Microbes and Infectious Diseases

Original article

Prevalence and antimicrobial resistance of *Escherichia coli* O157 from mutton meat in Khartoum State, Sudan

*Shahinaz Fadul Mohammed [1], Hassan Elsiddig Hag Elsafi [*2], Rayan Mohammed Abdalbage [1], Omeima Salih [3]*

1- Department of Food Safety and Veterinary Public Health, College of Veterinary Medicine, University of Bahri, Khartoum North, Sudan.
2- Ahfad Centre for Science and Technology, Ahfad University for Women, Omdurman, Sudan.
3- Public Health Department, School of Health Sciences, Ahfad University for Women, Omdurman. Sudan.

A R T I C L E   I N F O

A B S T R A C T

Background: *Escherichia coli* O157 is recognized as a major food-borne pathogen of great concern which can be transmitted by consumption of meat and meat products. **Aim:** This cross sectional study was conducted to determine the prevalence and antimicrobial resistance of *E. coli* O157 in mutton meat from butcher shops in Khartoum State, Sudan. **Methods**: A total of 500 mutton meat carcass swabs were collected in the period March-December 2021 and processed according to ISO16654:2001 methods. Six antibiotics were used to evaluate antimicrobial susceptibility by disc diffusion method according to the guidelines of CLSI. **Results:** The results showed that the prevalence of *E. coli* O157 was 1% (5/500) from mutton meat. All isolates were sensitive to amikacin 5 (100%), while all isolates were resistant to ampicillin 5 (100%). For other antimicrobials, the resistance was cotrimoxazole 4 (80%), cefuroxime and cefotaxime 3 (60%). Three out of five isolates (60%) showed multiple drug resistance (MDR). **Conclusions**: The findings of this study demonstrated that *E. coli* O157 was present in mutton meat and frequently exhibited multiple drug resistance. Proper manufacturing processes and regular antimicrobial resistance monitoring should be taken into account.

Introduction

Escherichia coli (*E. coli)* O157 is a common Shiga toxigenic *E. coli* (STEC). A significant zoonotic and foodborne pathogen in charge for the majority of serious cases of human enterohemorrhagic *E. coli* (EHEC) diseases in the world [1].

Escherichia coli O157 can cause several dangerous diseases for humans including hemolytic colitis (HC), hemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenia purpura (TTP) [2,3].

The main virulence factors of STEC are Shiga toxins, which can be divided into two subfamilies, Stx1 and Stx2, encoded by *stx1* and *stx2* genes [4].

The global burden of Shiga toxin *E. coli* (STEC) revealed that STEC causes about 2,8 million acute illnesses with an incidence rate of 43.1 cases per 100,000 person/year, 3,890 cases of HUS, 270 cases of end-stage renal disease (ESRD) and 230 deaths annually of which 10% can be attributed to *E. coli* O157. The incidence rate of STEC infections was estimated to range from 0.6 STEC illnesses per

* *Corresponding author:* Hassan Elsiddig Hag Elsafi
E-mail address: hassanelsafi@gmail.com

100,000 person-years in the African sub-regions, to 136 per 100,000 person-years in the Eastern Mediterranean sub-regions [5].

Human infections of *E. coli* O157 are associated with consumption of contaminated beef meat, raw milk, dairy products, vegetables, unpasteurized fruit juices and water [2].

Antimicrobial resistance has become a major health concern as it can lead to treatment failure which in turn can lead to death [6]. The use of antibiotics in animal production is thought to be a major contributor to the spread of antimicrobial resistant bacteria from livestock to humans [7]. Additionally, There have been increasing reports on the development of antimicrobial resistance to *E. coli* O157 [8]. Occurrence of resistant *E. coli* O157 strains isolated from animals, foods, and humans has been reported worldwide [9,10].

The lack of appropriate farm management practices and/or strict sanitary condition during the production process, handling and marketing of meat can lead to the transfer of *E. coli* O157 to the meat and its related food products, particularly, mutton meat. To our knowledge, there has been no prior study on prevalence of *E. coli* O157 from mutton meat in Sudan. This study aimed to determine prevalence and antimicrobial resistance of *E. coli* O157 from mutton meat in Khartoum state.

Material and methods

Sampling

This descriptive cross-sectional study was conducted between March-December 2021. A total of 500 mutton carcass swabs were randomly collected from Butcher shops in Khartoum State (Khartoum, Khartoum North and Omdurman). Sampling was done according to the methods of Cheesbrough [11]. The samples were kept in ice thermal box under hygienic condition and transported to the microbiology laboratory at Ahfad University for Women at the day of collection.

Isolation and identification of *E. coli* O157

Escherichia coli O157 was isolated according to the method described in ISO16654:2001

[12] with some modifications. Each swab was pre-enriched in 9 ml of modified tryptone soya broth (Oxoid, UK) and incubated at 37°C for 24 h. Enriched culture were streaked onto sorbitol MacConkey agar,supplemented with cefixime and potassium tellurite (Oxoid, UK). The plates were incubated at 37°C for 24 h and examined for typical *E. coli* O157 colonies. The suspected *E. coli* O157 (colorless, sorbitol negative colonies) were picked and sub-cultured onto nutrient agar slope and identified by indole test, growth in Kligler Iron agar and latex agglutination test (Oxoid, Uk).

Antimicrobial susceptibility testing:

The isolated *E. coli* O157 strains were tested for antimicrobial susceptibility according to guidelines Clinical and Laboratory Standards Institute (CLSI) [13] by using disc diffusion method in Muller-Hinton agar against six antimicrobial agents: amikacin (30 µg), ampicillin (10 µg), cefuroxime (30 µg), cotrimoxazol (25 µg), cefotaxime (30 µg) and tetracycline (30 µg).

Results

In present study five isolates (1%) of *E. coli* O157 were recovered from 500 swab samples of mutton meat in Khartoum State.

Testing of the five *E. coli* O157 isolates against six different antimicrobial agents showed variable results. All isolates were sensitive to amikacin 5 (100%), while all isolates were resistant to ampicillin 5 (100%). For other antimicrobials, the resistance was cotrimxazole 4 (80%), cefuroxime 3 (60%), cefotaxime 3 (60%), amikacin and tetracycline 0 (0%), respectively, while one isolate (20%) showed intermediate resistance to tetracycline **(Table 1)** and **(Figure 1)**. Three isolates showed multiple drug resistance (60%), MDR is recorded whenever, the isolate is resistant to there or more antimicrobial groups, The MDR pattern was same for the three isolates which was ampicillin, cefuroxime, cefotaxime and co-trimoxazole. (AM, CXM, CTX, COT)

Figure 1. Histogram of the percentage % resistance of *E. coli* O157 isolates to different antimicrobial agents
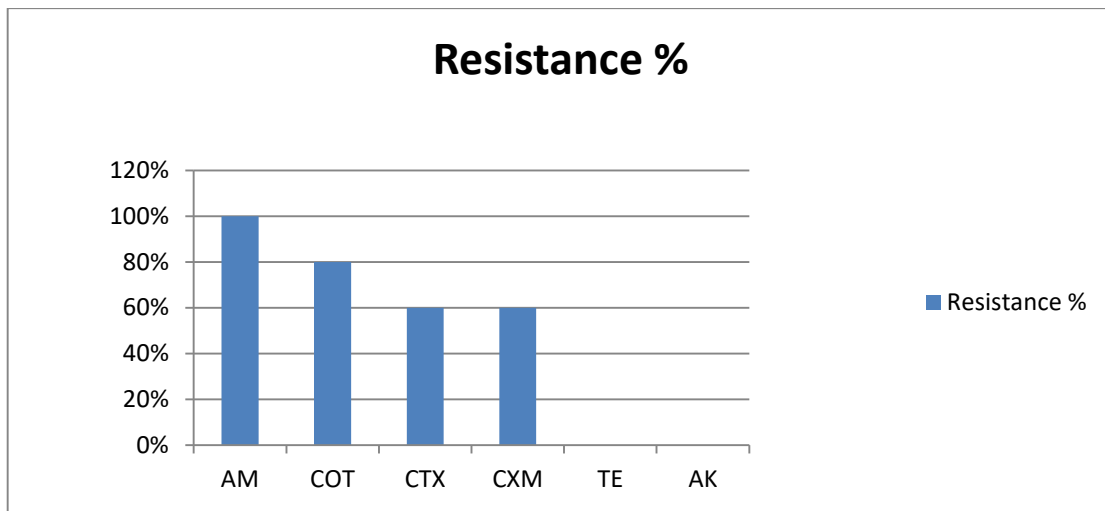
Key: AM: Ampicillin, COT: Cotrimoxazol, CTX: Cefotaxime, CXM: Cefuroxime, TE: Tetracycline, AK: Amikacin

Table 1. Antibiotic sensitivity/resistance pattern of *E. coli* O157 isolates

Antibiotic	Resistant	Intermediate	Sensitive
	No (%)	No (%)	No (%)
Amikacin	0 (0%)	0 (0%)	5 (100%)
Ampicillin	5 (100%)	0 (0%)	0 (0%)
Cefuroxime	3 (60%)	0 (0%)	2 (40%)
Cefotaxime	3 (60%)	0 (0%)	2 (40%)
Co-trimoxazole	4 (80 %)	0 (0%)	1 (20 %)
Tetracycline	0 (0%)	1 (20%)	4 (80%)

Discussion

Food borne infections are major health problem in developing countries, including Sudan. This study established the prevalence of 1% (5/500) of *E. coli* O157 from mutton meat in Khartoum State. This is the first detailed report of the prevalence *E. coli* O157 from mutton meat in Sudan.

The findings suggest the presence of *E. coli* O157 on carcass might be due to cross contamination with abattoir environment so the carcass contamination might come from potable water that the abattoir used for washing the carcass parts. The association of some risk factors with carcass contamination has been linked to feces [14,15] and skin [16]. *E. coli* O157 has been reported to spread easily onto carcass surfaces from the hide or during evisceration [14,17,18]. Meat is frequently found to be contaminated due to poor sanitary environment during slaughter, transportation and handling [19].

The findings of this study are similar to the results of **Duffy et al.** [20] in Australia who obtained a prevalence of 1.3% *E. coli* O157 from sheep meat. While **Battisti et al.** [21] in Italy 0.7% prevalence, which is lower than our results. In contrast, the results obtained are less than 2.5% of **Hiko et al.** [22] in Ethiopia and less than 4.8% of **Rahimi et al.** [23], 3.92% of **Shekarfroush et al.** [24] and 6.8% of **Jafaryan-Sedigh et al**. [25] in Iran. Also lower than 3.2% of **Messele et al.** [26] in Ethiopia. The variation in prevalence may be attributed to hygienic conditions and methods of isolation.

Antimicrobial susceptibility testing in this study showed that all isolates are sensitive to amikacin 5 (100%) and tetracycline 4 (80%) which is similar to the results of **Hiko et al.** [22] in Ethiopia and all of the isolates were resistant to ampicillin 5 (100%), co-trimoxazole 4 (80%) which is higher than the results of **Messele et al.** [26] in Ethiopia. In this study the results showed multidrug resistances was found in 60% of the isolate *E. coli* O157. The

occurrence of MDR was recorded previously ranging from 22.6% to 100% [22,27,28].

The wide spread and imprudent use of antibiotics in food animals is thought to be accountable for the emergence and wider spreading of antimicrobial resistant (AMR) bacteria in humans [29,30]. In humans, positive selection for drug resistant bacteria has also been reported in the normal microflora of exposed individuals or populations. This indicates that antibiotic resistance can be developed in both commensal and pathogenic bacterial strains and can even be transferred to other bacterial strains, including other pathogenic and environmental bacteria [31].

Conclusion

This study showed that *E. coli* O157 is present in mutton meat; our results showed high multiple drug resistance which poses risk to public health. In general, this study provides a baseline of *E. coli* O157 prevalence and antimicrobial resistance in mutton meat. Food handlers should be trained to apply good hygiene practices and good manufacturing practice to produce safe food for final consumer.

Conflicts of interest : None.

References

1- **Kim JS, Lee MS, Kim JH.** Recent Updates on Outbreaks of Shiga Toxin-Producing *Escherichia coli* and Its Potential Reservoirs. Front Cell Infect Microbiol 2020; 4, 10:273.

2- **Sima H, Ebrahim R, Hasan M.** A 3-year study of *Escherichia coli* O157:H7 in cattle, camel, sheep, goat, chicken and beef minced meat. International Conference on Food Engineering and Biotechnology 2009; 9, 162-166.

3- **Chileshe J, Ateba CN.** Molecular identification of *Escherichia coli* O145:H28 from beef in the North West Province, South Africa. Life Science Journal 2013; 10, 1171-1176.

4- **Fuller CA, Pellino CA, Flagler MJ, Strasser JE, Weiss AA.** Shiga toxin subtypes display dramatic differences in potency. Infect Immun 2011; 79, 1329-1337.

5- **Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, et al.** Global incidence of human Shiga toxin–producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog Dis 2014; 11, 447-455.

6- **CDC.** Antibiotic resistance threat in the United State. Atlanta, GA: U.S. Department of Health and Human Services, CDC 2019. Available at: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf. Accessed August 11, 2022.

7- **Hammerum AM, Heuer OE.** Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. Clin Infect. Dis 2009; 48, 916-921.

8- **Mir RA, Kudva IT.** Antibiotic-resistant Shiga toxin-producing *Escherichia coli*: An overview of prevalence and intervention strategies. Zoonosis Public Health 2019; 66, 1-13.

9- **Abdissa R, Haile W, Fite AT, Beyi AF, Agga GE, Edao BM, et al.** Prevalence of *Escherichia coli* O157: H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. BMC Infect. Dis 2017; 17(1):277.

10- **Mukherjee S, Mosci RE, Anderson CM, Snyder BA, Collins J, Rudrik JT, et al .** Antimicrobial Drug–Resistant Shiga Toxin–Producing *Escherichia coli* Infections, Michigan, USA. Emerg Infect Dis 2017; 23, 1609-1611.

11- **Cheesbrough M.** Medical Laboratory Manual for Tropical Countries. Vol. 2, Cambridge University press, U. K 2000.

12-**ISO 16654:2001.** Microbiology of food and animal feeding stuffs—Horizontal method for the detection of *Escherichia coli* O157. Geneva, Switzerland. International Organisation for Standardisation, 2001.

13-**Clinical Laboratory Standard Institute (CLSI).** Performance Standards for Antimicrobial Susceptibility Testing. 27thed. CLSI supplement M100S. Wayne, PA: CLSI 2017.

14-**Elder R, Keen J, Siragusa G, Barkocy G, Koohmaraie M, Laegreid W.** Correlation of Enterohemorrhagic *Escherichia coli* O157 prevalence in faeces, hides and carcasses of beef cattle during processing. Proc Natl Acad Sci USA 2000; 97:2999-3003.

15-**Gansheroff L, O'Brien A.** *Escherichia coli* O157:H7 in Beef Cattle Presented for Slaughter in the U.S. Higher Prevalence Rates than Previously Estimated. Proc Natl Acad Sci 2000; 97: 2959-2961.

16-**Reid C, Small A, Avery S, Buncic S.** Presence of foodborne pathogens on cattle hides. Food Control 2002; 13: 411-415.

17-**Arthur TM, Bosilevac JM, Brichta-Harhay DM, Guerini MN, Kalchayanand N, Shackelford SD, et al.** Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. J Food Prot 2007; 70: 280-286.

18-**Gun H, Yilmaz A, Turker S, Tanlasi A, Yilmaz H.** Contamination of bovine carcasses and abattoir environment by *E. coli* O157:H7 in Istanbul. Int J Food Microbiol 2003; 84: 339-44.

19-**Bassam Y, Khudaier B, Abbas A, Khulood A.** Prevalence and antimicrobial susceptibility of *E. coli* O157:H7 isolated from human and animal sources in Basrah province. J Vet Res 2012; 11: 2.

20-**Duffy LL, Small A, Fegan N.** Concentration and prevalence of *Escherichia coli* O157 and *Salmonella* serotypes in sheep during slaughter at two Australian abattoirs. Aust Vet J 2010; 88:399-404.

21-**Battisti A, Lovari S, Franco A, Di Egidio A, Tozzoli R, Caprioli A, et al.** Prevalence of *Escherichia coli* O157 in lambs at slaughter in Rome, Central Italy. Epidemiol Infect 2006; 134: 415-419.

22-**Hiko A, Asrat D, Zewde G.** Occurrence of *Escherichia coli* O157:H7 in retail raw meat products in Ethiopia. J Infect Dev Ctries 2008; 2, 389-393.

23-**Rahimi E, Kazemeini HR, Salajegheh M.** *Escherichia coli* O157:H7/NM prevalence in raw beef, camel, sheep, goat, and water buffalo meat in Fars and Khuzestan provinces, Iran. Veterinary Research Forum 2012; 3 (1) 15-17.

24-**Shekarforoush S, Tahamtan Y, Pourbakhsh A.** Detection and frequency of Stx2 gene in *Escherichia coli* O157 and O157: H7 strains isolated from sheep carcasses in Shiraz, Iran. Pak J Biol Sci 2008; 11(8):1085-1092.

25-**Jafareyan-Sedigh M, Rahimi E, Doosti A.** Isolation of *Escherichia coli* O157: H7 in sheep meats using cultural and PCR method. J Shahrekord U Med Sci 2011; 13(2)61-8.

26-**Messele YE, Abdi RD, Yalew ST, Tegegne DT, Emeru BA, Werid GM.** Molecular determination of antimicrobial resistance in *Escherichia coli* isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia. Ann Clin Microbiol Antimicrob 2017; 16(1):55.

27-**Haile AF, Kebede D, Wubshet AK.** Prevalence and antibiogram of *Escherichia coli* O157 isolated from bovine in Jimma, Ethiopia: Abattoir based survey. Ethiop Vet J 2017; 21:109-120.

28-**Mesele F, Leta S, Amenu K, Abunna F.** Occurrence of *Escherichia coli* O157:H7 in lactating cows and dairy farm environment and the antimicrobial susceptibility pattern at Adami Tulu Jido Kombolcha District, Ethiopia. BMC Vet Res 2023; 19(1):6.

29- **Molbak K.** Spread of resistant bacteria and resistance genes from animals to humans, the public health consequences. J Vet Med 2004; 51:364-9.

30- **Mooljuntee S, Chansiripornchai P, Chansiripornchai N.** Prevalence of the cellular and molecular antimicrobial resistance against *E. coli* isolated from Thai broilers. Thai J Vet Med 2010; 40:311-5.

31- **Lindsey RL, Frye SN, Thitaram RJ, Meinersmann PJ, Fedorka-Cray, Englen MD.** Characterization of multidrug-resistant *Escherichia coli* by antimicrobial resistance profiles, plasmid replicon typing, and pulsed-field gel electrophoresis. Microb Drug Resist 2011; 17:157-63.