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Research Article

Occurrence, antibiogram and MAR indices of *Escherichia coli* isolated from food of animal origin in and around Mhow, Indore, (M.P.)

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Abstract

Animal source foods (ASF) are wholesome meal with efficient source of bioavailable micronutrients. However, their contamination with food borne pathogen especially the strains with resistance against multiple antimicrobials is critical threat to human health. The present investigation was conducted with the objectives of studying the occurrence, antimicrobial susceptibility by disc diffusion method and risk level categorization based on AMR indices of *E. coli* isolated from milk and chicken meat samples. Amongst, a total of 158 samples, comprising milk (n=80) and chicken (n=78) collected from various retail shops in and around Mhow, Indore, M.P., overall, 63.29% samples have been found positive for occurrence of *E. coli*, with sample-wise occurrence of 65% and 61.53% in milk and chicken meat samples, respectively. The antibiogram revealed overall highest resistance against ciprofloxacin (65%) and the least (2%) towards amoxyclav and gentamicin. *E. coli* isolated from milk recorded highest resistance against ciprofloxacin (46.15%). Similarly, chicken meat isolates of *E. coli* showed highest resistance (85.42% each) against ciprofloxacin and tetracycline. The MAR indices of *E. coli* were found in the range of 0.11 to 0.94 suggesting overall 22.85% and 77.14% isolates to be likely originated from low and high-risk source of contamination.

Keywords: Antimicrobial resistance, Chicken, *E. coli*, MAR indices, Milk

Highlights

- Taken as a whole,63.29% samples of ASF were positive for occurrence of *E. coli*
- Highest AMR was observed against ciprofloxacin (65%) and lowest (2%) to amoxyclav and gentamicin.
- The MAR indices of isolates were found in the range of 0.11 to 0.94
- Overall, 22.85% isolates were traced to low, while 77.14% to high-risk sources of contamination

INTRODUCTION

Being a complete food for humans, Animal source foods (ASF) are a compact and effective source of micronutrients that are hard to obtain in suffice from plant-based foods alone (Schonfeldt et al., 2014). Nevertheless, they are perfect medium for the growth and proliferation of both commensal and pathogenic bacteria due to their higher nutritional content, particularly under unsanitary conditions. Consuming ASF tainted by pathogens has frequently been linked to severe outbreaks of food-borne illnesses (Cerva et al., 2014).

Increased consumer demand for ASF protein, has made utilization of antibiotics inevitable in livestock production (Van Boeckel et al., 2019). This significantly influences the spread of antimicrobial resistance (AMR). Improper dose and repetitive utilization of antimicrobials in animal production is most important factor in emergence, dissemination, and selection of AMR (Sayah et al., 2005; Tempini et al., 2018). ASF can create perfect conditions for establishment of novel resistant bacterial strains through acquisition of several resistance determinants (Kim et al., 2022). Antibacterial resistant pathogens can reach humans through consumption of ASF (Van den Bogaard et al., 2000; Silbergeld et al., 2008). Although *E. coli* is a commensal bacterium in the intestine of both animals and humans, it is also often utilized as an indicator organism for AMR (Moreno et al., 2000) because of its widespread

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distribution in the gut and high genomic plasticity, which allows it to readily acquire genes encoding AMR (Darwich et al., 2019).

However, there is dearth of knowledge on the carriage and antimicrobial susceptibility profile of *E. coli* in foods of animal origin in the region. Therefore, current investigation was carried out (i) to study occurrence of *E. coli* in animal source food sold in retail shops in and around Mhow, Indore, Madhya Pradesh (ii) to evaluate level of resistance of isolated strains against commonly used antimicrobial agents and (iii) to assess the level risk associated with contamination for each strain.

MATERIALS AND METHODS

Collection of samples: A total of 158 samples, comprising of milk (n=80) and chicken meat (n=78) from various retail shops in and around Mhow, Indore, Madhya Pradesh were collected aseptically in sterilized sample containers and transported to the laboratory in chilled condition in an icebox for further study.

Isolation and identification of *E. coli*: Isolation of *E. coli* from both milk and chicken meat samples followed by identification was conducted following the procedures drawn by Agarwal et al. (2003). The colonies exhibiting a blue-black appearance with a metallic sheen on Eosin Methylene Blue (EMB) agar (Hi-Media, Mumbai) were selected and identified based on cultural characteristics, morphological examination including Gram staining and motility, oxidase and catalase reactions, as well as the IMViC pattern.

Polymerase chain reaction (PCR) for confirmation of *E. coli*: The presumptive *E. coli* isolates obtained were further confirmed by PCR. Here the template DNA were prepared by boiling and snap-chill method (Nagappa et al., 2007). For confirmation of *E. coli,* primers targeting the species specific 16S rRNA gene were utilized, which produced an amplicon of 231bp as described by Sun et al. (2011). The sequences of forward and reverse oligonucleotides (5'-3') are as follows: E16S-Forward ATC AAC CGA GAT TCC CCC AGT and E16S-Reverse CAC TAT CGG TCA GTC AGG AG. PCR was set up in a final 25 µL volume reaction using 2X PCR mastermix (Qiagen, Germany Inc.) with 3 µL of the template DNA added to it. The PCR amplification was carried out in a thermal cycler (Eppendorf, Germany), with initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 50°C for 50 seconds, and extension at 72°C for 1minute. Final extension was setup at 72°C for 10 minutes. The amplified PCR products were subjected to submarine horizontal agarose gel electrophoresis with ethidium bromide fluorescence and visualized using a gel documentation system (Gel DocTMXR, Biorad, USA).

Antimicrobial susceptibility testing (AST) of *E. coli*: *In vitro* AST of the confirmed *E. coli* isolates was assessed by disc diffusion technique as outlined by Bauer et al. (1966) against 18 antimicrobial agents commonly used in human and veterinary medicine. Diameter of zone of inhibition was measured and graded as Sensitive, Intermediate and Resistant as per Clinical Laboratory Standard Institute [CLSI] (2024) and European Committee on Antimicrobial Susceptibility Testing [EUCAST] (2021) guidelines.

Determination of multiple antimicrobial resistance (MAR) index of *E. coli*: MAR index was calculated and interpreted for all *E. coli* isolates demonstrating resistance to multiple antimicrobials as per Krumperman, (1983).

$$\text{MAR index} = \frac{\text{The number of antibiotics to which an isolate is resistant}}{\text{The number of antibiotics to which the isolate exposed}}$$

Determination of risk potential of *E. coli* isolates: Following the calculation of MAR indices, *E. coli* isolates with a MAR index≤0.2 were categorised as originating from low-risk sources, whereas those with a MAR index≥0.2 were considered to have originated from high-risk sources of contamination where a variety of antibiotics are used for therapeutics or as growth promoters, indicating a high risk of antimicrobial resistance (Nandi & Mandal, 2016). Only, *E. coli* isolates demonstrating resistance, to more than one antimicrobial agent were included in this investigation for calculation of MAR indices.

RESULTS

Occurrence of *E. coli* in milk and chicken: In this study, out of 158 samples screened, 100 samples tested positive for the presence of *E. coli*, by conventional and molecular method, demonstrating an overall occurrence of 63.29%. The sample-wise analysis revealed the highest occurrence of 65% (52/80), in milk followed by 61.53% (48/78) in chicken meat samples (Table 1). The amplified PCR product of target 16S rRNA of the *E. coli* isolates (231bp), is depicted in Fig. 1.

Antibiogram of *E. coli* isolates from milk and chicken meat: *E. coli* isolates have been observed resistant to several antimicrobials used in this study in which the overall highest resistance was recorded against ciprofloxacin (65%) followed by tetracycline (54%), cefpodoxime (44%), ampicillin (39%), nalidixic acid and co-trimoxazole (33% each), cefotaxime (32%), sulphafurazole (31%), kanamycin (25%), azithromycin (23%), ceftazidime (16%), aztreonam (14%), streptomycin (12%), cefoxitin (11%), ceftriaxone (9%), chloramphenicol (8%), amoxyclav and gentamicin (2%).

In the sample wise analysis *E. coli* isolates obtained from milk had highest resistance to ciprofloxacin (46.15%) and the least towards gentamicin and chloramphenicol (1.92%), whereas in case of chicken meat isolates highest resistance was observed against both ciprofloxacin and tetracycline (85.42% each) and least resistance towards gentamicin (2.08%) (Table 2).

Table 1. Sample wise occurrence of *E. coli* isolates

Sr. No.	Sample type	No. of samples	No. of samples positive for *E. coli*
1	Milk	80	52 (65%)
2	Chicken meat	78	48 (61.53%)
Total		158	100(63.29 %)

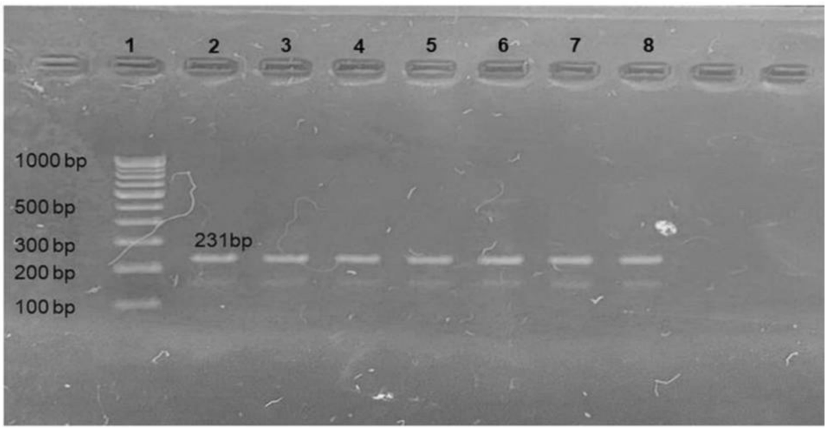

Figure 1: PCR amplification of 16S rRNA (231 bp) for identification of *E. coli*

Lane 1 : 100bp molecular marker
Lane 2 : Positive control (ATCC 25922)
Lane 3-8: Samples

Fig. 1. PCR amplification of 16S rRNA (231bp) for identification of *E. coli*

Table 2. Antibiogram of *E. coli* isolates from milk and chicken meat

Sr. No.	Antimicrobial agent	Concentration (mcg)	Pattern of antibiogram		
			Sensitive	Intermediate	Resistant
1	Cefotaxime (CTX)	30	10(10%)	58(58%)	32(32%)
2	Cefoxitin (CX)	30	58(58%)	31(31%)	11(11%)
3	Ceftazidime (CAZ)	30	39(39%)	45(45%)	16(16%)
4	Ceftriaxone (CTR)	30	67(67%)	24(24%)	09(9%)
5	Cefpodoxime (CPD)	10	37(37%)	19(19%)	44(44%)
6	Aztreonam (AT)	30	56(56%)	34(34%)	14(14%)
7	Amoxyclav (Amoxicilin/ Clavulanic acid) (AMC)	30 (20/10)	73(73%)	25(25%)	02(02%)
8	Ampicillin (AMP)	10	55(55%)	06(06%)	39(39%)
9	Azithromycin (AZM)	15	76(76%)	01(01%)	23(23%)
10	Ciprofloxacin (CIP)	5	16(16%)	19(19%)	65(65%)
11	Nalidixic acid (NA)	30	34(34%)	33(33%)	33(33%)
12	Gentamicin (GEN)	10	87(87%)	11(11%)	02(02%)
13	Kanamycin (K)	30	06(06%)	69(69%)	25(25%)
14	Streptomycin (S)	10	47(47%)	41(41%)	12(12%)
15	Co-Trimoxazole (Sulpha/ Trimethoprim) (COT)	25 (23.75/1.25)	60(60%)	07(07%)	33(33%)
16	Chloramphenicol (C)	30	80(80%)	12(12%)	08(08%)
17	Tetracycline (TE)	30	46(46%)	0(0%)	54(54%)
18	Sulphafurazole (Sulfisoxazole) (SF)	300	61(61%)	08(08%)	31(31%)

Table 3. Multiple antimicrobial resistance (MAR) indices of *E. coli* from milk and chicken meat

Nos. of antimicrobials	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
MAR indices	0.11	0.17	0.22	0.28	0.33	0.39	0.44	0.50	0.56	0.61	0.67	0.72	0.78	0.83	0.89	0.94	1.0
Milk	06	07	01	02	02	01	02	04	-	-	-	-	01	-	-	01	-
No. of isolates Chicken meat	-	03	04	11	04	06	07	03	02	-	-	03	-	-	-	-	-
Total	06	10	05	13	06	07	09	07	02	-	-	03	01	-	-	01	-

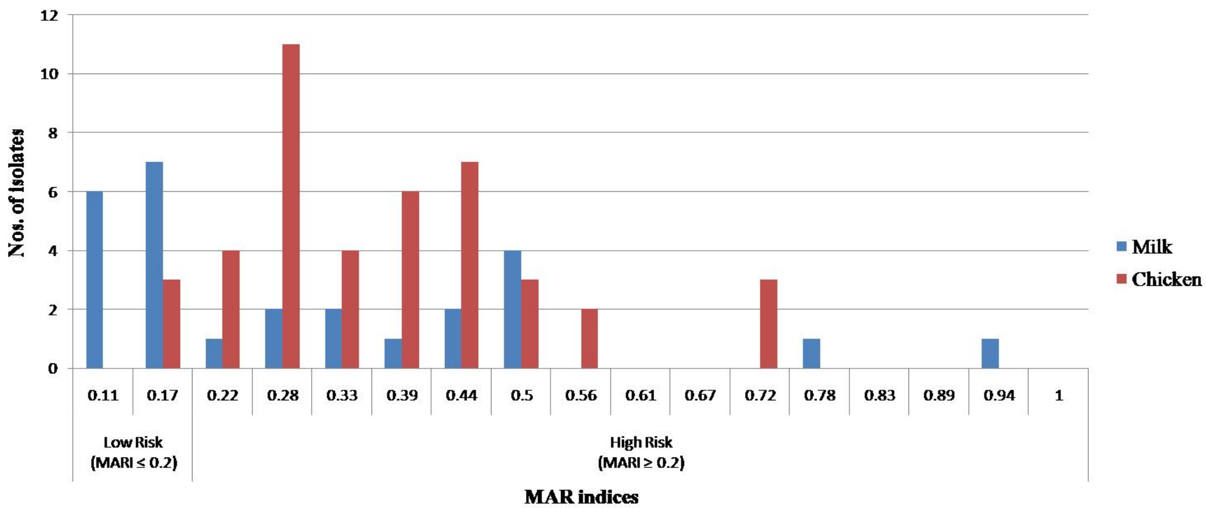

Fig. 2. Multiple antimicrobial resistance (MAR) indices of *E. coli* from milk and chicken meat with risk categorization

Table 4. Risk categorization of *E. coli* isolates from milk and chicken meat

Risk potential	**Low risk**		**High risk**	
Criteria (MAR index)	≤ 0.2		≥ 0.2	
Samples/isolates	Milk (n=27)	Chicken meat (n=43)	Milk (n=27)	Chicken meat (n=43)
Sample wise no. of isolates (%)	13 (48.14%)	03 (6.97%)	14 (51.85%)	40 (93.02%)
Total no. of isolates (%)	**16 (22.85%)**		**54 (77.14%)**	

MAR index of *E. coli* isolated from milk and chicken meat: In this investigation, a total of 20 out of the 100 *E. coli* isolates were observed to be sensitive to all the antimicrobials used (MARI= 0) and 10 isolates were resistant to only one antimicrobial; therefore, they were not considered for risk determination. However, out of the 70 *E. coli* isolates studied, 27 from milk and 43 from chicken meat were found to be resistant to multiple antimicrobials.MAR index of all *E. coli* isolates resistant to multiple antimicrobials in the present study had been found in the range of 0.11 to 0.94. Sample wise investigation revealed MAR indices of *E. coli* isolates of milk in the range of 0.11 to 0.94. Similarly, *E. coli* isolated from chicken meat samples showed MAR indices between 0.11 and 0.72. Overall highest MAR index of 0.94 and 0.72 was observed for milk and chicken meat isolate, respectively (Table 3, Fig. 2).

Risk potential of *E. coli* isolates: Overall, 77.14% (54/70) of the *E. coli* isolates had MAR index≥0.2 while 22.85% (16/70) had observed MAR index≤0.2 (Table 4).

DISCUSSION

In this study, overall, 63.29% samples tested positive for presence of *E. coli*. The sample wise investigation demonstrated that 65% of milk samples carried *E. coli*. In contrast, lower occurrence of 32%, and 40.02% was reported by Nema et al. (2015) and Bedasa et al. (2018), respectively. However, Bhoomika et al. (2016) recorded higher occurrence of 81.11% of *E. coli* in milk sample. With regard to chicken meat, present investigation observed 61.53% positivity, in concurrence with Gida et al. (2020) and Bhoomika et al. (2016) who recorded 61.33% and 66.32% occurrence of *E. coli*, respectively. In contrast, Younis et al. (2017) reported 11.66% positivity in chicken meat samples. Variation in occurrence of *E. coli* in the current research might be due to several factors, such as geographical location, sample size, sampling techniques, methods of isolation and testing, and hygienic standards followed in processing plants and retail outlets.

AMR has become a major threat to both human and animal health (Stella et al., 2018), and one of the hotspots linked to development and spread of AMR is animal production (Merle et al., 2012). This supports the necessity of ongoing surveillance of AMR strains in animals utilized for food production in order to comprehend and reduce AMR. Highest overall resistance had been observed against ciprofloxacin (65%) in this study. In corroboration to present findings Adzitey et al. (2020) also reported much higher resistance of 85% amongst *E. coli* isolated from meat samples. In contrast, only 6.6% isolates were reported to be resistant by Rasheed et al. (2014). Resistance against tetracycline among foodborne *E. coli* was also reported by Rasheed et al. (2014), Tark et al. (2017), Nema et al. (2015) and Adzitey et al. (2020) who reported 12.6%, 23.3%, 30.3% and 73.33% resistant isolates which differs from resistance (54%) observed in the present study. Current investigation also recorded 44% cefpodoxime resistant strains of *E. coli* compared to 66.30% reported by Gida et al. (2020). Limited studies are available regarding nalidixic acid. Nevertheless, Nema et al. (2015) recorded 46.4% resistance, comparable with 33% resistance as observed in the present study. Contrary to the findings of 39% ampicillin resistance in current study, Bedasa et al. (2018), Adzitey et al. (2020), and Nema et al. (2015) reported higher level of resistance *viz.,* 76.92%, 71.67%, and 62.5%, respectively. On the other hand, Rasheed et al. (2014) and Tark et al. (2017) reported lower 13.3% and 16.6% resistance to ampicillin. Thaker et al. (2012) reported cent percent ampicillin resistant strains of *E. coli* from ASF. Unlike previous studies, 33% *E. coli* isolates were found resistant against co-trimoxazole in present investigation. Adzitey et al. (2020) reported highest 85% resistance while 11.2%, 11.3%, 13.16% and 21.4% cotrimoxazole resistant isolates were observed by Tark et al. (2017), Rasheed et al. (2014), Thaker et al. (2012) and Nema et al. (2015), respectively. Reported cefotaxime resistance among *E. coli* isolates has varied widely, from 5.3% (Rasheed et al., 2014) to 83.9% (Nema et al., 2015). However, the current study observed an intermediate 32% resistance that closely aligns with 41.36% resistance documented by Bhoomika et al. (2016). Evidence on sulphafurazole and azithromycin resistance against food borne *E. coli* is scarce, impeding comprehensive comparison. However, present investigation revealed moderate 23% and 31% resistance against these antimicrobials, respectively. The kanamycin resistance reported by Jaja et al. (2020) in the formal (20.4%) and informal (19.6%) meat sectors corresponds closely with the 25% resistance observed in this study. The present investigation also demonstrates16% resistance against ceftazidime that aligns with previous reports of 12.5% and 13.08% by Nema et al. (2015) and Bhoomika et al. (2016), respectively. However, highest 66.30% ceftazidime resistance, inconsistent with the current study was also reported by Gida et al. (2020). The 12% streptomycin resistance observed in this study was in agreement with prior reports of 8% and 17.1% by Rasheed et al. (2014) and Tark et al. (2017); however, Bedasa et al. (2018) reported substantially much higher resistance (69.23%) in food-derived *E. coli* isolates. *E. coli* resistance to aztreonam (14%), cefoxitin (11%), and ceftriaxone (9%) in this study was markedly lower than the findings by Gida et al. (2020) viz., 35.86%, 29.34%, and 82% resistance, respectively. In case of chloramphenicol, 8% *E. coli* isolates recorded resistant in this investigation corroborates with earlier reports of 5.26% and 4.6% resistance by Thaker et al. (2012) and Rasheed et al. (2014). However, higher chloramphenicol resistance (83.33%) was reported by Adzitey et al. (2020). Only 2% isolates were found resistant against amoxyclav in this investigation and aligns with the findings of Rasheed et al. (2014) (4.6%). On contrary, 42.1% amoxyclav resistance was reported by Thaker et al. (2012). The least resistance amongst all the 18 antimicrobials tested was observed against gentamicin (2%), consistent with the findings of Rasheed et al. (2014) and Ranasinghe et al. (2022) who reported 4.6% and 4.79% resistance, respectively.

An effective technique for evaluating health concerns associated with resistance to antibiotics is MAR indexing. MAR indices of *E. coli* isolates in the present study had been found in the range of 0.11 to 0.94. Similarly, sample wise MARI of *E. coli* isolates from milk and chicken meat were also found in range between 0.11 to 0.94 and 0.11 to 0.72, respectively.

Earlier researchers also reported comparable MARI values ranging from 0.11 to 1 for *E. coli* isolated from distinct animal source foods (Varghese & Roymon, 2013; Adzitey et al., 2020; Ayandele et al., 2020).

MAR indexing is widely used to track the bacterial source of contamination (Ranasinghe et al., 2022). The MAR index of bacteria having value≥0.2, indicate that the contamination might have originated from a high-risk source, where numerous antibiotics are likely to be used for therapeutics or as growth promoters. While values≤0.2 demonstrate bacterial contamination from sources with less use of antimicrobials and presents a lower risk of development of drug resistance (Nandi & Mandal, 2016; Akande et al., 2019; Karthikeyan et al., 2023). In all, 77.14% isolates obtained in this study had MARI≥0.2 indicating that these isolates might have originated from high-risk source of contamination that potentiates risk of development of antimicrobial resistance. On contrary, Adenaike et al. (2016) and Ronald et al. (2023) recorded the MARI value ≥0.2 in 53% and 100% *E. coli* isolates respectively. However, only 22.85% isolates in current investigation were having MARI≤0.2 and believed to be originated from low-risk source of contamination. MARI values below or equal to 0.2 have also been reported by Lemlem et al. (2023). The sample wise investigation revealed that 48.14% and 51.85% isolates from milk were low and high-risk isolates, respectively. Conversely, only 6.97% (03/43) isolates from chicken were identified to be low risk whereas 93.02 % (40/43) were seems to be from high-risk source of contamination (Table 4). A dearth of studies on risk assessment of *E. coli* limits comparison with previous findings however, several authors interpreted MARI to offer baseline information about source of bacterial contaminants and origin of resistance in support of present findings (Tambekar et al., 2006; Chandran et al., 2007; Subramani & Vignesh, 2012; Maloo et al., 2014; Oluyege et al., 2014).

Based on present investigation it was concluded that *E. coli* isolates revealed resistance, against commonly used antimicrobials in the clinical practices. The evidence of the presence of MAR strains in ASF indicates likely contamination of the food from sources with higher and injudicious use of antibiotics. This emphasizes the need for epidemiological monitoring of resistance among bacterial contaminants along with use of better hygienic practices on production, processing and distribution ends so as to ensure safe animal source food and preserving public health.

Conflict of interest: Authors declare that there is no conflict of interest.

Author's contribution: R: Involved in investigation, data generation, preparing original draft; RST: Involved in conceptualization, data curation, supervision and final editing; VK: Monitored the research work; RS, JJ: Involved in methodology; AM, SK, PC: Involved in editing.

Data availability statement: The dataset generated during research is available with corresponding author and will be produced on reasonable request.

Ethical approval: Not applicable

Use of artificial intelligence tools: Artificial intelligence tools were not used during the preparation of this work.

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