Short Communication

ANTIBIOTIC SENSITIVITY PATTERN OF *YERSINIA ENTEROCOLITICA* ISOLATED FROM MILK AND DAIRY PRODUCTS*

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Received : 07-02-2013 Accepted : 13-07-2013

ABSTRACT

From a total of 2431 samples of milk and milk products analyzed at Chennai, twenty isolates of bacteria were identified as *Yersinia* species by the conventional biochemical method which includes nine *Yersinia enterocolitica* (0.37%), six *Y. frederiksenii* (0.25%) and five *Y. kristensenii* (0.21%). The sensitivity pattern of six antimicrobial agents *viz.* ampicillin, chloramphenicol, ciprofloxacin, gentamicin, penicillin and tetracycline were tested against nine isolates of *Y. enterocolitica*. The *invitro* antibiotic sensitivity testing showed that ciprofloxacin (5 mcg) and tetracycline (30 mcg) were the most effective antibiotics against *Y. enterocolitica*, whereas, penicillin (10 units) was the most ineffective one. Hence, analyzing the prevalence and antibiotic sensitivity pattern of *Y. enterocolitica* isolates from dairy products is essential.

Key words: Milk - *Yersinia enterocolitica* - antibiotic resistance

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* Part of the Ph.D. thesis of the first and corresponding author submitted to TANUVAS, Chennai.

INTRODUCTION

Spoilage of milk and milk products is of frequent occurrence in India, because of their perishable nature and the tropical environmental conditions prevailing in India. The genus *Yersinia* comprises three well known food borne human pathogens: *Yersinia enterocolitica, Y. pseudotuberculosis* and *Y. pestis*. Among these three, *Y. enterocolitica* is important milk borne pathogen and its presence in milk is of great public health importance by causing yersiniosis, an emerging disease (Bercovier and Mollaret, 1984), which is characterized by gastroenteritis with diarrhoea, vomiting, fever and abdominal pain (Bottone, 1999).

Although *Y. enterocolitica* is an ubiquitous microorganism and majority of the isolates recovered from food are non pathogenic, it is important to determine the control measures on the growth of *Yersinia enterocolitica* by antibiotics. Antimicrobials are used in food animals as therapeutic agents and as growth promoters. The use of effective drugs has been essential to guarantee the high indices of productivity reached in the last decades, propitiating a reduction of mortality and morbidity and the maintenance of animal well–being. However, the indiscriminate use of antimicrobials can lead to the selection of resistant bacteria in food animals that could subsequently be transmitted to humans, a serious public health problem (McEwen and Fedorka–Cray, 2002).

Antibiotic sensitivity of food borne pathogens is an important concern to the world today. Use of antibiotics to prevent diseases such as mastitis in cows may contribute to the emergence of microorganisms resistant to antibiotics currently used in human life.

MATERIALS AND METHODS

Isolation and identification of *Yersinia* species from aseptically collected 2431 milk and milk products samples including 1410 raw milk, 185 pasteurized milk, 308 buttermilk, 113 khoa, 205 ice cream and 210 paneer from various zones of Chennai during different seasons were carried out as per the procedure of standard methods for examination of dairy products (Robert, 1992 and Bottone *et al*., 2005). Nine isolates *of Y. enterocolitica* were identified based on various biochemical tests as well as molecular methods and were subjected to antibiogram.

Overnight grown pure cultures of *Y. enterocolitica* isolates (200 µl each) were spread-plated onto Mueller Hinton agar (Hi Media) aseptically. Antibiotic sensitivity pattern was determined by the disc diffusion method using commercially available discs ((M/s. Hi Media Laboratories Pvt. Ltd., Mumbai, India) with the following antibiotics with concentration noted against each: Ampicillin (A) – 10 mcg, Chloramphenicol (C) – 30 mcg, Ciprofloxacin (Cf) – 5 mcg, Gentamicin (G) – 10 mcg, Penicillin (P) – 10 units and Tetracycline (T) – 30 mcg. Antibiotic discs were placed at equidistance in each plate and incubated at 25^0 C for 24 hrs. The inhibition zone (including diameter of disc) was measured and interpreted as per the charts of Kushal and Anand, 2001. Isolates were considered resistant if no zone of inhibition could be seen around the disc. The statistical analysis was carried out as per the procedure adopted by Snedecor and Cochran, 1994.

RESULTS AND DISCUSSION

Twenty nine isolates (28.15%) were assumed as presumptive *Yersinia* species from a total of 2431 samples of milk and milk products collected and analysed from various sources in and around Chennai. Through various biochemical tests, 20 isolates were identified and confirmed as *Yersinia* sp. which includes nine *Y. enterocolitica* (0.37%), six *Y. frederiksenii* (0.25%) and five *Y. kristensenii* (0.21%). The same was also confirmed by molecular methods like Polymerase Chain Reaction (PCR) and multiplex PCR.

All the nine *Y. enterocolitica* isolates were tested for their susceptibility to various antibiotics and the susceptibility pattern of the isolates against these antibiotics were presented in Table 1. All the *Y. enterocolitica* isolates were sensitive to chloramphenicol, ciprofloxacin, gentamicin and tetracycline at varying levels. Ciprofloxacin (5 mcg) and tetracycline (30 mcg) were the most effective as all the isolates were completely sensitive to these antibiotics and penicillin (10 units) was the most ineffective as all the nine isolates were 100 % resistant to this antibiotic. Seven, six and five isolates of *Y. enterocolitica were* sensitive for gentamicin (10 mcg), chloramphenicol (30 mcg) and ampicillin respectively. None of the isolates was resistant, though few isolates showed intermediate effect to gentamicin and chloramphenicol.

These findings are in agreement with the studies of Pham *et al.* (1991) and Lal *et al.* (2003) who reported that *Y.enterocolitica* was sensitive to chloramphenicol, ciprofloxacin, gentamicin, tetracycline and trimethoprim and resistant to ampicillin and penicillin. Statistical analysis shows that there was highly significant difference (P d" 0.01) with regard to antibiotic sensitivity pattern on *Y. enterocolitica*.

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Table 1

Antibiotic resistance profile of *Yersinia enterocolitica*

***Y.enterocolitica* isolates** (sample No.)	**Particulars of antibiotic disc**					
	Ampicillin (10 mcg)	**Chloram Phenicol (30 mcg)**	**Ciprofloxacin (5 mcg)**	**Gentamicin (10 mcg)**	**Penicillin (10 units)**	**Tetracycline (30 mcg)**
Raw Milk - 96	S	S	**S**	S	**R**	**S**
Raw Milk - 222	**I**	**I**	**S**	**I**	**R**	**S**
Raw Milk - 655	S	S	**S**	S	**R**	**S**
Raw Milk - 762	**I**	S	**S**	S	**R**	**S**
Raw Milk-1354	**I**	**I**	**S**	**I**	**R**	**S**
Butter Milk -91	S	S	**S**	S	**R**	**S**
Butter Milk 301	S	S	**S**	S	**R**	**S**
Ice Cream - 48	**I**	**I**	**S**	S	**R**	**S**
Ice Cream - 167	S	S	**S**	S	**R**	**S**
χ^2 = 64. 67**						

** : Highly Significant (P d" 0.01)

R – Resistant S - Sensitive I - Intermediate