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ISOLATION AND IDENTIFICATION OF ENTEROPATHOGENIC BACTERIA FROM MARES MILK SAMPLES

Murathan M., Researcher
Kazakh Agrotechnical University, named after S.Seifullin, Nur-Sultan

Milk is a typical mammary secretion of milking animals, which is used for the nutritional purpose and is a good source of sugar, lipid, proteins, vitamins, calcium and some other minerals. Turkic-speaking peoples, including Kazakhs, have a tradition of drinking raw mare's milk

The aim of this study was to isolate the aforementioned bacteria and compare the risk of foodborne pathogens in different milk sources in the Kazakhstan region. Collect samples from different dairy stores and farmhouses, then isolate and characterize these pathogens through culture and different biochemical tests to identify whether raw mare milk contains microorganisms that are harmful to human health.

Methods. Sample collection. This study involved collection of samples from different milk outlets in East Kazakhstan and sAkmola areas of the city (Astana, Koiandy and Koktal) aseptically. A total of 20 samples were collected randomly from Dairy shops, Farms, houses and mares milk collection point. Test samples were taken in sterile UV irradiated disposable plastic tubes and transported aseptically in ice box within four hours at 4 ºC for further processing.

Sterilization of Test tubes and Petri plates. Test tube and Petri dishes were washed with tap water and left for drying. The washed tubes and Petri plates were covered with aluminium foil and then for 15 minutes and autoclaved at 121 oC under 15 psi. After this, they were placed in a laminar flow hood for further processing.

Culture media preparation. Separated flasks were used for the ingredient of each media and then mixed by heating and shaking on a shaker at 1000 rpm for 10 minutes at 100°C. The flasks were plugged with cotton, covered by aluminium foil and autoclaved. After sterilization by autoclave, media flasks were cooled. UV

lamp was lit in a biosafety cabinet for 5 minutes and ethanol was sprayed, and media was transferred.

Culture media pouring. In biosafety cabinet, the same quantity (20 milliliters per petri plate) was poured in every plate and allowed for solidification. Petri dishes were covered with aluminium foil in order to avoid contamination of air and incubated at 37 oC for 24 hours for checking media's sterility.

Incubation of Petri plates. After 24 hours of incubation of Petri dishes, contamination of microbes was checked. Petri plates with no microbial contamination were taken and used for further processing.

Dilution preparation. Samples were aseptically taken through pipette from sample bottles by using sterilized tips. Milk samples were diluted in tubes containing 9ml buffer sulphate in each tube. 1ml of raw milk sample was added to 9ml buffer sulphate tube, making 1/10 (10-1) dilution, and then 1ml was transferred from that tube to 9ml tube containing buffer sulphate to make 1/100 (10-2) dilution. In the same way dilutions were made up to 8/10 (10-8).

Samples processing. 1ml samples were taken aseptically from each dilution and poured on salmonella-shigella agar (SSA) and MacConkey agar for detection of shigella spp, salmonella spp and E. coli, respectively. The plates were placed in the incubator in an inverted position at 37°C for 24-48 hours for bacterial growth. After 24-48 hours, colonies appeared on some plates, the colonies were counted using CFU/ml by the standard microbiological method through colony counter device.

Purification. The colonies on, MacConkey agar and SS agar were obtained and differentiated on biochemical properties and morphology basis. Restreaking of the isolates was done for organism purification and incubated at 37°C for 24hrs.

Morphological identification. Identification on the basis of colony. After 24 hours some of the plates showed bacterial growth in colony form. Identification of different bacteria was performed on the basis of colony colour, surface texture, size, elevations, edges and shape. Colonies of E. coli on MacConkey agar were smooth, transparent and their colour was pink. On SS agar, two different types of colonies appeared. Smooth round and opaque colourless colonies with black centers which were suspected to be Salmonella, and transparent colourless with no black center colonies were identified as Shigella. Using sterile loop, colonies from both plates (MacConkey and SS agar plates) were picked and spread on EMB agar and incubated at 37 oC for 24hrs for further confirmation. After incubation, EMB showed green metallic shine colonies which were E. coli. Pink coloured colonies and semi-transparent colourless colonies also appeared that showed to be Salmonella and Shigella respectively.

Further Morphological identification of E, coli, Salmonella and Shigella was done through Gram-staining. First of all, a clean slide was taken. One drop of normal saline was dropped on the slide. To prepare the smear a loop full of the sample was placed on the slide, dried in air and fixed through heat. Then crystal Violet was used for thirty seconds to one minute and washed with water. Then Gram iodine was used for 60 seconds and rinsed with water. After this, 95 percent alcohol (10 to 20 seconds) was used for washing and then cleaned with water. In the last step, safranin was used for sixty seconds and rinsed through the water. In the end, the slide was dried in air and then checked under a microscope using oil emersion. E coli, Salmonella and Shigella were Gram-negative and rod-shaped in single and chain form.

Biochemical identification. Catalase test. Catalase test was used for identification of bacteria that secrete an enzyme known as catalase. Catalase breaks hydrogen-peroxide into oxygen and water. The bacterial smear was made on a sterile cleaned glass slide from MacConkey agar plates and SS agar plates and then 3% Hydrogen-peroxide was added. Then foam appearance was observed which indicated a positive test.

Bubbles were produced by E. coli and Salmonella because of oxygen production by catalase while Shigella did not produce bubbles and was catalase negative.

Oxidase test. Two drops of oxidase reagents (N, N-dimethyl-p-phenylenediamine (DMPD) were put on clean filter papers placed in sterilized petri dish. Then a colony was with sterilized wire loop from MacConkey Agar plates and SS agar plates and mix with oxidase reagents. Then appearance of purple colour on filter paper of petri dish was noted. Purple colour is an indicator of a positive test. No change in colour on filter paper was indicated and showed that E.coli, Salmonella and Shigella were oxidase negative.

Results. Frequency of E. coli, Salmonella and Shigella in mares milk samples collected from different locations

All of these samples were tested for detection of above-mentioned bacteria using three growth media. For the detection of E. coli, MacConkey agar and nutrient agar were used. For Salmonella and Shigella, SS agar and nutrient agar were used. No microorganism was identified in any of these samples (Table 2.1).

Table 2.1 - Frequency of *E. coli*, *Salmonella a*nd *Shigella* in different mares milk samples collected from different locations

Media used	Number of brand samples	Positive Samples	Negative samples

E. coli			
MacConkkey Agar	11	0	11
Nutrient Agar	11	0	11
Salmonella			
SS Agar	11	0	11
Nutrient Agar	11	0	11
Shigella			
SS Agar	11	0	11
Nutrient Agar	11	0	11

2.2 CFU count of different bacterial species in raw milk samples at district locations

From different areas, raw mares milk samples were taken, diluted and then poured on plates. After incubation colonies countable (30-300CFU) for three bacterial species E.coli, Salmonella and Shigella were taken and CFU/ml was counted. Results showed Mean CFU/ml of E.

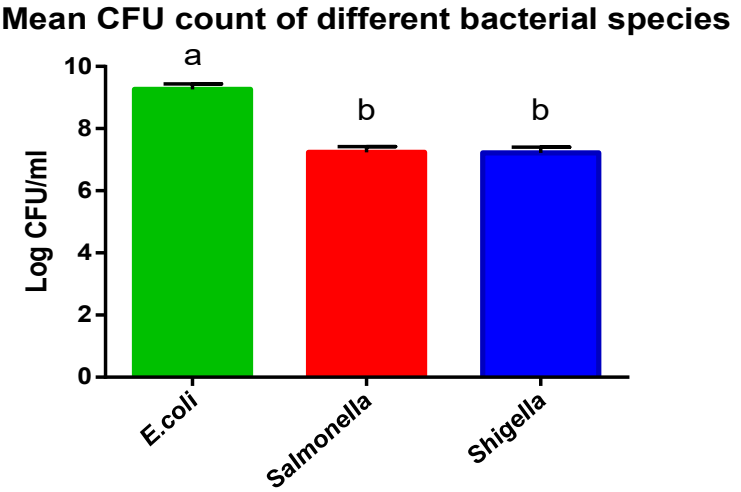

Figure 1.1: CFU count of different bacterial species in raw milk samples. Common alphabetic letters showed no statistical difference while different alphabets were significantly different. Results are Mean±SEM of selected number of samples.

List of used literature