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PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *PROTEUS* IN RAW MILK, MILK PRODUCTS AND UTI PATIENTS

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ABSTRACT

A total of 360 samples of raw milk and milk products were collected from different dairies of different locations of Dehradun and 640 urine samples of (UTI patients) from Doon Hospital & Mahant Indresh Hospital, Dehradun. The samples were collected under aseptic conditions. The samples were inoculated in enrichment media for 24 hrs. The enriched milk, milk products (Curd and ice-cream) and urine samples were streaked on selective media MacConkey Agar and Hektoen Enteric Agar with the help of inoculating loop and incubated at 37°C for 24 hrs for recovery of isolates of *Proteus*. The isolates were characterized & identified on the basis of their morphological & biochemical characterization.

It was observed that raw milk & milk products had the prevalence of many strains of *E.coli*, *Salmonella sp.*, *Staphylococcus sp.*, *Proteus sp.*, & *Bacillus sp.* But the prevalence of ESBL producing *Proteus sp.* in milk samples was 11% and in urine samples of urinary tract infective patients was 13%.

Key Words: Raw Milk, Aseptic, Bacterial Strains, Agar, Urinary Tract Infection

INTRODUCTION

Milk and dairy products are complete food for human health and nutrition; also provide an ideal medium for growth and multiplication of microorganisms. Raw milk is almost sterile from healthy udder but subsequent milking and post milking operations contaminate the milk. Number and types of micro flora present in milk depend upon the extent and nature of unhygienic conditions prevalent at different level. Thus raw milk often contains microorganisms which may cause food borne diseases (Adesiyun *et al.*, 1995; Steele *et al.*, 1997). The micro floras entering the milk during milking and further processing are of two type i.e. spoilage and pathogenic type. Most common food poisoning microbes associated with milk and its products including *Staphylococcus*, *Salmonella sp.*, *Shigella dysenteries*, *Proteus sp.*, and *Vibrio cholerae*. Among all these *Proteus* plays an important role but at low level as compared to *E.coli*, *Klebsiella*, *Salmonella*, *Shigella*. Gram negative pathogens harbouring extended spectrum β -lactamases (ESBL) have caused numerous outbreaks of infection and are responsible for causing therapeutic problems in many countries. The incidence of ESBL producing strains among the clinical isolates have been steadily increasing over the past years resulting in limitations of therapeutic options (Podschun and Cellmann, 1998). Over the last 15 years numerous out breaks of infections with organism producing extended spectrum β -lactamases (ESBL) have been observed worldwide. More than 80% of human urinary tract infections (UTI) are due to the bacterium, *Escherchia coli*, but urinary infection due to *Proteus mirabilis* are also well documented. *P.mirabilis* once attached to urinary tract, infects the kidney more commonly than *E.coli* and *P.mirabilis* belongs to enterobacteriaceae and is gram-negative, motile swarmer bacterium. *P.mirabilis* are often found as free living organisms in soil and water but they are also parasitic in the upper urinary tract of human beings (Walker *et al.*, 1999).

MATERIALS AND METHODS

Collection of Samples

360 samples of raw milk and milk products (curd and ice-cream) were collected from different dairies of different locations of Dehradun and 640 urine samples of (UTI patients) from Doon Hospital & Mahant Indresh Hospital, Dehradun. The samples were collected aseptically in 50 ml Oakridge tubes.

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Enrichment of Samples

The samples were inoculated in Buffer Peptone water for 24 hrs. for enrichment. One ml of each sample was added to 9 ml of sterile peptone water for sufficient enrichment and incubated at 37°C for 24 hrs.

Recovery of Isolates of *Proteus* sp.

The enriched milk, milk products (Curd and ice-cream) and urine samples were streaked on selective media MacConkey Agar and Hektoen Enteric Agar with the help of inoculating loop and incubated at 37°C for 24 hrs. for recovery of isolates of *Proteus*.

Morphological Characterization

Morphological characteristics of recovered isolates e.g. Colony morphology (color, shape, margin, elevation and surface) and cell morphology (shape, arrangement and gram reaction) were studied.

Biochemical Characterization

The various biochemical tests e.g. Indole, Methyl Red, Voges Proskauer, Citrate Utilization test (IMViC), Catalase, Triple Sugar Iron Agar (TSI) test, Urease test, Phenylalanine deaminase test of ESBL producing *Proteus* were carried out according to Cappuccino and Sherman (1992).



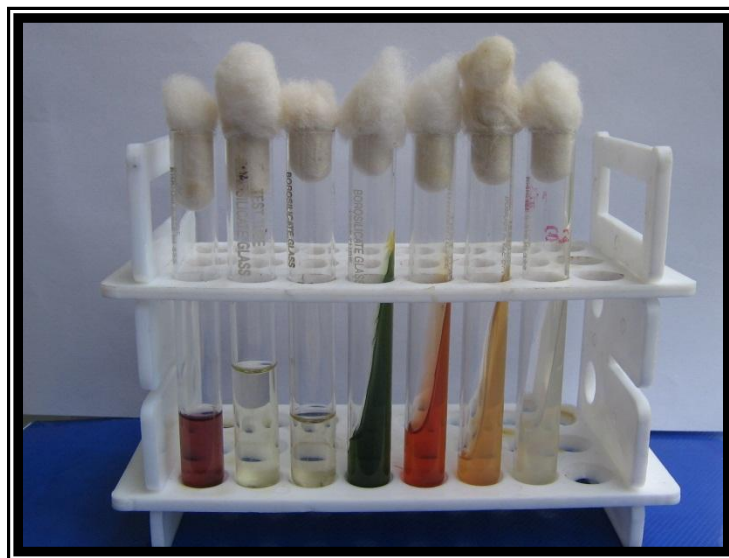
(A)



(B)

Figure 1: Colony morphology of *Proteus* on (A) MacConkey agar; (B) Hektoen enteric agar

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(A)



(B)

Figure 2: Biochemical Characterizations (A) Control left to right -Indole, MR, VP, Citrate, TSI, Urease, Phenylalanine; (B) *Proteus* 1- Indole (+) 2- MR (+) 3- VP (-) 4- Citrate (+) 5- TSI (K/H₂S) 6- Urease (+) 7- Ph (+)

Indole Test

The isolates were stab inoculated in SIM medium and incubated at 37°C for 24 hours. After 24 hrs, a few drops of Kovac's reagent were added. The cherry red colour was appeared of indicate Indole positive test.

Methyl Red

The isolates were inoculated in MR-VP medium and incubated at 37°C for 24 hours. After 24 hrs, a few drops of methyl red reagent were added.

The red colour was appeared of indicate positive test.

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Voges-Proskauer Test

The isolates were inoculated in MR-VP medium and incubated at 37°C for 24 hours. After 24 hrs, 3 drops of 40% KOH and 9 drops of α -Naphthol reagent were added. The deep rose colour was not appeared of indicate negative test.

Citrate Utilization Test

The isolates were streaked on Simmons citrate agar slant (Appendix) and incubated at 37°C for 24 hours. After 24 hrs., the green colour of the Simmons citrate agar slant change into blue colour indicate positive test.

Triple Sugar Iron Agar Test

The isolates were streaked in Triple Sugar Iron Agar slants and incubated at 37°C for 24 hours. After 24 hrs., the slants were red and butt were yellow with bubbles and blackening of media occurs, conditions were alkaline/acid H_2S production.

Urease Test

The isolates were streaked on Urease agar slants and incubated at 37°C for 24 hours. After 24 hrs, the deep pink colour was appeared of indicate positive test.

Phenylalanine Deaminase Test

The isolates were streaked on Phenylalanine agar slants and incubated at 37°C for 24 hours. After 24 hrs, 5 drops of 10% ferric chloride solution were added. The deep green colour was appeared after the addition of ferric chloride indicate positive test.

RESULTS AND DISCUSSION

Proteus is one of the most common bacteria present in the raw milk and milk products. It is an opportunistic pathogen which can cause nosocomial infection mainly in immunocompromised patients (Chow *et al.*, 1979). These bacteria also play an important role in the urinary tract infection (Warren, 1996). The enzyme urease catalyzes urea into NH_3 and CO_2 causes the pH of urine to rise and unchecked growth of the bacteria. The higher pH, which is also toxic to renal cells and the formation of the urinary stones.

Our study was planned to find out the prevalence of ESBL producing *Proteus* in raw milk, milk products and urinary tract infection patients (urine sample) of Doon Valley. A total of 65 consecutive *Proteus* were recovered during the study in 360 samples of milk and milk products, 40 isolate were ESBL producers and 25 isolates were non ESBL producers, 192 consecutive *Proteus* were recovered in 640 urine samples of UTI patients, 83 isolates were ESBL producers and 109 isolates were non ESBL producers. The prevalence of ESBL producing *Proteus* in raw milk and milk products was 11% and urine (UTI patients) sample was 13 % (Table 1).

Table 1: Prevalence of *Proteus* in raw milk, milk products and urine Samples

Total Sample	Total isolates		% of ESBL producing <i>Proteus</i> .
	ESBL	Non-ESBL	
360 (milk and milk products)	40	25	11%
640 (urine)	83	109	13%

Clinicians should consider ESBL production as a possibility in case of treatment failure with β -lactam antimicrobial. ESBL refers to β -lactamase enzyme produced by gram negative organisms that confer resistance against broad spectrum β -lactam antibiotics, normally having activity against gram negative bacilli. Examples of such antibiotics are Cefotaxime, Ceftriaxone, Ceftazidime and Aztreonam (Fridkin *et al.*, 1999). The prevalence of ESBL producing bacteria in most hospitals remains unknown in spite of numerous reports of nosocomial outbreaks of infection due to these organisms. Important ESBL

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producing Gram negative bacilli include *K. pneumoniae*, *E. coli* and *P. mirabilis*, *Enterobacter sp*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Stenotrophomonas maltophilia* (Coudron *et al.*, 1997).

In our study the relative frequency of ESBL producing *Proteus sp.* was found to be 11% and 13% (In milk, milk product and urine sample) which is greater than frequency reported from France (6.9%), U.S. (9.5%) and Italy (8.8%). Sander *et al.*, (1992) study showed that besides other pathogenic microorganism, *Proteus* also produce ESBL due to which these are becoming resistant to the first and second generation Cephalosporin and Penicillin.

Because of developing resistance toward first and second generation antibiotics, the third generation Cephalosporin came in the form to encounter such infections. But increasing resistance towards them developed the use of antibiotic inhibitor combination.

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