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Research Article

MICROBIAL CONTAMINANTS OF RAW MILK AND THE RISK OF MILK BORNE ZOONOSES AMONG MILKMEN IN SINGUR, WEST BENGAL, INDIA

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ABSTRACT: The present study was conducted to assess the microbial contamination in raw milk emphasizing their potential public health risks in Singur, Hooghly district, West Bengal, India during the period December 2018 to August 2019. A total of 56 samples (21 raw milk, 17 hand swabs of milkmen, 18 udder swabs) were collected to assess the microbial contamination. The samples from milk, hand and udder showed contamination with *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas spp.*, *Proteus spp.* and non-typhoidal *Salmonella spp*. Antibiogram profile of confirmed isolates was found to be 100% resistant to Ampicillin, Cefprozil, Cefixime, and 50% resistant to Erythromycin respectively. All the isolates showed 100% sensitivity to Ciprofloxacin and Co-trimoxazole. The presence of bacteria in the samples indicates that the milk has been contaminated due to unhygienic and poor milking practices. Unhygienic milking practices can be correlated with poor knowledge among the milkmen.

Key words: Antimicrobial resistance, Microbial contamination, Raw Milk, Zoonoses

INTRODUCTION

Milk is a highly perishable commodity that provides macro and micronutrients to improve the nutritional status of the population. With the increase in population, the requirement for milk and milk products has also increased. India contributes approximately 16% of global milk production and is one of the most economical milk producers in the world (Ohlan *et al*. 2016, Seru *et al*. 2019). Milk production is a livestock enterprise that can successfully improve the livelihood of small-scale farmers. Since 2001, India has been the world leader in milk production followed by the USA (Hemme *et al*. 2003). So far, India is estimated to contain the highest number of small-scale dairy farmers. Many factors like farm management, changes in prices, and marketing strategies influence the small-scale milk production systems. India's milk production has increased from 55.6 million tonnes in 1991-92 to 187.7 million tonnes as reported in 2018-19 (NDDB 2019). At present, the per capita availability of milk is 394 grams per day. More than 50% of marketable surplus milk is handled by the unorganized sector. The market of unorganized milk and milk products in India is about Rs. 470 billion per annum (Ondeieki *et al*. 2015). A large number of farm families, workers and a growing segment of the general population still consume raw milk as they still believe that the raw milk is not only safe but also imparts beneficial health effects which are destroyed by pasteurization and boiling of the milk.

To improve the quantity of milk, enormous research has been conducted for neglecting the quality of milk. There are various possible direct and indirect routes through which the pathogens can enter the milk and some of them are through infected animals, milk handlers, contaminated farm environments etc. The milk produced in unhygienic environments reduces the quality and nutritive value. The presence of pathogenic organisms in milk further poses a public health risk and also economic losses due to its spoilage (Kumar *et al*. 2013). Milk-borne zoonotic infections account for 2–6% of bacterial food-borne outbreaks reported by surveillance systems from several countries (De Buyser *et al*. 2001). The most common pathogenic and food-borne organisms found in milk include *Escherichia coli*, *Klebsiella pneumoniae*,

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Staphylococcus aureus, Mycobacterium bovis, Bacillus cereus, Salmonella spp., Clostridium perfringens, Listeria monocytogenes, Coxiella burnetii, Brucella abortus etc. (Costanzo *et al*. 2020). Following the excess use of antibiotics, antimicrobial resistance is a significant public health threat. The cross-resistance of antimicrobials occurs in humans due to the use of similar antibiotics in veterinary practice (Ondeieki *et al*. 2015, Kumar *et al.* 2021). The multi-drug resistant strains of bacteria possibly enter humans through the consumption of unprocessed animal products (Toth *et al*. 2019). Keeping the above things in mind, we designed this study to gather knowledge about milk-borne zoonoses, practices regarding clean milk production and the quality of raw milk along with multiple antibiotic-resistant indices of the isolates.

MATERIALS AND METHODS

A cross-sectional study was conducted at Singur block of Hooghly district, West Bengal from December 2018 to August 2019.

The samples were collected from milkmen who were selected using a simple random sampling method. A total of 56 samples (21 raw milk, 17 hand swabs of milkmen, and 18 udder swabs) were collected for microbial assessment. Overall, 17 groups of samples were collected aseptically using sterile test tubes and swab sticks from the same cow milk and its udder, and milkman who milked the cow. They were transported to the laboratory maintaining cold chain at 4-8ºC within 3-4 hours. The samples were processed for isolation of zoonotic pathogens like *E. coli, Salmonella spp., Pseudomonas spp., Staphylococcus aureus* followed by identification using biochemical methods at the National Institute of Cholera and Enteric Diseases and All India Institute of Hygiene and Public Health, Kolkata on the same day of receiving the samples.

RESULTS AND DISCUSSION

Microbial assessment

Out of 56 samples, we were able to isolate 10 isolates. The isolates were cultured on various media like MacConkey, Xylose Lysine Deoxycholate, Hektoen Enteric agars for purity. After isolation, strains were tested for antimicrobial sensitivity and resistance pattern. The isolated bacteria were identified based on colony characteristics followed by confirmation using biochemical methods.

E. coli was the most commonly isolated organism from all the samples. It was visible as Gram-negative with pink stained bacilli and lactose fermenting on MacConkey agar having colony characteristics of circular, flat, and pink colour colonies. Biochemical test results are as shown in Table 1.

The majority of the *E. coli* isolates were recovered from udder samples where fecal contamination was observed. *E. coli* was isolated from vendors' hands' samples which shows that this may pose a potential source of contamination. In resource deficient tropical countries where refrigeration facilities are not accessible, *E. coli* can multiply easily in raw milk which corresponds to a high occurrence of *E. coli* in our investigation. A similar finding was reported by Batabyal *et al*. (2018). The presence of *E. coli* in milk indicates not only fecal contamination but also other contributing factors which can be potential public health concerns. The presence of *E. coli* near the udder region can also contribute to the incidence of coliform mastitis.

The second most isolated organism was *Klebsiella pneumonia* (2/56) with the morphology of Gram-negative, rod-shaped, and lactose fermenting with mucoid colonies on MacConkey agar. The biochemical test results are shown in Table 1.

The presence of *Klebsiella pneumoniae* correlates with the findings of Langoni *et al*. (2015) who isolated *K.*

Table 1. Biochemical tests for the identification of bacterial isolates.

Sl. No.	Biochemical Tests	*Escherichia coli*	*Klebsiella pneumonia*	*Proteus spp.*	*Pseudomonas spp.*	*Salmonella spp.*
1.	Citrate test	Negative	Positive	Positive	Positive	Positive
2.	Indole test	Positive	Negative	Negative	Negative	Negative
3.	Lysine Iron Agar	Positive	Positive	Negative	Positive	Negative
4.	Urease	Negative	Positive	Positive	Negative	Negative
5.	Triple Sugar Iron agar	A/A, gas (+), H_2S negative	A/A, gas (+), H_2S Negative	K/A, gas (-), H_2S ++	K/K, gas (-), H_2S(-)	K/A, gas (+), H_2S (+++)
6.	Motility Indole Ornithine	Positive	Negative	Positive	Positive	Positive

pneumonia from the dairy environment. There are even reports of the presence of *Klebsiella pneumoniae* in milk products such as Khoa in India (Bobbadi *et al*. 2019). The presence of *K. pneumoniae* on the milker's hand can be transmitted to the other cows thus spreading the infection. *Klebsiella pneumoniae* causes mastitis which is more severe than mastitis caused by *E. coli*. *K. pneumoniae* also causes urinary tract infections, pneumonia, wound infections etc. in humans, which poses potential risks to milkers, vendors and others associated with handling raw milk (Navon-Venezoa *et al*. 2017). The adverse effects are due to poor response to antibiotic therapy thus causing huge economic losses to the livestock owners. *Klebsiella pneumoniae* is known for its notoriety in carrying various antibiotic resistance genes (Lagoni *et al*. 2015, Navon-Venezoa *et al*. 2017).

Proteus spp. were Gram-negative, rod-shaped, non-lactose fermenting on MacConkey agar and produced H_2S. They were urease positive and motile. The biochemical test results were as shown in Table 1. We could isolate one *Proteus* isolate from milker's hand that warrants the chance of spreading the infection to cows while milking. This poses a potential reverse zoonoses threat and stresses the importance of milk hygiene awareness.

Proteus spp. are uncommon pathogens found in cow's environment (bedding, feed, and water). Despite *Proteus spp.* are rarely associated with food-borne infections, their presence can cause wound and urinary tract infections in the milk handlers. The Antibiogram profile revealed that *Proteus* isolate is resistant to Ampicillin, Cefixime and Trimethoprim. These results are not in accordance with the findings of Prasastha *et al.* (2021), who found that Proteus was highly sensitive to Ampicillin.

Pseudomonas isolates were gram-negative rod showing motility on MIO semi-solid agar and showed alkaline slant and alkaline butt on TSI agar. The biochemical test results are shown in Table 1.

Pseudomonas spp. are ubiquitous and psychotropic organisms that can grow over a temperature range of 4-42ºC. They are frequently linked with the spoilage of raw milk and are present in different environments (Meng *et al*. 2017). The potential source of contamination could be the environment due to its vast presence in nature. Presence of *Pseudomonas spp*. correlates with the findings of Vidal *et al.* (2017) who demonstrated the presence of the organism on milker's hands, the surface of cow's teats, and utensils. *Pseudomonas spp*. is known to harbour genes for various multi-drug efflux pumps and is therefore usually multi-drug resistant.

Salmonella isolates could be isolated from an udder sample. Identification was done based on Gram staining, colony characteristics on HEA, and biochemical reactions. The isolates showed greenish colonies with a black centre on HEA agar, were positive for H_2S, citrate, and flaring from the line of inoculation indicating motility. The biochemical test results are shown in Table 1.

Table 2. No. of microorganisms isolated from the samples (m= milk, h= milker's hand, u= udder).

Sl. No.	Isolate No.	Microorganism
1.	3 m	*Klebsiella pneumonia*
2.	21 m	*Escherichia coli*
3.	3 h	*Klebsiella pneumonia*
4.	5 h	*Escherichia coli*
5.	6 h	*Vibrio spp.*
6.	7 h	*Proteus spp.*
7.	10 h	*Pseudomonas spp.*
8.	3 u	*Non-typhoidal Salmonella*
9.	7 u	*Escherichia coli*
10.	5 u	*Staphylococcus aureus*

The presence of *Salmonella spp.* on the udder can contaminate the milk as well as the milker while milking. About 15-100 CFU of *Salmonella spp.* can pose a public health risk (Pooja *et al*. 2018). Due to the high nutritive value of milk, it is consumed by every age group especially children whose nutrient requirement for growth is much higher. *Salmonella spp.* is one of the common food-borne zoonotic infections which affect children below 5 years and immuno-compromised individuals. Unpasteurized milk can be a possible source of infection in this group of people.

Staphylococcus aureus isolate showed Gram-positive cocci on Gram's staining and coagulase-positive. *Staphylococcus aureu*s was isolated from milk, milker's hands as well as udder. The results are in correlation with the findings of Tasnim *et al*. (2015) who found *Staphylococcus* in all the raw milk samples. The presence indicates the improper milking and hygienic conditions of the farm. The other non-pathogenic *Vibrio cholerae* was isolated on TCBS agar which showed agglutination with polyvalent sera.

Sample-wise results

From the total 56 samples, we could isolate 7 different types of pathogens. The majority isolated were *E. coli*

Table 3. *In vitro* antibiotic sensitivity pattern of the isolates (m= milk, h= milker's hand, u= udder; S= Sensitive, I= Intermediate, R=Resistant).

Sl. No.	Class of antibiotics	Name of Antibiotics	3m	21m	3h	5h	6h	7h	10h	3u	5u	7u
1.	Quinolones	Ciprofloxacin (CF)	S	S	S	S	S	S	S	I	S	S
2.		Lomefloxacin (Lo)	S	R	S	R	S	S	S	S	S	I
3.		Nalidixic acid (NA)	S	S	S	R	S	S	S	S	S	S
4.		Norfloxacin (Nx)	S	S	S	I	S	S	S	S	S	S
5.	Tetracycline	Tetracycline (T)	S	R	S	R	I	I	S	I	I	I
6.	Macrolides	Erythromycin (E)	I	R	I	R	R	I	R	R	I	I
7.	Aminoglycoside	Neomycin (N)	I	I	S	I	S	I	I	I	R	R
8.		Kanamycin (K)	S	I	S	I	I	S	S	I	S	I
9.	Beta-lactams	Ampicillin (A)	R	R	R	R	R	R	R	R	R	R
10.		Ceftriaxone (Ci)	R	I	R	S	S	S	S	S	S	S
11.		Cefprozil (CFZ)	R	R	R	R	R	R	R	R	R	R
12.		Cefixime (CFX)	R	R	R	R	R	R	R	R	R	R
13.	Chloramphenicol	Chloramphenicol (C)	I	S	I	R	S	S	S	S	S	S
14.	Sulphonamides	Trimethoprim (TR)	S	R	I	I	S	R	S	S	S	S

(3/56) from milk and udder followed by *K. pneumoniae* (2/56) from milk and milker's hand respectively. Surprisingly we could isolate *Vibrio spp.* from milker's hand but on serotyping, we found that as non-pathogenic. We could isolate *Proteus spp.* and *Pseudomonas spp.* from the milkers' hands. From the udder sample, we could isolate *Salmonella spp.* and we could suspect it as non-typhoidal Salmonella based on excessive production of H_2S on triple sugar iron agar. Data are shown in Table 2.

Antibiogram of the isolates

The antibiotic susceptibility of 14 commonly used antibiotics in human and veterinary medicine in Singur was used. The isolates showed varied pattern of resistance and susceptibility to the antibiotic discs used (Table 3). The majority of the isolates showed 100% resistance to Ampicillin (A), Cefrozil (CFZ), and Cefixime (CFX) followed by Erythromycin (E). About 90% of the isolates showed sensitivity to Nalidixic acid (NA), Norfloxacin (Nx) followed by Ceftriaxone (Ci), Chloramphenicol (C) (70%), and Trimethoprim (TR) (60%). The isolates of about 60% showed an intermediate response to Neomycin (N) and 50% against Kanamycin (K), Erythromycin (E), and Tetracycline (T). Two of the isolates were found to be resistant to 8 antibiotics (Lo-NA-T-E-A-CFZ-CFX-

Table 4. MAR indices of the isolates (n=10).

Isolate No.	No. of antibiotic isolate subjected to face resistance	Antibiotics resistance	MAR index
3m	14/4	A, Ci, CFX, CFZ	0.2
21m	14/7	Lo, T, E, A, CFX, CFZ, TR	0.5
3h	14/4	A, Ci, CFX, CFZ	0.2
5h	14/4	E, A, CFX, CFZ	0.2
6h	14/8	Lo, NA, T, E, A, CFX, CFZ, C	0.57
7h	14/4	E, A, CFX, CFZ	0.2
10h	14/4	A, CFX, CFZ, TR	0.2
3u	14/4	E, A, CPX, CPZ	0.2
5u	14/4	N, A, CFX, CFZ	0.2
7u	14/4	N, A, CFX, CFZ	0.2

(A: Ampicillin, Ci: Ceftriaxone, CFX: Cefixime, CFZ: Cefprozil, Lo: Lomefloxacin, T: Tetracycline, E: Erythromycin, TR: Trimethoprim, N: Neomycin, NA: Nalidixic acid, C: Ciprofloxacin; m= milk, h= milkers hand, u= udder).

C) and 7 antibiotics (Lo-T-E-A-CFZ-CFX-TR) *i.e* 5 classes of antibiotics. These two isolates could be multi-drug resistant. The majority of resistance was observed for the Beta-lactam group of antibiotics followed by Macrolides. Most of the isolates showed intermediate reactions against Tetracycline and Aminoglycosides. There is a potential risk of antibiotic resistance gene transfer between animals and humans through horizontal gene transfer while consumption of raw milk. The multidrug resistance of the isolates can pose a high risk to animals as well as humans who are at immediate risk of infection.

Table 4 shows the information regarding the multiple antibiotic resistances against 14 antibiotics used in the study. Among the 10 isolates, 2 isolates had a MAR index of more than 0.2, and 8 isolates had a MAR index of 0.2. The isolates from milk (21m) and hand swab (6h) showed a MAR index of more than 0.2 representing the different levels of risk. About 80% of the isolates were resistant to similar antibiotics suggesting plasmid exchange within them. This assumption is in harmony with observations made by Krumperman (1983).

Overall, the isolates identified were *E. coli, Klebsiella spp, Proteus spp, Staphylococcus aureus, Pseudomonas spp, and Salmonella spp.* Among the isolated organisms, the majority of them were enterobacteria which indicates probable fecal contamination of the milk which may be due to poor knowledge among the milkmen. The common practice usually followed in the locality (if the milk was spoiled) was the preparation of Chhena (Paneer or cheese curds). The consumption of spoiled milk can cause nausea, vomiting, stomach ache, and diarrhea. Due to lack of refrigeration, there are more chances of milk getting spoiled due to the above-mentioned organisms. This study suggests a probable association between the poor quality of milk and less knowledge among the dairy farmers and vendors.

CONCLUSION

Based on the observations, we conclude that the contamination of milk with the bacteria may be due to a lack of knowledge regarding milk hygiene, poor facilities for the preservation, and transport of raw milk. It is recommended that extensive awareness campaigns are periodically needed to promote food safety in rural areas. In the present scenario, zoonotic infections should also be given importance and the communities should be made aware of the preventive and control measures.

REFERENCES

Batabyal K, Banerjee A, Pal S, Dey S, Joardar SN *et al.* (2018) Detection, characterization and antibiogram of extended-spectrum beta-lactamase *Escherichia coli* isolated from bovine milk samples in West Bengal, India. Vet World 11(10): 1423-1427.

Bobbadi S, Bindu Kiranmay C, Nelapati S, Tumati SR, Srinivas K *et al.* (2019) Occurrence and genetic diversity of ESBL producing *Klebsiella* species isolated from livestock and livestock products. J Food Safety 40(1): e12738.

Costanzo N, Ceniti C, Santoro A, Clausi MT, Casalinovo F (2020) Foodborne pathogen assessment in raw milk cheeses. Int J Food Sci 2020:3616713.

De Buyser ML, Dufour B, Maire M, Lafarge V (2001) Implications of milk and milk products in food-borne disease in France and different industrialized countries. Int J Food Microbiol 67(1-2): 1-17.

Hemme T, Garcia O, Saha A (2003) A review of milk production in India with particular emphasis on small-scale producers. FAO PPLPI Working Papers No. 2. Intern Farm Compar Network (1Fon).

Krumperman PH (1983) Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of foods. App Environ Microbiol 46(1): 165-170.

Kumar A, Nagal KB, Panda AK (2013) Bacteriological quality and safety of raw milk and milk products with special reference to coliforms in Palampur (Himachal Pradesh). Himachal Vet J 6(1): 98-101.

Kumar A, Panda AK, Sharma N (2021) Determination of antibiotic residues in bovine milk by HPLC-DAD and assessment of human health risks in Northwestern Himalayan region, India. J Food Science 1-10.

Lagoni H, Guiduce MVS, Nobrega DB, Silva RC, Pereira VBR *et al.* (2015) Research of *Klebsiella pneumoniae* in dairy herds. Pesq Vet Bras 35(01): 9-12.

Meng L, Zhang Y, Liu H, Zhao S, Wang J *et al.* (2017) Characterization of Pseudomonas spp. and associated proteolytic properties in raw milk stored at low temperatures. Front Microbiol 8: 2158.

Navon-Venezia S, Kondratyeva K, Carotti A (2017) *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 41(3): 252-275.

NDDB (2019) Milk production and per capita availability of milk in India. https://www.nddb.coop/sites/default/files/statistics/Mp%20India-ENG-2019.pdf

Ondieki GK, Ombui JN, Obonyo M, Gura Z, Githuku J *et al.* (2017). Antimicrobial residues and compositional quality of informally marketed raw cow milk, Lamu West Sub-County, Kenya, 2015. The Pan Afr Med J 28(1): 5.

Ohlan R (2016) Dairy economy of India: structural changes in consumption and production. South Asia Res 36(2): 241-260.

Pooja S, Singh RV, Bhavana G, Tripathi SS, Tomar KS *et al.* (2018) Prevalence study of *Salmonella* spp. in milk and milk products. Asian J Dairy Food Res 37(1): 7-12.

Prasastha RV, Venkateswara Rao L, Srinivas Rao T, Subramanyam KV, Srinivas K (2018) Prevalence and virulence gene profiles of Proteus mirabilis isolates from animal, human and water samples in Krishna District, Andhra Pradesh, India. The Pharma Innovation J 8(6): 19-23.

Prasastha RV, Venkateswara Rao L, Srinivas Rao T, Subramanyam KV, Srinivas K (2021) A study on Antibiogram and beta-lactam resistance *of Proteus mirabilis* isolated from animals and humans in Andhra Pradesh. Ind J Ani Res B4184: 1-6.

Seru LV, Chendu BP, Atul R, Bhattacharya S, Banik A (2019) A study on milking practices among dairy owners in a rural community of Singur block, Hooghly District, West Bengal, India. South Asian R J Biol App Bioscienc 1(1): 33-37.

Tasnim UT, Islam MT (2015) Pathogenic and drug resistant bacteria in raw milk of Jessore city: A potential food safety threat. Bangladesh J Vet Med 13(1): 71-78.

Toth AG, Csabai I, Kriko E, Tozser D, Maroti G *et al.* (2019) Raw milk for human consumption may carry antimicrobial resistance genes. bioRxiv preprint 2019: 1-7.

Vidal AMC, Netto AS, Vaz ACN, Copodifoglio E, Goncalves ACS *et al.* (2017) *Pseudomonas* spp.: contamination sources in bulk tanks of dairy farms. Pesq Vet Bras 37(9): 941-948.

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