



Research Article

Assessment of third generation cephalosporin resistant *Escherichia coli* in raw beef samples from retail market of Faisalabad

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ABSTRACT

Beef is a crucial source of protein, vitamins and daily require nutrients. To maintain its safety and quality constant monitoring is required. Because of the higher danger of foodborne diseases, meat is a major issue globally related to health because of contamination of the meat through different bacterial contaminants. *Escherichia coli* is mostly present globally in livestock, particularly cattle, which serve as a significant reservoir of this bacteria and also a major hazard source for raw beef. Third-generation cephalosporin-resistant *E. coli* is one of the major disease causing bacteria in humans. The present study was designed to assess the third-generation cephalosporin-resistant *E. coli* contaminant of raw beef. Total 120 raw beef samples were collected from different butcher shops in retail market of Faisalabad, Pakistan. For sample preparation 25g raw beef samples were separated from every 120 samples and placed all these separated 25g samples on separate ziplock plastic bags having 225ml brain heart infusion broth in it and homogenised using a stomacher at 230rpm for 2 minutes. Isolation of *E. coli* was done by plating of 100 microliter sample on MacConkey agar, for pure culture, subcultured on Blood agar and for identification Grams staining, oxidase test, Indole test, Methyl Red test, Voges Proskauer test and catalase test were performed. Kirby-Baur disc diffusion method was performed for assessment of third generation cephalosporin resistant *E. coli*, antimicrobial discs such as ceftazidime, cefotaxime, ceftizoxime, ceftriaxone, cefixime, cefpodoxime, cefdinir and ceftibuten were placed on *E. coli* swabbed Mueller Hinton Agar. Out of 120 samples 80.83% samples were contaminated with *E. coli* from which *E. coli* showed resistant to Ceftriaxone 57.7%, Ceftazidime 61.8%, Cefotaxime 55.5%, Ceftibuten 41.2%, Cefixime 53.6%, Ceftizoxime 39.2%, Cefdinir 17.5% and Cefpodoxime 25.8%. Beef samples from local market of Faisalabad were significantly ($p < 0.05$) more frequently contaminated with third generation cephalosporin resistant *E. coli*.

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Introduction

Beef is a crucial source of food that fulfils many nutrient requirements of the human body which include protein, crucial amino acids, and different vitamins (Hocquette et al., 2018). Meat which consider regular food source in rich countries while in case it cannot use as regular

one in poor states (Zerabruk et al., 2019). Those butcher shops which is not clean enough due to

which contamination of the beef results, which leads to the different disease in different people who consume it by purchasing beef from these butcher shops different bacteria also cause contamination of the beef and may spoil it and

make the beef unable to consume (Rani et al., 2017). Different nutrient which is present on beef support different kinds of bacteria which may grow and cause spoilage of beef texture and their taste as well as ruined meat, which is horrendous and unsatisfactory for consumption, causing colossal financial misfortune (Azumah et al., 2018). Poor water source also cause the defilement of beef and leads to the growth of different harmful bacteria such as *E. coli* and this bacterial contaminated beef when consume by different people around the world leads to different major disease (Li et al., 2019). Because of the high danger of foodborne diseases, meat become the major challenge related to different people health due to defilement of meat with germs from a variety of illnesses (Tesson et al., 2020). Antimicrobial resistant bacteria also spread among humans through contaminated beef, Gram negative bacteria, in particular, have become a growing threat due to their resistance to major antibiotics which regularly consume by human to treat different diseases (Zhang et al., 2020).

Different dietary patterns of different peoples around the world, mind-boggling with extensive transmission of food methodologies as expanded global development to helpless cleanliness practices are major contributing elements (Hille et al., 2017). As 3rd generation cephalosporin antibiotics are considered important to treat different human diseases caused by Gram negative bacteria (e.g. *E. coli*) but due to having greater prevalence cases related to the *E. coli* resistant to these antibiotics in many developed as well as in developing countries in raw beef become the global public health concern issue (Cohen et al., 2012).

Due to the presence of resistant to different antibiotics among bacteria treatment of different diseases become lesser and effectiveness of different antibiotics are also affected. Thus antimicrobial resistant bacteria cause major health risk in humans as well as in animals (Ventola et al., 2015). The danger of the transmission of zoonotic diseases is additionally connected to tainted meat and also those meat which having bacterial contaminant, which may lead towards the major diseases in to different people, globally, different organization around the world set rules about the contamination and different contaminant level of beef, if the meat cannot fulfill these rules then this meat consider as spoilage meat, which may be unable to eat (Heydari et al., 2020).

Despite a lack of basic facilities and conditions require to diagnose different infections, much research related to this major issue have been conducted that reveal outbreaks of diseases linked to inadequate safety and cleanliness, although majority of the data which receive from

different lab unable to tell about the true concentration of infection as different people reports show that many *E. coli* outbreaks are common in Pakistan (Siddiqui et al., 2008). *E. coli* which becomes major disease causing bacteria from food day by day, while those bacteria which comes from raw beef also cause serious consequences and these contaminants are due to the butchers who cannot disinfect their instruments, different types of bacteria may cause contamination of the beef surface which may arrive from different sources these contaminants may cause spoilage of the beef surface due to which different diseases originates and the majority of beef samples were contaminated with coliform and other pathogenic microorganisms, to limit the danger of food-borne bacterial illness butcher shops and workers must be educated and informed about proper sanitation and safe meat handling practices (Rao et al., 2021).

Materials and Methods

Sample collection

Total 120 raw beef samples were collected from different butcher shops in retail market of Faisalabad from different location such as from Ghulam Muhammad Abad, Samanabad and D-Ground (collected 40 samples from each location). The samples were kept in sample bags (sterile ziplock bags). Then the samples were transported to the cell culture laboratory, Institute of Microbiology, University of Agriculture Faisalabad, by maintaining the suitable temperature required for isolation of the bacteria.

Preparation of samples

Almost 25g raw beef samples were separated from every 120 samples and placed all these separated 25g samples on separate ziplock plastic bags having 225ml brain heart infusion broth in it and homogenised using a Stomacher at 230rpm for 2 minutes.

Isolation of *E. coli*

Hundred microliter sample from the homogenate was plated on MacConkey agar through micropipette for isolation of *E. coli* and incubated at 37°C for 18 to 24 hours. After incubation typical colonies were streaked on Blood agar for isolation of pure culture. For confirmation of *E. coli* Gram staining, Indole test, Methyl Red test, Voges-Proskauer test, Oxidase test and Catalase test were performed.

Antimicrobial susceptibility testing

Kirby-Baur disk diffusion method was used for antimicrobial susceptibility testing for third generation cephalosporins resistant *E. coli*.

Samples were inoculated on Mueller Hinton Agar in Kirby-Baur disk diffusion Method. Antimicrobial discs such as ceftazidime (30µg), cefotaxime (30µg), Ceftriaxone (30µg), cefixime (10µg), cefpodoxime (10µg), cefdinir (5µg) and ceftibutin (30µg) were placed on agar as per CLSI manual. Agar plates were incubated for 16-24 hour at 37°C. After incubation zone of inhibition were measured by using the Vernier calipers or by simple ruler. According to the CLSI guidelines if the ceftazidime, cefotaxime, ceftizoxime, ceftriaxone, cefixime, cefpodoxime, cefdinir and ceftibutin zone of inhibition measurements less than 20, 22, 21, 19, 15, 17, 16 and 17 mm respectively then the isolate will be resistant to third generation cephalosporin.

Statistical analysis

Statistical analysis was performed by using SPSS software 18 (IBM, Armonk, USA) and Percentage, Average was performed by using this software. Through Microsoft Excel (Data analysis) z-test was performed and the value of P was less than 0.05 ($P < 0.05$).

Results

Prevalence of *E. coli*

Total 120 raw beef samples were collected from different butcher shops in retail market of Faisalabad from different location such as from Ghulam Muhammad Abad, Samanabad and from D-Ground (Collected 40 samples from each location) from which 97/120 (80.83%) raw beef samples were contaminated with *E. coli* bacteria while 23 raw beef samples were negative for *E. coli* contamination. Out of 80.83% (97/120), isolated *E. coli* bacteria showed highest resistant to Ceftazidime 61.8% (60/97) while 70.1% (68/97) isolated *E. coli* were susceptible to Cefdinir. From Ghulam Muhammad Abad total 40 raw beef samples were collected from retail market, out of which 35 samples were contaminated with *E. coli* while 5 samples were not contaminated with *E. coli* bacteria which showed almost 87.5% contamination in Ghulam Muhammad Abad. In

case of Samanabad, total 40 raw beef samples were collected out of which 33 samples were contaminated with *E. coli* while seven samples were not contaminated with *E. coli* which showed almost 82.5% contamination. While From D-Ground total 40 samples were collected among which 29 samples were contaminated while 11 samples were negative for *E. coli* contamination which showed 72.5% contamination.

Antimicrobial susceptibility testing

Kirby-Baur disk diffusion method was used for antimicrobial susceptibility testing for isolation of third-generation cephalosporins-resistant *E. coli*. Total 97 isolated *E. coli* showed resistant to Ceftriaxone 56/97 (57.7%), Ceftazidime 60/97 (61.8%), Cefotaxime 54/97 (55.5%), Ceftibuten 40/97 (41.2%), Cefixime 52/97 (53.6%), Ceftizoxime 32/97 (39.2%), Cefdinir 17/97 (17.5%) and to Cefpodoxime 25/97 (25.8%). While in 15/97 (15.4%) isolated *E. coli* Ceftriaxone showed intermediate results. Ceftazidime showed intermediate results in 20/97 (20.6%) isolated *E. coli*. While Cefotaxime, Ceftibuten, Cefixime, Ceftizoxime, Cefdinir and cefpodoxime showed intermediate results in 14/97 (14.4%), 21/97 (21.7%), 19/97 (19.6%), 15/97 (15.5%), 12/97 (12.4%) and in 11/97 (11.3%), respectively. Cefdinir and Cefpodoxime both showed susceptible result to 68/97 (70.1%) and 61/97 (62.8%) respectively in isolated *E. coli*. Isolated *E. coli* showed higher resistance to Ceftazidime (61.8%), Cefotaxime (55.5%) and to Cefixime (53.6%). While isolated *E. coli* showed higher susceptibility to Cefdinir (70.1%) and to Cefpodoxime (62.8%).

Table 1: Distribution of samples and contamination of *E. coli*

Sample location	Collected samples	<i>E. coli</i> positive samples	<i>E. coli</i> negative Samples
Ghulam Muhammad Abad	n = 40	35	5
Samanabad	n = 40	33	7
D- Ground	n = 40	29	11

Total	n = 120	97	23
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Table 2: Interpretation of Kirby-Baur disc diffusion method

Antibiotics name	Susceptible	Intermediate	Resistant
Ceftriaxone (CRO) 30µg	26.8% (26/97)	15.4% (15/97)	57.7% (56/97)
Ceftazidime (CAZ) 30µg	17.5% (17/97)	20.6% (20/97)	61.8% (60/97)
Cefotaxime (CTX) 30µg	29.9% (29/97)	14.4% (14/97)	55.5% (54/97)
Ceftibuten (CTB) 30µg	37.1% (36/97)	21.7% (21/97)	41.2% (40/97)
Cefixime (CFM) 10µg	26.8% (26/97)	19.6% (19/97)	53.6% (52/97)
Ceftizoxime (CTZ) 30µg	45.3% (44/97)	15.5% (15/97)	39.2% (32/97)
Cefdinir (CD) 5µg	70.1% (68/97)	12.4% (12/97)	17.5% (17/97)
Cefpodoxime (CPD) 10µg	62.8% (61/97)	11.3% (11/97)	25.8% (25/97)

Discussion

In this research, higher contamination of *E. coli* observed in different sites of local market of Faisalabad such as from Ghulam Muhammad Abad 87.5%, Samanabad 82.5% and in D-Ground 72.5%. Overall 80.83% *E. coli* isolated from raw beef samples collected from retail market of Faisalabad (From three major sites), this bacteria identified through different biochemical tests such as Indole, Methyl Red, Voges Proskauer, Catalase and Oxidase test as well as through microscopy (Gram Staining). While out of 97/120 (80.83%) isolated *E. coli* were showed resistant to Ceftriaxone 56/97 (57.7%), Ceftazidime 60/97 (61.8%), Cefotaxime 54/97 (55.5%), Cefixime 52/97 (53.6%), Ceftibuten 40/97 (41.2%) and to Ceftizoxime 32/97 (39.2%) cephalosporin-third generation antibiotics. These results were related, to a bit, with those results elaborated by (Sebsibe *et al.*, 2020), (Dsani *et al.*, 2020), (Osemwowa *et al.*, 2021), (Momtaz *et al.*, 2011) and (Irshad *et al.*, 2020).

In raw beef bacterial defilements such as *E. coli* contamination is due to not apply safety procedure at different processing level, contaminated surface where the animal slaughter, from different instruments used to skinning of animals and from using unhygienic water which having different pathogenic forms of live (Vargas *et al.*, 2016). Bacterial contamination of raw beef may also be occurred through butcher hands as well as buyer hands which may not clean through soap or other sanitizers (Teshome *et al.*, 2020). Another important source through which *E. coli* may contaminate the raw beef is a fecal defiled water either of human or animal feces which is the major defilement source of *E. coli* bacteria (Nag *et al.*, 2021).

When the fecal defiled water used for sanitation purpose then it lead to the transfer of *E. coli* bacteria on the surface of beef. From this surface transfer to the other surface which contact with these defiled beef (Lenart *et al.*, 2020). Resistant genes transfer through a plasmid which after entering into the other bacteria

(which do not produce resistant previously) also produce resistant in human or animals for different antibiotics (Ramos *et al.*, 2020). Overuse of cephalosporin in humans can cause to develop resistant in *E. coli* after which there is no effect of these antibiotic on *E. coli* bacteria and lead to the major and harmful consequences in humans globally (Alonso *et al.*, 2017). Third-generation cephalosporin-resistant *E. coli* is one of the major issue spread through beef globally which cause major disease in children such as Diarrhea which may lead to the death of the children, while in aged person this bacteria cause harmful effect such as bacteremia (Dambrosio *et al.*, 2007) Shedding of animal pathogens in slaughter house can cause contamination of the raw beef surface through which these pathogens also transfer to human by eating (Hepburn *et al.*, 2002).

Majority of raw beef contamination in Pakistan occurred due to poor water conditions used in different butcher shops and in different slaughter houses from which defilement of different pathogenic microorganisms can occur and make it unsuitable for eating purposes (Zafar *et al.*, 2016). To overcome foodborne diseases related to beef, there is need of proper handling of beef products. In local market of Faisalabad at different butcher shops there is need to provide education and awareness related to the bacterial contamination and their origin and how to overcome the contamination of raw beef. Proper sanitation conditions are required in slaughter houses to overcome the fecal related defilements of beef. Proper storage or handling temperature is required to stop further growth of bacteria such as *E. coli*. Proper testing system for raw beef is the big requirements to stop the defilement of pathogenic microbes. There is need of proper handling of animal waste on slaughter house to prevent transmission of pathogens directly.

Conclusion

Contamination of *E. coli* in raw beef was higher in local market of Faisalabad, indicating poor working conditions in butcher shops. Cephalosporin-resistant *E. coli* was highly identified in local market of Faisalabad which indicated third-generation cephalosporin usage among livestock was higher.

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Author Contribution: All authors contributed to the study conception, design and analysis.

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