

PREVALENCE AND SENSITIVITY OF *STAPHYLOCOCCUS AUREUS*  
CAUSING MASTITIS IN BUFFALOES AROUND LAHORE, PAKISTAN

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**ABSTRACT**

The present study determined the prevalence and antibiotic sensitivity of *Staphylococcus aureus* causing mastitis in buffaloes around Lahore, Pakistan. Clinical mastitis was diagnosed on the basis of physical examination of the udder, teat and the physical appearance of the milk. Subclinical mastitis was diagnosed on the basis of the California Mastitis Test. Among a total of 598 buffaloes, 332 were found to have mastitis (55.5%). A total of 449 mastitic milk samples from different animal quarters were collected from different livestock farms from peri-urban area of Lahore of which 16% (71/449) and 84% (378/449) were clinical and subclinical, respectively. The animal prevalence of mastitis was 55.5% with 95% Confidence interval of 51.5 to 59.4, while prevalence per quarter was 18.7% with 95% Confidence interval of 17.4 to 20.3. From these 449 milk samples, 257 isolates of Staphylococci were obtained. A Staphytest plus test confirmed 65% (95% CI 58.9 to 70.6) isolates as coagulase positive

*Staphylococcus aureus*. Antibiotic sensitivity of all coagulase positive *Staphylococcus aureus* was performed by disk diffusion method. The zone of inhibition of antibiotics trimethoprim, erythromycin, ciprofloxacin, doxycycline, streptomycin, gentamycin, tylosin, kanamycin, amoxicillin and chloramphenicol were measured as 96.5%, 87%, 84.5%, 80.5%, 73.3%, 73.2%, 71.5%, 69.5%, 58% and 56.5%, respectively.

**Keywords:** *Bubalus bubalis*, buffaloes, mastitis, *Staphylococcus aureus*, california mastitis test, antibiotics

**INTRODUCTION**

Pakistan is rich in livestock based production, which contributes 11.9% of total GDP with 35 million rural population engaged in raising livestock (Anonymous, 2013). The buffalo population in this country is 33.7 million heads (Anonymous, 2013). Buffaloes are recognized

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as the second most important milk producing species around the world (FAO, 2010). Among the various diseases which affect milk production, mastitis is a major one (Shakoor, 2006). Mastitis is basically a tenderness of the mammary gland and is characterized by physical, chemical and pathological changes in the milk and glandular tissue. In the case of subclinical mastitis, milk is apparently normal with an increase in the somatic cell count (Radostits *et al.*, 2000). In Pakistan, mastitis eradication may not be possible, but hygienic management practices can reduce the incidence (Rahman *et al.*, 2009). It has been observed by Mustafa *et al.* (2011) that the prevalence of mastitis, both clinical and subclinical in buffalo was 40.4%, 59.6%, respectively.

A wide variety of organisms are associated with mastitis, however the most frequent pathogens are bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus dysagalactiae*, *Streptococcus uberis*, *Streptococcus agalactiae* (Radostits *et al.*, 2000). However, more than 50% of cases of mastitis are caused by *Staphylococcus aureus* (Shakoor, 2006). *Staphylococcus aureus* creates pockets in the inner lining of mammary gland, which is bounded by fibrous tissue and where antibiotics cannot respond. Dry period antibiotic therapy and teat dipping are not in practice in Pakistan, which might result in losses due to mastitis to be higher (Arshad, 1999).

Changes in the nature of proteins expressed by the organism, mutation or horizontal changes in its genome may lead to bacteria becoming resistant. It is common that with the passage of time bacteria may develop resistance to currently available antibacterial drugs by either new mutations or the exchange of genetic information, that is, putting old resistance genes into new hosts (Tenover, 2006). For example, methicillin resistance of

*Staphylococcus aureus* is primarily due to changes in the penicillin binding protein that consequently inhibit cell wall synthesis. Expression of the *mecA* gene results in an alternative penicillin binding protein that has a low affinity for most  $\beta$ -lactam antibiotics, thereby allowing these strains to replicate in the presence of methicillin and related antibiotics. The objectives of present study were to determine the prevalence of mastitis in buffalo and the evaluation of commonly used antibiotics in the treatment of mastitic buffaloes.

## MATERIALS AND METHODS

### Sampling procedure and sampling area

A cross sectional study was carried out between January and June for investigation the prevalence of mastitis in dairy buffaloes. The present study was conducted in the peri-urban area of district Lahore, Punjab. All lactating buffaloes were selected as study unit and the study population was examined by cluster sampling technique.

### Selection criteria and diagnosis of mastitis

Only lactating buffaloes were incorporated in the present study and those which were pregnant, in the dry period and heifers were excluded. Animals suffering with clinical mastitis were diagnosed upon physical examination by the presence of clinical signs such as swelling of the udder and teat, udder oedema, udder and teat injury, abnormal milk secretion etc. Animals with normal udder quarters were screened by California Mastitis Test (CMT) (Varatanovic *et al.*, 2010). A total of twenty three hundred and ninety two (2392) quarter milk samples from 598 buffaloes was examined by CMT. Animal and quarter wise prevalence of mastitis was recorded in a

questionnaire.

### **Collection, transportation and culturing of samples**

The recommendation of National Mastitis Council (1990) was considered for the collection transportation, culturing and isolation of *Staphylococcus aureus*. All milk samples were collected in sterilized screw capped vials and samples transported by placing them on the crushed ice to the Microbiology Laboratory in the Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore. Conventional methods were used for the isolation and identification of *Staphylococcus aureus*. Staph. 110 medium and 5% sheep blood agar was used for growth and scrutiny of the hemolytic properties of Staphylococcal species. Biochemical tests performed were gram's staining, catalase test, coagulase test and Staphytest plus test (Oxoid UK). After the biochemical test pure culture of *Staphylococcus aureus* was preserved in 50% glycerol for further study.

### **Antibiotic sensitivity of *Staphylococcus aureus***

According to the guideline of the Clinical Laboratory Standard Institute (CLSI, 2005) the disk diffusion method was performed for antibiotic sensitivity. *Staphylococcus aureus* ATCC 25923 was used as the quality control organism and trimethoprim, erythromycin, ciprofloxacin, doxycycline, amoxicillin, streptomycin, tetracycline, tylosin, kanamycin, chloramphenicol were used in antibiotics sensitivity testing, taking into account the previous historical records over one decade that these antibiotics were commonly used in the treatment of buffaloes mastitis (Hussain *et al.*, 2013; Hameed *et al.*, 2008).

## **RESULTS AND DISCUSSION**

A total of 449 milk samples from 598 lactating buffaloes were collected. The animal wise prevalence of mastitis was 55.5% with 95% confidence interval 51.5 to 59.4. While the prevalence of clinical and subclinical mastitis was 11.8% (95% CI of 9.4 to 14.6) and 43.6% (95% CI of 39.7 to 47.6), respectively (Table 1). On quarter level the prevalence of mastitis per quarter was 18.7% with 95% confidence interval of 17.4 to 20.3. Among these total mastitic quarters (449), the prevalence of clinical mastitis was 16% (71/449) while prevalence of subclinical 84% (378/449) were recorded. The quarter wise prevalence of mastitis was recorded as 11%, 30%, 10.5% and 22.57 in left front, left rare, right front and right rare, respectively (Table 2). Among these total milk samples, 257 isolates of Staphylococci were obtained. All these 257 isolates of *Staphylococci* were gram positive and catalase positive and shows hemolytic property on 5% sheep blood agar. Among these 167 were coagulase positive and 90 were negative and the Staphytest plus test confirmed 65% (95% CI of 58.9 to 70.6) isolates as coagulase positive *Staphylococcus aureus*. All these one 167 isolates of *Staphylococcus aureus* were subjected to antibiotic sensitivity testing by disk diffusion method. The zone of inhibition of antibiotics were recorded and shown in Figure 1.

The results of the present study are similar to those of Sharif and Ahmed (2007); Akhtar *et al.* (2012) who reported that the animal prevalence of mastitis was 51% and 45.7%, respectively. On the other hand, the present results are lower than those reported by Buchaya *et al.* (2005) who recorded an overall animal prevalence of 78%. The lower prevalence of mastitis in our study may be due to improved farming practices and awareness

of dairy farmers. The present study subclinical mastitis prevalence (43.6%) was close to findings of Ali *et al.* (2011) who reported the prevalence of subclinical mastitis as 44%. In the present study the frequency of *Staphylococcus aureus* mastitis was 65%, higher than the 45% reported by Khan *et al.* (2005).

In present study the prevalence of mastitis at the quarter's level LF, LR, RF and RR was 11%, 30%, 10.5% and 22.7%, respectively. The mastitis was highest in hind quarters as compared to forequarters. The association of quarter wise prevalence on mastitis was highly significant ( $P < .000$ ) in hind quarters. Present study was in line with previous studies Bilal and Muhammad (2004); Nigo *et al.* (2013); Gebrekrustos *et al.* (2012). Similarly, Chishty *et al.* (2007) who reported that location of mastitis in the hindquarters was more frequent than that of front quarters. In our view, hind quarters are more exposed to dirt when the animal lies down on the floor as well as they are more contaminated with fecal materials. The difference in quarter wise prevalence of mastitis may also be due to the fact that predisposing factors like injury, defective sphincters, and so forth could vary from quarter to quarter.

In the present study trimethoprim proved to be the most suitable drug for treatment of *Staphylococcus aureus* mastitis in accordance with many studies (Fazal-ur-Rehman, 1995; Khan *et al.*, 2004; Hussain *et al.*, 2013). In our study *Staphylococcus aureus* isolates were 87% sensitive to erythromycin in line with many previous studies (Khan *et al.*, 2004; Alekish *et al.*, 2013; Brinda *et al.*, 2010) who reported erythromycin sensitivity ranging from 78 to 95%. Ciprofloxacin was against *Staphylococcus aureus* was 84.4% which is in line with the studies (Khan *et al.*, 2005; Hussain *et al.*, 2013) who reported ciprofloxacin as drug of choice

and its sensitivity was from 80 to 100%. In present study doxycycline sensitivity was 80.5% which appose to previous study Alekish *et al.* (2013) who reported <50% sensitivity it may be due to use of doxycycline against *Staphylococcus aureus* from locality to locality. Amoxicillin sensitivity in present study was 58%, similarly in previous studies (Farooq *et al.*, 2008; Khan *et al.*, 2004) who reported amoxicillin as drug of choice in mastitis. In present study streptomycin sensitivity against *Staphylococcus aureus* mastitis was 73.2% same as in previous studies (Idiress *et al.*, 2014; Kurjogi *et al.*, 2011; Farooq *et al.*, 2008) reported their sensitivity from 65 to 80%. *Staphylococcus aureus* sensitivity against antibiotic may be attributed to the extent from locality to locality. Gentamycin has been shown as drug of choice against *Staphylococcus aureus* similarly, many studies (Akram *et al.*, 2013; Anjum *et al.*, 2010) who reported gentamycin sensitivity 80%. Tylosin was 71.6% sensitive in present study which was not in line with the study of Brinda *et al.*, (2010) who reported its 28% sensitivity. This might be due to frequent use to tylosin in their localities.

In our study *Staphylococcus aureus* was 69.5% sensitive to kanamycin similarly, in previous studies (Idiress *et al.*, 2014; Farooq *et al.*, 2008; Anjum *et al.*, 2010) who described kanamycin as drug of choice in mastitis. Some others studies (Mustafa *et al.*, 2014; Anjum *et al.*, 2010) who reported their sensitivity less than 40%. *Staphylococcus aureus* in buffalo mastitis was 56.5% sensitive to chloramphenicol which is close to previous finding Wang *et al.* (2015); Li *et al.* (2009); Khan *et al.* (2004) who reported 52%, 70% and 91% respectively.

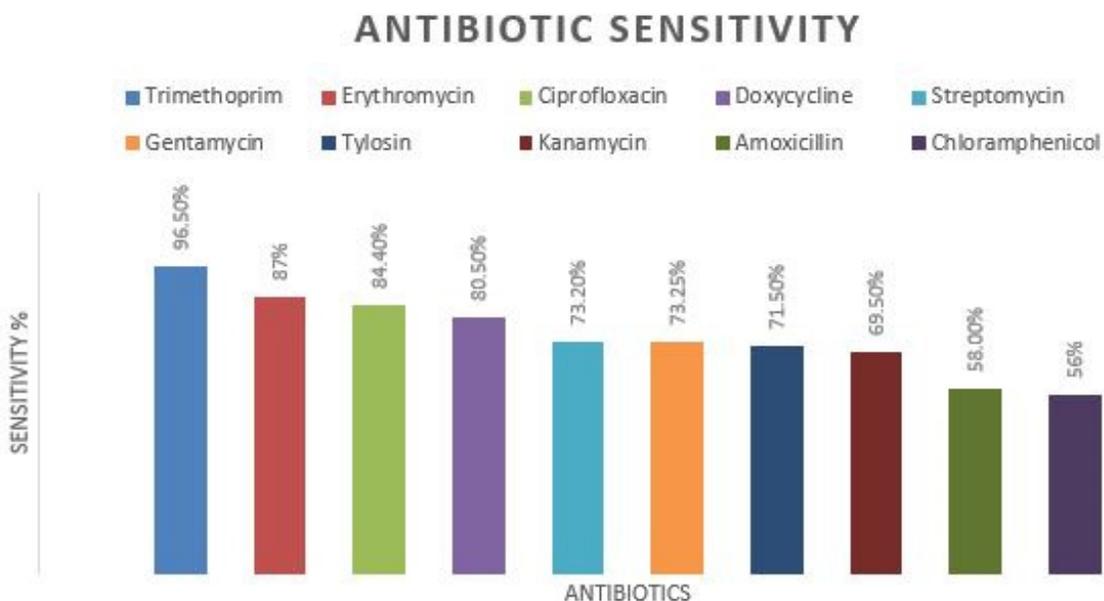


Figure 1. *Staphylococcus aureus* sensitivity against antibiotics.

Table 1. Prevalence of overall, clinical and subclinical mastitis in Lahore.

Mastitis	Buffalo examined	Total positive buffalo	% Prevalence	95% CI
Overall mastitis	598	332	55.5	51.5-59.4
Subclinical mastitis	598	261	43.6	39.7-47.6
Clinical mastitis	598	71	11.8	9.4-14.6

Table 2. Quarter wise prevalence of mastitis in Lahore.

Quarters	Quarters examined	Total positive quarters	Prevalence %	95% CI
LF	598	66	11	8.7-13.7
LR	598	184	30	27.1-34.5
RF	598	63	10.5	8.2-13.9
RR	598	136	22.7	19.5-26.2
Total	2392	449	18.7	17.4-20.3

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