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The Occurrence, Antibiotic Susceptibility Pattern and Multiple Drug Resistance Index Studies of *E. coli* from Fresh Meats in Marathwada Region of Maharashtra

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ABSTRACT

Background: Foodborne diseases are a huge social burden in both developed and underdeveloped countries. *Escherichia coli* is a natural inhabitant of the human and warm-blooded animal digestive tracts. It is considered as an indicator organism for direct or indirect faecal contamination of raw meats. The presence of antibiotic-resistant microorganisms in meat accounts for a public health risk.

Methods: In current study, all 39 characterized *E. coli* isolates recovered on screening 405 fresh meat samples collected from different butcher shops in the Udgir city, Marathwada region of Maharashtra were also tested for Congo red dye binding assay and haemolysis assay. Antimicrobial resistance profiling of recovered isolates was carried out using the disc diffusion method.

Result: Overall occurrence of *E. coli* in fresh meats was noted to the tune of 9.63% and the corresponding group wise occurrence in chicken and chevon samples were 12% and 7.32%, respectively. All E. *coli* tested positive in the Congo red dye binding assay and haemolysis assay, suggesting virulent nature of isolated organisms. On screening for antimicrobial resistance, these isolates were found resistant to mainly â-lactams (94.87%), lincosamides (84.62%), 4th Generation cephalosporins (82.05% each), DHFR Inhibitors (64.10%), glycopeptides (51.28%). The multidrug resistant isolates showed resistance to a minimum of 4 and maximum of 12 antibiotics with MAR index ranging from 0.27 to 0.8 and 9 resistance patterns.

Key words: Chevon, Chicken, *E. coli*, Meat, Multidrug resistance.

INTRODUCTION

The indiscriminate usage and misuse of different antibiotics have led to the emergence of resistance, which has a negative impact on the efficacy of bacterial infection treatment and prevention (Deshmukh *et al*., 2023). *Escherichia coli* has become one of the microorganisms that are frequently resistant to antimicrobials due to its ubiquity in humans and animals, as well as its roles as a pathogenic and commensal bacterium (Zhao *et al*., 2012). According to several investigations, infections with drug-resistant *E. coli* in humans have been caused by strains from animals and those infectious agents had the same mobile resistance genes in different species of bacteria originating from diverse animal sources (von Baum and Marre, 2005; Hammerum and Heuer, 2009; Johnson *et al*., 2009). Although antimicrobial-resistant *E. coli* strains have been found from a variety of foods, a large number of resistant strains have been isolated from common retail meats and poultry (Schroeder *et al*., 2004).

In response to the increasing public concern over food safety, the current study focused on the precise identification, characterization and antimicrobial resistance of bacterial food-borne pathogens. Its objective was to isolate, identify and confirm pathogenic *E. coli* from fresh meat samples obtained from meat shops in Udgir, Maharashtra's Marathwada region.

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MATERIALS AND METHODS

Samples collection and transportation

A total of 405 fresh meat samples, including 200 samples of chicken and 205 samples of chevon were collected at random from different butcher shops in the Udgir city, Maharashtra. All raw meat samples were collected in sterile

sample containers (HiMedia, India). All samples were labeled and immediately transported to the laboratory of Department of Veterinary Public Health and Epidemiology, College of Veterinary and Animal Sciences, Udgir by maintaining cold chain for isolation and identification of *E. coli*.

Isolation, identification and *in vitro* pathogenicity assessment of *E. coli*

The cultural isolation and identification of *E. coli* was carried out according to the conventional procedure given by Feng *et al*., (2002). Pure cultures of recovered *E. coli* isolates were grown on Brain Heart Infusion (BHI) agar slants. These slant cultures were kept at 4-8°C for biochemical characterization and in vitro pathogenicity assays. Gram staining of presumptive *E. coli* isolates was performed as per Preston and Morel (1962) and pink Gram-negative rods were identified under a binocular microscope (Olympus, Japan). The biochemical characterization was carried out using the technique outlined by Quinn *et al*., (1994). IMViC (Indole, Methyl Red, Voges Proskauer and Citrate) tests were employed for determining *E. coli*.

The *in vitro* pathogenicity assays *viz*. Congo red dye binding and haemolysis assays (on 5% defibrinated sheep blood agar) were used to determine the virulence potential of the presumptive isolates of *E. coli*. The Congo red dye binding assay was performed as per the procedure given by Berkhoff and Vinal (1986). In case of haemolysis assay, the freshly grown *E. coli* isolates were streaked and cultured on 5% Sheep blood agar for 24-48 hours at 37°C following the procedure described by (Beutin *et al*., 1989). The zone of haemolysis production was recorded.

Antimicrobial susceptibility testing

Antimicrobial resistance profiling of recovered isolates was carried out using the disc diffusion method, as described by Bauer *et al*., (1966). Using an antibiotic zone scale, zones of inhibition were measured after 18 hours and again after 48 hours of incubation. The isolates were classified as sensitive, intermediate sensitive or resistant based on the diameter of the zone of growth inhibition (CLSI, 2020). The isolates were evaluated against a panel of 15 unique antibiotics belonging to different classes and which are routinely used in human and animal treatment as shown in Table 1.

Determination of the multiple drug resistance (MAR) index

The MAR index (b) was calculated using the method described by Krumperman (1983), which divides the number of antibiotics used in the investigation by the number of antibiotics an isolate is resistant to (a). The calculating formula is as follows: MAR index equals a/b.

RESULTS AND DISCUSSION

Occurrence of *E. coli* isolates

In this investigation, on screening a total of 405 raw fresh meat samples collected from several butcher shops in the Udgir city, Maharashtra altogether 39 samples were found positive for *E. coli* suggesting the overall occurrence of *E. coli* to the tune of 9.63%. Chicken and chevon samples exhibited 12% (24/200) and 7.32% (15/205) occurrences of target organism, respectively.

***In vitro* pathogenicity assessment**

All 39 presumed *E. coli* isolates tested positive in the Congo red dye binding experiment and haemolysis assay used to assess the pathogenicity of bacteria. The positive isolates developed intense brick red colonies on Congo red agar and showed haemolysis in haemolysis assay.

Table 1: Antimicrobial susceptibility pattern of *E. coli.*

Antibiotics	Antibiotic class	No. of isolates			Percentage %		
		S	M	R	S	M	R
Amoxicillin/Clavulanic acid	β lactam	1	1	37	2.56	2.56	94.87
Tetracycline	Tetracyclines	12	8	19	30.77	20.51	48.72
Cefalexin	1st Generation Cephalosporins	13	12	14	33.33	30.77	35.90
Cefaclor	2nd Generation Cephalosporins	10	10	19	25.64	25.64	48.72
Cefotaxime	3rd Generation Cephalosporins	6	14	19	15.38	35.90	48.72
Cefepime	4th Generation Cephalosporins	5	2	32	12.82	5.13	82.05
Ciprofloxacin	fluoroquinolones	19	7	13	48.72	17.95	33.33
Enrofloxacin	fluoroquinolones	17	8	14	43.59	20.51	35.90
Ofloxacin	fluoroquinolones	9	11	19	23.08	28.21	48.72
Azithromycin	Macrolides	30	2	7	76.92	5.13	17.95
Gentamicin	Aminoglycosides	29	7	3	74.36	17.95	7.69
Lincomycin	Lincosamides	3	3	33	7.69	7.69	84.62
Nalidixic Acid	Quinolones	13	7	19	33.33	17.95	48.72
Trimethoprim	DHFR Inhibitors	8	6	25	20.51	15.38	64.10
Vancomycin	Glycopeptides	11	8	20	28.21	20.51	51.28

Where, S- Sensitive, M- Intermediate sensitive, R- Resistant.

Antibiogram profiling of recovered *E. coli* isolates

The results showed that the majority of the isolates had multidrug resistance characteristics (Table 3). Two individual isolates recovered from raw chicken meat sample demonstrated resistance against 12 and 11 antibiotics respectively. while, two other isolates recovered from chicken samples and 3 isolates recovered from chevon samples showed resistance against 10 antibiotics (Table 3). Similar trends were also seen in the current study's observation of sample group-wise antibiotic resistance of *E. coli* isolates. As shown in Table 2, a significant percentage of *E. coli* isolates obtained from chicken and chevon exhibited antibiotic resistance to several drugs. The majority of these isolates were found to be resistant to antibiotic classes based on the data obtained are β-lactams (94.87%), lincosamides (84.62%), 4th Generation cephalosporins (82.05% each), DHFR Inhibitors (64.10%), glycopeptides (51.28%) followed by fluoroquinolones, quinolones, 2nd and 3rd generation cephalosporins (48.72% each) (Table 1).

Multiple drug resistance (MAR) index

The multi-drug resistant (MDR) isolates in the present study showed resistance to a minimum of 4 and a maximum of 12 antibiotics. Thus, 9 resistance patterns were observed ranging from 4 to 12 antibiotics with maximum multiple antibiotic resistance (MAR) index of 0.8 and a minimum MAR index of 0.27 in MDR isolates as depicted in Table 3. A MAR greater than 0.2 indicates that places with high usage of antibiotics are the source of contamination (Davis and Brown, 2016).

Escherichia coli organism is also regarded as an indicator of faecal contamination in food and water. It is a commonly found commensal bacterium in humans and the environment (Rahman *et al*., 2017). In this study, the overall occurrence of *E. coli* was observed to the tune of 9.63% in raw fresh meat samples collected from different butcher shops in the Udgir city, Maharashtra. These findings can be corroborated with Suryawanshi *et al*. (2023) and Deshmukh *et al*. (2023) who made similar observations, reporting an overall prevalence of *E. coli* of 9.17% and 12%, respectively, in raw fresh meats collected from same geographic area.

In current investigation, chicken and chevon samples revealed 12% (24/200) and 7.32% (15/205) occurrence of target organism. Suryawanshi *et al*., (2023) screened 425 samples of fresh meats comprising chicken, mutton, chevon and carabeef. Researchers found that the occurrence of *E. coli* was 12.50% (15/120) in chicken and 9.52% (10/105) in chevon, which is consistent with the results of the present study. However, these results contradict with the findings reported by Bhoomika *et al*., (2016) and Mawia *et al*., (2012). Bhoomika *et al*., (2016) reported 66.32% (65/98) and 46.34% (38/82) occurrence of *E. coli* in chicken meat and chevon samples collected in Chhattisgarh, India. While, Mawia *et al*., (2012) also obtained 47 (28.14%) *E. coli* isolates comprising 22 (25.88%) from chevon samples and 25 (30.49%) from chicken on screening 167 meat samples collected from local markets of Jammu, India. These values of higher prevalence of *E. coli* from chicken and chevon samples reported can be attributed to the variation of sampling methods, detection protocols, poor sanitary practices adopted during handling of meat with the use of microbiologically contaminated water and difference of area of sample collection.

Berkhoff and Vinal (1986) and Roy *et al.* (2006) confirmed the recovery of 100% Congo red dye-binding isolates in their investigations. Based on their findings, researchers suggested using the Congo red binding assay

Table 2: Sample group wise antibiotic resistance of *E. coli* isolates.

Antimicrobial agents	Chicken isolates N=24	%	Chevon isolates N=15	%
Amoxicillin/ Clavulanic acid	23	95.83	14	93.33
Tetracycline	13	54.17	06	40.00
Cefalexin	07	29.17	07	46.67
Cefaclor	10	41.67	08	53.33
Cefotaxime	13	54.17	06	40.00
Cefepime	20	83.33	12	80.00
Ciprofloxacin	10	41.67	03	20.00
Enrofloxacin	10	41.67	04	26.67
Ofloxacin	13	54.17	06	40.00
Azithromycin	05	20.83	02	13.33
Gentamicin	03	12.50	00	0.00
Lincomycin	19	79.17	14	93.33
Nalidixic Acid	10	41.67	09	60.00
Trimethoprim	15	62.50	10	66.67
Vancomycin	13	54.17	07	46.67

Where, N- Number, %- Percentage.

as a phenotypic marker to discriminate between pathogenic and non-pathogenic isolates. In current investigation, all 39 *E. coli* isolates were tested positive in the Congo red dye binding experiment and also showed haemolysis in haemolysis assay, suggesting their invasive character.

Microbiologists and public health veterinarians are urged all over the world to survey the antibiotic resistances of key foodborne pathogens in order to deliver epidemiological data to the professionals in charge of public health in order to make recommendations on the appropriate use of antibiotics. For current experiment, the most frequently used antibiotics in both human and animal health were chosen to be tested against recovered *E. coli* isolates. The investigation's findings showed that the majority of the isolates had a pattern of multidrug resistance. All 39 isolates recovered were found to be resistant to at

Table 3: Multiple drug resistance (MAR) index of *E. coli* isolates from different type of samples.

Isolate number	Type of sample	Resistant to antibiotics	Resistance to number of antibiotics	MAR index
AP1	Chicken	AMC, TE, CPM, CIP, EX, OF, L, TR	8	0.53
AP2	Chicken	AMC, TE, CTX, EX, AZM, L, TR, VA	9	0.6
AP3	Chicken	AMC, TE, CTX, CPM, CIP, OF, GEN, L, NA	10	0.66
AP4	Chicken	AMC, TE, CPM, EX, OF, TR	7	0.47
AP5	Chicken	AMC, TE, CN, CTX, CPM, CIP, EX, AZM, L, TR, VA	12	0.8
AP6	Chicken	AMC, TE, CN, EX, OF, AZM, L	8	0.53
AP7	Chicken	AMC, TE, CN, CTX, CPM, CIP, AZM, L, TR	10	0.66
AP8	Chicken	AMC, TE, CN, CTX, CPM, CIP, OF, NA	9	0.6
AP9	Chicken	AMC, CN, CPM, EX, GEN, L, TR, VA	9	0.6
AP10	Chicken	AMC, CN, CPM, OF, GEN, L, NA	8	0.53
AP11	Chicken	AMC, TE, CN, CTX, CPM, CIP, TR, VA	9	0.6
AP12	Chicken	AMC, CPM, CIP, OF, L, VA	6	0.4
AP13	Chicken	AMC, TE, CTX, EX, OF, L, VA	7	0.47
AP14	Chicken	AMC, CTX, NA, VA	4	0.27
AP15	Chicken	AMC, TE, CTX, CPM, OF, TR	6	0.4
AP16	Chicken	AMC, CTX, CPM, EX, OF, AZM, L, NA, TR, VA	11	0.73
AP17	Chicken	AMC, CPM, L, TR, VA	6	0.4
AP18	Chicken	AMC, TE, CPM, OF, L, NA, TR, VA	8	0.53
AP19	Chicken	AMC, CTX, CPM, CIP, EX, L, TR, VA	8	0.53
AP20	Chicken	AMC, CPM, EX, L, NA, TR	6	0.4
AP21	Chicken	AMC, CTX, CPM, OF, L, VA	6	0.4
AP22	Chicken	AMC, CPM, CIP, L, NA	5	0.33
AP23	Chicken	AMC, TE, CPM, L, NA, TR	5	0.33
AP24	Chicken	CTX, CPM, CIP, OF, L, NA, TR, VA	8	0.53
AC1	Chevon	CTX, CPM, EX, L, NA, TR	6	0.4
AC2	Chevon	AMC, TE, CPM, OF, L, VA	6	0.4
AC3	Chevon	AMC, CPM, CIP, L, TR, VA	6	0.4
AC4	Chevon	AMC, CN, CTX, CPM, EX, VA	7	0.47
AC5	Chevon	AMC, CN, CPM, CIP, EX, OF, L, NA, TR	10	0.66
AC6	Chevon	AMC, CN, CPM, AZM, L, TR, VA	8	0.53
AC7	Chevon	AMC, TE, CN, CPM, EX, OF, L, NA, TR	10	0.66
AC8	Chevon	AMC, CN, L, NA, TR, VA	8	0.53
AC9	Chevon	AMC, TE, CN, CTX, OF, AZM, L, TR, VA	10	0.66
AC10	Chevon	AMC, TE, CN, CPM, CIP, L, NA	8	0.53
AC11	Chevon	AMC, CPM, OF, L, NA, TR	7	0.47
AC12	Chevon	AMC, CTX, L, TR	4	
AC13	Chevon	AMC, TE, CTX, CPM, L, NA	6	0.4
AC14	Chevon	AMC, CTX, CPM, L, NA, TR	6	0.4
AC15	Chevon	AMC, TE, CPM, OF, L, NA, VA	7	0.47

Where, AMC- Amoxicillin/Clavulanic acid, TE- Tetracycline, CN- Cefalexin, CF- Cefaclor, CTX-Cefotaxime, CPM- Cefepime, CIP- Ciprofloxacin, EX- Enrofloxacin, OF- Ofloxacin, AZM- Azithromycin, GEN- Gentamicin, L- Lincomycin, NA- Nalidixic acid, TR- Trimethoprim, VA- Vancomycin, MAR- Multiple drug resistance index.

least four of the antibiotics that were tested against them. Occurrence of two isolates from chicken showing resistance against 12 and 11 antibiotics respectively was also noted. While, 06 isolates including 03 from chicken and remaining 03 from chevon, displayed resistance against 10 different antibiotics. In our investigation, a very high percentage of antibiotic resistance was noted against amoxycillin-clavulanic acid (94.87%), lincomycin (84.62%), cefepime (82.05%), trimethoprim (64.10%), vancomycin (51.28%), ofloxacin, nalidixic acid, cefotaxime, cefaclor and tetracycline (48.72% each) followed by other antibiotics with resistance levels under 40%. Similar high multidrug resistance pattern was also reported by other researchers like Deshmukh *et al*. (2023), Adzitey (2015) and Uzeh *et al*., (2021). The results of present study are consistent with that of observed by Deshmukh *et al*. (2023) who screened *E. coli* isolates recovered from poultry farms environment, chicken meat retailers shop, raw chickens from Udgir city of Maharashtra, wherein they reported high percentage of antibiotic resistance against lincomycin and tetracycline (85.29%), nalidixic acid and vancomycin (82.35% each), ofloxacin (67.64%), amoxycillin-clavulanic acid (61.76%), cefepime (41.17%), trimethoprim (38.23%) and cefaclor (47.05%) with similar MDR pattern of each isolate showing resistance to at least four antibiotics. Adzitey (2015) screened 45 *E. coli* isolates from Beef and its related samples of Ghana and observed a very high resistance to amoxycillin-clavulanic acid (86.67%), trimethoprim (82.22%) and vancomycin (88.89%). Similarly, Uzeh *et al*., (2021) studied *E. coli* isolates from raw meats and revealed MDR in 22% of the isolates, as well as resistance against ampicillin (57%), tetracycline (45%) and sulfamethoxazole-trimethoprim (21%).

Despite the lack of antibiotic usage histories to link with susceptibility data, findings of this study might be interpreted as reflecting, at least in part, the selective pressures exerted by antimicrobial use in food animal production and processing contexts. It is hypothesised that increasing levels of resistance in food animals are attributable in part to modern production practises, in which antibiotics for disease prevention and control are provided through water as well as feed. Resistance to expanded-spectrum cephalosporins, as observed in current experiment, is of particular concern because these antimicrobials are used as first-line therapy for a variety of Gram-negative infections, especially systemic and paediatric salmonellosis.

In this study, it was noted that a noteworthy percentage of *E. coli* isolates recovered from chicken as well as chevon samples exhibiting virtually identical trend of MDR pattern to set of antibiotics It may be the result of a meat-selling strategy used by retailers in India, in which the same shops sell both chicken and chevon meat, based on the preferences of the customers., It additionally introduces the chance that *E. coli* bacteria may appear as a secondary contamination while cutting chicken and chevon meats with the same contaminated cutting boards or knives and/or washing water. Insufficiently cleaned chopping boards and post-processing meat handling equipment have been linked to cross-contamination, according to Suryawanshi *et al*., (2023). Uzeh *et al*., (2021) also speculated the possibility of contamination of raw meats with antibiotic resistant pathogenic organisms at unhygienic slaughter as well as sale points.

Finding regarding MDR isolates in the present study showing resistance to a minimum of 4 and a maximum of 12 antibiotics with minimum MAR index of 0.27 and maximum MAR index of 0.8 and 9 resistance patterns are comparable with Adzitey (2015), who observed MAR index ranging from 0.11to 0.78 shown by *E. coli* isolates recovered from meats. Multidrug resistant *E. coli* was also discovered by Hassanien *et al*., (2016), Adzitey *et al*., (2020), Abass *et al*., (2020), Jaja *et al*., (2020), Mir *et al*., (2022) Ahmed *et al*., (2023), with MAR indices ranging from 0.14-1, 0.13-1, 0.22-0.78, 0.2–0.5, 0.45-0.81 and 0.32-0.95, respectively.

CONCLUSION

In conclusion, the current analysis shows a high occurrence of *E. coli* in retail meats, showing that faecal contamination at slaughter and processing is prevalent and emphasizing the significance of consumer awareness about proper food handling and cooking. Retail meats are an exposure site close to the customer, so it's important to keep track of the occurrence of antimicrobial resistance among commensal and pathogenic microorganisms that are found in such products in order to identify emerging resistance issues in the food supply chain.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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