Bangladesh Journal of Veterinary and Animal Sciences, Vol. 7, No. 2, July-December 2019

ISSN 2227-6416

Bangladesh Journal of Veterinary and Animal Sciences

Journal home page: www.bjvas.cvasu.ac.bd

Research Article

Prevalence and multidrug resistance pattern of *Escherichia coli* isolated from street food

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ARTICLE INFO

Article history :
Received : 05/07/2019
Accepted : 19/09/2019

Keywords :
Escherichia coli, Street food, Chatpoti, Multi drug resistance.

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ABSTRACT

Street food is one of the most consumable food items in the countries like Bangladesh, which could pose public health concern because of their preparation and vending scheme. To address the public health issues, the present study was targeted the isolation of *Escherichia coli*, a public health threatened bacteria from a highly consumable street food items chatpoti and its various components including chatpoti salad, chatpoti water from five randomly selected markets such as Foy's lake, Jhautala Bazar, G.E.C, Bahaddarhat Bazar and Reazuddin Bazar of Chattogram City Corporation (CCC), Bangladesh. The collected samples were screened for *E. coli* using classical bacteriological examination. Chatpoti samples showed 30% individual colonies for *E. coli* whereas other ingredients of chatpoti including water and salad showed reasonably higher (50%) prevalence of *E. coli*. The collected samples were tested for antimicrobial susceptibility against 8 different selective antimicrobial agents for bacteriological examination. Here, the *E. coli* isolates recovered from chatpoti were found 100% resistant to Ampicillin, Amoxicillin, Tetracycline but was 100% sensitive to Doxycycline and Gentamicin with few of intermediate. Similarly, the *E. coli* isolated from chatpoti salad and chatpoti water were also showed similar resistance and sensitivity pattern and additionally Cephalexin resistance observed in chatpoti salad. In summary, all isolates of *E. coli* showed an evidence of multi-drug resistance which is a global alarming issue because this level of antimicrobial resistance in those bacteria isolated from food sample may act as media for antimicrobial resistance transmission and could pose zoonotic significance. Therefore, it is important to monitor the occurrence of antimicrobial resistance in other street food items, which is very essential for public health context.

To cite this paper : *Akter, H., Shaikat, A. H., Imtiaz, M. A., Islam, A., Khan, S. A. and Hassan, M. M. 2019. Prevalence and multidrug resistance pattern of Escherichia coli isolated from street food. Bangladesh Journal of Veterinary and Animal Sciences, 7 (2): 69-74*

1. INTRODUCTION

Street foods are defined as a variety of ready-to eat foods and beverages prepared and sold by vendors in streets for immediate consumption by the people. Street foods consumption is very popular in this part of the world (Mensah *et al.*, 2002). In Bangladesh, the vending shops were mostly located on the footpath (68%) irrespective of areas surveyed and a significant percentage (30%) vending carts were placed near municipal drains and around 18% near sewage (Faruque *et al.*, 2010). Therefore, microbiological study on different street foods items, drinking water and vendors hand swab samples revealed the prevalence of overwhelmingly high numbers of aerobic bacteria and coliform bacteria (Faruque *et al.*, 2010). However, antibiotics have saved the life of human and animal from the infectious diseases for many decades, antimicrobial resistance has been recognized as a serious public health concern globally now a days. Resistance to antimicrobial agents is a natural phenomenon because microorganisms evolve new mechanisms to cope up with the action of antimicrobials used against them and drug residues that persist in the food of animal origin pose the multiple drug resistance (Sattar *et al.*, 2014; Chowdhury et al., 2015) against the organisms. However, antibiotic-resistant organisms have emerged and spread among human and animal populations worldwide through different routes (Ahaduzzaman *et al.*, 2014; Hassan *et al.*, 2014; Hassan *et al.*, 2015). Foodborne diseases are among the most widespread global public health problems of recent times, and their implication for health and economy is increasingly recognized (Gomez *et al.*, 1997). The majority of foodborne outbreaks are caused by *E. coli*, *Salmonella*, *Listeria monocytogenes* and *Campylobacter* strains (Velusamy *et al.*, 2010). However, studies made elsewhere indicated that street foods are important sources of *E. coli* particularly among those raw consumers (Freimuth *et al.*, 2000). *E. coli* and *Salmonella* was recorded from street foods like Ragda-Petis, Bhel and Panipuri available in India (Kharel *et al.*, 2016). A recent study found that 37 (74%) out of the 50 food samples, had *E. coli* contamination and that foods containing vegetables had *E. coli* at a high percentage (91%), and these organisms also existed in fish, meat and cereals through vendors (Biswas *et al.*, 2010). A previous study on bacteriological quality of street food (chatpoti) samples collected from 7 different locations of Dhaka University campus were subjected to antimicrobial susceptibility testing against *E. coli* isolated from the food samples shown 16.67 to 66.67% (Multidrug Resistance Index%) and majority of the isolates showed resistance against Ampicillin, Colistin, Chloramphenicol, Ceftriaxone, Ciprofloxacin and Nitrofurantoin. An investigation which was previously done on the microbiological quality of different street food (Hog Plum, Fusca, Chanachur and Guavas) sold by various street vendor at Dinajpur. Bangladesh showed the prevalence of *E. coli* (35%), *Staphylococcus* spp. (30%), *Klebsiella* spp. (25%), *Salmonella* spp. (5%) and *Shigella* spp. (5%) whereas the *E. coli* samples were sensitive to ciprofloxacin and *Salmonella* spp. were resistance to Gentamicin (Khalif *et al.*, 2018). All isolates showed resistant to Cefixime, Cefalexin, Erythromycin, Cefuroxime and Aztreonam. All samples indicate the presence of multidrug resistant bacteria which might cause public health hazards if those antibiotic resistance genes transfer to human food chain (Khalif *et al.*, 2018). In Chattogram, Bangladesh, street foods samples such as fuska, chicken biriani, sugarcane juice and egg toast-were studied previously for the presence of *E. coli* and antimicrobial resistance pattern showed that *E. coli* sample was (100%) resistance to Oxytetracycline, Ciprofloxacin, Amoxicillin, and Streptomycin and sensitive (100%) to Cephalexin and Gentamicin. There is a very few numbers of research data available on street food safety from microbiological perspective in Chattogram. Besides a good number of city people consumed varieties of street food items that are not yet screened through microbiological assay. Therefore, the organism originated from this food chain could pose a serious public health issue. In this perspective, we aimed to determine the prevalence and distribution of the *E. coli* on street foods by conventional culture methods and biochemical assays, and to assess the prevalence of antimicrobial resistance of the isolates from the street food samples in Chattogram, Bangladesh.

2. MATERIALS AND METHODS

Study Design

Five markets such as Foy's lake, Jhautala Bazar, G.E.C, Bahaddarhat Bazar and Reazuddin Bazar of Chattogram City Corporation (CCC) were randomly selected for the present study to investigate the prevalence of *E. coli* and find out the antimicrobial resistance pattern against *E. coli* isolated from chatpoti, chatpoti salad, and chatpoti water. A total of 150 samples from chatpoti (n=50), chatpoti salad (n=50), and chatpoti water (n=50) were collected through random sampling (10 from each site).

Isolation and identification of *E. coli*

Sample was inoculated into a test tube containing buffer peptone water (Oxoid Ltd, Basingstoke, Hampshire, UK), and for primary enrichment, incubated at 37°C. After that, the culture was streaked onto MacConkey agar medium (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 hours. The growth of *E. coli* was suspected when bright pink-colored large colonies yielded on a MacConkey agar plate. Then such colonies were streaked onto EMB agar plate (Merck, pH: 7.1±0.2) and incubated at 37°C for 24 hours. "Green metallic sheen" colony morphology yielded on this medium was taken as the growth of *E. coli*.

Storage of isolated *E. coli*

At the final stage all *E. coli* isolates were cultured in brain heart infusion (BHI) (Oxoid Ltd., England) broth and incubated overnight at 37°C. For each isolate, 700 µl BHI broth culture was added to 300 µl 15% glycerol in 2 ml sterile in an eppendorf tube and stored at -80°C for further investigation.

Antimicrobial test

Commercially available antimicrobial discs (OXOID, Hampshire, England) (CL= Cephalexin; GEN= Gentamicin; CT= Colistin sulphate; AMP= Ampicillin; CIP= Ciprofloxacin; TEC= Tetracycline; DO= Doxycycline; AMX= Amoxicillin) were used to observe the antimicrobial sensitivity of *E. coli* by measuring the diameter of zone of inhibition. The antimicrobial sensitivity test of the isolated bacteria from the sample of chatpoti, chatpoti salad and chatpoti water were performed on Muller-Hinton Agar (Liofilchem, Italy) by Kerby-Bauer micro-disc diffusion techniques (Bauer *et al.*, 1966). For the interpretation of antimicrobial sensitivity, we measured the zone of inhibition following standard methods (Table 1).

Data analysis

Data were stored in MS excel-2007 and sorted for analysis. Initial descriptive analysis were done to identify the prevalence of *E. coli* with 95% CI in collected samples with further detection of percentages (%) status of resistance, intermediate and sensitive organisms.

3. RESULTS

Chatpoti sample showed 30% individual colonies of *E. coli*. On the other hand, chatpoti salad and water showed similar prevalence (50%) of *E. coli* (Table 2). *E. coli* isolates recovered from chatpoti sample showed 100% resistance to Ampicillin, Amoxicillin, Tetracycline but 100% sensitive to Doxycycline and Gentamicin (Table 3). However, certain percentages of Ciprofloxacin, Colistin sulphate and Cephalexin were intermediately

Table 1. Standard measurement of diameter of zone of inhibition

Sl. No.	Antimicrobial agent	Diameter of zone of inhibition (millimeter)		
		Resistant	Intermediate	Sensitive
1	Ampicillin	≤13	14-16	≥17
2	Amoxicillin	≤13	14-17	≥18
3	Doxycycline	≤10	11–13	≥14
4	Ciprofloxacin	≤15	16-20	≥21
5	Gentamicin	≤12	13-14	≥15
6	Colistin Sulphate	≤10	11-13	≥14
7	Tetracycline	≤11	12-14	≥15
8	Cephalexin	≤14	15-17	≥18

Table 2. An overview of samples collected from chatpoti, chatpoti salad and chatpoti water and frequency of isolated *E. coli*

Kind of sample	Number of samples	Positive(%; 95%CI)
Chatpoti	50	15 (30; 17.8-44.6)
Chatpoti salad	50	25 (50; 35.5-64.5)
Chatpoti water	50	25 (50; 35.5-64.5)

Table 3. Antimicrobial resistance pattern against *E. coli* isolated from chatpoti

Antimicrobials	No. of isolates	Sensitive N (%)	Intermediate N (%)	Resistance N (%)
Ampicillin	15	0	0	15(100)
Amoxicillin	15	0	0	15(100)
Ciprofloxacin	15	0	10 (75)	1(25)
Tetracycline	15	0	0	15(100)
ColistinSulphate	15	0	5(25)	10(75)
Doxycycline	15	15(100)	0	0
Cephalexin	15	0	1(25)	10(75)
Gentamicin	15	15(100)	0	0

Table 4. Antimicrobial resistance pattern against *E. coli* isolated from chatpoti salad

Antimicrobials	No. of isolates	Sensitive N (%)	Intermediate N (%)	Resistance N (%)
Ampicillin	25	0	0	25(100)
Amoxicillin	25	0	0	25(100)
Ciprofloxacin	25	15 (60)	10 (40)	0
Tetracycline	25	0	0	25(100)
Colistin Sulphate	25	0	20(80)	5 (20)
Doxycycline	25	25(100)	0	0
Cephalexin	25	0	0	25(100)
Gentamicin	25	25(100)	0	0

Table 5. Antimicrobial resistance pattern against *E. coli* isolated from chatpoti water

Antimicrobials	No. of isolates	Sensitive N (%)	Intermediate N (%)	Resistance N (%)
Ampicillin	25	0	0	25(100)
Amoxicillin	25	0	0	25(100)
Ciprofloxacin	25	20 (80)	5 (20)	0
Tetracycline	25	0	0	25(100)
ColistinSulphate	25	25 (100)	0	0
Doxycycline	25	25(100)	0	0
Cephalexin	25	0	0	25(100)
Gentamicin	25	25(100)	0	0

sensitive or resistance. Table 4 has showed that *E. coli* isolates recovered from chatpoti salad were 100% resistant to Ampicillin, Amoxicillin, Tetracycline, Cephalexin but 100% sensitive to Doxycycline and Gentamicin. Moreover, certain level of Ciprofloxacin, and Colistin sulphate was intermediately sensitive or resistance. Besides, *E. coli* isolates from Chatpoti water was 100% resistant to Ampicillin, Amoxicillin, Tetracycline and Cephalexin and 100% sensitive to Colistin sulphate and Doxycycline (Table 5). Furthermore, certain percentages of Ciprofloxacin were found intermediately sensitive or resistance.

4. DISCUSSION

Previous studies identified positive isolates for *E. coli* in chatpoti, a lucrative street food item in major city of Bangladesh including Dhaka, Chattogram and they further investigated their MDR (multi-drug resistance) pattern (Ali *et al.*, 2011; Hassan *et al.*, 2018). We found higher percentage of *E. coli* isolates in chatpoti, salad and water. Therefore, the unhygienic water could be a source of those transmissions. Similar study was conducted with similar findings to determine the contamination sources of *E. coli* from different types of street foods in Chattogram city area where the samples were positive for *E. coli* (28.75%) (Khan *et al.*, 2013). Excluding chatpoti, 67.5 % of other vending street food items were contaminated with one or more bacteria (Plabon *et al.*, 2018). A latest study was conducted to examine microbiological quality of raw salad vegetables of Chattogram City and their role as a source of antibiotic resistant bacteria which showed a higher prevalence of *E. coli* (40.62%) than our study (Nipa *et al.*, 2011). *E. coli* organism isolated from the street food samples have the evidence of MDR which is also supported by previous studies (Nipa *et al.*, 2011; Khan and Saha, 2015; Plabon *et al.*, 2018; Sabuj *et al.*, 2018). Tetracycline, Ampicillin and Amoxicillin were 100% resistant in case of *E. coli* isolated from the street food which is supported by previous studies (Plabon *et al.*, 2018; Sabuj *et al.*, 2018) whereas Ciprofloxacin and Colistin Sulphate are less resistant in the *E. coli* samples isolated from street food. On the other hand, Gentamicin is completely (100%) sensitive to *E. coli* isolated from street food sample which is opposite to the finding of previous study (Plabon *et al.*, 2018) and Doxycycline is completely sensitive (100%) (Islam *et al.*, 2014; Rahman *et al.*, 2017). Our Study found that, significant level of MDR *E. coli* present in street food which is an alarming issue for public health because, bacteria isolated from such food samples may act as media for transmission of antimicrobial resistance gene to human and the environment.

5. CONCLUSIONS

E. coli is a leading foodborne pathogen worldwide. A wide range of foods has been implicated in such pathogen and cause of human disease. From the study it can be concluded chatpoti, chatpoti salad, chatpoti water could be a good harbor of *E. coli* and could be an excellent source of transmission to human. Present research findings also showed that, the source of single and multiple antimicrobial- resistant of *E. coli* isolates is the frequently used antimicrobials including Tetracycline, Ampicillin and Amoxicillin. Therefore, it is important to monitor the occurrence of resistance among bacteria from animals and food, as these bacteria are able to spread through food product to human.

5. ACKNOWLEDGEMENT

The author expresses cordial thanks to National Science and Technology (NST) Fellowship 2017-2018 of Ministry of Science and Technology, Bangladesh and Research and Extension, CVASU, Bangladesh for the financial help provides during the study period.

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