Prevalence of *Klebsiella oxytoca* in milk samples of metropolitan city Kolkata: A study

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ABSTRACT

Background: *Klebsiella oxytoca* is an emerging pathogen and can cause a plethora of infections in man. It is often found in food. **Objective:** The objective of the study was to assess the prevalence of *K. oxytoca* in milk samples and study their antibiogram. **Methods:** A total of 100 milk samples collected from various sources were cultured. **Results:** A total of seven isolates of *K. oxytoca* were found in 100 samples and none were multidrug-resistant. Isolates were uniformly susceptible to ofloxacin and chloramphenicol. They coexisted with other coliforms in some of these samples. **Conclusion:** Raw milk is an important source of *K. oxytoca* which can be pathogenic by oral route.

Key words: *Indole, Klebsiella oxytoca, Milk*

Klebsiella oxytoca is a Gram-negative, rod-shaped bacterium found normally in human gut. They are usually capsulated and can be recognized by their large, grayish-white, mucoid colonies on laboratory medium, especially if it has high sugar content. It was first described in the year 1886, when it was isolated for the first time from sour milk and named *Bacillus oxytocus* perniciosus, from the Greek word, oxus meaning "sour" and tokos meaning "producing" [1]. It is very closely related to *Klebsiella pneumoniae*, however, is differentiated by being indole-positive and by having slightly different growth characteristics and features. These organisms can grow on melezitose, but not 3-hydroxybutyrate.

K. oxytoca is considered to be a notoriously drug-resistant bacterium which can cause various infections such as bronchopneumonia, colitis, and sepsis [2]. Outbreaks of antibiotic-resistant *K. oxytoca* have occurred in many hospitals and intensive care units (ICUs) throughout the world, and handwashing stations have been recognized in literature as a potentially important environmental reservoir of the bacterium [3]. It is also an important cause of mastitis and hence can be frequently isolated from cow and buffalo milk [4]. Hence, the present study was planned with an objective to study the presence of *K. oxytoca* in milk samples and also to study its antibiotic susceptibility.

METHODS

The present laboratory-based observational study was carried out in the laboratory of the Department of Microbiology, All India Institute of Hygiene and Public Health, Kolkata, for a period of 6 months from July 2019 to December 2019. A total of 100 milk samples consisting of 75 samples of raw milk from the market and 25 samples of pasteurized milk were collected from 25 different sources such as vendors, farms, and packed milk (pasteurized) which were assessed during the study period. From each vendor, four random milk samples were randomly selected for the study. The collected milk samples were transported aseptically to the laboratory in the icebox and subsequently processed for laboratory tests for the identification of bacterial species. The samples were preserved at 4–8°C in the refrigerator for 24 h whenever necessary, after the process followed by Anderson *et al*. [5].

All the samples were inoculated on Nutrient agar, MacConkey agar, and 10% Sheep Blood Agar plates and incubated at 37°C for 24 h and on Robertson's cooked meat medium for 48 h for anaerobic bacteria. Methylene blue reduction test was also carried out with these milk samples directly and incubated at 37°C for 5 h [6]. Further identification of the bacteria was carried out by biochemical and phenotypic characterization of isolates from the colonies by tests such as Gram reaction, catalase production, oxidase production, oxidation/fermentation test and production of indole, utilization of citrate, urease, and triple sugar iron. Methylene blue reduction test was done and changes were observed. In addition, lactose fermentation, motility, and growth on MacConkey agar were examined. Antibiotic susceptibility test was carried out on Mueller-Hinton agar by the Kirby-Bauer method of disk diffusion following CLSI protocol [7]. The following antibiotic disks were used for susceptibility testing: Amikacin (30 µg), erythromycin (15 µg,) amoxiclav (25 µg), ofloxacin (5 µg), cefotaxime (30 µg), tetracycline (30 µg), and

chloramphenicol (30 µg). We could not perform quantitative culture tests due to a lack of facility in our department laboratory. The obtained data were analyzed using descriptive statistical methods.

RESULTS

In our study of 100 samples, seven isolates appeared as Gram-negative, encapsulated, indole positive, urease positive, and nonmotile rods from the colonies and were hence confirmed to be *K. oxytoca*. All the *K. oxytoca* strains were recovered from raw milk samples. Methylene blue reduction test was positive in all those samples in which *K. oxytoca* isolates were observed. The biochemical and phenotypic test results of the identified isolates are shown in Table 1 and Fig. 1. Results of antibiotic susceptibility are shown in Table 2. Along with *K. oxytoca*, other enteric bacteria were also isolated from some of the samples. *Escherichia coli* were isolated from 33 samples, followed by *K. pneumoniae* in 26 samples, *Citrobacter koseri* in 12 samples, *Enterobacter aerogenes* in eight samples, and *Pseudomonas aeruginosa* were isolated from three samples. Seven strains of *K. pneumoniae* were recovered followed by five strains of *E. coli* and 3 strains of *Acinetobacter lwoffii.* We also noted presence of three different bacteria in a single sample.

DISCUSSION

Unpasteurized milk is a very important source of transmission of multidrug-resistant pathogenic bacteria such as *P. aeruginosa* and others [8]. *K. oxytoca*, apart from causing urinary tract infection and septicemia in man and being an important pathogen in immunocompromised and debilitated patients in ICUs, can also cause severe antibiotic and nonsteroidal anti-inflammatory drugs associated hemorrhagic colitis [2,9,10]. This is characterized by segmental hemorrhagic lesions in the colon and is believed to be caused by the cytotoxin (tillivaline) released by the bacterium [9,10]. Results of animal studies conclude that antibiotic-associated hemorrhagic colitis caused by *K. oxytoca* affects mainly the transverse and right colon and hence colonoscopy instead of sigmoidoscopy is advised [10]. *K. oxytoca* hemorrhagic colitis has been found most commonly in younger individuals, in contrast with antibiotic-associated diarrhea caused by *Clostridium difficile* [11].

Figure 1: Biochemical reactions of *Klebsiella oxytoca* strain

Bacteria of genera *Klebsiella*, especially *K. oxytoca* and *Raoultella ornithinolytica*, are found in food very commonly and are capable of causing many health hazards [12]. This entity is found more commonly in the Japanese population and rarer in other countries like United States [13]. Its presence in raw milk is very important and an issue of great public health concern, since milk is one of the most common consumed nutritious foods in India. In a study by Singh *et al.*, *K. oxytoca* isolates were found to be highly resistant to amikacin and more susceptible to ciprofloxacin [2]. Our study has also found similar results, where isolates were uniformly resistant to amikacin and erythromycin, and very highly resistant to amoxiclav. Our isolates were uniformly susceptible to ofloxacin. However, there was no occurrence of any multidrug-resistant strains (resistant to three or more groups of drugs) as found in our observations.

K. oxytoca was earlier called by various names, like *Bacterium oxytocum* as propounded by Flugge in 1886, and is a bacterium that forms indole, has a positive Voges–Proskauer reaction, and can liquefy gelatin along with other characteristics of *Klebsiella* species [2]. *K. oxytoca* is normally acquired from various environmental sources and comes in milk from infected mammary glands of cows and buffaloes [2]. Among species in the genus *Klebsiella* spp., *K. oxytoca* is lesser common cause of all infections as compared to *K. pneumonia. K. oxytoca* causes about 13–25% of all infections caused by members in genus *Klebsiella* [14]. We found that this pathogen exists in milk along with other pathogens like *E. coli* and hence its presence should always be looked for in milk samples available in the market. A study by Gaffer *et al*. from Egypt has shown that *K. oxytoca* was found in only about 8.5% of the total of milk product samples

Table 1: Phenotypic characteristics of *Klebsiella oxytoca* isolates

Name	Catalase	Oxidase	Motility in semisolid agar	Citrate	Urease	TSI	Indole
Klebsiella oxytoca	+	Negative	Negative	Utilized	++	a/a, H_2S-ve, gas++	Positive

TSI: Triple sugar iron

Table 2: Antibiotic susceptibility of *Klebsiella oxytoca* isolates

Name	Amikacin	Amoxiclav	Erythromycin	Ofloxacin	Cefotaxime	Chloramphenicol	Tetracycline
Klebsiella oxytoca, 7 isolates	(R) in all	(R) in all except 1	(R) in all	(S) in all	(S) in all	(S) in all	(M.S) in 3, (R) in 4

S: Susceptible, R: Resistant, M.S: Moderately susceptible

such as milk, cheese, and ice-cream samples and was highly resistant to beta-lactam antibiotics [15].

This study was the first attempt from this part of the country to search for *K. oxytoca* and other bacteria as foodborne pathogens in milk. Our study had limited sample size and non-availability of resources limited molecular characterization of typing. Knowing the antibiogram was important because sometimes empiric antibiotic therapy is needed to treat the hemorrhagic colitis if it occurs. Further studies are eagerly awaited in this regard and should be carried out.

CONCLUSION

K. oxytoca is an emerging pathogen, especially through the oral route in food matter, and hence should be searched for in raw milk. Milk should, hence, always be boiled or pasteurized before consumption.

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