ISOLATION, CHARACTERIZATION AND ANTIBIOGRAM OF *Enterococcus faecalis* IN BOVINE MILK SAMPLES FROM CASES OF RECURRENT MASTITIS IN A FARM

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Enterococci are ubiquitous in nature and normal commensals in the digestive tract, but they are one of the leading causes of nasocomial infections causing significant threat to public health because of the problem of antibiotic resistance. Majority of the isolates are *Enterococcus faecalis* and *Enterococcus faecium* but the incidence of other species shows an alarming rise (Prakash *et al*., 2005). Enterococcal spp. isolated from animals also showed considerable diversity (Jung *et al*., 2007). Enterococci can cause many economically important animal diseases including bovine mastitis and are considered as environmental pathogens as they are transmitted between the environment and the animal rather than from animal to animal (Rossitto *et al*., 2002). Enterococcal spp. are very often used as indicator bacteria for development of antimicrobial resistance and it provides accurate information on precise antibiotic treatment of the animals (CDC, 2002).

Bovine mastitis is the most common reason for the usage of antibiotics in livestock industry. Adequate knowledge regarding their resistance profiles in mastitic milk sample could be useful for assessing the extent of antibiotic resistance problem. The current study investigates the cause of recurrent mastitis in a cattle farm in Thrissur district. The animals were frequently been affected with mastitis and were blindly treated with a wide range of antibiotics. There was improvement in condition after antibiotic therapy for few days but with decrease in milk production, thereafter cases recurred.

Four milk samples from cows belonging to a private farm in Thrissur were submitted to the Department of Veterinary Microbiology, CVAS, Mannuthy. The samples were inoculated onto Brain hear infusion agar (BHIA) and McConkey's agar and incubated at 37°C for 24-48 h. Samples were also inoculated onto Blood agar and incubated at 37°C under 5-10 per cent carbon dioxide tension for 48-72 h. The colonies were subjected to further characterization based on cultural, morphological and biochemical characterization (Carter and Chengappa, 1981). *In vitro* antimicrobial susceptibility testing was conducted using the disc diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002).

On BHIA, no visible growth could be detected even after 48 h of incubation at 37°C. On McConkey's agar, small pin point colonies were observed. Small, non-haemolytic colonies were observed on Blood agar after 72 h of incubation. On staining, gram positive cocci in pairs and short chains were observed. On phenotypic characterization, catalase and oxidase test were negative. Growth could be

detected in 6.5 per cent sodium chloride and at pH 9.6. The VP reaction was found to be negative. On sugar fermentation test, the organism was found positive for Lactose, Mannitol, Salicin, Sorbitol, Trehalose, Aesculin hydrolysis and negative for Inulin and Raffinose.

On antibiogram, the isolate was found sensitive to only Cefriaxone-Tazobactum, but resistant to Amoxycillin, Ampicillin, Gentamicin, Chloramphenicol, Enrofloxacin, Tetracycline, Ceftriaxone, Bacitracin, Cefotaxim and Sulpha-Trimethoprim.

Based on the cultural, morphological and biochemical characterization, the isolate was identified as *Enterococcus faecalis*. The isolate responded to the susceptible antibiotic and the condition was treated successfully. But, the results of current study showed that the enterococcal isolate was resistant to almost all the commonly used antibiotics available in field against mastitis. They can get into human food chain via raw milk products or other forms of contamination (Tenhagen *et al*., 2006). The resistance genes contained within antimicrobial resistant enterococci could be transferred to other bacteria including those that are implicated in human diseases. The extent of antimicrobial resistance in enterococci from food animals should be monitored carefully in order to assess the role of these animals as reservoirs of resistant bacteria and their subsequent impact on human health.

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