Antibacterial sensitivity of *Escherichia coli* isolated from milk and milk products in Jabalpur, MP, India

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**Abstract:** Milk and milk products are an essential element of the diet. Apart from its nutritional value, it can also serve as a carrier for multidrug-resistant bacteria attributed to many infectious diseases. The present study was conducted to detect antibiotic-resistant bacteria from milk and milk products (Raw milk, pasteurized milk, and cottage cheese) marketed in Jabalpur city of Madhya Pradesh, India. A total of 640 samples of pasteurized milk, raw milk, and cottage cheese were collected in a sterile container from different dairies and shops of Jabalpur city. Immediately after collection, samples were brought to the microbiology lab within 1 hour. The *Escherichia coli* were isolated from milk and cottage cheese samples, based on cultural and molecular tests. These isolates were further subjected to antibacterial susceptibility against commonly used antibiotics by the disk diffusion methods. Out of 640 samples examined, 118 (18.44%) were positive for *Escherichia coli*. The highest isolation of *Escherichia coli* was from cottage cheese (32%), followed by raw milk (22.5%) and pasteurized milk (0%). Antibiotic susceptibility profile showed that *Escherichia coli* were resistant for nitrofurantoin (61.8%), nalidixic acid (37.2%) and cefotaxime (30.50%). The analysis showed that 89.8 % of isolates showed multidrug resistance comprising 2-3 antimicrobials. The presence of *Escherichia coli* with multiple antibiotic resistances poses a significant threat to public health and food safety. These findings stress the need for better sanitation practices in the production and consumption of milk and milk products and strict monitoring of uses and misuses of antibiotics in humans and food animals.

**Keywords:** Antibiotic resistance, Antibiotic-resistant bacteria, Milk and milk products

**Introduction**

Milk and milk products are an indispensable part of the Indian diet. When milk is secreted from mammary glands to alveoli of the udder, it is generally free from microbes (Tolle, 1980). However, later on, different sources might contribute to the contamination of milk with a wide variety of microbial populations (Mennane et al. 2007). Unhygienic practices in pre milking preparation of udder, substandard hygiene of milk handlers, and poor sanitation practices related to milking and storage equipments are the responsible factors for contamination of raw milk at different critical points (Gardew et al. 2012). Milk and milk products are rich in various nutrients such as; proteins, fats, carbohydrates, minerals, and vitamins. These nutritional contents work as a perfect medium for the growth of microbes. Microbial quality of the milk and milk products also depends upon production procedures and post-production processing, handling, packaging, and storage of products (Kumar et al. 2014). In India, several studies reported contamination of dairy products with various pathogenic microorganisms that could cause disease in humans (Desale et al. 2009; Godbole et al. 2013). According to Elmoir et al. (2018), up to 5% of foodborne infections are attributed to the consumption of milk and dairy products at different critical points (Gardew et al. 2012). Milk and milk products are an efficient vehicle for transmission of diseases causing agents to human beings (Garedew et al. 2012).

*Escherichia coli* bacteria are frequently used as an indicator of fecal contamination of milk and dairy products and may impose the presence of disease-causing serotypes for humans. Various strains of *E. coli* have been associated with several life-threatening food-borne outbreaks worldwide (Elmoir et al. 2018).

Antibacterial resistance can further increase the mortality rate as various resistant strains of *Escherichia coli* have been reported globally (Bell et al. 2002). Being referred to as ‘AMR Capital’
condition for India is challenging as other infectious diseases such as Malaria, Tuberculosis, and Cholera are still prevalent in the communities (Chaudhry et al. 2017). Due to a lack of awareness about communicable diseases and poor access to health sectors, the Indian population, usually self medicate with antimicrobial agents, having no knowledge of doses and duration of treatment (Morgan et al. 2011). Various other factors, such as poverty, illiteracy, malnutrition, and overcrowding, further increase the problem (Swaminathan et al. 2016).

In milk production, India acquires the first position in the world (Awan et al. 2014). It is estimated that India’s milk production will reach up to 180-200 million by 2021-2022, with a growth rate of 5% per annum (Parekh, 2011). However, with the amplifying production of milk, the demands of milk have also been increased over the years due to exponential population growth rate, increased urbanization, and scattered colonization (Awan et al. 2014). Still, there exists a gap in supply and demand. Because of this gap and poorly organized non-regulatory marketing system, the quality of milk is being compromised. Therefore, this study aimed to investigates the prevalence of Escherichia coli as a principal foodborne agent in milk and dairy products collected from markets in Jabalpur town of India. Antibiotic resistance of this pathogen was also studied.

Material and Methods

Study area

This study was conducted in the Jabalpur city of Madhya Pradesh, India. This city is purposively selected because it is one of three most populous city of Madhya Pradesh (Jabalpur ranks third after Indore and Bhopal with a population of 1,268,848 in 2011 survey) making it more prone to faulty practices in milk distribution to fulfill the demand of such a large population.

Experimental design

A cross-sectional study was conducted from October 2015 to March 2016 to determine the incidence and antibacterial resistance of Escherichia coli in milk and milk products (Raw milk, pasteurized milk, and cottage cheese) samples. In the present study, 640 samples (Raw milk = 240, pasteurized milk = 200 and cottage cheese = 200) were collected. Dairy outlets, shops, and supermarkets that had a high level of consumers were included in this study.

Collection and analysis of samples for laboratory analysis

Samples were collected according to the instructions introduced by the International Dairy Federation. Until the analysis was performed, the samples had kept at 4°C.

Analysis of samples

Isolation and Identification of Escherichia coli

Enrichment of the sample was done by using EC-broth at 37°C for 24 hours, after that, Mac Conkey agar media were streaked by enriched sample and incubated at 37°C for 24 hours. The single pink colony was then picked up and gram stained for morphological identification and further transferred on Eosin Methylene Blue agar to get typical metallic sheen colonies of Escherichia coli. These metallic sheen colonies were transferred to nutrient agar to conduct further confirmatory biochemical tests (IMViC).

Testing for antibacterial susceptibility

The disc diffusion method was used to determine the antibacterial susceptibility. Mueller-Hinton agar media was used for this purpose as per the criteria defined by the National Committee for Clinical Laboratory Standards (Kiehlabuch et al. 2000). The isolated Escherichia coli were tested for sensitivity to the most commonly used antibiotics including, Ciprofloxacin (CIP) (05 mcg), Cefazidime (CAZ) (30 mcg), Cefotaxime (CTX) (30 mcg), Netilin (NET) (30 mcg), Ofloxacin (OF) (01 mcg), Norfloxacin (NX) (50 mcg), Nalidixic acid (NA) (30 mcg), Nitrofurantoin (NIT) (300 mcg), Gentamicin (GEN) (30 mcg). Antibiotics discs were procured from HiMedia Laboratories Pvt Ltd, Mumbai, and Maharashtra.

Results and Discussion

The results of analysis for prevalence and antibacterial sensitivity of Escherichia coli are presented in Tables 1, 2, and 3. The present research revealed that Escherichia coli were isolated from 18.44% of milk and milk products (raw milk, pasteurized milk, and cottage cheese). Meanwhile, this study also confirmed that Escherichia coli were not present in pasteurized milk. The presence of Escherichia coli in pasteurized milk does not necessarily show that organism can survive the pasteurization temperature. Poor hygienic handling after the milk is pasteurized might contributes to milk contamination. Our findings simulate with the results obtained by Bedasa et al. (2018), where they observed the absence of Escherichia coli in pasteurized milk.

In the current research, 22.5% of raw milk samples were found to harbor Escherichia coli, which is somewhat in agreement with the report of 21.66% by Bonyadian et al. (2014). However, prevalence in current study is much lower when compared to 44.5%, 100%, 51.66%, and 83% prevalence reported by Tadeesi et al. (2018) from Ethiopia, Swai and Schoonman (2011) from Tanzania, Soomro et al. (2002) from Tandozam Pakistan (51.66%) and Kilango et al. (2012) from Deres Sallam, Tanzania respectively and far higher when compared to 14.65% prevalence reported by Younis et al. (2018). These variations might be due to differences...
in animal management, milking system, and milk handling and storage practices in different countries.

Further, in the present study, 32% isolation rate of *Escherichia coli* was recorded from cottage cheese (Cottage cheese) samples. This prevalence is slightly higher than the report of Ombark et al. (2018) (29.7%) and De Campos et al. (2018) (19.8%). Though, when Indian cottage cheese (Cottage cheese) is manufactured under strict conditions, it may not contain any pathogens, but unsanitary practices during handling, storage, and packaging, after the product is prepared, might contribute to the growth of these organisms (Rao et al. 1992; Kumar et al. 2014). Several studies reported a high prevalence of *Escherichia coli* (Kumar et al. 2010; Ahmadi and Panda, 2015; Selvamalar et al. 2018) because other than the contamination during handing, *Escherichia coli* was also found to survive the manufacturing of cottage cheese. Unpasteurized and improperly pasteurized milk could be a vital source for the transmission of this pathogen (Wahi et al. 2006)

Most of the foodborne illness is associated with foods of animal origin. Now a day, the drug-resistant pathogen in milk and milk products is becoming an increasing public health problem worldwide due to the excessive use of antibiotics in animal feed (Pérez-Rodríguez et al. 2019). Resistance towards drugs also emerges from the extensive use of antibacterial in humans and animals and consequent transfer of resistance genes among animals, human beings, animal products, and surroundings (Tadeesi et al. 2018).

In India, there have been reports on drug resistance of *Escherichia coli* isolates from milk and milk products (Selvamalar et al. 2018; Singh et al. 2018). Other researchers have previously reported the link between the use of antibacterial drugs in animal farming and the incidence of antibacterial resistant organisms in the food products obtained from these animals (Aaresbeep, 2000; Asai et al. 2005; Van den Boogard et al. 2001).

The high antibacterial resistance observed in this research might be due to the extensive use of antibiotics in animals to treat different diseases. In the current study, maximum numbers of isolates were resistant towards nitrofurantoin (61.8%), nalidixic acid (37.2%) and cefotaxime (30.50%). Meanwhile, this study also revealed that all the *Escherichia coli* isolates were sensitive to ofloxacin. Similarly, Esquivel et al. (2008) and Bhatt & Lakhy (2008) also reported the sensitivity of *Escherichia coli* towards ofloxacin. Reported resistance of nitrofurantoin and nalidixic acid in this study was similar to the findings of Uddin et al. (2011). However, various researchers reported that *Escherichia coli* is

### Table 1 Prevalence of *Escherichia coli* in different sources (n=640)

| Products          | No. of samples | No. of samples Positive | Percentage of sample positive |
|-------------------|----------------|--------------------------|------------------------------|
| Pasteurized Milk  | 200            | 0                        | 0%                           |
| Raw Milk          | 240            | 54                       | 22.5%                        |
| Cottage Cheese    | 200            | 64                       | 31.6%                        |
| Total             | 640            | 118                      | 18.43%                       |

### Table 2 Antibiotic sensitivity of *Escherichia coli* isolated from Milk and Milk Products (n=118)

| S.No. | Name of Antibacterial Agent | No. of isolates screened | Resistant | Intermediate | Sensitive |
|-------|----------------------------|--------------------------|-----------|--------------|-----------|
| 1     | Norfloxacin(10mcg)         | 118                      | 04 (3.38%)| 06 (5.08%)  | 108 (91.5%)|
| 2     | Ofloxacin(5 mcg)           | 118                      | 0(0%)     | 0(0%)        | 118 (100%)|
| 3     | Cefazidime(30mcg)          | 118                      | 15 (12.71%)| 09 (7.63 %) | 94 (79.66%)|
| 4     | Ciprofloxacin(5mcg)        | 118                      | 09 (7.63%)| 19 (16.10%) | 90 (76.27%)|
| 5     | Cefotaxime(30 mcg)         | 118                      | 36 (30.51%)| 35 (29.66%) | 47 (39.83%)|
| 6     | Nalidixic acid(30 mcg)     | 118                      | 44 (37.29%)| 04 (3.39%) | 70 (59.32%)|
| 7     | Nitrofurantoin(300mcg)     | 118                      | 73 (61.86%)| 22 (18.64%) | 23(19.49%)|
| 8     | Gentamicin(30 mcg)         | 118                      | 14(11.86%) | 0 (0%)      | 104(88.13%)|

### Table 3 Multiple drug resistance in *E coli* isolates

| Number of Antimicrobials | Number of resistant Isolates | Percentage of resistant Isolates |
|--------------------------|------------------------------|---------------------------------|
| One drug                 | 12                           | 10.16                           |
| Two drug                 | 71                           | 60.17                           |
| Three drug               | 35                           | 29.66                           |
| Multi drug resistant isolates | 106                     | 89.83                           |
highly susceptible to nitrofurantoin (Hafsa et al. 2013; Ntuli et al. 2016; and Abike et al. 2015), which is contrary to the results of our research. But in Egypt, Elmonir et al. (2018) reported that *Escherichia coli* isolates were resistant to nitrofurantoin, which is in line with the findings of the present study.

Furthermore, the current study also revealed that *Escherichia coli* showed resistance to gentamicin, ciprofloxacin, norfloxacin, ceftriaxone. However, the percentage of resistance varied with the antibiotics. These variations could be a manifestation of the use and misuse of these antibiotics in the population. These findings are not shocking because, in India, the general population has easy access to various antibiotics at any drug store without any prescription from a medical practitioner.

Multidrug resistance analysis showed that 89.8% of tested isolates were resistant to two to three antibiotics. These results are in line with the finding of Mude et al. (2017), who showed 92% of multidrug-resistance. Moreover, various researchers (Bekele et al. 2014; Iweriebor et al. 2015; Atnafie et al. 2017) from the different countries recorded multidrug resistance pattern. According to Aarestrup (1999) and Levin et al. (1997), multiple resistances are capable of regional dissemination and can develop as a result of antibacterial selection pressure in either live stocks or humans. Several pieces of evidence suggest that transmission of a resistant pathogen can occur in humans through food too (Oosterom, 1991; Khachatourians, 1998).

**Conclusions**

Milk and milk products collected from Jabalpur city were contaminated with *Escherichia coli*, and these bacteria showed resistance to various antibiotics. Contamination may originate from infected animals or unsanitary practices during processing, handling, and distribution of these products. Importantly, the incidence of *Escherichia coli* and its multiple antibiotics resistant profile reveals a risk for public health and food safety (Ulukanli et al. 2006). Therefore, good hygiene and sanitation practices should be mandated in all the farms and dairy outlets. Furthermore, there is a need for stricter laws to limit the sale of antibiotics to the population with a valid prescription from qualified medical professionals only.

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