PREVALENCE AND ANTIBIOGRAM OF *PSEUDOMONAS* FROM BOVINE MASTITIS IN THRISSUR DISTRICT*

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Abstract

Two hundred and eighty nine milk samples of dairy cows with mastitis from various small and medium sized dairy farms of Thrissur were subjected to cultural examination and antibiogram studies. Of the 231 isolates obtained, 10 of the isolates were Pseudomonas based on cultural characteristics and biochemical test. The isolates were subjected to antibiotic sensitivity test to 12 antibiotics by disc diffusion techniques. All the Pseudomonas isolates were sensitive to gentamicin and ampicillin- cloxacillin was found to be least sensitive. This study highlights Pseudomonas spp. as a pathogen of bovine mastitis in Thrissur district.

Keywords: *Pseudomonas, Bovine mastitis, Antibiogram*

One of the major limitations in prevention and control of mastitis is the diversity of the organisms causing the disease. Although the coliforms and staphylococci are considered to be the major pathogens of mastitis, many other bacterial agents are also involved in its causation. *Pseu*domonas spp. is one such Gram negative bacterial etiology. The organism occurs frequently in the environment including water supply systems, contaminated drugs, infusion equipments etc. and is known to cause environmental mastitis. *Pseudomonas aeruginosa* and *P.fluorescens* form the frequently isolated species in this genus. Mastitis due to *Pseu*domonas can be less severe when the number of invading organisms in less, but can be lethal if a large number of organisms invade the mammary gland. As pseudomonas are the most refractory Gram negative pathogen to antibacterial therapy (Bannermann *et al.,* 2005) and considered as an important contaminant in milk and milk products leading to public health hazards (Yu-Cheng *et al.,* 2012). The present study focuses on the prevalence of mastitis due to *Pseudomonas aeruginosa* in Thrissur district and its antibiotic sensitivity pattern.

Materials and Methods

A total of 289 milk samples from animals, clinically affected with mastitis were subjected to cultural examination. The milk samples were obtained from dairy farms and dairying households in and around Mannuthy, Thrissur District and in nearby villages. Animals were observed for clinical signs with detailed clinical examination. Midstream milk samples from the affected cattle were collected based on strict aseptic protocols in sterile glass vials to ensure contamination free samples for further processing.

Milk sample (0.1 mL) was spread on Brain heart Infusion Agar plates and incubated at 37^{0}C for 24 to 48h. The colonies were

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subjected to identification based on colony morphology, Gram staining and biochemical tests.

Antimicrobial susceptibility patterns of all the isolates were checked using agar disc diffusion method against 12 different antibiotics: enrofloxacin (10ug), ciprofloxacin (10ug), gentamicin (30ug), tetracycline (30ug), streptomycin (10ug), cefotaxime (10ug), ceftizoxime (30ug), ampicillin/sulbactam (10/10ug), ceftriaxone (10ug), sulpha – trimethoprim (25ug), amoxicillin– clavulanate (30ug), and amplicillin (10ug). The sensitivity was measured in terms of the diameter of the zone of inhibition surrounding the disc.

Results and Discussion

Out of the 289 samples cultured, 231 samples yielded bacterial isolates. Pseudomonas was isolated from 10 samples. *Staphylococcus aureus*, with 46 isolates formed the commonest organism. Other organisms obtained were E. coli (15%), Klebsiella spp. (10%) and streptococci (10%). The results are comparable to the findings of Dutta *et al.* (2007), Ranjan *et al.* (2011) and Awandhkar *et al.* (2009) who studied the prevalence of various bacterial etiology and reported the prevalence of pseudomonas to be ranging from one to four percent.

It was observed that animals infected with pseudomonas were having normal feed intake and pale, roseate mucous membrane, Pyrexia was not noticed in any case. Milk was watery to creamy in consistency and there were flakes present in two cases. Colour of milk was cream coloured in all cases. There was mild swelling of udder in all cases presented.

Pseudomonas mastitis was reported to be of acute and usually lethal form in any organized dairy farms in developed countries (Zadoks *et al.,* 2011). This type of disease manifestation was not observed in any of the cases where etiology was diagnosed as Pseudomonas. This may be because; the manifestation of pseudomonas mastitis is depended on the amount of organisms invading the udder. The quantity of organisms entering during outbreaks in advanced farms could be more because of accidental heavy contaminations during machine milking, dry cow therapy etc. (Dally *et al.,* 1999). In the present study, none of the farms where Pseudomonas could be isolated, practised any intensive farming technologies and hence quantity of organisms invading the udder was less.

Pseudomonas were identified based on colony morphology, Gram staining and biochemical rests which revealed negatively staining coccobacillary to small bacilli, oxidase test (positive) and OF (strictly oxidative) tests. The species level identification were not attempted as number of cases with pseudomonas mastitis was far less compared to other organisms. Pseudomonas were isolated alone, and not

Table 1. Number of sensitive and resistant isolates of Pseudomonas to various antibiotics

Antibiotic	No.of Sensitive isolates (out of 10)	No.of Resistant Isolates (out of 10)
Enrofloxacin	7	3
Gentamicin	9	1
Chloramphenicol	5	5
Tetracycline	5	5
Streptomycin	3	7
Ampicillin – Sulbactam	6	4
Cefriaxone	3	7
Amoxycillin-Clavulanate	2	8
Cefotaxim	7	3
Ampicillin	3	7
Co-trimoxazole	5	5
Ciproflozxacin	4	6
Cefoperaxone	3	7
Amoxycillin-Cloxacillin	4	6

in combination with any other organisms in all the cases. Gentamicin was found to be the most sensitive antibiotic with nine isolates out of them being sensitive. Enrofloxacin and cefotaxim were found to be sensitive for seven isolates. Ampicillin- sulbactam were sensitive for six isolates. Ciporofloxacin and ampicillin-cloxsacillin were sensitive for four isolates. Results are summarised in Table 1.

Gentamicin was used in four cases and entrofloxacin was used in six cases for treatment. Swelling of udder subsided and the colour of milk returned to normal by 24h of treatment. Milk yield improved by the third day after treatment.

In the cases discussed presently, although animals were able to recover clinically because of the low grade infections, *in-vitro* antibiogram showed that isolates obtained were resistant to the commonly used antibiotics such as tetracycline, amoxycillin-cloxacillin and streptomycin. Treatment of pseudomonas mastitis is usually considered unrewarding because of the persistence of the organisms in the udder and poor response to antibiotics (Krik and Bartlett, 1984). Recurrences of mastitis due to pseudomonas in these cases are unknown as they were not presented again during the study period. Detailed investigation on the occurrence of pseudomonas, based on better selective methods like enrichment and molecular typing of the isolates will prove to be an important tool in better understanding and control of bovine mastitis in general and pseudomonas mastitis in particular.

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