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Original article

Isolation ESBL-Producing Enterobacteriaceae Species in Dhaka City's Red Meat: Phenotypic Analysis and Antibiotic Resistance Profiles

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Abstract

Background: It is generally acknowledged that one of the most significant issues facing modern human medicine is the fast and irreversible rise of antibiotic-resistant pathogenic bacteria, which has been documented over the past two decades. This circumstance raises concerns in many areas. When treating different types of infections, there are resistance mechanisms that prevent the use of last-resort medications. Many resistance mechanisms that develop in bacterial populations and propagate are those with broad activity spectra that render all or most medicines in a particular treatment category ineffective. ***Objective:*** The goal of this study is to evaluate the microbiological quality of raw meat,the frequency of production of Extended- spectrum-beta-lactamases (ESBL) in Enterobacteriaceae and the antibiotic susceptibility of these isolates in vitro. ***Methodology:*** A comparative cross-sectional approach was employed in this study to examine 40 food samples of beef and mutton from different areas of Dhaka. For pure culture, Mac Conkey agar plates were utilized after sample collection. Muller-Hinton agar media (MHA) was used for antibiotic susceptibility testing after completion of organism detection test on Eosin-methylene blue (EMB) agar media. ***Results:*** Among 40 samples, 95% of the bacteria discovered belonged to the Enterobacteriaceae family. Five (or 25%) of the beef samples showed resistance against the combination of Ceftazidime (CAZ) and Amoxicillin-Clavulanic acid (AMC). About the mutton, 20 (100%) cases were found resistant to CAZ+AMC (100%), and 12 instances were resistant to CTX (Cefotaxime) + AMC (60%). Several strains (17.5%) in our investigations demonstrated phenotypic positive for producing the ESBL enzyme. Of 40 dietary samples, 35% of the strains that create ESBL are identified in beef and mutton, respectively, with 30% and 5% 0f theses strains producing ESBL. ***Conclusion:*** Antibiotic resistance is an important health issue due to the potential pathogenicity of antibiotic-resistant bacteria associated with animals, their case of transmission to mankind through food chains, and their extensive environmental dispersion through animal wastes. These might cause patient to develop complicated, long-lasting, incurable illnesses as well as animal drug resistance gene might be transferred from livestock to human being, which could decrease the life expectancy and increase the cost of treatment even death.

Keywords: ESBL, phenotypic characteristics, AMC

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Introduction:

Antimicrobial resistance is a burgeoning global concern. Annually, 33,000 individuals succumb fatal infections caused by bacterial antibiotic resistance within the European region. According to published article, it has been projected that the global population is going to face a significant threat to public health and economic stability in the coming years due to anti-microbial resistance.[1] By the year 2050, it is estimated that approximately 10 million lives per annum and a cumulative financial output of 100 trillion USD could be jeopardized due to the emergence of drug-resistant infections. We must urgently seek proactive measures to mitigate the progression of drug resistance to safeguard public health and economic prosperity.[2] It is worth noting that the production of ESBLs is often associated with the emergence of multi-drug resistance.[3] Meat is a good substrate for many organisms to thrive because it is high in moisture, rich in nitrogenous compounds (such as amino acids, peptides, and proteins),and abundantly provided with mineral & auxiliary growth agents.[4] Food continue to endanger both human and animal health, despite the fact that industrialized nations have improved the hygienic of all meat production procedures. Food borne illnesses spread to humans when tainted food is consumed and toxins enter the body. This becomes one of the major global public health issues & both social and economic stability will be endangered.[5]

Generally speaking, unclean slaughter and sale facilities can harbor microorganisms resistant to antibiotics that infect raw meat. Eating meat tainted with microorganisms that are resistant to antibiotics can have serious health effect[6] and because of cultural and religious concerns, many here consume red meat.

This study proposes to assess the microbiological quality of raw meat, the incidence of Extended spectrum-beta-lactamases (ESBL) Production in *Enterobacteriaceae* isolates from raw meat, and the susceptibility of these isolates to antibiotics.

Materials and methods

Samples of beef and mutton were utilized in this cross-sectional investigation.To properly isolate the bacteria, beef and mutton samples were obtained straight from the slaughterhouse, placed in a sterile Ziploc bag, and then delivered to the lab immediately without freezing. There were 40 tissue samples collected from various portions of cows and goats. After that, samples were immediately swabbed directly with a sterile cotton swab over MacConkey agar using the direct cotton swab technique. After that, overnight incubation was performed at 37 degrees Celsius aerobically & maintaining the same procedures subculture was done. For stock preparation, Luria-Bertani broth, a nutrient-rich medium frequently used for the growth of bacteria and 500 microliters of 50% glycerol were used. At last, -20 and -80 degrees Celsius were utilized to store two copies of the stock for each of these copies. Organism detection tests were done on Eosin-methylene blue media and Kirby-Bauer, a double-disk diffusion test was done over Mueller-Hinton agar media using two different antibiotics along with beta-lactamase inhibitor.[7] Bauer used the disc diffusion technique to assess the susceptibility of ESBL-producing *E. Coli* by Clinical Laboratory Standards Institute recommendations (CLSI 2018). Microsoft Excel was used for the statistical analysis.

Result:

Among 40 samples, 95% of the pieces exhibited strain positivity & 5% no growth over MacConkey agar plates (Figure 1). Eosin methylene blue (EMB) medium organism detection test findings show that all 20 samples of beef are Klebsiella spp, whereas 10 and 8 samples of mutton are *E.coli* and *Klebsiella* spp (Table 1).

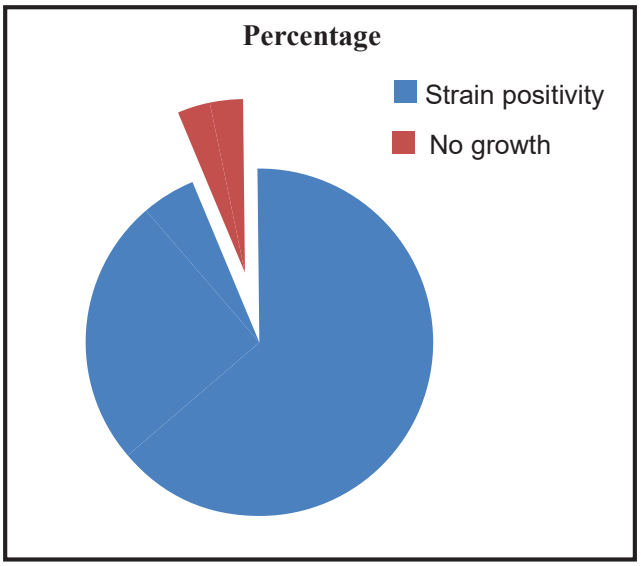

Figure 1: A graphic illustrates the appearance of *Entero-bacteriaceae* in total samples onMacConkeyagar plates by direct swab approach.

Table 1: Distribution of micro-organisms in beef & mutton samples.

Name of organism	Beef (n=20)	Mutton(n=20)
E.coli	0	8
klebsiella	20	10
No growth	0	2

During the screening process, it was observed that a total of 7 strains exhibited positive for ESBL producing bacteria among the 40 overall meat samples obtained (Table 2) & Five (or 25%) resisted the CAZ and AMC combos in case of beef (All are *klebsiella* spp).Regarding the mutton, there were 20 that were resistant to CAZ+AMC (100%), and 12 were resistant to CTX+AMC (60%) (Figure 2).

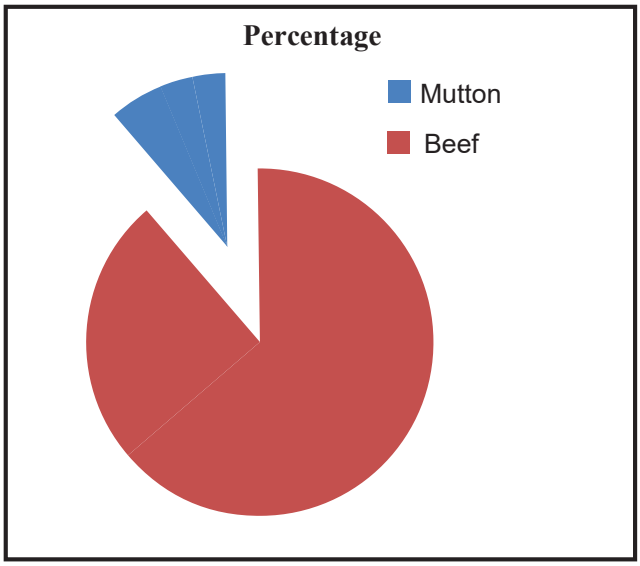

Figure 3: This pie chart represents percentage distribution of ESBL producers in animal based foods after Double-disc synergy tests.

Table 2: Double-disk diffusion test results only for ESBL producing bacteria (Zone diameter-mm).

Sample ID	Gram-negative bacteria	CAZ (30µg/ml)	CAZ(30µg/ml) + AMC(30µg/ml)	CTX (30µg/ml)	CTX(30µg/ml) + AMC (30µg/ml)	Results
P3B	*Klebsiella* spp.	0	25	28	25	ESBL
P7B	*Klebsiella* spp.	23	25	15	27	ESBL
P9A	*Klebsiella* spp.	23	23	20	26	ESBL
P9B	*Klebsiella* spp.	25	27	15	29	ESBL
P10A	*Klebsiella* spp.	30	30	7	30	ESBL
P10B	*Klebsiella* spp.	30	30	11	30	ESBL
BaPA	*E.coli*	22	24	4	20	ESBL

* CAZ = Ceftazidime, CTX= Cefotaxime, AMC= Amoxicillin- Clavulenic acid

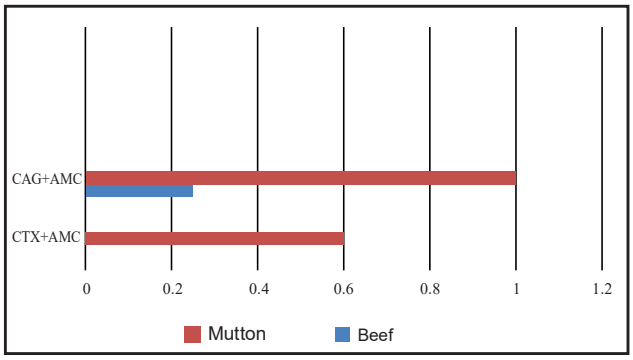

Figure 2: This column chart represents AMC resistance results in overall samples.

The distribution of ESBL-producing bacteria among various food types .The strains that produce ESBL account for 35%, of which 30% and 5% are found in beef and mutton, respectively among 40 food samples (Figure 3).

Table 3: Shows the antimicrobial drug resistance profile of microorganisms (*klebsiella* Species) isolated from beef samples in several Dhaka market locations

Sl No.	Strain name	CAZ (30µg/ml)	CAZ (30µg/ml) + AMC(30µg/ml)	CTX (30µg/ml)	CTX (30µg/ml) + AMC (30µg/ml)	Results
1.	P1A	30	25	35	35	None
2.	P1B	0	0	33	33	None
3.	P2A	26	26	30	28	None
4.	P2B	24	23	27	30	None
5.	P3A	0,	0	27	28	None
6.	P3B	1	25	28	25	None
7.	P4A	0	0	30	30	None
8.	P4B	0	0	30	30	None
9.	P5A	0	0	30	29	None
10.	P5B	0	0	25	20	None
11.	P6A	25	25	27	27	None
12.	P6B	28	28	15	20	None
13.	P7A	25	22	31	32	None
14.	P7B	23	25	15	27	ESBL
15.	P8A	32	30	30	32	None
16.	P8B	25	25	34	34	None
17.	P9A	23	23	20	26	ESBL
18.	P9B	25	27	15	29	ESBL
19.	P10A	30	30	7	30	ESBL
20.	P10B	30	30	11	30	ESBL

- P=Piece of sample, A= Anterior side of the sample, B= Posterior side of the sample
- Antibiotics susceptibility zone diameter in mm.
- On EMB media Klebsiella species purple in color

Tables 3, 4a, and 4b list 40 distinct samples, their sensitivity to cefotaxime (CTX), ceftazidime (CAZ), and clavulanic acid, along with the strain name and abbreviations for each.

Table 4 (a): Strain abbreviations & their meanings.

Strain name	Full form	Strain name	Full form
T	Thigh	B	Belly
Ta	Tail	Lu	Lungs
H	Head	C	Chest
L	Liver	Ba	Back
LA	Leg anterior	LB	Leg back

Table 4 (b): Shows the antimicrobial drug resistance profile of microorganisms isolated from mutton samples in several Dhaka market locations

Sl No.	Strain name	Organism	CAZ(30 µg/ml)	CAZ(30µg/ml) + AMC(30µg/ml)	CTX(30µg/ml	CTX(30µg/ml) + AMC (30µg/ml)	Results
1	LBPA	*E.coli*	0	0	25	27	None
2	BaPA	*E.coli*	0	0	4	20	ESBL
3	BPA	*E.coli*	0	0	0	0	None
4	BPB	*E.coli*	0	0	0	0	None
5	LuPA	*Klebsiella* spp.	0	0	20	15	None
6	LuPB	*E.coli*	0	0	25	25	None
7	LAPA	*Klebsiella spp*.	0	0	0	0	None
8	LAPB	*Klebsiella* spp.	0	0	0	0	None
9	LPA	*Klebsiella* spp.	0	0	0	0	None
10	LPB	*E.coli*	0	0	0	0	None
11	CPA	*Klebsiella* spp.	0	0	13	13	None
12	CPB	*Klebsiella* spp.	0	0	22	22	None
13.	TaPA	*E.coli*	0	0	0	0	None
14	TaPB	*E.coli*	0	0	0	0	None
15	HPA	*Klebsiella* spp.	0	0	22	22	None
16	HPB	*Klebsiella* spp.	0	0	20	20	None
17	TPA	*Klebsiella* spp.	0	0	0	0	None
18	TPB	*Klebsiella* spp.	0	0	0	0	None
19	LBPB	Nil	0	0	0	0	None
20	BaPB	Nil	0	0	0	0	None

- P=Piece of the sample, A=Anterior side of the sample, B=Posterior side of the sample.
- On EMB media *Klebsiella* species purple in color & *E.coli* metallic sheen green in colour.

Discussion:

In the Dhaka metropolitan area, specifically in the neighborhood markets of Dhaka, a total of 40 animal-based foods were assessed to assess ESBL production. According to the Dhaka Tribune, red meat accounts for more than 50% of all meat consumption in Bangladesh. Additionally, it is believed that food obtained from the local markets in Dhaka may harbor pathogens capable of causing food borne illnesses. The published article, [8] brought attention to the potential health risks associated with retail beef due to inadequate sanitation and hygiene practices during processing and handling. *Escherichia coli (E. coli)* is thought to spread through meat because of the people and tools used to process it, such as knives and cutting boards, as well as poor hygiene and sanitation practic-

es.[9] The transmission of contamination during the slaughtering process may occur through various means, including the hands of the slaughter men, water, and equipment utilized. Among the 40 samples derived from the red meat, 95% belong to the family Enterobacteriaceae, which supports the study in Ghana in 2012,[10] where 65% Enterobacteriaceae was found in food samples. Among all the strains, 100% and 50% of *Klebsiella* spp were found in beef and mutton samples, respectively, however, this study strongly aligns with the investigations in Athens.[11] In this study, a number of strains (17.5%) showed phenotypic positivity for making the ESBL enzyme. This is different from other studies, which found conflicting results (45.9%) for this phenomenon, which was seen in China.[12] Five (or 25%) resisted the CAZ and AMC combos in case of beef (All are *klebsiella* spp). Regarding the mutton, there were 20 that were resistant to CAZ+AMC (100%), and 12 were resistant to CTX+AMC (60%). The mutton findings closely match the Nigerian study, [13] where 91 samples (82.7%) were resistant to AMC.

A variety of enteric illnesses, including diarrhea and endocarditis, as well as infections of the skin, soft tissues, joints, bones, eyes, respiratory tract, and urinary tract, are caused by the *Enterobacteriaceae* family.Consequently, it is now a growing problem. We only got 40 pieces of meats, so the sample size was really small. The sample was not collected from throughout the nation but rather from different parts of Dhaka city. The weight, size, and nutritional value of the meats are not taken into account in this investigation. We are unable to utilize human samples due to a lack of resources and time. The World Health organization has designated several Enterobacteriaceae superbugs because of their increased production and spread of Carbapenamaeses & ESBL.This study aims to assess the incidence of Enterobacteriaceae microorganisms that cause ESBL and their phenotypic characterization & antibiotic resistance pattern.

Conclusion:

This study shows that the ESBL-producing Enterobacteriaceae found in raw meat in Dhaka, Bangladesh, have high antibiotic resistance. The rising rate of antibiotic resistance is highlighted by the need to enact policies to rationalize antibiotic usage and build a national resistance surveillance system. These policies will help local communities produce antibiotic therapy guidelines. The most significant degree of public health and laboratory techniques must be significantly advanced to stop the spread of bacteria resistant to medications.

Conflict of interest:

The authors hereby declare that no conflict of interest exists.

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