ANTIBIOGRAM PATTERN OF BACTERIAL ISOLATES FROM SUBCLINICAL MASTITIS IN COWS

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ABSTRACT

From 69 lactating cows of University livestock farm , a total of 266 quarters were screened for subclinical mastitis (SCM), 89 quarters were found to be positive which resulted in the recovery of 190 bacterial isolates. Of these, 50 (56.17%) quarters yielded monomicrobic isolates whereas 39 (43.82%) quarters harboured mixed infection. On bacteriological culture examination, a total of 190 bacterial isolates were recovered which comprised of 54 isolates of CPS, 115 isolates of CNS 2 isolates of *Micrococcous varians,* 11 isolates of *Str. agalactiae* and 8 isolates of coliforms.

In the present study a total of 67 isolates comprising of 57 gram positive and 10 gram-negative isolates were studied for their antibiogram pattern against 20 antibacterial drugs . Results of *in-vitro* drug susceptibility testing on gram-positive and gram-negative bacterial isolates showed that most of the broad-spectrum antibacterial drugs were found highly effective against isolates recovered from cases of subclinical mastitis. The sensitivity profile of isolates showed poor sensitivity towards commonly used antibacterial drugs *penicillin, ampicillin* and *amoxyclav*.

KEY WORDS: Subclinical mastitis, bacterial isolates, antibiogram pattern.

INTRODUCTION

Sub Clinical Mastitis causes huge economic losses to milk production and remains a continuous source of infection to other herd mates. Most episodes of SCM are caused by bacteria.. Indiscriminate use of antibacterial without prior drug susceptibility testing is a major problem in controlling intramammary infection. Such antimicrobial resistant organisms can pose serious health menace to animal as well as human beings. Therefore, determination of drugs' efficacy by *in-vitro* drug sensitivity testing has been suggested as vital factor for the strategic use of antibacterials against mastitis pathogens to control intramammary infection. Antibiotic sensitivity testing (ABST) aims to determine the susceptibility of an isolate to a range of potential therapeutic agents.

MATERIALS AND METHODS

The study was conducted on a total of 69 lactating cows of Live Stock Research Station (LRS) comprising of 35 Triple cross (Kankrej x Jersey x HF), 26 Kankrej and 8 Gir, from July-December, 2011 at the College of Veterinary Science and Animal Husbandry, Anand. All the cows had apparently healthy quarters during the sampling period. The foremilk samples were collected, after discarding first 2-3 streams of milk from each quarter of individual cow in sterile screw-capped vials.

Bacteriological Culture Examination

Loopfull of milk from foremilk sample from each quarter was streaked on blood agar plate (containing 5% sheep blood) and simultaneously on MacConkey agar plate for primary bacterial isolation and the plates were incubated at 37°C for 24 hours. Following incubation, the plates were examined for bacterial growth and the morphological characteristics of bacterial colonies were recorded. Characterization of bacterial isolates was performed as per the method described by Cowan and Steel (1970). The antibiotic sensitivity Icosa G-I Plus disc (Icosa 002 and 003 plus, HiMedia Ltd.

Mumbai) were placed with the help of flamed forceps on the plates. The plates were incubated overnight at 37ºC. Diameters of the clear zone of inhibition were measured and the interpretation of the results was made in accordance with the instructions supplied by the manufacturer.

RESULTS AND DISCUSSIONS

In-*vitro* antibiotic sensitivity testing of bacterial isolates recovered from cases of SCM.

In the present study a total of 67 isolates, comprising of 57 gram positive and 10 gram-negative isolates, were studied for their antibiogram pattern. Gram positive organisms were tested against 20 antibacterial drugs viz. *cephalothin, clindamycin, co-trimoxazole, erythromycin, gentamicin, ofloxacin, chloramphenicol, oxacillin, linezolid, azithromycin, amikacin, clarithromycin, teicoplanin, methicillin, novobiocin, vancomycin, tetracycline, penicillin, ampicillin and amoxyclav* for their antibiogram profile. Gram negative organisms were also tested against 20 antibacterial drugs viz. *imipenem, ciprofloxacin, tobramycin, moxifloxacin, ofloxacin, sparfloxacin, levofloxacin, norfloxacin, co-trimoxazole, colistin, nalidixic acid, augmentin, kanamycin, gatifloxacin, gentamicin, amikacin, streptomycin, ceftriaxone, cefpodoxime* and *ticarcillin* for their antibiogram profile.

1. Gram-positive organisms

Gram-positive organisms recovered from subclinical infections of udder exhibited sensitivity ranging between 92-99 per cent to 12 antibacterial drugs *(cephalothin, clindamycin, co-trimoxazole, gentamicin, chloramphenicol, oxacillin, linezolid, azithromycin, amikacin, teicoplanin, methicillin, and tetracycline).* Further, the isolates showed 80-88 per cent sensitivity to *erythromycin, vancomycin, clarithromycin, novobiocin* and *ofloxacin.* Low sensitivity was reported to *penicillin* and *ampicillin* (58%), and *amoxyclav* (53%).

Coagulase-positive staphylococci (CPS)

Antibiogram pattern of 23 CPS isolates (8-*Staph. aureus*, 12-*Staph. hyicus and* 3-*Staph. intermedius*) was studied. CPS isolates recovered from subclinical infections of udder showed 80-100 per cent sensitivity to 14 antibiotics *(cephalothin, oxacillin, clindamycin, gentamicin, co-trimoxazole, methicillin, amikacin, azithromycin, tetracycline, erythromycin, chloramphenicol, clarithromycin, teicoplanin* and *linezolid)*. The sensitivity shown to *ofloxacin, novobiocin, vancomycin* and *penicillin* was between 60-80 per cent whereas low (30-50%) sensitivity was exhibited to ampicillin and amoxyclav. Verma and Nauriyal (2007), have earlier reported high sensitivity of CPS organisms to *gentamicin,* t*etracycline, cephalothin, co-trimoxazole.* Munda *et al.* (2009) observed appreciable sensitivity of CPS to *chloramphenicol.* The CPS have earlier been reported to respond poorly to *penicillin* (Verma and Nauriyal, 2007) and ampicillin (Munda *et al.*, 2009).

Coagulase-negative staphylococci (CNS)

Twenty four CNS isolates (15-*Staph. chromogenes*, 9-*Staph. epidermidis*) were used to assess their antibiogram pattern. The CNS isolates elicited 80-100 per cent sensitivity to 17 antibacterials *(cephalothin, clindamycin, co-trimoxazole, erythromycin, gentamicin, ofloxacin, chloramphenicol, oxacillin, linezolid, vancomycin, azithromycin, amikacin, novobiocin, tetracycline, clarithromycin, teicoplanin* and *methicillin).* The susceptibility of isolates declined in case of *ampicillin* (54%), *amoxyclav* (47%), and *penicillin* (30%). Many earlier reports have indicated high susceptibility shown by CNS to *tetracycline,* (Edward *et al.*, 2002; Enyew, 2004), *erythromycin, methicillin,* (Kaliwal *et al.*, 2011), *erythromycin* (Luthje and Schwarz 2006), *oxacillin, clindamycin* and *chloramphenicol* (Enyew, 2004) which concur with the observations of present study. Earlier workers have also noted poor response of CNS to *ampicillin* (Luthje and Schwarz 2006) and *penicillin* (Persson *et al.,* 2009) corroborating the findings of present study.

Ten out of 20 antibacterial drugs used in the *in-vitro* drug sensitivity testing has not reported for bovine diseases in India. These antibacterials include *clindamycin, linezolid, ofloxacin, oxacillin, azithromycin, clarithromycin, teicoplanin, methicillin, novobiocin and vancomycin.*

Rampant use of antibacterials drugs like penicillin and ampicillin, particularity in the treatment of the mastitis in the absence of bacterial isolation and subsequently evaluation of their *in-vitro* antibiogram pattern can be attributed to the poor sensitivity as observed in the study under report.

2. Gram-negative organisms

Ten isolates of gram-negative organisms used in the present study for their antibiogram pattern belonged to coliform organisms.

Coliform organisms

All the isolates of coliform organisms exhibited cent per cent sensitivity to 12 antibiotics (*ciprofloxacin, augmentin, kanamycin, gatifloxacin, colistin, moxifloxacin, ofloxacin, sparfloxacin, levofloxacin, norfloxacin, streptomycin and ticarcillin*) besides eliciting 90% susceptibility to imipenem, *gentamicin, co-trimoxazole, tobramycin* and *ceftriaxone,* and 80% sensitivity to *nalidixic* acid and *amikacin,* and 30% sensitivity to *cefpodoxime.*

Out of twenty antibacterial drugs used for *in-vitro* drug sensitivity of gram-negative organisms, only *ciprofloxacin* (precisely enrofloxacin), *co-trimoxazole, colistin, augmentin, gentamicin, streptomycin, amikacin* and *ceftriaxone* are reported to have been used for therapeutic purpose in bovines under Indian conditions.

The perusal of treatment records of cows at LRS farm revealed that among these drugs *enrofloxacin, ceftriaxone* and *streptomycin* (in combination with *penicillin*) were used frequently for therapeutic purpose whereas *gentamicin, co-trimoxazole* and *augmentin* were used infrequently. Colistin has been used as an intramammary preparation in combination with ampicillin for mastitis therapy.

Unlike gram-positive organisms, the coliform did not show poor sensitivity to *augmentin (amoxyclav)*. However, the surprising revealation was resistance shown by 70% isolates to cefpodoxime, which has not been reported to have been used in veterinary practice.

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