The plates were incubated at 37^0C for 18-24hours.

2.3 Growth on Eosin Methylene Blue agar

The incubated plates were checked for growth and colonies with green metallic sheen were picked and sub cultured on already prepared plates of EMB agar by streaking. The plates were labelled properly and then incubated for 18-24hours. Pure and distinct colonies with green metallic sheen were picked for further tests.

2.4 Identification of isolates

Preliminary identification of bacterial isolates was performed using colonial and morphological characteristics of each isolates. Bacterial isolates were further characterized by physiological characteristics through biochemical reactions of the bacterial isolates to some reagents and media. Bacterial isolates were identified using the Bergey's Manual of Determinative Bacteriology (Olutiola *et al*., 2000).

2.5 Antibiotic susceptibility test

This was used to determine the antibiotic susceptibility pattern of the *E. coli* isolates. Antibiotic susceptibility of the *E. coli* isolate was done using the Kirby-Bauer's disc diffusion method and interpreted according to the guidelines of Clinical Laboratory Standard (CLSI, 2013). The antibiotics (Oxoid Ltd, UK) of known concentration; Augmentin (30 µg), Ceftriazone (30 µg), Nitrofurantoin (300 µg), Gentamycin (10 µg), Cotrimozazole (25 µg), Ofloxacin (10 µg),

Amoxicillin (25 µg), Ciprofloxacin (10 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Cefixime (5 µg), Choramphenicol (30 µg), Streptomycin (10 µg), Erythromycin (5 µg) and Pefloxacin (5 µg) were firmly placed on the agar plates previously seeded with the test organisms and incubated at 37^{o}C for 18-24 h. After incubation, the diameter of the zones of inhibition were measured with a transparent calibrated ruler to the nearest millimeter and recorded. The results were recorded as resistant, intermediate and susceptible according to the guideline of Clinical Laboratory Standard Institute (CLSI, 2013).

3.0 RESULTS

3.1 Microbial Load in Meat Samples

Table 1 shows the mean colony count of *E. coli* isolated from the 5 meat sampled. The mean microbial load ranged from 8.85 x 10^2cfu/ml to 5.77 x 10^4cfu/ml. *E. coli* were isolated from the growth on the plates after incubation at 37°C for 24 hours.

Table 1: Mean Colony Count of *E. coli* Isolates (cfu/ml)

Sample Code	Mean Colony Count (cfu/ml)
MSA	8.97 x 10^3
MSB	8.85 x 10^2
MSC	1.94 x 10^4
MSD	1.0 x 10^3
MSE	5.77 x 10^4

Key: MSA=Meat Sample A, MSB=Meat Sample B, MSC=Meat Sample C, MSD=Meat Sample D, MSE=Meat Sample E

3.2 Frequency of Antibiotic Susceptibility of the *E. coli* isolated

Table 2 shows the frequency of the antibiotic susceptibility of the isolates. 100% of the *E. coli* isolates were resistant to augmentin, ceftriaxone, nitrofurantoin and gentamycin. 98.55% of *E. coli* isolated was resistant to amoxillin and the least resistant was recorded in ofloxacin with 8.70%. However, 91.30% of *E. coli* was sensitive to ciprofloxacin, 81.16% of the *E. coli* was sensitive to ciprofloxacin, and 36.23% was sensitive to pefloxacin while none was sensitive to augmentin, ceftriaxone, nitrofurantoin and gentamycin.

Table 2 Frequency of Antibiotic Susceptibility of the *E. coli* Isolated

Antibiotics with disc potency	Frequency (%)			
	Resistance	Intermediate	Susceptibility	Total
Augmentin (30µg)	100	0	0	100

Ceftriaxone (30µg)	100	0	0	100
Nitrofurantoin (200µg)	100	0	0	100
Gentamycin (10µg)	100	0	0	100
Cotrimoxazole (25µg)	81.16	14.49	4.35	100
Ofloxacin (5µg)	8.70	0	91.30	100
Amoxillin (25µg)	98.55	1.45	0	100
Ciprofloxacin (10µg)	18.84	20.29	60.87	100
Tetracycline (30µg)	94.20	5.80	0	100
Pefloxacin (5µg)	63.77	21.74	14.49	100

3.3 Multiple Antibiotic Resistance Patterns of the *E. coli* isolated

The multiple antibiotic resistance patterns of the 69 isolates are displayed in Table 3. Thirty isolates (43.48%) were resistant to 7 different antibiotics and this was the highest multiple resistance pattern. The lowest multiple resistance patterns were recorded for 5 and 10 different antibiotics with one isolate each (1.45%). The total multiple resistance patterns recorded was 19.

Table 3: Multiple Antibiotics Resistance Pattern of *E. coli* isolated from beef

Number of antibiotics	Antibiotic resistance pattern	Frequency (%)	Total number (%)
5	AUG/CRO/NIT/GEN/AMX	1 (1.45%)	1 (1.45%)
	AUG/CRO/NIT/GEN/COT/AMX	1 (1.45%)	
6	AUG/CRO/NIT/GEN/CPX/TET	1 (1.45%)	6 (8.70%)
	AUG/CRO/NIT/GEN/AMX/TET	4 (5.80%)	
	AUG/CRO/NIT/GEN/COT/AMX/TET	24 (34.78%)	
	AUG/CRO/NIT/GEN/AMX/TET/PFX	2 (2.90%)	
	AUG/CRO/NIT/GEN/AMX/CPX/TET	1(1.45%)	30 (43.48%)
	AUG/CRO/NIT/GEN/AMX/TET/PFX	1(1.45%)	
7	AUG/CRO/NIT/GEN/COT/AMX/PFX	1(1.45%)	
	AUG/CRO/NIT/GEN/AMX/TET/PFX	1(1.45%)	
	AUG/CRO/NIT/GEN/COT/AMX/TET/PFX	18 (26.09%)	
	AUG/CRO/NIT/GEN/AMX/CPX/TET/PFX	3 (4.35%)	23 (33.34%)
8	AUG/CRO/NIT/GEN/OFL/AMX/TET/PFX	1 (1.45%)	
	AUG/CRO/NIT/GEN/COT/AMX/CPX/TET	1 (1.45%)	

	AUG/CRO/NIT/GEN/COT/AMX/CPX/TET/PFX	4 (5.80%)	
	AUG/CRO/NIT/GEN/COT/OFL/AMX/CPX/PFX	1 (1.45%)	
9	AUG/CRO/NIT/GEN/OFL/AMX/CPX/TET/PFX	1 (1.45%)	8 (11.60%)
	AUG/CRO/NIT/GEN/COT/OFL/AMX/TET/PFX	2 (2.90%)	
10	AUG/CRO/NIT/GEN/COT/OFL/AMX/CPX/TET/PFX	1 (1.45%)	1 (1.45%)
	TOTAL	69 (100.02%)	69 (100.02%)

Key: AUG=AUGMENTIN, CRO=CEFTRIAXONE, NIT=NITROFURANTOIN

GEN=GENTAMYCIN, COT=COTRIMOXAZOLE, OFL=OFLOXACIN

AMX=AMOXILLIN, CPX=CIPROFLOXACIN, TET=TETRACYCLINE

PFX=PEFLOXACIN

4.0 Discussion

The presence of *E. coli* in the meat sampled coupled with the high microbial load indicates that the meat sold in the market places were highly contaminated, dangerous and have various health consequences. The presence of bacteria in meat has been widely reported from different parts of the world (Holds *et al*., 2007; Kinsella *et al*., 2008). Some groups recognized the presence of bacteria especially gram-negative organisms as an indicator of open air meat spoilage

The results presented showed the antimicrobial susceptibility and resistance levels of the isolates to antibiotics. According to the results obtained from this study, *E. coli* showed a higher percentage of resistance to augmentin 100%, ceftriaxone 100%, nitrofurantoin 100%, gentamycin 100%, cotrimoxazole 81.16%, amoxicillin 98.55% and tetracycline 94.20%, while it showed a higher percentage of sensitivity to ofloxacin 91.30%, ciprofloxacin 81.16% and pefloxacin 36.23%. This contradicts the report by Shekh *et al*. (2012) who reported the sensitivity of *E. coli* to Gentamycin. Based on this statistics, ofloxacin and ciprofloxacin may be used for the effective treatment of *E. coli* infections.

All isolates showed multiple resistance to a number of the antibiotics ranging from 5 to 10 antibiotics. This implies that if these antibiotics are used, they will not be effective against the microorganism. If the antibiotics are even combined, there will be little or no effect against the microorganism. The use of these antibiotics will make the organisms build resistance to the antibiotics over time.

The multiple resistance of the organisms may arise from cross contamination between animals to animals or between animals and man. It could also be caused by the use of antibiotics for prophylaxis. It should be noted that misuse of these antibiotics may lead to development of

resistant strains of *E. coli* to them. These misuses could be excessive use of antibiotics in animal husbandry practices, uncompleted dosage of antibiotics etc.

Antibiotics have been helpful in combating bacterial infections since their discovery. The use of antibiotics leads to a healthier life and prevents the death or incapacitation of patients. They not only help to cure and prevent infections but also aid growth.

4.1 Conclusion

The meat sold in market places are highly contaminated from different sources and could be causes of infection to the general public and if proper measures are not taken, could lead to an epidemic.

The use ofloxacin and ciprofloxacin which were the antibiotics to which *E. coli* showed a high percentage of sensitivity to, should be encouraged for the treatment of *E. coli* infections. The isolates were resistant to many antibiotics and can cause high mortality in cows.

Indiscriminate use of antibiotics should be avoided because it may lead to the development of drug-resistant strains of bacteria**.** There must also be a regulated use of antibiotics for livestock so as to ensure that the resistant strains do not become established.

When an infection shows a sign of resistance to a particular drug, it is advisable that the use of the drug should be discontinued. To prevent the danger of drug resistance, two drugs can be used simultaneously. This is called synergism in which case each of these drugs cannot be as effective on their own as they would be if used together.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

CLSI. (2013). Performance standards for antimicrobial susceptibility testing; eighteen informational supplements 23: 62-64.

Cohen N., Ennaji H., Bouchrif B., Hassar M., Karib H.: Comparative study of microbiological quality of raw poultry meat at various seasons and different

slaughtering process in Casablanca (Morocco). J. Appl. Poultry Res. 2007, 16, 502-508.

CSIRO (The Commonwealth Scientific and Industrial Research Organisation) Food and Nutritional Sciences, Meat technology update: How useful are microbiological criteria for fresh meat? Newsletter 02/1:2002. Clayton South-Australia 2002.

Ercolin, D., Russo, F., Torrieri, E., Masi, P. and Villani, F. (2006) Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl. Environ. Microbiol., 72(7): 4663-4671.

Gayathri Thanigaivel, AnuSwedha Anandhan (2015).Isolation and Characterization of Microorganisms from Raw Meat Obtained from Different Market Places in and Around Chennai *Journal of Pharmaceutical, Chemical and Biological Sciences*; 3 (2): 2 9 5-301

Holds G, Pointon A, Lorimer M, Kiermeier A, Raven G, sumner J. (2007). Microbial profiles of carcasses and minced meat from Kangaroos processed in South Australia Int. J. Food. Microbiol. 123: 88-92.

Kinsella, K. J, Prendergast, D. M, McCann, M. S, Blair, I. S, McDowell, D. A, Sheridan, J. J (2008). The survival of

Matias, B. G., Pinto P. S. A., Cossi M. V and Nero, L. A. (2010). *Salmonella* spp. and hygiene indicator microorganisms in chicken carcasses obtained at different processing stages in two slaughter houses. Foodborne Pathog. Dis. 7, 313-318.

Ray, B. (2004): Fundamental Food Microbiology. CRC Press, Boca Raton, Florida. *Salmonella enteric* serovar Typhimurium DT 104 and total viable counts on beef surfaces at different relative humidities and temperatures J. App. Microbiol. 106: 171-180

Schroeder, C. M., White D. G and Meng, J. (2004): Retail meat and poultry as a reservior of antimicrobial-resistant Escherichia coli. *Food Microbiology* 21, 249-255.

Shekh, C.S, Deshmukh V. V, Waghamare, R. N, Markandeya, N. M and Vaidya, M. S (2013) Isolation of pathogenic E. coli from buffalo meat sold in Parbhani city, Maharashtra, India, Vet.

Soepranianondo, K, Wardhana, D. K, Budiarto, Diyantoro (2019) Analysis of bacterial contamination and antibiotic residue of beef meat from city slaughterhouses in East Java Province, Indonesia, Veterinary World, 12(2): 243-248.

Weigand, I., Geiss, H., Mack, D., Sturenburg, E. and Seifert, H. (2007) Detection of extended-spectrum beta-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. J. Clin. Microbiol., 45(4): 1167-1174.

Zhao C., Ge B., Villena de J., Sudler R., Yeh E., Zhao S., White D. G., Wagner D., Meng J.: Prevalence of Campylobacter spp., Escherichia coli, and Salmonella serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. Appl. Environ. Microbiol. 2001, 67, 5431-5436