Original Research Article

IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF PATHOGENIC *Escherichia coli* IN RAW BEEF SOLD IN OTUOKE MARKET, BAYELSA STATE, NIGERIA

ABSTRACT

Meat and meat products are a very important category of food consumed widely to meet the nutritional requirements of humans. Due to the high nutrient and moisture content of meat, they readily support the growth of diverse microorganisms. The consumption of these products, when contaminated by pathogenic microorganisms, can pose a risk to health leading to possible food poisoning, with *Escherichia coli* being the most implicated organism. Thus, this research focused on the isolation of *Escherichia coli* from raw beef (*Bos t*aurus) retailed in Otuoke market, its biochemical identification, pathogenicity testing and antibiogram. Total 90 raw beef samples were collected from three retail points (30 samples per point) over 3 months and cultured on Eosin-Methylene Blue (EMB) agar for the elucidation of *E. coli.* Conventional biochemical tests were performed on isolates to identify *E. coli*. The isolates were subjected to Congo-red assay to test for pathogenicity and the agar-diffusion assay to test sensitivity to commonly utilized antibiotics. A total of 51 samples (56%) were contaminated with *E. coli* of which 24 samples (26.6%) had to mean aerobic bacteria counts greater than 5.0 log cfu/gm which is above the European Commission Regulation No. 2073/2005 guideline for fresh beef. All *E. coli* isolates tested positive to the Congo-red assay thus indicating their potential pathogenicity. Antimicrobial sensitivity assay indicates the resistance of isolates to Tetracycline (60%), Erythromycin (80%) and Amoxicillin (85%). However, the isolates were sensitive to Nitrofurantoin (90%), Gentamicin (78%) and Ciprofloxacin (82%). The results obtained highlights the high level of contamination by potentially pathogenic *E. coli* in retailed fresh meats which are highly resistant to some of the commonly used antibiotics. The results obtained from this study is of public health significance as it indicates possible risks of infection to people through the consumption of inadequately cooked meat or the cross-contamination of other food items by the meat products which may lead to outbreaks of food poisoning.

Keywords: Coliform; Meat; Antibiogram

INTRODUCTION

Escherichia coli (E. coli) is a normal flora of the intestinal tract of humans and many healthy animals but can be found in soil, water and vegetation [1]. Worldwide, *E. coli* is the most common causative agent of food and water-borne human diarrhoea, causing numerous deaths in children under five years of age in developing countries [2]. A variety of foods and food products are associated with food poisonings by *E. coli.* However, approximately 52% is through the consumption of beef and bovine products [3]. After consumption, *E. coli* proliferate in the human body and can cause several illnesses including hemolytic uremic syndrome, which can be fatal, with a mortality rate of 2-10% [4]. The potential pathogenicity of *E. coli* makes its presence in food of high concern. Furthermore, *E. coli* is an indicator organism whose

presence in food may indicate inadequate handling and possible contamination by faecal matter. Thus, its detection in food is important in determining sanitary indices [5].

There is an increase worldwide in the rate of resistance of *E. coli* to numerous antibiotics and this is of growing concern as this leads to complications in the treatment of infections [6]. Research has indicated the ability of normal intestinal microflora, specifically commensal *E. coli* strains to serve as a reservoir of resistance genes under specific conditions that can be transferred to other pathogenic organisms [7]. This growing problem of antibiotics resistance by *E. coli* is of public health concern especially with curli-producing *E. coli* which can secrete extracellular adhesive amyloid fibres termed curli which is utilized by the organism in adhesion to surfaces, as a structural scaffold and for cell-cell or cell-surface interactions which all promote biofilm formation and thus pathogenicity [8,9]. Because of lack of information, meat and meat products are usually not adequately cooked, therefore increasing the risk of transfer of these bacterial pathogens to the finished product or through cross-contamination with raw meat. There are far-reaching consequences in the consumption of beef products contaminated by pathogenic *E. coli* which is resistant to commonly administered antibiotics as this will contribute to disease burden, reduction of productivity due to ill health and high morbidity and mortality rates especially in rural areas without access to adequate health care services.

This study was aimed at evaluating the rate of contamination of raw beef retailed in Otuoke, Nigeria by *E. coli,* investigation of their pathogenicity and susceptibility/resistance to commonly used antibiotics.

MATERIALS AND METHODS

Samples

A total of 90 beef (*Bos taurus*) samples were collected between August and October 2018 from three retail points (30 samples per point) in Otuoke Market, Bayelsa State of Nigeria. All samples were collected randomly and placed individually in sterile plastic bags to prevent cross-contamination. They were immediately transported to the Microbiology laboratory of the Federal University Otuoke for analysis.

Isolation and conventional identification of *E. coli*

Isolation, identification and enumeration of *E. coli* were undertaken by classical plating methods [10]. 10g of each meat sample was homogenized for 2 minutes in 90ml of normal saline solution (pH 7.2). Ten-fold serial dilution was undertaken using normal saline up to 10^{-3}. A volume of 0.1ml inoculum was introduced onto Eosin-Methylene Blue (EMB) agar by the spread plate technique in duplicates. After inoculation, the plates were incubated at 37®C for 24 hours.

The occurrence of typical colonial morphology of *E. coli* on EMB agar (greenish colonies with a metallic sheen) gives preliminary identification and such colonies were counted using a colony counter. Resultant colonies were sub-cultured onto nutrient agar for subsequent biochemical testing [11].

In-vitro pathogenic test of *E. coli*

The pathogenicity of isolated *E. coli* was investigated by the Congo-red binding assay [12]. The isolates were inoculated on Congo-red medium and incubated overnight. Colonies that produced an intense brick red/orange colour were considered as positive while colonies exhibiting a greyish/white appearance were recorded as negative.

Antibiogram of *E. coli* isolates

The susceptibility of the *E. coli* isolates to commonly utilized antibiotics (Tetracycline, erythromycin, amoxicillin, nitrofurantoin, gentamicin and ciprofloxacin) was analyzed using the agar-diffusion assay [13]. Isolates adjusted to the Mac-Farlands standard was inoculated onto Muller-Hinton agar by the spread plate method in duplicates. Antibiotics discs were placed onto the inoculated plates and incubated overnight at 37®C. After overnight incubation, resulting zones of inhibition was measured with the aid of a calibrated ruler and classified as sensitive, intermediate or resistant.

Statistical analysis

Chi-square test was employed in the comparison of microbial load and antimicrobial sensitivity/resistance. P-value of <0.05 was considered to indicate a statistically significant difference.

RESULTS

A total of 51 *E. coli* isolates were obtained from the 90 beef samples. Identification of the isolates was based on their colonial morphology comprising of bluish-green colonies with a metallic sheen and biochemical testing. The average count of *E. coli* on the samples was 2.31 ± 0.12 $\log_{10}$cfu/gm. However, 24 samples (26.6%) had total counts exceeding 5.0 $\log_{10}$ cfu/gm which is above the European Commission Regulation No. 2073/2005 guideline for fresh beef [14].

Upon culturing on Congo-red media, all isolates (100%) were positive to the Congo-red binding assay, producing an intense orange/brick-red colour on Congo-red media, thus showing their potential pathogenicity.

Antimicrobial susceptibility assay showed that isolated *E. coli* had high resistance to Tetracycline (60%), Erythromycin (80%) and Amoxicillin (85%). However, sensitivity to Nitrofurantoin (90%), Gentamicin (78%) and Ciprofloxacin (82%) were recorded ($p<0.05$) by the isolates.

Table 1. Antibiogram of *E. coli* isolates

Antibiotics	Sensitive	Intermediate	Resistant
Tetracycline	20	1	30
Erythromycin	10	1	40
Amoxicillin	7	0	44
Nitrofurantoin	45	1	5
Gentamicin	39	1	11
Ciprofloxacin	41	1	9

Discussions

In the present study, *E. coli* was isolated from beef samples. The bacteria occurred in 51 (56%) of samples, thus indicating gross contamination. This value is comparable to previous studies [15] that reported values of 67.5% in retail beef from South Africa and 65% [16] in open butcher shops in Saudi Arabia but lower than a study in Nigeria which had contamination rates of 85.65% [17]. The high rate of contamination of the samples may point to the use of water that is not sufficiently clean/portable and unsanitary measures during slaughter and meat preparation [18]. Furthermore, of the contaminated samples, 24 (26.6%) had a coliform count greater than 5.0 $\log_{10}$ cfu/gm. These values exceed the

maximum permissible limit for fresh beef as set out in European Commission Regulation No. 2073/2005 [14]. This means that the beef samples are unacceptable for consumption even when adequately cooked. Such beef cannot be accepted for trade within the European Union.

Many pathogenic organisms can produce functional amyloids termed curli that is utilized in adhesion and biofilm formation, thus promoting pathogenicity [19]. The congo-red dye can be used in binding curled *E. coli* without inhibiting growth [19]. Therefore, the ability of the organisms to bind congo-red dye on Congo-red media indicates their pathogenicity. All *E. coli* isolated from retail fresh beef in Otuoke Nigeria were able to form brick-red colouration on Congo-red media, thus indicating their potential pathogenicity. This corresponds to another finding that extrapolated pathogenicity through the congo-red binding assay [18].

Results from the agar-diffusion assay using 6 antibiotics indicates rising resistance by the *E. coli* isolates to conventionally utilized first-line antibiotics. Resistance ranged from 60% in tetracycline to 80% in erythromycin and 85% in amoxicillin. These high rates of resistance are of a public health concern as organisms with resistant plasmids and genes can pass on the resistance to previously susceptible organisms [20] thus, leading to a clone of multi-drug resistant superbugs which are difficult to control. Conversely, the antibiotics; nitrofurantoin, gentamicin and ciprofloxacin proofed to be effective in the inhibiting the growth of *E. coli.* These results are in line with the findings of [1] who recorded resistance to tetracycline (72.6%), erythromycin (89.4%) and amoxicillin (86.0%) in *E. coli* isolated from clinical samples in Ethiopia while the isolates were sensitive to nitrofurantoin (96.4%), gentamicin (79.6%) and ciprofloxacin (79.6%).

Conclusion

In this study, *E. coli* was isolated from retail raw beef, using eosin-methylene blue agar. The contamination rate of the samples was 56%, with 26.6% of the samples having microbial loads above 5.0 log cfu/gm, exceeding the maximum acceptable limit as set by European Commission Regulation No. 2073/2005 for fresh beef and all the *E. coli* isolates were potentially pathogenic based on the Congo-red assay. Furthermore, the isolates proofed resistant to some of the antibiotics assayed (Tetracycline, Erythromycin and Amoxicillin), thus indicating that foodborne infections/intoxications resulting from the consumption of such beef in the raw or undercooked state may be difficult to treat using conventional antibiotics. Therefore, there is need for public health intervention and adequate sanitary measures to be employed at the point of meat slaughter and retail to minimize contamination of meat products by pathogenic *E. coli* and the use of portable water in all steps of meat processing.

Highlights

- There are potentially pathogenic *E. coli* present in raw beef retailed in the market.
- These pathogens are resistant to some commercially available antibiotics.
- Consumption of inadequately cooked beef can pose a risk to health, leading to possible food poisoning.

References

[1]. Kibret, M. and Abera, B. (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *African Health Sciences*; 11(S1): S40 - S45

[2]. Turner SM, Scott-Tucker A, Cooper LM, Henderson IR. Weapons of mass destruction: virulence factors of the global killer enterotoxigenic *Escherichia coli*. *FEMS Microbial Letters*. 2006; 263(1): 10-20.

[3]. World Health Organization. 1997. Consultation on prevention and control of enterohaemorrhagic *Escherichia coli* (EHEC) infections. World Health Organization, Geneva, Switzerland.

[4]. Law D (2000) Virulence factors of *E. coli* O157 and other Shiga – toxin-producing E. coli - A review. J. Appl. Microbiol 88: 729 – 745.

[5]. Sherikar A. T., V. N. Bachil and D. C. Thaplial (2011) Textbook of Elements of Veterinary Public Health. Indian Council of Agriculture Research, New Delhi.

[6]. Dromigny JA, Nabeth P, Juergens-Behr A, Perrier-Gros-Claude JD. Risk factors for antibiotic resistant *Escherichia coli* isolated from community-acquired urinary tract infections in Dakar, Senegal. *J Antimicrobial Chemother*. 2005;56: 236-239.

[7]. Ajiboye R., O. Solberg, B. Lee, E. Raphael, C. DebRoy and L. Riley (2009) Global spread of mobile antimicrobial drug resistance determinant in human and animal Escherichia coli and Salmonella strains causing community-Acquired infections. Clin. Infect Dis. 49(3): 365-371.

[8]. Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, et al. Role of Escherichia coli curli operons in directing amyloid fiber formation. Science. 2002; 295(5556):851–5.

[9]. Barnhart MM, Chapman MR. Curli biogenesis and function. Annu Rev Microbiol. 2006; 60:131–47.

[10]. BAM (1998) Bacteriological Analytical Manual, 8th edition publication by FDA, U.S.

[11]. Islam, M.M., Islam, M.N., Sharifuzzaman and Fakhruzzaman, M. (2014). Isolation and identification of *Escherichia coli* and *Salmonella* from poultry litter and feed. *International Journal of Natural and Social Sciences*. 1: 1-7

[12]. Agarwal R. K., K. N. Bhilegaokar, D. K. Singh, A. Kumar and R. S. Ratore (2003) Laboratory manual for the isolation and identification of food borne Pathogens. 1st Edn. ICAR, New Delhi, pp. 55-69.

[13]. Zige, D.V. and Anumudu, C.K. (2019). Prevalence and multi-drug resistance pattern of food poisoning enteric bacteria associated with diarrhoea patients. *American Journal of Biomedical and life Sciences.* 7(3):63-67

[14]. European Commission (2005). Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

[15]. Tanih NF, Sekwadi E, Ndip RN, Bessong PO (2015) Detection of pathogenic *Escherichia coli* and Staphylococcus aureus from cattle and pigs slaughtered in abattoirs in Vhembe District, South Africa. Scientific World Journal 2015: 1-8

183 [16]. Iyer A, Kumosani T, Yaghmoor S, Barbour E, Azhar E, Harakeh S (2013) *Escherichia coli* and
184 Salmonella spp. in meat in Jeddah, Saudi Arabia. The Journal of Infection in Developing Countries 11 :
185 812-818.

186 [17]. Enabulele SA, Uraih N (2009) Enterohaemorrhagic *Escherichia coli* O157: H7 Prevalence in meat
187 and vegetables sold in Benin City, Nigeria. African Journal of Microbiology Research 5: 276-279.

188 [18]. Shekh CS, Deshmukh VV, Waghamare RN, NM Markandeya and Vaidya MS (2013) Isolation of
189 pathogenic *E. coli* from buffalo meat sold in Parbhani city, Maharashtra, India, *Vet. World* 6(5):277-279

190 [19]. Reichhardt C, Jacobson AN, Maher MC, Uang J, McCrate OA, Eckart M, et al. (2015) Congo Red
191 Interactions with Curli-Producing E. coli and Native Curli Amyloid Fibers. PLoS ONE 10(10): e0140388.

192 [20]. Lerminiaux, N.A. and Cameron, A.D.S. (2019). Horizontal transfer of antibiotic resistance genes in
193 clinical environments. *Canadian journal of Microbiology* 65(1): 34-44.