ANTIBIOTIC RESISTANCE PATTERN OF *E. COLI* AND *STAPHYLOCOCCUS AUREUS* ASSOCIATED WITH CLINICAL AND SUBCLINICAL MASTITIS IN RAJASTHAN

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ABSTRACT

Majority of mastitis cases are a result of bacterial infection by small group of microorganisms. Mastitis reduces milk yield, degrades milk composition and causes heavy economic losses to farmers. Keeping these points in mind the common microbial pathogens were isolated from the milk of cows (n=45) from different farms in Jaipur showing subclinical and clinical signs of mastitis and were subjected to drug sensitivity test with routinely used 15 antibiotics. The pathogens detected were *E. coli* (42) and *Staphylococcus aureus* (28). The *E. coli* were found most resistant to majority of antibiotics like Ampicillin, Streptomycin, Tetracycline, Gentamicin, Vancomycin, Penicillin, Cefipime, Amoxicillin- clavulanate while *S. aureus* isolates were found resistant to Vancomycin, Penicillin, Imipenem, Tetracycline, Aztreonam, Azithromycin, Streptomycin and Ampicillin. Most *E. coli* isolates were sensitive towards Fosfomycin and Enrofloxacin while *S. aureus* isolates were sensitive towards Fosfomycin and intermediate sensitive towards Trimethoprim. The rampant use of antibiotics in dairy sector for improving milk-meat yield and to combat diseases is the main cause of an increased resistance amongst pathogens. Therefore, the strategies for prudent use of antibiotics in dairy industry should be implemented and the line of treatment for mastitis must be suggested only after antibiotic sensitivity test.

Key words:Antibiotics, cows, mastitis, microorganisms, resistance

Introduction

Mastitis is one of the most common diseases of dairy cattle throughout the world leading to great financial losses (Almeida *et al*., 2011; Sakemi *et al*., 2011). Economic losses are due to loss in milk production, degrading of milk quality and price, increased risk of subsequent mastitis, herd replacement, costs of drugs, veterinary services and labour (LeBlanc *et al*., 2006; Getahun *et al*., 2008; Momtaz *et al*., 2010). Early diagnosis of causative bacterial agents is always desirable for initiating specific treatment and management of mastitis. The increased administration of antibiotics in feeds to promote health and control diseases could lead to selection and emergence of resistant bacterial strains. The injudicious and imprudent use of antibiotics in livestock has posed an increased risk of emergence of resistant bacterial strains that may enter the food chain. The objectives of this study was to isolate *E. coli* and *Staphylococcus aureus* from milk samples of cows suffering from symptoms of clinical and sub clinical mastitis and evaluate their antibiotic resistance pattern.

Materials and Methods

Collection of samples

The milk samples were collected from different farms in Jaipur from cows suffering from the symptoms of mastitis. A total of 45 milk samples of about 5-10 ml in amount from cattle were collected in sterilized falcon tubes after disinfecting teats with 70% ethanol and discarding initial 3-4 streams of milk. The samples were collected in the morning and were immediately transported to the laboratory on ice for further processing.

Isolation and characterization of bacteria

The samples were enriched in nutrient broth overnight followed by the selective isolation of *Escherichia coli* and *Staphylococcus* spp. The enriched samples were cultured on MacConkey agar (MCA) and Mannitol Salt agar (MSA).

Antibiotic sensitivity test

The confirmed *E. coli* and *Staphylococcus aureus* isolates were screened for representative antibiotics (n=15) from all important classes as recommended by CLSI 2017. The disks were procured from Himedia Pvt. Ltd., Mumbai, according to the recommended guidelines of CLSI. The following antibiotic was used- penicillin, Tetracycline, Amoxicillin-clavulanate, Cefipime, Trimethoprim- Sulfamethoxazole, Ceftazidime, Aztreonam, Imipenem, Vancomycin, Enrofloxacin, Gentamicin, Azithromycin, Fosfomycin, Ampicillin and Streptomycin. The standard agar disc diffusion method described by Bauer- Kirby (Bauer *et al*., 1966) on Mueller Hinton agar was followed to determine the antibiotic resistance pattern.

Results and Discussion

Out of the 45 milk samples that were processed for selective isolation of microorganisms, 42 *E. coli* and 28 *Staphylococcus aureus* were isolated. All the samples (n=45) tested were positive for at least one species.Twenty six samples were found to contain both microorganisms. The overall incidence of *E. coli* and *S. aureus* in clinical and subclinical mastitis are 93.33% and 62.22%, respectively. Red pinpoints, lactose fermenting, well separated colonies were observed on MCA. Similarly, all *S. aureus* isolates were gram positive (clusters of cocci), glistening, smooth and round colonies with variable degree of pigmentation (golden yellow) on MSA. These isolates did not show growth on Mac Conkey agar.Most of the pathogens were resistant to multiple antibiotics (multi drug resistant). Maximum *E. coli* isolates were resistant to ampicillin (95.23%), streptomycin and tetracycline (92.85%), gentamicin (88.09%), Vancomycin

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(85.71%), Penicillin (83.33%), Cefipime (76.19%) and amoxicillin-clavulanate (71.42%). Nearly 90.47% of *E. coli* isolates were found only sensitive to antibiotic Fosfomycin while 47.61% isolates were sensitive towards enrofloxacin (Table 1). Of the 28 *Staphylococcus aureus* isolated, maximum were resistant to vancomycin (92.85%), penicillin and imipenem (89.28%), tetracycline (85.71%), aztreonam and azithromycin (82.14%), streptomycin (78.57%), ampicillin, gentamicin and ceftazidime (71.42%). Maximum *Staphylococcus* isolates (89.28%) were sensitive to fosfomycin (Table 2).

Mastitis is one of the most common and important disease of dairy cattle causing significant economical loss to the dairy industry (Tollersrud *et al*., 2000; Gillespie and Oliver, 2005).At least, 137 infectious causes of bovine mastitis are known and the commonest pathogens are *S. aureus, S. agalactiae,* other *Streptococcus* spp. and Coliforms (Sumathi *et al*., 2008).The most common mastitis pathogens*S. aureus, S. agalactiae, S. dysgalactiae* and *S. uberis* (Phuektes *et al.,* 2001). Besides being a predominant and recurrent pathogen responsible for causing mastitic infection in cattle worldwide, *E. coli* is also reservoir of many antibiotic resistance genes.Many developing countries have

Table 1: Antibiogram of *E. coli* isolates

Antibiotics	Resistant	Intermediate	Sensitive
Penicillin	83.33%	7.14%	9.52%
Tetracycline	92.85%	7.14%	-
Amoxicillin	71.42%	23.80%	4.76%
Cefipime	76.19%	19.04%	4.76%
Trimethoprim-sulfamethoxazole	59.52%	38.09%	2.38%
Ceftazidime	59.52%	28.51%	11.90%
Aztreonam	66.66%	16.66%	16.66%
Imipenem	54.76%	23.80%	21.42%
Vancomycin	85.71%	14.28%	-
Enrofloxacin	28.57%	23.80%	47.61%
Gentamicin	88.09%	11.90%	-
Azithromycin	59.52%	21.42%	19.04%
Fosfomycin	-	9.52%	90.47%
Ampicillin	95.23%	4.76%	-
Streptomycin	92.85%	4.76%	2.38%

Table 2: Antibiogram of *S. aureus* isolates

Antibiotics	Resistant	Intermediate	Sensitive
Penicillin	83.33%	7.14%	9.52%
Tetracycline	92.85%	7.14%	-
Amoxicillin	71.42%	23.80%	4.76%
Cefipime	76.19%	19.04%	4.76%
Trimethoprim-sulfamethoxazole	59.52%	38.09%	2.38%
Ceftazidime	59.52%	28.51%	11.90%
Aztreonam	66.66%	16.66%	16.66%
Imipenem	54.76%	23.80%	21.42%
Vancomycin	85.71%	14.28%	-
Enrofloxacin	28.57%	23.80%	47.61%
Gentamicin	88.09%	11.90%	-
Azithromycin	59.52%	21.42%	19.04%
Fosfomycin	-	9.52%	90.47%
Ampicillin	95.23%	4.76%	-
Streptomycin	92.85%	4.76%	2.38%

reported the presence of *E. coli* in subclinical cases of mastitis like Uruguay, Turkey, Brazil, Mexico, China and Thailand (Gianneechini *et al*., 2002; Guler and Gunduz, 2007; Olivares-Perez *et al*., 2015; Wang *et al*., 2015; Hinthong *et al*., 2017). In a study in Algeria related to clinical mastitis of dairy cows, *E. coli* was most resistant for amoxicillin (86.5%), followed by tetracycline (75%), which correlates to the findings of our study (Tahar *et al*., 2020). *E. coli* isolated from sub clinical mastitis also showed highest resistance towards Tetracycline (92.2%) and Streptomycin (90.47%) in a study from Brazil (Rangel and Marin, 2009). Similar incidence of *E. coli* resistant to ampicillin isolated from sub clinical mastitis in dairy cows in Thailand has also been reported (Hinthong *et al*., 2017). In a study that conducted antimicrobial susceptibility of *E. coli* strains isolated from clinical bovine mastitis in Finland and Israel, high susceptibility towards various antimicrobials were shown where only 15% and 10% percent of the Israeli isolates were resistant to tetracycline and ampicillin, respectively and 14% and 7% of the Finnish isolates were resistant to tetracycline and ampicillin, respectively (Lehtolainen *et al*., 2003).

Staphylococcus aureus is also the most notable causative agent of mastitis infections in cattle all over the world. Similar to the results obtained in this work, *S. aureus* isolated from mastitis also showed high resistance against penicillin G and tetracyclinein an study conducted in Iran, Algeria (Jamali *et al*., 2014; Saidi *et al*., 2019). Our study is well in agreement with those reported for *E. coli* and *S. aureus* isolated from mastitic animals where bacteria was found most resistant to amoxicillin and gentamicin(Singh *et al*., 2018). However, the use of fosfomycin and enrofloxacin for treatment of mastitis caused by *E. coli* and *Staphylococcus aureus* remains to be a good option for a successful treatment. Similar efficacy of enroflaxacin has been reported by Jeykumar *et al*. (2013). Thus, from the present study it could be concluded that there is mixed infection of microorganisms in mastitis and a frequent use of an *in vitro* antimicrobial sensitivity tests are recommended for treatment of clinical or subclinical mastitis pathogens. Also, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed in developing countries.

Conclusion

Mastitis, a complex and costly to manage disease of dairy herds cause huge economic losses to farmers. The use of antimicrobials is rampant among both farmers and vets not only to combat Gram-positive and Gram-negative bacteria causing mastitis in dairy cows but also to improve animal performance. The indiscriminate use of antibiotics increases the possibility of their residues in milk giving rise to zoonotic threats and increases bacterial resistance to antimicrobials. Therefore, the present study recommends combining the results of microbial cultures and antimicrobial susceptibility testing to the clinicians to decide the line of treatment and to limit the imprudent use of antibiotics to shun the development of resistance among bacteria causing mastitis. The dosage of antibiotics for mastitis treatment can be recommended on the basis of

results of this study.

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