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Isolation of *Staphylococcus* from bovine mastitis and their antibiotic sensitivity pattern

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Abstract

A total of 300 milk samples from cows and buffaloes were evaluated for mastitis and incidence of *Staphylococcus*. Overall, 131 milk samples (43.6%) were found positive for mastitis and out of these positive isolates, 47 bacterial isolates (35.87%) were identified as *Staphylococcus.* All the 47 isolates of *Staphylococcus* were tested for *in vitro* sensitivity towards 15 antibacterial drugs. The highest resistance was attributed towards antibiotic Ampicillin followed by Methicillin, Oxacillin, Ceftriaxone, Rifampicin, Vancomycin, Amoxyclave, Oxytetracycline, Erythromycin, Nitrofurantoin, Cefuroxime, Gentamicin, Norfloxacin, Ciprofloxacin and Levofloxacin. The sensitivity of bacterial isolates against various groups of antibiotics revealed Quinolones to be most efficacious.

Key words: AST, antibiotics, mastitis.

Introduction

Mastitis is an inflammation of the mammary gland characterized by physical, chemical, bacteriological and cytological changes in milk. Pathological changes in glandular tissues of the udder and effects on the quality and quantity of milk have been observed (Amir, 2013). This disease is mainly caused by microorganisms usually gram-negative and gram-positive bacteria, mycoplasmas, yeasts and algae (Zadoks *et al.*, 2011, Jyotishree and Sourabh, 2017). Staphylococcal bacterial mastitis is the commonest and economically the greatest concern wherever dairy farming is practiced. Different species of *Staphylococcus* especially *S. aureus* is capable to develop severe drug resistance with multiple mechanisms of resistance.

Antimicrobials are routinely used for treatment of dairy animals affected with mastitis. The uses of antibiotics have, over time, increased the number of antimicrobial-resistant microbes globally. Inappropriate usage of antimicrobials such as wrong dose, drug or duration may increase in antimicrobial resistance without improving the outcome of treatment (Williams, 2000). Hence the present work was undertaken on isolation of Staphylococcus from the boine mastitis and their antibiotic sensitivity pattern.

Materials and Methods

A total of 300 milk samples were collected from cows and buffaloes (150 each) and animals found positive for clinical and subclinical mastitis (SCM) were included in the present study. The cases

of clinical mastitis were diagnosed on the basis of physical examination of udder (swelling, redness, pain on palpation, induration) and milk color- (yellow or blood tinged and consistency- watery, flakes, etc.). The subclinical mastitis was diagnosed by California mastitis test (Schalm *et al*., 1971).

Isolation of Bacteria

Milk samples from clinical cases and those showing ++ (distinct) /+++ (strongly positive) reaction in CMT were cultured bacteriologically to isolate and identify the *Staphylococcus* bacteria (Kalorey *et al*., 2007). Positive samples were inoculated in BHI and incubated aerobically at 37^{O}C for 24 hrs and then streaked on nutrient agar media and incubated aerobically at 37^{O}C for 24 hrs to obtain isolated single bacterial colonies. The gross cultural characteristics of the colonies were observed followed by Gram staining to study the morphology of bacteria.

Identification of *Staphylococcus* Bacteria

The identification was done by biochemical tests using readymade kits (HiStaph identification kits, Hi Media). The selective media like Baired parker agar and Mannitol salt agar were used (Ollis *et al*., 1995, Collee *et al*., 1996).

Antibiotic Sensitivity Test

In vitro antibiotic sensitivity test of the *Staphylococcus* isolates towards 15 antibacterial drugs was conducted as per the method of Bauer *et al*. (1966). Antibiotics used in the present study were: Ampicillin, Amoxyclave, Cefuroxime, Ciprofloxacin, Ceftriaxone, Erythromycin, Gentamicin, Levofloxacin, Methicillin, Nitrofurantoin, Norfloxacin, Oxacillin, Oxyteyracycline, Rifampicin and Vancomycin. A loopful of the growth from slant was inoculated in BHI and incubated at 37 ^{O}C for 18- 24 hrs. and then the surface of preincubated and sterile Muller Hinton agar (Hi Media) was uniformly smeared with the culture swab and the plate was kept at room temperature for 30 min to allow the inoculums to be adsorbed on the surface and then the antibiotic discs (Hi Media) were placed on the media surface at equal distances and sufficiently separated from each other. The plates were incubated overnight at 37 ^{O}C. Diameters of clear zone of inhibition around antibiotic discs were measured in mm. The interpretation of result was made in accordance with the instruction supplied by manufacturer.

Results and Discussion

Staphylococcal mastitis is the most common and costly mammary disease of dairy cattle worldwide. A total of 300 milk samples from cows and buffaloes were evaluated for mastitis and incidence of *Staphylococcus*. Overall, 131 milk samples (43.6%) were positive for mastitis and out of these positive isolates, 47 bacterial isolates (35.87%) were identified as *Staphylococcus.* This is in close agreement with the 38.81% *Staphylococcus* spp. reported by Sharma and Sindhu (2007).

Antimicrobials are routinely used for treatment of dairy cattle affected with clinical and subclinical infections. Over the years, antimicrobial susceptibility testing has become under scrutiny because of concerns about antimicrobial resistance, changes in methodology and the relationship between *in vitro* results and on-farm clinical outcomes (Idriss *et al*., 2014).

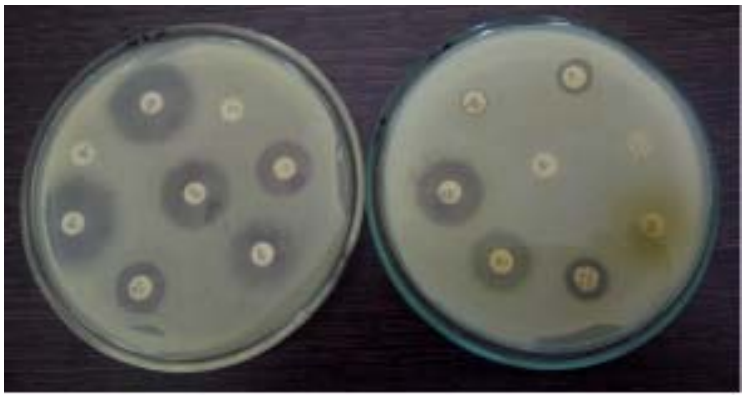

Plate 1: Antibiotic sensitivity of *Staphylococcus* isolate.

Recently, there has been increased concern about antibiotic resistant strains of genus *Staphylococcus.* In

the present study 47 isolates of *Staphylococcus* were tested for *in vitro* sensitivity towards 15 antibacterial drugs (Plate 1). Sensitivity for individual isolates to various drugs was interpreted according to the manufacturer's (Hi Media) instructions. The highest resistance was attributed towards antibiotic Ampicillin followed by Methicillin, Oxacillin, Ceftriaxone, Rifampicin, Vancomycin, Amoxyclave, Oxytetracycline, Erythromycin, Nitrofurantoin, Cefuroxime, Gentamicin, Norfloxacin, Ciprofloxacin and Levofloxacin (Plate 1). The highest resistance to antibiotics of Penicillin group was also recorded by Abera *et al*., (2010) and Wang *et al*., (2016). These antibiotics belonged to the groups viz. Fluoroquinolones, Macrolides, Aminoglycosides, Tetracycline, Cephalosporins, Penicillin, Miscellaneous Glycopeptide and Miscellaneous synthetic antibiotics were used. Similar groups were used by Seixas *et al*. (2014) for AST.

The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semisynthetic Penicillins (Methicillin, Oxacillin, and Nafcillin), Macrolides, Tetracyclines, and Aminoglycosides has made the therapy of staphylococcal disease a global challenge. The Tetracycline resistance detected can be attributed to the large use of this antibiotic in mastitis treatment and in the water of the herd as a prophylactic measure aimed at reducing infections.

In this study, the *Staphylococcus* isolates, simultaneously resistant to 2 to 11 antibacterial agents, was observed in 45 (95.74%) out of total 47 isolates. The maximum no. of isolates was resistant to 3 drugs. None of the isolate was resistant to all the 15 antimicrobial agents. Multidrug resistance was also reported by Hana Muftah,(2011) and Mehmeti *et al*. (2016) . A high percent of multiple drug resistance was observed in the isolates. Hence, it is recommended that antibiotics should be used in standard dosage and for appropriate time prescribed by the clinicians, preferably after performing the *in vitro* sensitivity testing. Injudicious and rampant use of antibiotics is not advisable and should be discouraged.

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Conflict of Interest: All authors declare no conflict of interest.

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