RESEARCH ARTICLE

Microbiological Contamination of Retail Meat from Mizoram (India) with Special Reference to Molecular Detection and Multi-Drug Resistance of *Escherichia coli*

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Abstract

Food articles serve as a common vehicle for transmitting many pathogenic and non-pathogenic microorganisms to humans, and many of these organisms may have developed multidrug resistance. Meat may act as a vehicle for transferring multi-drug-resistant organisms to the consumers. In the present study, a total of 180 (retail beef, N = 90 and chicken meat, N = 90) samples were collected from 3 districts of Mizoram and analyzed for the level of microbial contamination, isolation, and molecular detection of *Escherichia coli* (*E. coli)* and its drug resistance pattern. Unacceptable levels of total viable count (TVC) and *Escherichia coli* count (ECC) were recorded in retail beef and chicken. On PCR assay-based confirmation, *E. coli* was detected in beef (83.33%) and chicken (80.00%). Significantly ($p \leq 0.01$) lower prevalence of *E. coli* was recorded in chicken meat from Champhai district (63.33%) than Aizawl (86.67%) and Kolasib (90.00%) districts. Resistance of *E. coli* strains to amoxicillin was highest with detection of MDR *E. coli* from beef (42.67%) and chicken (56.94%), indicating public health concerns.

Keywords: *E. coli*, Microbial Contamination, Molecular Detection, Multidrug Resistance, Retail meat.

Ind J Vet Sci and Biotech (2022): 10.21887/ijvsbt.18.2.6

Introduction

The members of the Enterobacteriaceae group of bacteria are the most prominent bacterial contaminant in raw and processed meat products worldwide. The antibiotic resistance determinants can be transferred from one bacterium to another bacteria of clinical significance. The prevalence of MDR among food-borne pathogens has been increased during recent decades, and treatment has been complicated by the emergence of resistance to most first-line antimicrobial agents (Akbar *et al*., 2014). A large proportion of the world's population relies on meat as a source of protein (Bradeeba and Sivakumaar, 2013). In the North-East region of India, the majority of the tribal populations consume meat (Saikia and Joshi, 2012). Although chicken is the most commonly consumed meat in India and 95% of the meat is obtained from retail shops, chicken and beef are predominantly consumed besides pork in northeast India, including Mizoram. The meat available at retail outlets goes through poor hygienic practices during unorganized slaughtering, transportation, and selling, which may cause microbial contamination of raw meat. There is scanty literature from Mizoram in this aspect; hence the present study was undertaken to detect the level of microbial contamination of retail beef and chicken meat, molecular detection of *E. coli*, and its MDR pattern from three districts of Mizoram.

Materials and Methods

Collection of Meat Samples and Determination of Microbial Contamination

A total of 180 retail meat samples, 30 each of beef and chicken, were aseptically collected from Aizawl, Kolasib, and Champhai districts of Mizoram. The microbial load of meat samples was assessed by TVC and ECC (Fawole and Oso, 2001). The colonies were counted as a total number of bacterial colony (cfu/g) = Average number bacterial colony count on the plates x 10 x dilution factor. Isolation and identification of *E. coli* were carried out by the method of Quinn *et al*. (2004).

Molecular confirmation of *E. coli*

All the presumptive *E. coli* isolates were subjected to *16S-rRNA* gene amplification by using published

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How to cite this article: Debbarma, M., Deka, D., Tolenkhomba, T.C., & Rajesh, J.B. (2022). Microbiological Contamination of Retail Meat from Mizoram (India) with Special Reference to Molecular Detection and Multi-Drug Resistance of *Escherichia coli*. Ind J Vet Sci and Biotech. 18(2), 32-35.

Source of support: Nil

Conflict of interest: None.

Submitted: 20/11/2021 **Accepted:** 12/02/2022 **Published:** 10/04/2022

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primers (F: GACCTCGGTTTAGTTCACAGA and R: CACACGCTGACGCTGACCA) following the method of Candrian *et al*. (1991). The bacterial lysate was prepared by using boiling and snap chilling method. The PCR amplification of bacterial DNA was done in a thermal cycler (Biorad™) and the steps involved 30 cycles of initial denaturation (95°C for 5 min), denaturation (94°C for 45 sec), annealing (59°C for 45 sec), extension (72°C for 45 sec) and final extension (72°C for 6 min). All the amplified PCR products were analyzed by agarose gel electrophoresis, and 100 bp DNA ladder was used as a reference to compare the size of amplified products.

Antimicrobial susceptibility of *E. coli*

All the PCR confirmed *E. coli* strains were subjected to *in vitro* antibiotic susceptibility test by disc diffusion method (Bauer *et al*.,1966) in Muller Hinton agar plate against a panel of 12 commonly used antibiotics namely ampicillin (AMP, 10 mcg), amoxycillin (AMX, 10 mcg), norfloxacin (NX, 10 mcg), amoxyclav (AMC, 30 mcg), tetracycline (TE,30 mcg), cefazolin (CZ,30 mcg), ceftriaxone (CTR,30 mcg), cefotaxime (CTX,30 mcg), ciprofloxacin (CIP,5 mcg), cotrimoxazole (COT,25 mcg), gentamicin (GEN,10 mcg) and imipenem (IPM,10 mcg).

Statistical Analysis

Statistical analysis of data was done using the software of SPSS (Version 16.0). One-way ANOVA and normal deviation tests were used at $P \leq 0.05$ and $P \leq 0.01$.

Results and Discussion

Microbial Contamination of Retail Meat

Microbiological contamination of retail beef and chicken from 3 districts of Mizoram is presented in Table 1. The mean TVC and ECC of retail beef and chicken from three districts of Mizoram revealed that there was no significant variation in beef and chicken microbiological contamination among the three districts. 62.22% and 65.56% beef samples exceeded the maximum permissible limit of TVC (10^7cfu/g) and ECC (10^2cfu/g) whereas 56.67% and 58.89% chicken samples exceeded the maximum permissible limit of TVC (10^7cfu/g) and ECC (10^2cfu/g) (ICMSF, 1974) (Table 2).

The TVC and ECC are hygienic indicators in the selling of raw meat. Various workers reported higher TVC in beef (Ahmed *et al*., 2013; Bradeeba and Shivakumar, 2013; Singh *et al.,* 2014; Huges *et al*., 2015) and chicken meat (Patyal *et al*., 2012; Ahmed *et al*., 2013; Singh *et al*., 2014; Ramya *et al*., 2015) from retail outlets in different parts of the country.

Detection of *E. coli* from Retail Meat

PCR assay based 16-r RNA gene detection (Fig. 1) *16S-rRNA* gene detection (Fig. 1) confirmed *E. coli* in147 (81.67%) meat samples contributing to 75 (83.33%) and 72 (80.00%) from beef and chicken, respectively. The occurrence of *E. coli* was significantly ($p \leq 0.01$) lower in chicken meat from Champhai district (63.33%) than Aizawl (86.67%) and Kolasib (90.00%)

Table 1: Microbiological contamination (TVC and ECC) of retail beef and chicken

	Bacteriological criteria in log_{10} scale			
	Beef		*Chicken*	
District	*TVC ± SE*	*ECC ± SE*	*TVC ± SE*	*ECC ± SE*
Aizawl	6.91 ± 0.13 (Range 5.91-8.18)	3.09 ± 0.15 (Range 1.84-3.14)	7.01 ± 0.11 (Range 5.56- 8.11)	2.92 ± 0.07 (Range 1.84-3.16)
Kolasib	7.09 ± 0.13 (Range 5.98- 8.16)	3.09 ± 0.20 (Range 1.95-3.17)	6.92 ± 0.10 (Range 5.84-8.10)	2.99 ± 0.02 (Range 1.77-3.11)
Champhai	7.01 ± 0.92 (Range 5.12-8.23)	3.04 ± 0.30 (Range 1.95-3.17)	6.90 ± 0.13 (Range 5.69- 8.20)	3.05 ± 0.02 (Range 1.92-3.14)

Table 2: Level of TVC and ECC in retail beef and chicken

		No of samples analyzed (N = 180)							
		Aizawl		*Kolasib*		*Champhai*		*Total*	
Criteria	*Log_{10} (cfu/g)*	*Beef (n = 30)*	*Chicken (n = 30)*	*Beef (n = 30)*	*Chicken (n = 30)*	*Beef (n = 30)*	*Chicken (n = 30)*	*Beef (N = 90)*	*Chicken N*
TVC	5 - 6	1	5	1	2	2	4	4 (4.44)	11 (12.22)
	6-7	12	10	8	10	10	8	30 (33.33)	28 (31.11)
	>7	17	15	21	18	18	18	56 (62.22)	51 (56.67)
ECC	ND	5	4	4	3	6	11	15 (16.67)	18 (20)
	Upto2	7	7	6	7	3	5	16 (17.77)	19 (26.38)
	>2	18	19	20	20	21	14	59 (65.56)	53 (58.89)

Table 3: Detection of *E. coli* (%) in retail beef and chicken

Sample	*Aizawl (n = 30)*	*Kolasib (n = 30)*	*Champhai (n = 30)*	*Total*
Beef	25/30 (83.33)	26/30 (86.67)	24/30 (80.00)	75/90 (83.33) ^{NS}
Chicken	26/30 (86.67) ^{a}	27/30 (90.00) ^{a}	19/30 (63.33) ^{b}	72/90 (80.00) **
Overall	51/60 (85.00)	53/60 (88.33)	43/60 (71.67)	147/180 (81.67)

Values bearing different superscripts ($^{a, b}$) in a row (**) denotes significant difference at ≤ 0.01 and NS = Non-significant

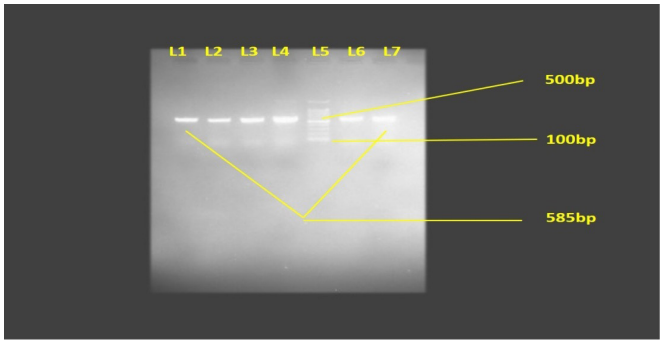

Fig. 1: *16 S-r RNA* gene amplicon of *E. coli* (585 bp); L1-4 and L 6-7: positive samples and L5: 100 bp DNA ladder

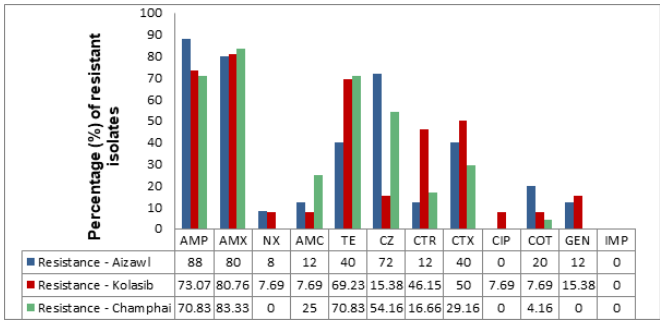

Fig. 2: Antibiotic resistance pattern of *E. coli* isolated from retail beef in 3 districts of Mizoram

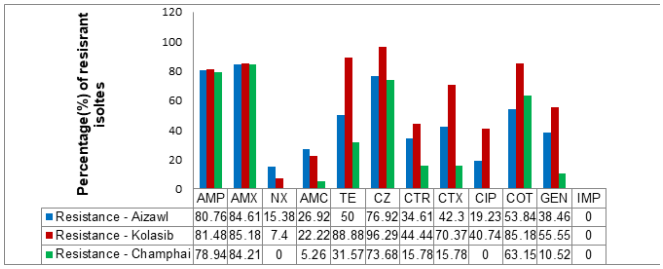

Fig. 3: Antibiotic resistance pattern of *E. coli* isolated from retail chicken in 3 districts of Mizoram

districts (Table 3). Meat acts as an important vehicle for the transmission of various pathogens that can cause food-borne diseases in humans (Heredia and García, 2018), consequently creating food safety hazards (Kshirsagar *et al*., 2014; Abebe *et al*., 2020).

Antimicrobial Resistance Pattern of *E. coli* from Retail Meat

The *E. coli* strains isolated from retail beef of 3 districts were sensitive to imipenem and highly resistant to amoxicillin (81.33%), followed by ampicillin (77.33%), tetracycline (60.00%), cefazolin (46.66%), cefotaxime (40.00%), ceftriaxone (25.33%), amoxyclavulanic acid (14.60%), cotrimoxazole (10.66%), gentamicin (9.33%), norfloxacin (5.33%) and ciprofloxacin (2.66%). There are variations in the resistance pattern of E.coli strains to different antibiotics among three districts under study Figs. 2 and 3.

Table 4: Multidrug resistance pattern of *E. coli* strains from retail beef and chicken

	Number of resistant E. coli strains	
Resistance to 3 or 3< numbers of Antibiotics	*Beef (n = 75)*	*Chicken (n = 72)*
3	11	8
4	12	10
5	5	9
6	3	6
7	0	3
8	1	3
9	0	2
Multi-drug-resistant *E. coli* strains	32 (42.67%)	41 (56.94%)

The *E. coli* strains isolated from retail chicken from the 3 districts were sensitive to imipenem and highest resistant to amoxicillin (84.72%) followed by cefazolin (83.33%), ampicillin (80.55%), cotrimoxazole (68.05%), tetracycline (59.72%), cefotaxime (45.83%), gentamicin (37.50%), ceftriaxone (33.33%), ciprofloxacin (22.22%), amoxyclavulanic acid (19.44%) and norfloxacin (8.33%).

Detection of Multi-drug-resistant *E. coli* from Retail Meat

The *E. coli* strains showing resistance to three or more antibiotics were considered multi-drug-resistant. The *E. coli* strains isolated from beef (75) and chicken (72) of 3 districts showed multidrug (3-9 antibiotics) resistance patterns with 42.67 and 56.94%, respectively (Table 4).

Multi-drug-resistant *E. coli* strains from animals have been a major concern worldwide. Saud *et al.* (2019) reported overall 52.50% MDR *E. coli* in raw meat from retail shops in Nepal, contributing to 50% from chicken and 21.90% from buffalo meat.

The high prevalence of multi-drug-resistant *E. coli* in beef and chicken recorded during the present study is of concern from the public health point of view as it may transfer the antibiotic resistance determinants not only to other strains of *E. coli*, but also to other bacteria in the gastrointestinal tract and also to the human being through the consumption of contaminated meat.

Conclusion

The bacteriological contamination of retail meat exceeding the prescribed standards in terms of TVC and ECC and the presence of MDR *E. coli* has been an indicator of improper hygienic quality in the conventional meat production system and selling in retail markets Mizoram. Moreover, the resistance of the *E. coli* strains to some of the commonly used antibiotics in animals and humans might lead to critical health situations in the lack of alternative antimicrobials against MDR bacteria. Therefore, it is imperative to develop hygienic meat production standards and measures to limit the development of antimicrobial-resistant indicators and pathogenic microorganisms.

Acknowledgements

This study forms a part of MVSc research work of the first author. The authors are thankful to the Dean, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl, Mizoram: 796015 and vice-Chancellor, Central Agricultural University, Imphal for granting permission to conduct the research work.

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