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Article

Identification and antibiogram study of bacterial species isolated from milk samples of different locations in Bangladesh

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Abstract: Cow's milk containing pathogenic bacteria is an important threat to the consumers. The objectives of the present study were to identify the bacterial agents of public health importance in milk samples (n=35) of different locations and to determine their sensitivity to different antibiotics. The milk samples were collected and transported aseptically and subsequently allowed for culture in bacteriological media, Gram's staining and biochemical tests for the identification of bacterial species. The bacteria identified were *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*, and their prevalence, in case of vendor milk specimens (n=28), were 96.43%, 53.57% and 35.71% respectively, and of brand milk specimens (n=7), were 42.86 %, 28.57% and 0%, respectively. This suggests that cautionary measures should be taken for quality milk production and consumption. The antibiotic sensitivity test was done by disc diffusion method and the average inhibition zones, in case of *Staphylococcus aureus*, were 32 mm for oxytetracycline, 26 mm for amoxicillin, 35 mm for ciprofloxacin, 27 mm for cefotaxime, 30 mm for ceftriaxone, 30 mm for azithromycin, and 26 mm for erythromycin; in case of *Escherichia coli*, were 5 mm for oxytetracycline, 9 mm for amoxicillin, 22 mm for ciprofloxacin, 30 mm for cefotaxime, 31 mm for ceftriaxone, 15 mm for azithromycin, and 0 mm for erythromycin; in case of *Salmonella typhi.*, were 25 mm for oxytetracycline, 24 mm for amoxicillin, 38 mm for ciprofloxacin, 31 mm for cefotaxime, 34 mm for ceftriaxone, 24 mm for azithromycin, and 0 mm for erythromycin. Therefore, ciprofloxacin and ceftriaxone may be the antibiotics of first choice, and cefotaxime and azithromycin may be the second choice among the test antibiotics for the treatment of illness caused by these bacteria.

Keywords: public health; cow's milk; bacterial species; identification; antibiotic sensitivity

1. Introduction

Cow's milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms and for the multiplication of several bacteria of various genera. Milk-borne and milk-product borne outbreaks represent 2–6% of bacterial food-borne outbreaks reported by surveillance systems from several countries (De Buyser *et al*., 2001).

Various bacteria of public health concern such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli*, *Salmonella* spp., *Vibrio* spp., and enterotoxigenic strains of *Staphylococcus aureus* may be found in milk (Sharma and Malik, 2012; CDC, 2003a;

CDC, 2002c; Murinda *et al*., 2002b; Murinda *et al*., 2002a; Ackers *et al*., 2000; Castro *et al*., 1986; Bunning *et al*., 1986).

General infections such as typhoid fever, diphtheria, scarlet fever, bacillary dysentery, anthrax and mastitis-related enterotoxaemia are also often transmitted through milk, whilst the most severe zoonoses transmitted from animals to humans via milk are tuberculosis and brucellosis, campylobacteriosis, salmonellosis, hemorrhagic colitis, Brainerd diarrhoea, Q fever, listeriosis, yersiniosis, and toxoplasmosis (Plotter, 2002; Heuvelink *et al*., 1998; Ruegg, 1999).

Food spoilage is an enormous economic problem worldwide. Approximately one-fourth of the world's food supply is lost through microbial activity alone. Nowadays, public health concern associated with microbial food safety has arisen. Numerous epidemiological reports have implicated non-heat treated milk and raw-milk products as the major factors responsible for illnesses caused by food-borne pathogens. Therefore, the objectives of the present study were to identify the bacterial species of public health importance from milk samples (n=35) and to observe their sensitivity to different antibiotics.

2. Materials and Methods

2.1. Laboratory and duration of the experiment

This investigation was performed in the bacteriological laboratory of Bangladesh Livestock Research Institute (BLRI) and the duration of the experiment was 6 months (January to June/2014).

2.2. Sample collection

The vendor (n=28) milk samples were collected from different districts such as Joypurhat, Rangpur, Pabna, Sirajganj, Dhaka, Gazipur and Chittagong, and the brand (n=7) milk samples were collected from different supermarkets of Dhaka district. The milk samples were collected and transported aseptically in laboratory cool box and subsequently allowed for laboratory tests immediately upon arrival for the identification of bacterial species. The samples were preserved at 4^0C in the refrigerator for 24 hours when necessary.

2.3. Laboratory tests for identification of bacteria

2.3.1. Culture in bacteriological media

After tenfold dilution, the collected samples were allowed for culture in bacteriological media by spread plate method. The media used were MacConkey agar, mannitol salt agar, blood agar, nutrient broth, nutrient agar, Salmonella-Shigella (SS) agar, thiosulfate citrate bile salt sucrose (TCBS) agar and eosine methylene blue (EMB) agar. The amount of sample taken in each plate was 0.5 ml. All plates were incubated for 24 hours at 37^oC temperature.

2.3.2. Gram's staining

Gram's staining was done as per Cowan (1985) to study the morphological properties of bacteria.

2.3.3. Biochemical tests

Different biochemical tests like oxidase test, catalase test, indole test, methyl red test, Voges Proskauer test, citrate test and carbohydrate fermentation test were performed with the isolated bacteria. The biochemical characteristics of bacterial isolates were observed on the basis of color, bubble, acid and/or gas formation.

2.4. Antibiotic sensitivity test

The antibiotic sensitivity test was done by using disc diffusion method (Cowan and Steel, 1965). Commercially available antibiotic discs such as, oxytetracycline (30µg/disc, Oxoid), amoxicillin (30µg/disc, Oxoid), ciprofloxacin (5µg/disc, Oxoid), cefotaxime (30µg/disc, Oxoid), ceftriaxone (30µg/disc, Oxoid), azithromycin (15µg/disc, Oxoid) and erythromycin (15µg/disc, Oxoid) were used to know the sensitivity of the identified bacteria to these drugs.

2.5. Data analysis

The data were analysed by using Microsoft Excel programme to determine the prevalence of a particular organism identified.

3. Results

The isolates were identified as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, on the basis of morphology, cultural characteristics and biochemical characteristics. In case of cultural properties, *Staphylococcus aureus* produced yellow and small colonies on mannitol salt agar, *Escherichia coli* produced pink, small or large raised and convex colonies on Mac Conkey agar, and *Salmonella typhi* produced blackish and medium sized colonies on SS agar (Table 1, Figures 1, 2 & 3). In case of morphological characteristics, the *Staphylococcus aureus* isolates appeared Gram positive round shaped, the *Escherichia coli* and *Salmonella typhi* isolates appeared Gram negative rod shaped (Table 1, Figure 4). In case of biochemical properties, *Staphylococcus aureus* showed oxidase, indole and methyl red tests negative, citrate, catalase and Voges Proskauer tests positive, and fermented glucose, sucrose, maltose, lactose and mannitol (Table 1), whereas *Escherichia coli* showed oxidase, citrate and Voges Proskauer tests negative, indole, methyl red and catalase tests positive, and fermented glucose, sucrose, maltose, lactose and mannitol (Table 1), while *Salmonella typhi* exhibited oxidase, indole, Voges Proskauer and citrate tests negative, methyl red and catalase tests positive, and fermented glucose, maltose, mannitol and lactose but did not ferment sucrose (Table 1). Out of 28 vendor milk samples 27 (96.43 %) were positive for *S. aureus*, 15 (53.57 %) were positive for *E. coli* and 10 (35.71 %) were positive for *Salmonella typhi* (Table 2).

On the other hand, out of 7 brand milk samples 3 (42.86 %) were positive for *S. aureus*, 2 (28.57 %) were positive for *E. coli* and no sample (0%) was positive for *Salmonella typhi* (Table 3).

Regarding antibiotic sensitivity, most of the antibiotics among the seven antibiotics (oxytetracycline, amoxicillin, ciprofloxacin, cefotaxime, ceftriaxone, azithromycin and erythromycin) used in this study were found highly sensitive (Table 4).

Table 1. Identifying features of the bacteria detected in this study.

Sl. No.	Characters	*E. coli*	*S. aureus*	*S. typhi*
1.	Colony characters	Pink, small or large raised, convex colonies	Yellow, small	Blackish, medium
2.	Motility	Motile	Non motile	Motile
3.	Morphological characters	Rod	Round	Rod
4.	Gram's staining	-ve	+ve	-ve
5.	Biochemical tests			
	a. Oxidase test	-ve	-ve	-ve
	b. Indole test	+ve	-ve	-ve
	c. Methyl red test	+ve	-ve	+ve
	d. Citrate test	-ve	+ve	-ve
	e. Catalase test	+ve	+ve	+ve
	f. Voges Proskauer test	-ve	+ve	-ve
	g. Glucose fermentation	+ve*	+ve*	+ve*
	h. Sucrose fermentation	+ve*	+ve	-ve
	i. Maltose fermentation	+ve*	+ve*	+ve*
	j. Lactose fermentation	+ve*	+ve*	+ve*
	k. Mannitol fermentation	+ve*	+ve*	+ve*

N.B.: +ve* indicates sugar fermentation with the production of both acid and gas.

Table 2. Bacteria identified in the vendor milk samples.

Bacteria identified	Total number of samples tested	No. of positive samples	Percentage of positive samples
S. aureus	28	27	96.43 %
E. coli	28	15	53.57 %
Salmonella typhi	28	10	35.71 %

Table 3. Bacteria identified in the brand milk samples.

Bacteria identified	Total number of samples tested	No. of positive samples	Percentage of positive samples
S. aureus	7	3	42.86 %
E. coli	7	2	28.57 %
Salmonella typhi	7	0	0 %

Table 4. Inhibition zones produced by antibiotics used against *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* in the sensitivity test.

Antibiotics discs used	Bacterial species	Zone of inhibition (in diameter)	Result
Oxytetracycline (30µg/disc, Oxoid)	*S. aureus*	32 mm	Highly sensitive
	E. coli	5 mm	Resistant
	Salmonella typhi	25 mm	Highly sensitive
Amoxycillin (30µg/disc, Oxoid)	*S. aureus*	26 mm	Highly sensitive
	E. coli	9 mm	Resistant
	Salmonella typhi	24 mm	Highly sensitive
Ciprofloxacin (5µg/disc, Oxoid)	*S. aureus*	35 mm	Highly sensitive
	E. coli	22 mm	Highly sensitive
	Salmonella typhi	38 mm	Highly sensitive
Cefotaxime (30µg/disc, Oxoid)	*S. aureus*	27 mm	Highly sensitive
	E. coli	30 mm	Highly sensitive
	Salmonella typhi	31 mm	Highly sensitive
Ceftriaxone (30µg/disc, Oxoid)	*S. aureus*	30 mm	Highly sensitive
	E. coli	31 mm	Highly sensitive
	Salmonella typhi	34 mm	Highly sensitive
Azithromycin (15µg/disc, Oxoid)	*S. aureus*	30 mm	Highly sensitive
	E. coli	15 mm	Moderately sensitive
	Salmonella typhi	24 mm	Highly sensitive
Erythromycin (15µg/disc, Oxoid)	*S. aureus*	26 mm	Highly sensitive
	E. coli	0 mm	Resistant
	Salmonella typhi	0 mm	Resistant

N.B.: The interpretation was done as resistant (≤10 mm), less sensitive (11-14 mm), moderately sensitive (15-18 mm) and highly sensitive (≥19 mm) according to Bauer *et al.* (1966).

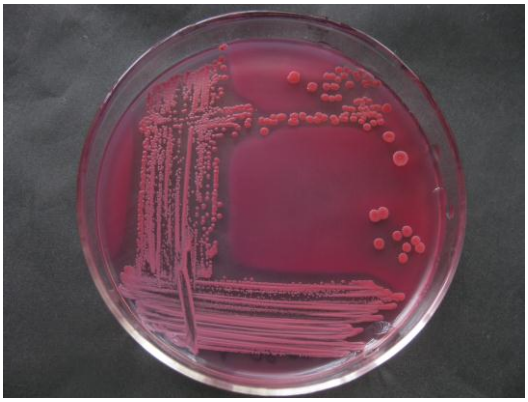

Figure 1. *E. coli* showing pink color colony on Mac Conkey agar medium.

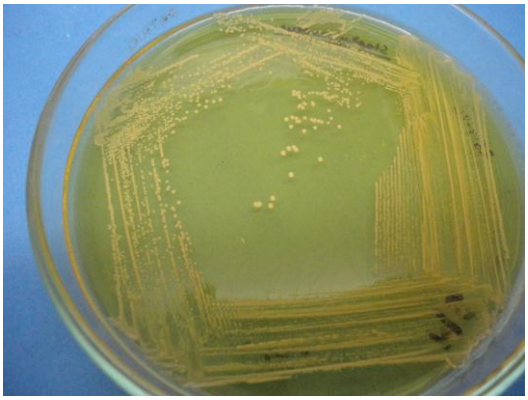

Figure 2. *S. aureus* showing yellow color colony on MS agar medium.

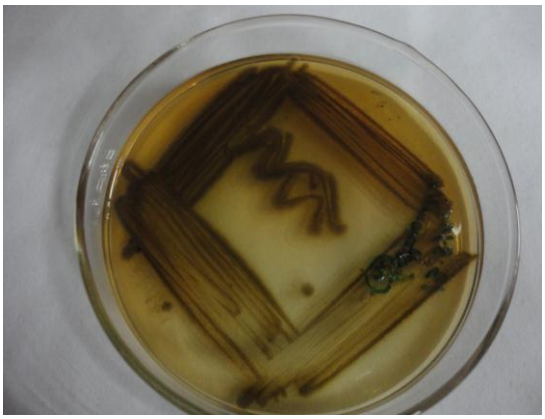

Figure 3. *Salmonella typhi* on SS agar medium.

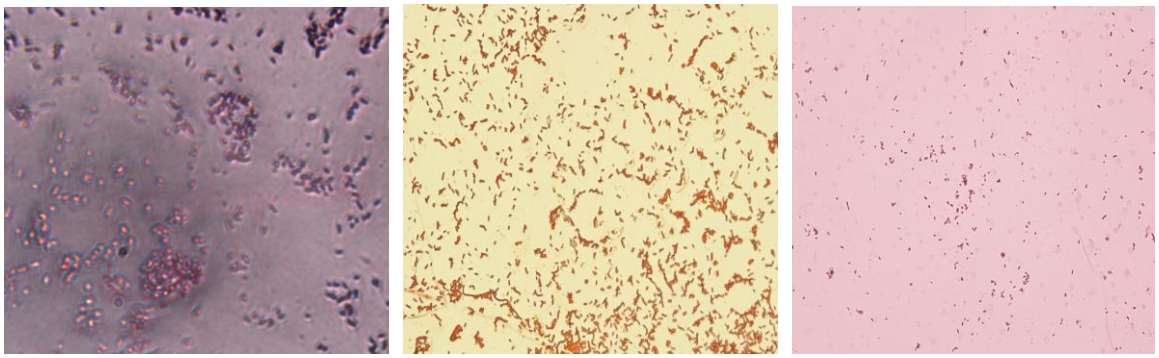

Figure 4. Gram positive *S. aureus* (left), Gram negative *E. coli* (middle) and Gram negative *S. typhi* (right).

4. Discussion

In the present study, out of 28 vendor milk samples 27 samples were positive for *S. aureus*, 15 samples were positive for *E. coli* and 10 samples were positive for *Salmonella typhi*, whereas the prevalence of the organisms was 96.43 %, 53.57% and 35.71%, respectively (Table 2). On the other hand, 7 brand milk samples were tested out of which 3 samples were positive for *S. aureus*, 2 samples were positive for *E. coli* and no sample was positive for *Salmonella typhi*, whereas the prevalence of the organisms was 42.86 %, 28.57% and 0%, respectively (Table 3).

In this study, the prevalence of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* in milk samples has received considerable attention which is in support of several scientists (Zecconi and Hahn, 2000; Mrema *et al*., 2006; Jay, 2000; Watts, 1989; Jones, 1990; Dabassa and Bacha, 2012; Hasan *et al*., 2015).

E. coli may be considered an indicator microorganism of faecal contamination and other enteric pathogens. The presence of large number of coliform bacteria are suggestive of unsanitary conditions or practices during production, processing, distribution or storage (Thomas *et al.*, 1971).

Pathogenic bacteria may also be present in raw milk as a direct consequence of clinical or subclinical mastitis (Giesecke *et al*., 1994).

The present study showed that *E. coli* isolates were resistant to several antibiotics like erythromycin, amoxycillin and oxytetracycline. These findings are in agreement with Ershaduzzaman *et al.* 2007.

According to Bauer *et al.* (1996), antimicrobial resistance is currently the greatest challenge to the effective treatment of infections throughout the world. Globally, the three main causes of antimicrobial resistance have been identified as use of antimicrobial agents in agriculture, over-prescribing by physicians and misuse by patients (Dabassa and bacha, 2012).

In this investigation some modern antibiotics have been found sensitive to the bacteria identified (Table 4), where the highest sensitivity was recorded for ciprofloxacin, ceftriaxone, cefotaxime and azithromycin because these are new generations of antibiotics and have not been used by the doctors for long time.

5. Conclusions

The study revealed that *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* were the major pathogenic bacteria found in milk available in the market. Furthermore, ciprofloxacin and ceftriaxone may be the antibiotics of first choice, and cefotaxime and azithromycin may be the second choice among the test antibiotics for the treatment of bacterial infection or food poisoning related to market milk consumption in human beings.

Conflict of interest

None to declare.

References

Ackers ML, S Schoenfeld, J Markman, MG Smith, MA Nicholson, W DeWitt, DN Cameron, PM Griffin and L Slutsker, 2000. An outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk . J. Infect. Dis., 181: 1834-1837.

Bauer AW, WM Kirby, JC Sherris and M Turck, 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Am. J. Clin. Pathol., 45: 493-496.

Bunning, VK, RG Crawford, JG Bradshaw, JT Peeler, JT Tierney and RM Twedt, 1986. Thermal resistance of intracellular *Listeria monocytogenes* cells suspended in raw bovine milk. Appl. Environ. Microbiol., 52: 1398-1402.

Castro R, R Schoebitz, L Montes and MS Bergdoll, 1986. Enterotoxigenicity of Staphylococcus aureus strains isolated from cheese made from unpasteurized milk. Lebensmittel-Wissenschaft und -Technologie, 1 9: 401 -402.

CDC, 2002c. Outbreak of *Campylobacter jejuni* infections associated with drinking unpasteruized milk procured through a cow-leasing program-Wisconsin, 2001. Morb. Mortal. Wkly Rep., 51: 548-549.

CDC, 2003a. Multistate outbreak of *Salmonella* serotype Typhimurium infections associated with drinking unpasteurized milk-Illinois, Indiana, Ohio, and Tennessee, 2002-2003. Morb. Mortal. Wkly Rep., 52: 613-615.

Cowan ST, 1985. Cowan and Steel's Manual for Identification of Medical Bacteria. 2nd Edn., Cambridge University Press, London. pp: 138-139.

Cowan ST and KJ Steel, 1965. Manual for the Identification of Medical Bacteria. Cambridge University Press, Cambridge.

Dabassa A, K Bacha, 2012. The Prevalence and Antibiogram of *Salmonella* and *Shigella* Isolated from Abattoir, Jimma Town, South West Ethiopia. International Journal of Pharmaco-Biological Research, 3: 143-148.

De Buyser, ML Dufour, BM Marie and V Lafarage, 2001. Implications of Milk and Milk Products in Food Borne Diseases in France and in Different Industrialized Countries. Int. J. Food Microbiol., 67:1-17.

Ershaduzzaman M, MJFA Taimur, and MN Munsi, 2007. Bacteriopathology of Pneumoenteritis and Antibiotic Sensitivity of the Organisms Isolated from Black Bengal Kids Affected with the Diseases. Bang. J. Livs. Res., 14:59-66.

Foster G, B Holmes, AG Steigerwalt, PA Lawson, P Thorne and DE Byrer, 2004. *Campylobacter insulaenigrae* Isolated from Marine Mammals. Int. J. Syst. Evol. Microbiol., 54: part 6, pp. 2369 – 2373.

Giesecke WH, JHDU Prreez and TM Petzer, 1994. Practical mastitis control in dairy herds. Butterworth, Durban.Thomas SB, RG Druce and M Jones, 1971. Influence of Production Conditions on the Bacteriological Quality of Refrigerating Farm Bulk Tank Milk-a Review. J. Appl. Bacteriol., 34: 659-677.

Hasan MA, MA Islam, MS Mahmud, ASMA Uddin and S Ahmed, 2015. Microbial analysis of raw and pasteurized milk from selected areas of Dinajpur, Bangladesh. Asian J. Med. Biol. Res., 1: 292-296.

Heuvelink AE, FL van den Biggelaar, E de Boer, RG Herbes, WJ Melchers, JH Huis in't Veld and Monnens, L.A. 1998. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. J. Clin. Microbiol., 36:878–882.

Jay JM, 2000. Food-Borne Gastroenteritis Caused by *Salmonella* and *Shigella*. In: Modern Food Microbiology, James, JM (Ed.). 6th Edn. Aspen Publishers, Inc., Maryland, USA. pp: 511-530.

Jones TO, 1990. *Escherichia coli* mastitis in dairy cattle-A review of the literature. Vet. Bull., 60: 205-220.

Mrema NS, BA Mpuchane and Gashe, 2006. Prevalence of *Salmonella* in raw meat minced meat, raw fresh sausage and burger patties from retail outlets in Gaborone, Botswana. Food Control, 17: 207-212.

Murinda SE, LT Nguyen, SJ Ivey, BE Gillespie, RA Almeida, FA Draughon and SP Oliver, 2002a. Molecular characterization of *Salmonella spp.* isolated from bulk tank milk and cull dairy cow fecal samples. J. Food Prot., 65:1100.

Murinda SE, LT Nguyen, SJ Ivey, BE Gillespie, RA Almeida, FA Draughon and SP Oliver, 2002b. Prevalence and molecular characterization of *Escherichia coli* O157:H7 in bulk tank milk and fecal samples from cull cows: a 12-month survey of dairy farms in east Tennessee. J. Food Prot., 65:752.

Plotter HM, 2002. Raw milk and milk products for human consumption. Dairy Division, Indiana State Board of Animal Health, Indianapolis, IN.

Ruegg P, 1999. *Salmonella, Listeria, E. coli* and *Mycobacterium paratuberculosis* in milk. Is there cause for concern? [online] [1 April]. Available from worldwide web:http/www.wisc.edu/sysci/uwex /milk/pubs/zoo.pdf

Sharma D and A Malik, 2012. Incidence and prevalence of antimicrobial resistant *Vibrio cholerae* from dairy farms. Afr. J. Microbiol. Res., 6: 5331-5334.

Watts JL, 1989. Etiological agents of bovine mastitis. Vet. Microbiol., 16: 41-66.

Zecconi, A.; Hahn, G. 2000. *Staphylococcus aureus* in raw milk and human health risk. Buletin IDF, 345:15-18.