MULTIDRUG RESISTANCE PATTERN OF *E. COLI* FOUND IN MILK AND MILK PRODUCTS SAMPLES

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ABSTRACT

Milk is wholesome nourishment for humans, milk and it's by products offer the perfect conditions for the growth of microorganisms. Consuming such microbe-contaminated items can lead to serious infectious disorders including typhoid, gastrointestinal issues, etc. Numerous microbiological techniques were used to isolate and characterize pathogens during the study. 90 samples of different milk and milk products were gathered from different local markets. Following their initial enrichment in the appropriate enrichment medium, each sample was sent traditional investigation. As per results, using a selective medium, 20 different species of *E.Coli* were recovered from the entire number of samples.

All isolated thermo tolerant *E.coli* was subjected to analyses antibiotic resistance.

This study concludes that there are significant health concerns associated with dairy products. The source of pathogenic bacterial contamination in dairy products varies depending on the type of product and how it is produced and processed, including sanitary milk processing methods, careful product handling, and how long the products are stored.

(Key Words: dairy products, food-borne illnesses, and enteric pathogen, antibiotic resistance pattern)

I. INTRODUCTION

Milk is a very perishable product that can get contaminated at every stage, from the farm to the customer's plate. The purpose of the current study was to evaluate the milk quality offered in Katol City by examining the microbiological load and the frequency of thermotolerant E. coli. More nutrients, including proteins, fats, carbs, vitamins, and minerals, are supplied in milk's easily digestible form than in any other diet, and it also offers immunogenic protection (Ramya P. et al 2015). Food borne infections are the most common and serious health problems affecting human health. Among such infections, salmonellosis is the most common food-borne bacterial infection occurring worldwide. *E. coli* has been of significant interest to food microbiologists as a leading cause of food borne infections (T.A.Nassib et.al. 2003).

Because milk and its byproducts provide a favorable habitat for various microorganisms to flourish, the products get contaminated. Products made from dairy, including butter, curd, khoa, yoghurt, chaka, and paneer, are prone to microbial contamination. However, eating raw milk and its byproducts is thought to be potentially dangerous and has been linked to a number of infectious illnesses, including brucellosis, salmonellosis, infections with Escherichia coli, and poisoning from staphylococcal enterotoxins. (Olawale et. al.), (Delgado C.L. 2003).

Particularly in developing countries, the shelf-life of milk and milk products tends to be shorter as the production of milk and it's processing into different milk products takes place under unsanitary conditions and poor production practices (Mogessie A., 1990). Coliforms are invariably found in raw milk but with good manufacturing practices, their number can be kept very low (Boor K.J, 1998).

Their presence is an indication of unsanitary production practices and/or improper handling of either milk or milk utensils (El Zubeir I.E. M, 2007).

Escherichia coli (*E. coli*) is one of these bacteria that is commonly linked to foodborne illness. *E. coli* is a Gram-negative, non-spore-forming, flagellated, rod-shaped, and facultative anaerobic bacterium belonging to the family *Enterobacteriaceae*. Milk or dairy products are nutritious foods that billions of people consume every day5. Ingestion of raw milk and milk products is one of the sources for foodborne illnesses (Boor K.J. et. al. 1998) (Ei. Zubeir I.E.M. et. al. 2007). In industrialized nations, diarrheal illness due to tainted food affects 33 percent of the population; in underdeveloped nations, it affects almost 70, percent. (Nirwal S. 2013). Little attention has been paid to the connection between antimicrobial resistance and the overuse of antimicrobial medications in food processing, both in people and animals (Faraz A et al. 2013). Research indicates that E. coli multi-drug resistance is a major global concern; yet, little research has been done on this topic, especially in developing nations (Mwangi A, 2000). Therefore, the purpose of this study was to examine the frequency, associated risk factors, and patterns of antimicrobial susceptibility of E. coli in milk and milk products; and to evaluate the hygienic quality of raw milk and the detection of *E. coli and its* antibiotic resistance pattern from milk and milk products as an indicator of revel the possible public health risk.

II. MATERIALS AND METHODS

A total of 90 raw milk and milk product samples were collected from 25 different locations in and around Katol Town were subjected to quality evaluation based on the methylene blue reduction test (MBRT), standard plate count, and coliform count as per the standard procedure.A total of 90 milk and milk product samples (approximately 200 mL) were collected aseptically in sterile containers from bulk milk cans from 25 different locations and markets in and around Katol Town. The samples were transported immediately to the laboratory, maintaining the cold chain and thereafter subjected to bacteriological examination within 3-4 h of collection.

A. METHYLENE BLUE REDUCTION TEST (MBRT)

The MBRT was carried out as per the standard method (M. Karimuthu M. et. al, 2013). One milliliter of methylene blue was added to 10 mL of milk in a sterilized test tube followed by sealing it with a rubber stopper and slowly inverted it 3 times to mix the content thoroughly. The tube was then placed in a water bath maintained at 35°C and examined at regular intervals up to 8 h. Time taken for the sample to decolorize, that is, reduction time, was noted based on the time taken by dye from a definite color (blue) to colorless form (white), and the result was interpreted. The quality of milk was graded as excellent (no decolonization within 8 h), good (decolonization between 5 to and 8 h), fair (decolonization between 2 to and 5 h), poor (decolonization between 20 min and 2 h), and very poor, respectively.

B. BACTERIOLOGICAL ANALYSIS ISOLATION AND IDENTIFICATION OF *E. COLI*

A sample of 25 gmyoghurt, 25 g of cottage cheese, and 25 mL of raw milk were each diluted in 250 ml of buffered saline water. Every sample supplemented with saline water was incubated for 24 hours at 37 °C. Every pre-enriched sample was infected for 24 hours at 37 °C on Mac-Conkey agar (Hi-Media Pvt. Ltd., Mumbai, India). Gram's stain was applied to typical pink colonies grown on Mac-Conkey agar in order to observe their staining and morphological traits. Next, colonies of E. coli that were suspicious were moved to Eosin-Methylene-Blue (EMB) agar, which was provided by Hi-media Pvt. Ltd. in Mumbai, India. Typical E. coli colonies are those that have a metallic sheen on EMB agar. Further confirmatory biochemical assays, such as methyl-red, citrate utilization, Voges-Proskauer, and indole synthesis, as well as lactose and glucose fermentation employing triple sugar iron agar (Quinn et. al. 2002).

C. ANTIMICROBIAL SUSCEPTIBILITY TESTING

The Kirby-Bauer disc diffusion method was used to determine the antimicrobial susceptibility profile of *E. Coli* strains (CLSI, 2017). In order to manufacture Mueller-Hinton

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agar plates (Hi-Media, India), commonly used antimicrobials were taken into account. Subsequently, isolates with bacterial suspension equivalent to 0.5 McFarland standards were equally streaked onto Mueller Hinton agar plates containing antibiotic discs, and the plates were incubated for 18 to 24 hours. Lastly, the diameter of the inhibitory zone was estimated using a black surface and a clear ruler. The results were classified as sensitive, moderate and resistant based on CLSI.

III. RESULTS AND DISCUSSION

In the present study total 90 samples were collected and processed on the basis of morphological such as the gram nature,cultural characters that are the colonies on selective media, biochemical characterization and sugar fermentation,32 possible *salmonella* isolates were recovered. Out of it 9 isolates from curd , 4 isolates from paneer , 5 from butter , 4 from khoa , 4 from chakka and 6 isolates from tak(Butter Milk).

Based on the MBRT results, almost 50% of the 90 samples that were tested were rated as being of poor to extremely poor quality. Just 14.5% of the samples received a good quality rating, and none of the samples could receive an excellent quality rating.The results of present study showed that out of 92 samples of raw milk, 18 milk samples were showed positive for presence of *E.Coli*.

Of these, eleven milk samples were obtained directly from milkmen who delivered the milk to town residents' doorsteps. The existence of *E. Coli* demonstrated the unsanitary state of the distribution tank, storage area, and cow yard. The milk was traditionally dispensed manually from a milk tank. Hand contact with milk samples has the potential to contaminate

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Seven of the eighteen milk samples were obtained from neighborhood milk vendors who worked the morning and evening shifts at the dairy business. Seven milk samples were positive for the presence of *E. Coli* when the samples were examined for the bacteria. The milk was kept cold by the seller in stainless steel containers (Table No. 1).

Table: 01- Antibiotics Used in Study and Its Concentration
Azithromycin (AZM) (30 µg),
Amphicillin (AMP) (10 µg),
Erythromycin (E) (30 µg),
Amikacin (AMK) (30 µg),
Ceftriaxone (CTR) (5 µg),
Tetracycline (TET) (30 µg),
Amoxycclave (AMC)(300
Amoxicillin (AMX). (20 µg),
Gentamycin (GEN) (10 µg),
Ciprofloxacin (CPR) (5 µg),
Choremphinecol (C) (30) µg),

Bacteriological Quality of Milk (MBRT):The raw milk sample results from 20 various locations in and around Katol Town are reported based on the MBRT analysis. Based on the amount of time needed to decrease the dye to a colorless form, only 25% of the samples were deemed to be of good quality; none of the samples could be rated as exceptional quality. Of the ninety milk samples, forty-one percent could reduce the dye in two to five hours and were graded as fair, twenty-three percent were poor (dye reduction time: 20 minutes to two hours), and eleven percent were very poor quality (dye reduction time: less than twenty minutes), regardless of where the samples were located. The highest percentages of samples were graded as poor quality.

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Similarly out of 92, 20 milk products samples were contaminated with *E. Coli*. Out of These 20, E.coli found in 3 Curd, # Butter Milk (Tak), 4 Paneer , 2 Butter 1 form Chakka (Shrikhand Base) and 4 Khoa Samples. All *E. Coli* positive samples were collected from the local Dairy Shop and vendors of Katol and nearby Town. When the results were compare with the hygienic condition of these shops it was found that the hygienic condition was not up to the marks, the probable contamination sources might be storage tank, milk product handling as well as packaging (Table 2).

Table No.02 :- *E.coli* isolated from total milk samples .

Sr. No.	Sample No.	Sample Types	Collected From	Colonies On EMB	Isolated Pathogen
2	M2	Milk	Local milkmen Karanja (GH)	Green metallic sheen	*Escherichia coli*
3	M3	Milk	Local milkmen Karanja (GH)	Green metallic sheen	*Escherichia coli*
4	M4	Milk	Local milk vendor Karanja (GH)	Green metallic sheen	*Escherichia coli*
5	M5	Milk	Local milk men Karanja (GH)	Green metallic sheen	*Escherichia coli*
6	M6	Milk	Local milkmen, Wardha	Green metallic sheen	*Escherichia coli*
7	M7	Milk	Local milkmen, Wardha	Green metallic sheen	*Escherichia coli*
8	M8	Milk	Local milkmen, Thanegoan	Green metallic sheen	*Escherichia coli*
9	M9	Milk	Local milkmen Karanja (GH)	Green metallic sheen	*Escherichia coli*
10	M10	Milk	Local seller , karanja (GH)	Green metallic sheen	*Escherichia coli*
11	M11	Milk	Local seller, katol	Green metallic sheen	*Escherichia coli*
12	M12	Milk	Local seller ,katol .	Green metallic sheen	*Escherichia coli*
13	M13	Milk	Local seller , Karanja(GH)	Green metallic sheen	*Escherichia coli*
15	M15	Milk	Local milk men Mendla	Green metallic sheen	*Escherichia coli*
16	M16	Milk	Local milk men , Ladgoan	Green metallic sheen	*Escherichia coli*
17	M17	Milk	Local milk men ,Kondhali .	Green metallic sheen	*Escherichia coli*
18	M18	Milk	Local milk vendor , Katol	Green metallic sheen	*Escherichia coli*
19	M19	Milk	Local milk men , Katol	Green metallic sheen	*Escherichia coli*
20	M 20	Khoa	Local seller ,Kalmeshwar	Greenmetallic sheen	*Escherichia coli*

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It could be assessed from the present study that most of the milk sold in and around Guwahati might have a poor shelf-life unless adequate intervention measures for processing are adopted at the earliest. The variation in the grading of milk samples collected from 20 different locations might be due to location specificity, seasonal variation, unhygienic production practices, and inadequate maintenance of cold chain, milk composition, and initial contamination (Faraz A. et. al. 2013).Because the pathogenic bacteria, *E. Coli*, produce particular toxins that may withstand heat during the pasteurization process, their presence increases the risk of food poisoning. Certain environmental factors, such as time, temperature, storage conditions, the quality of the water used to rinse utensils and produce dairy products, moisture contents, ingredients used in the production process, the product handler's personal hygiene, relative humidity, and associated organisms and fecal coliforms, can also affect the production and multiplication of pathogens like *E. Coli*.

Table No.03:- *E.coli* isolated from total dairy products

Sr. no.	Sample no.	Samples	Collected from	Colonies on EMB	Isolated pathogen
1	MP1	Curd	Satguru Dairy, Katol	Green metallic sheen	*Escherichia coli*
2	MP 2	Curd	Local seller ,Kalmeshwar	Green metallic sheen	*Escherichia coli*
3	MP 3	Tak	Satguru Dairy, Katol	Green metallic sheen	*Escherichia coli*
4	MP 4	Tak	Local seller, Kalmeshwar	Green metallic sheen	*Escherichia coli*
5	MP 5	Paneer	Satguru Dairy, Katol	Green metallic sheen	*Escherichia coli*
6	MP 6	Paneer	Local seller ,Kalmeshwar	Green metallic sheen	*Escherichia coli*
8	MP 8	Chakka	Satguru Dairy, Katol	Green metallic sheen	*Escherichia coli*
9	MP 9	Khoa	Satguru Dairy, Katol	Green metallic sheen	*Escherichia coli*
10	MP 10	Khoa	Shri gajanan Dairy , Katol	Green metallic sheen	*Escherichia coli*
11	MP 11	Curd	Shri gajanan Dairy , Katol	Green metallic sheen	*Escherichia coli*
12	MP 12	Tak	Shri gajanan Dairy , Katol	Green metallic sheen	*Escherichia coli*
14	MP 14	Paneer	Local seller karanja	Green metallic sheen	*Escherichia coli*
15	MP 15	Paneer	Renuka Dairy , Katol	Green metallic sheen	*Escherichia coli*
16	MP 16	Butter	Renuka Dairy , Katol	Green metallic sheen	*Escherichia coli*
17	MP 17	Butter	Shri sai dairy, Katol	Green metallic sheen	*Escherichia coli*
18	MP 18	Chakka	Satguru Dairy, Katol	Green metallic sheen	*Escherichia coli*
19	MP 19	Khoa	Renuka Dairy , Katol	Green metallic sheen	*Escherichia coli*
20	MP 20	Khoa	Local seller ,Kalmeshwar	Green metallic sheen	*Escherichia coli*

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In the present study total 90 samples were collected and processed on the basis of morphological such as the gram nature,cultural characters that are the Colonies on selective media, biochemical characterization and sugar fermentation,20 possible*E. Coli*isolates were recovered. Out of it 4 isolates from curd , 3 isolates from paneer , 4 from butter , 2 from khoa , 1 from chakka and 6 isolates from tak Butter Milk .The results can be explained by the fact that the method used for the production of these dairy products is still traditional or primitive as compared to modern ways of food processing and production. The major risk increasing factors are the use of contaminated and raw materials , lack of pasteurization , use of poorly controlled natural fermentation , improper storage and maturation conditions , the major factor is the poor hygiene of the product handler (PatilS.S, 2014).

Table No. 05 :- Antibiotic Susceptibility Test against isolates from Raw Milk

Sr. no	Sample No.	Antibiotics used and zones of inhibition (mm)									
		Gentamicin	Tetracycline	Ampicillin	Ciprofloxacin	Erythromycin	Amoxyclav	Chloramphenicol	Streptomycin	Amikacin	Azithromycin
1	M 7	19	0	21	16	14	12	19	11	0	18
2	M 16	16	12	21	16	14	16	23	12	18	14
3	M 39	16	13	16	20	21	0	23	0	18	12
4	M 42	18	16	17	21	19	15	19	19	16	16
5	M 74	18	20	18	0	19	18	16	21	18	0

The results were concluding that the isolates from milk samples were showed highest resistance against tetracycline, Amikacin, Amoxyclav streptomycin and ciprofloxin and azithromycin. Whereas the moderate resistance showed by these isolates against streptomycin and tetracycline (Fig 1.)

ANTIMICROBIAL SUSCEPTIBILITY TESTING FOR *E. COLI*

Escherichia coli contamination was significantly higher in milk samples from farms that had inadequate hygienic standard, those using unclean milking utensils, and not properly washing udders and teats.

When the samples were analyses for antibiotic susceptibility test the results were showed that sample no. R7 showed highest zone of Inhibition (27 mm) for ampicillinwhereasM16 and 39 showed 23 mm for chloramphenicol. Other samples M 42 and M 74 showed the moderate zone of inhibition. The result analysis showed that M7. M 16, M 39, M42 and M74 isolates showed the potential resistance against Gentamycin Tetracycline Ampicillin, Ciprofloxacin, Erythromycin, Amoxyclav, Chloramphenicol, Streptomycin, Amikacin and Azithromycin (Table 5).

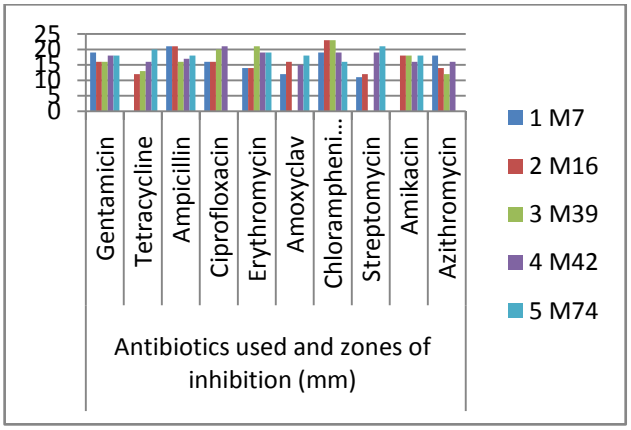

Fig. 1 Antibiotic resistance pattern of Isolated *E.coli* from milk samples

Study showed that isolate of E.coli those were isolated from milk products were showed more resistance as compare to raw milk isolates. E.coli from sample MP 22 showed the highest sensitivity (28 mm) for Chloramphenicol , whereas this isolate showed the highest resistance against MP4 (23) For tetracycline.Isolate from MP 4 showed 20 mm, 23 mm 24mm 19mm, 17, 23mm 21mm, 24mm and 22 mm zone of inhibition against gentamycin, tetracycline, ampicillin, erythromycin, Amoxyclav, chloramphenicol streptomycin Amikacin and azithromycin.

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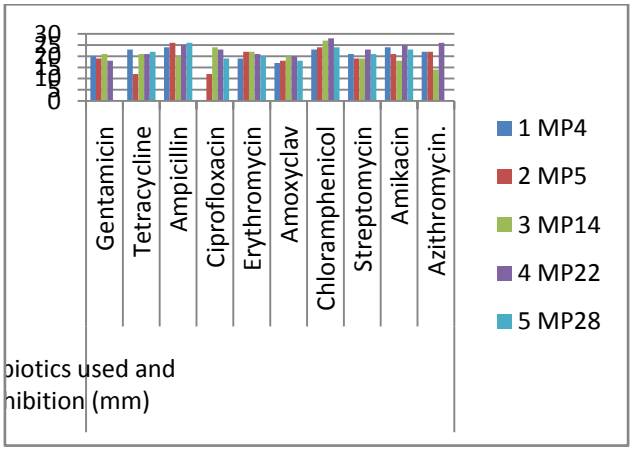
E.coli from MP4 showed maximum resistance for ciprofloxin (Zone of inhibition was zero mm). MP 5 showed highest resistance (12 mm) against Tetracycline and ciprofloxin. Isolates from MP 14 showed highest resistance against ampicillin and Amoxyclav, 20 mm respectively. Isolate MP 22 showed highest resistance against Amoxyclav (20 mm) and Gentamycin (18 mm). When results were analyzed for MP 28 showed highest antibiotic resistance against gentamycin and azithromycin (No zone of Inhibition). The results ere concluded that *E.coli* isolates form milk products showed highest resistance against ciprofloxin and gentamycin and azithromycin.

Table No. 06:- Antibiotic **Susceptibility Test against isolates from Milk Products.**

Sr.no.	Sample no.	Antibiotics used and zones of inhibition (mm)									
		Gentamicin	Tetracycline	Ampicillin	Ciprofloxacin	Erythromycin	Amoxyclav	Chlorampheni	Streptomycin	Amikacin	Azithromycin.
1	MP4	20	23	24	0	19	17	23	21	24	22
2	MP5	19	12	26	12	22	18	24	19	21	22
3	MP14	21	21	20	24	22	20	27	19	18	14
4	MP22	18	21	25	23	21	20	28	23	25	26
5	MP28	0	22	26	19	20	18	24	21	23	0

The above figure shows that the isolates no. R4, R5, R14, R22, R28showed the highest zone of inhibition against the antibiotics gentamicin, Tetracycline, Ampicillin, Ciprofloxacin, Erythromycin, Amoxyclav, Chloramphenicol, Streptomycin, Amikacin, Azithromycin.



In general, cleaning of the dairy farm environment including washing the cows and milking barn regularly, practicing good personal hygiene and washing milking and milk handling utensils are needed to avoid bacterial contamination in the current study areas.

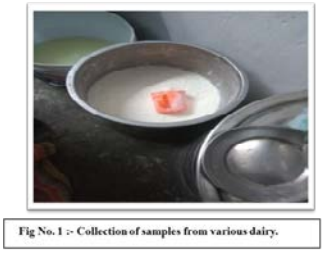


Fig No. 1 :- Collection of samples from various dairy.


Fig No. 2 :- Collected samples

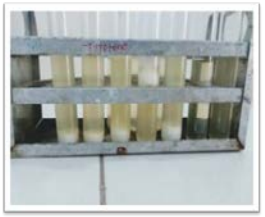


Fig No. 3:- Enrichment in bacteriological peptone water.

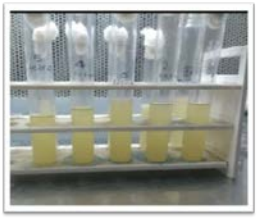


Fig No. 4 :- Enrichment in Tetrathionate broth.

As a limitation, surveillance, and antimicrobial susceptibility of *E. coli* isolates among food handlers using stool samples for comparisons with milk, milk product isolates, and enhanced public health intervention was not done.

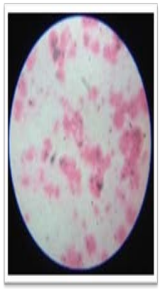


Fig No. 6:-Gram nature of isolated pathogen.


Fig No. 7:-Biochemical characteristics of isolated pathogen.

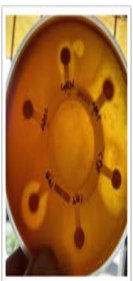


Fig No. 8:-Antibiotic assay of isolated pathogen.

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The small number of farms and bulk tank samples made it difficult to assess the risk factors for contamination of milk using multivariable regression. Due to the absence of flagella (H) antigen latex kit, the *E. coli* was tested only for somatic (O) antigen. In addition, molecular and other advanced studies were not carried out due to the lack of materials and suitable laboratories.

IV. CONCLUSION

The prevalence of *E. coli* was greater in this investigation. The identification of E. Coli in milk and milk products indicates a possible concern to food safety and public health due to the presence of fecal contamination. The study areas' identified risk factors were dirty milking containers and utensils, unclean dairy farms, and unsanitary milking and handling practices. The results of this study suggest that raw milk marketed in the majority of Katol town does not meet the legal microbiological standard and may provide a significant danger of milk-borne illness to public.

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