Isolation and Identification of *Proteus* SPP from retailed milk with special reference to multidrug resistant strains

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Abstract: Background: Extended spectrum beta-lactamase (ESβL) producing *Proteus* spp are emerging pathogens that have mounting public health significance and constitute great challenge in the human medicine. Much remains unknown about the epidemiology and zoonosis of such bacteria. Therefore, the present study was carried out to investigate the possible role of raw milk in the epidemiology of ESβL producing *Proteus* spp. **Methods:** 151 raw and pasteurized milk samples were collected from farms and milk pending shops. Milk samples were cultured for isolation and identification of ESβL producing *Proteus* spp. **Results:** A total of 13 (8.6%) consecutive *Proteus* recovered during the study period in 151 milk samples of different area of Egypt, Antimicrobial sensitivity testing against 8 antibiotic agents revealed that all of isolates (100%) were sensitive to imipenem, while 92% of the isolates were sensitive to nalidixic acid and ceftazidime, 84.6% to ampicillin/ sulbactam, 70.9% to sulphamethoxazole/ trimethoprime, 69.2% to cefotaxime On the other hand,61% of isolates were resistant to ampicillin followed by 53.8% to cephalexin. 12 (7.9%) isolates were ESBL producer and 1(0.7%) isolates were non-ESBL producers. **Conclusions:** The occurrence of ESβL producing *Proteus* in milk is higher rates in milk vending house of milk may be lowered when it is contaminated by a number of factors such as adulteration, contamination during and after milking, presence of udder infection, mastitis disease and drugs residues used for treatment of disease which is considered to be public health concern and one of the most important causes of economic losses in the dairy industry worldwide. Otherwise Clinicians should consider ESBL production as a possibility in case of treatment failure with β-lactam antimicrobials.

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Key words: raw milk, ESβL, *Proteus*, antibiotic.

1. Introduction

Proteus spp. consists of Gram-negative, motile, aerobic rod-shaped bacilli belonging to the family Enterobacteriaceae. Members of the Enterobacteriaceae family generally range from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length. They are urease positive and form swarmer cells which allow for swarming motility on solid media[1]. *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 immune compromised patients[2]. These bacteria also play an important role in the urinary tract infection.The enzyme urease catalyzes urea into NH3 and CO2 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 [3].

ESβL refers to extended spectrum beta lactamases which are plasmid mediated enzymes having the capability to hydrolyze beta-lactams and thus inactivate a wide range of antibiotics[4]. In 1980s, ESβL producing members of family *Enterobacteriaceae* were first introduced to the scientific community when ESβL producing *Klebsiellapneumoniae* has emerged to be the cause of dangerous nosocomial infections[5,6]. Since this date, ESβL producing members of family *Enterobacteriaceae* has become the focus of many clinicians, epidemiologists, and microbiologists. As the time advances, these pathogens are becoming a critical worry as they constituted a great challenge in human medicine and public health, and became one major concern associated with nosocomial infections[7].

2. Materials and methods

Samples:

151 raw and pasteurized milk samples were collected from farms and milk vending shops.

Culture and identification of *Proteus* of members of family *Enterobacteriaceae*:

Samples were cultured on MacConkey Agar (BD, Maryland, USA) and Xylose lysine decarboxylase agar (XLD) (BD, Maryland, USA) and incubated at 37°C for 24 hours. The colonies showed red color with black center due to H_2S production on XLD, and colorless non-lactose fermenting colonies on MacConkey agar. Suspected colonies were then inoculated into Triple Sugar Iron Agar (BD, Maryland, USA), Lysine Iron Agar (BD, Maryland, USA), Motility Indole Ornithine

Agar (BD, Maryland, USA), Citrate Agar (BD, Maryland, USA), and Urea broth (BD, Maryland, USA). API 20 E (BioMerieux SA, F-69280 Marcy l'Etoile, France) strips were used for confirmation of isolates.

Initial screening of ESβL by disk diffusion test[8]:

Disk diffusion test was applied to detect ESβL producing isolates. Muller Hinton agar was swabbed by a suspension of a pure culture and antibiotic discs were then loaded. ampicillin (10 µg), ceftazidime (30 µg), cephalexine (30 µg), cefotaxime (30 µg), Nalidixic acid (30 µg), Imipenem (10 µg), sulphamethoxazole/ trimethoprim (25 µg) and ampicillin/sulbactam (20µg). While Cefotaxime (30 µg), ceftazidime (30 µg), Ceftazidime/ clavulanic acid (30/10 µg) and Cefotaxime/ clavulanic acid (30/10 µg) were used for screening of ESβL producing *Proteus spp*. Interpretation was occurred relying on the instructions of CLSI, 2013.

Confirmation test for ESβL[8]:

Confirmatory test was applied by using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid (cefotaxime 30 µg, cefotaxime/ claculanic acid 30/10 µg) and (ceftazidime 30 µg, ceftazidime/claculanic acid 30/10 µg). A ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus the zone diameter of the agent when tested alone equals ESβL.

3. Results

A total of 13(8.6%) consecutive *Proteus* recovered during the study period in 151 milk samples of different area of Egypt, 12(7.9%) isolates were ESBL producer and 1(0.7%) isolates were non-ESBL producers (table 1).

Table 1: Occurrence of the extended spectrum beta-lactamase producing *Proteus spp* in raw and pasteurized milk.

Type of milk samples	Positive Proteus/Total no	%	Positive ESβL/Total no	%
Dairy farms	1/79	1.2	1/79	1.2
Milk vending shops	12/42	28.5	11/42	26.1
Pasteurized milk	0/30	0	0	0
Total	13/151	8.6	12/151	7.9

Antimicrobial sensitivity testing against 8 antibiotic agents revealed that all of isolates (100%) were sensitive to imipenem, while 92% of the isolates were sensitive to nalidixic acid and ceftazidime, 84.6% to ampicillin/sulbactam, 70.9% to sulphamethoxazole/ trimethoprime, 69.2% to cefotaxime.

On the other hand, 61% of isolates were resistant to ampicillin followed by 53.8% to cephalexin.

4. Discussion

This study highlights that detection of ESBLs strain of Proteus in raw milk and milk products becomes necessary, as strains with susceptible zone in vitro may not respond in vivo to the antibiotic leading to treatment failure. The rapid and irrepressible increase in resistance of pathogenic bacteria (especially ESBL's against ß-lactam antibiotics) that has been observed over the last two decades is widely considered to be one of the major problems in human infections. The development and spread of ESBL in raw milk is most likely caused by the overuse of antibiotics to treat cattle disease like mastitis [9]. The data generated from such studies provide physicians in these countries as important scale and scientific community as a whole and understanding of resistance rate on a global scale. In addition, it also helps in formulating effective guideline in therapy and appropriate antibiotics policy for the hospital and prevents further development and spread of resistant strain. Proper infection control practices and barriers are essential to prevent spreading and break of ESBL producing bacteria.

Summery and conclusion

This article discusses the possibility of milk to disseminate ESβL-producing *Proteus* spp from mastitis or contamination after milking. Therefore, people consume raw milk without pasteurization may be under hazard from acquiring this infection.

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