



## *In vitro* Antimicrobial Susceptibility Profile of Sub-Clinical Mastitis Isolates from Dairy Goats

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**Abstract** | Sub-clinical mastitis is a major problem that causes huge economic loss due to occurrence of antimicrobial resistant organisms. The present study was designed to observe the antimicrobial susceptibility pattern of sub-clinical mastitis bacterial isolates in dairy goats. Bacterial species (n=9) caused sub-clinical mastitis in dairy goats isolated from milk samples were subjected towards the disc diffusion method for antimicrobial sensitivity against twenty six different antimicrobial agents. The results of antimicrobial susceptibility testing revealed that *Staphylococcus aureus* and *Micrococcus luteus* were recorded highly sensitive to oxytetracycline with 100% susceptibility, whereas *Bacillus cereus* was observed highly susceptible (100%) to amoxicillin/clavulanic acid, cephalexin and norfloxacin. *Citrobacter species* were observed 100% susceptible to ceftriaxone. Likewise, 100% susceptibility of *Escherichia coli* was detected to ceftriaxone and chloramphenicol; *Micrococcus luteus* to oxytetracycline; *Proteus vulgaris* to amikacin, erythromycin and sulphamethoxazole/trimethoprim; *Bacillus subtilis* to norfloxacin; *Staphylococcus epidermidis* to doxycycline; and *Streptococcus agalactiae* to doxycycline and amoxicillin/clavulanic acid. These results suggested that bacterial species caused caprine sub-clinical mastitis could be treated through antibiotics. Moreover, enrofloxacin and doxycycline were observed the most effective antimicrobial agents against the sub-clinical mastitis pathogens.

**Keywords** | sub-clinical mastitis, bacterial isolates, antimicrobials, susceptibility, goats

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## INTRODUCTION

Mastitis is the inflammation of mammary gland of the dairy animals causes huge economic losses till date. Mammary gland affected by bacterial infection have great influence on dairy goats' milk production in terms of decreased production and increased somatic cell count (Boscos et al., 1996). Mastitis is caused by a wide range of microorganism and is characterized by poor quality milk and pathological changes in mammary glands (Radostits et al., 2008). For treatment and prevention of mastitis, indiscriminate use of antimicrobial agents in dairy animals

is a serious problem that leads to appearance of resistant bacterial agents of zoonotic importance (Pidcock, 1996). It has been suggested that antimicrobial susceptibility testing should be carried out prior to the use of antimicrobials for mastitis treatment.

Importance of goats in Pakistan is considerable for providing milk and meat to peoples of Pakistan (Haenlein, 2004). These usually are called as "Poor Man's Cow" in Pakistan, as goat milk is the main dietary as well as source of income in rural areas. Goat farming provides the only means of subsistence to poor rural population. Goat milk

is cheap, wholesome, easily digestible and nutritious. Due to colossal position of dairy goat milk, it is important to supply disease free and healthful milk to the users. Globally dairy goats with mastitis, like dairy cattle with mastitis is a disease of significance importance. In Pakistan losses due to mastitis may be higher due to lack of proper reports on mastitis and poor disease prevention and managements. According to Samiullah et al. (2000) in Khyber Pakhtunkhwa (Pakistan), a massive damage to livestock by culling large number of sick animals is due to mastitis. Bad hygienic environments and poor managements, lack of advanced farming practices like dipping of teat prior and post to milking are some main aspects of this disease development in goats. The prevalence of sub-clinical mastitis in dairy goats ranges from 5 - 30% (Contreras et al., 2007). The sub-clinical mastitis is characterized by having no visible signs either in the udder or in the milk.

Keeping in view the above facts, the present study was therefore designed to determine the *in vitro* susceptibility of bacterial isolates of sub-clinical mastitis of dairy goats against the common antimicrobial agents. Furthermore, it would be helpful in formulation of effective treatment plans against the bacteriological sub-clinical mastitis of goats.

## MATERIALS AND METHODS

### BACTERIAL ISOLATES

A total of nine bacterial organisms were isolated from sub-clinical mastitis milk samples (n= 200) of goats that were collected from surroundings of Tandojam. The samples were processed according to standard bacteriological procedures to recover the bacterial pathogens (Pirzada et al., 2016). The bacterial isolates included were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Citrobacter species*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Bacillus subtilis*, *Streptococcus agalactiae* and *Bacillus cereus*.

### IN VITRO SUSCEPTIBILITY OF BACTERIAL ISOLATES AGAINST VARIOUS ANTIMICROBIAL AGENTS

The antimicrobial sensitivity of the bacterial isolates was investigated using disk diffusion method as described by National Committee for Clinical Laboratory Standards (NCCLS, 1993). A suspension of the test organism was prepared in sterile saline equivalent to 0.5 McFarland turbidity standard and incubated at 37°C for 2 hours. With the help of sterile cotton swab the organism was inoculated on Mueller Hinton agar plate and spread evenly over entire surface while using a sterile needle antimicrobial disks were placed over the agar surface at an equal distance from each other. Twenty six different antimicrobial agents were applied and sensitivity was observed. The antimicrobials used were amoxicillin/clavulanic acid (30ug), amikacin

(30ug), bacitracin (10 IU), colistin sulphate (10ug), ceftriaxone (30ug), chloramphenicol (30ug), cephalexin (30ug), ciprofloxacin (5ug), cefpirome (30ug), doxycycline (30ug), erythromycin (15ug), enrofloxacin (5ug), gentamycin (10ug), kanamycin (5ug & 30ug), Metronidazole (10ug), neomycin (10ug), norfloxacin (10ug), nystatin (100 IU), nalidixic acid (30ug), oxytetracycline (30ug), penicillin G (10 IU), streptomycin (10ug), sulphamethoxazole/trimethoprim (25ug), trimethoprim (5ug) and vancomycin (5ug).

## RESULTS AND DISCUSSION

### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Staphylococcus aureus* ISOLATES

The bacterial species *Staphylococcus aureus* was found highly sensitive (100%) to oxytetracycline while the quite effective antimicrobials against the organism were erythromycin, enrofloxacin, gentamycin and kanamycin, and their efficacy was recorded as 63.33, 73.33, 63.33 and 50% respectively. However, the efficacy of other drugs tested against the species is given in Table 1. Gentilini et al. (2000) isolated a total of 206 strains of *Staphylococcus aureus* from bovine clinical and sub-clinical mastitis in Argentina and tested for their *in vitro* susceptibility to several antimicrobial agents. Minimum Inhibitory Concentrations (MIC) that inhibited 90% of the strains tested per millilitres were 1.5, 0.5, 0.75, 1.50, 0.75, 1.0 and 0.125 for penicillin, oxacillin, cephalotin, gentamycin, erythromycin, clindamycin, and ampicillin-sulbactam, respectively. The resistance against the drugs was detected in 83 (40.3%), 24 (11.6%), 16 (7.7%) and 7 (3.4%) *Staphylococcus aureus* isolates for penicillin, erythromycin, pirlimycin and gentamycin. Sol et al. (1997) and Moroni et al. (2006) carried-out a study on the antimicrobial susceptibility against 68 *Staphylococcus aureus* isolates collected from milk of cows affected by sub-clinical mastitis. The antimicrobial agents tested were the  $\beta$ -lactamase penicillin G, amoxicillin, ampicillin, cloxacillin, amoxicillin + clavulanate, cephalonium, and cefoperazone; and other drugs including lincomycin, oxytetracycline, doxycycline, and kanamycin. MIC recorded only certain  $\beta$ -lactamase-resistant penicillin (specifically cloxacillin) or penicillin combinations (amoxicillin + clavulanate) effective against *Staphylococcus aureus*, whereas the other  $\beta$ -lactam derivatives and drugs from other pharmacological groups were found either moderately effective or ineffective. Thus,  $\beta$ -lactamase-resistant penicillin's are considered to be the antimicrobial agents of choice for the treatment of bovine mastitis resulting from infection by *Staphylococcus aureus*.

*Staphylococcus aureus* are known to cause a number of diseases in farm animals including non-ruminants. Our previous study have reported the multidrug resistant *Staphylococcus aureus* as a causative agent of septic arthritis in poultry birds

**Table 1:** The antimicrobial sensitivity (%) of various organisms identified from sub-clinical mastitic milk samples of goats

Antimicrobial Agents	Antimicrobial sensitivity (%) of bacterial species*								
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>Citrobacter species</i>	<i>E. coli</i>	<i>M. luteus</i>	<i>P. vulgaris</i>	<i>B. subtilis</i>	<i>S. agalactiae</i>	<i>B. cereus</i>
Amoxicillin/ clavulanic acid (30ug)	83.33	3.12	80	73.33	51.72	70	57.89	100	100
Amikacin (30ug)	53.33	68.75	80	3.33	37.93	100	71.05	50	70.58
Bacitracin(10 IU)	40	37.5	0	0	34.48	0	0	46.66	0
Colistin sulphate (10 ug)	3.33	0	53.33	33.33	0	0	39.47	43.33	41.17
Ceftriaxone (30 ug)	0	40.62	100	100	0	10	5.26	96.66	32.35
Chloramphenicol(30 ug)	43.33	75	3.33	100	68.96	50	86.84	80	76.47
Cephalexin (30 ug)	0	93.75	50	40	43.48	3.33	5.26	43.33	100
Ciprofloxacin (5 ug)	83.33	0	0	0	0	0	0	0	0
Cefpirome (30 ug)	10	68.75	40	6.66	34.48	83.33	5.26	96.66	97.05
Doxycycline (30 ug)	86.66	100	40	60	6.89	50	97.36	100	88.23
Erythromycin (15 units)	63.33	81.25	0	0	41.37	100	94.73	50	44.11
Enrofloxacin (5 ug)	73.33	75	3.33	40	86.20	90	94.73	86.66	79.41
Gentamycin (10 ug)	63.33	3.12	46.66	40	55.17	53.33	65.78	3.33	2.94
Kanamycin (5µg)	33.33	40.62	0	6.66	62.06	6.66	0	43.33	52.94
Kanamycin (30µg)	50	81.25	53.33	46.66	68.96	50	2.63	80	64.70
Metronidazole (10 ug)	0	0	0	0	51.72	0	78.94	0	0
Neomycin (10 ug)	56.66	3.12	43.33	30	41.37	46.66	0	60	26.47
Norfloxacin(10 ug)	50	3.13	0	3.33	89.65	0	100	80	100
Nystatin ( 100 IU)	0	0	0	0	0	0	0	0	0
Nalidixic acid (30µg)	33.33	56.25	0	3.33	48.27	33.33	60.52	56.66	2.94
Oxytetracyclin (30µg)	100	93.75	70	0	100	3.33	2.63	93.33	14.70
Penicillin G (10 IU)	50	100	0	0	10.34	0	55.26	63.66	0
Streptomycin10 ug)	0	3.31	26.66	0	31.03	6.66	55.26	60	64.70
Sulphamethoxazole/ Trimethoprim (25 ug)	3.33	56.25	83.33	0	62.06	100	92.10	83.33	52.94
Trimethoprim (5 ug)	0	0	3.33	0	27.58	83.33	68.42	30	0
Vancomycin (5 ug)	56.66	3.31	0	0	41.37	0	68.42	60	14.70

\**S. aureus*: *Staphylococcus aureus*; *S. epidermidis*: *Staphylococcus epidermidis*; *E. coli*: *Escherichia coli*; *M. luteus*: *Micrococcus luteus*; *P. vulgaris*: *Proteus vulgaris*; *B. subtilis*: *Bacillus subtilis*; *S. agalactiae*: *Streptococcus agalactiae*; *B. cereus*: *Bacillus cereus*

(Nazia et al., 2015). A recent study has demonstrated the host-related differences in susceptibility levels of *Staphylococcus aureus* isolates from different farm animals, thus indicated a host-microbe interaction and probable role of host in development of antimicrobial resistance in *Staphylococcus aureus* organisms (Habib et al., 2015).

### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Bacillus cereus* ISOLATES

The results regarding the efficacy of antimicrobials against *Bacillus cereus* isolates are summarized in the Table 1. *Bacillus cereus* was observed as highly sensitive to cephaloxin and amoxicillin/clavulanic acid and their efficacy against tested organism was recorded as 100%. Dogruer et al. (2010) car-

ried-out a study to determine the efficacy of three treatment protocols for *Bacillus cereus* during lactation in Damascus goats. For this purpose, intramammary ampicillin dicloxacillin, intramuscular amoxicillin + clavulanic acid and the combination of intramammary ampicillin + dicloxacillin and intramuscular amoxicillin+ clavulanic acid were used in goats with subclinical mastitis during lactation. The microbiological treatment rates in the intramammary ampicillin + dicloxacillin, intramuscular amoxicillin + clavulanic acid and the combination of intramammary ampicillin + dicloxacillin and intramuscular amoxicillin+ clavulanic acid groups in the 7th and 21st days after the treatment were 62.5% and 92.5%, 62.5% and 70%, 87.5% and 87.5%, respectively. The intramammary and intramuscular com-

bination group was found statistically higher effective rate ( $P < 0.05$ ) than other two groups on day 7th. On day 21st intramammary and combination groups were found to have statistically better treatment rates ( $P < 0.05$ ) than that of intramuscular group. The study concluded that the goat subclinical mastitis could be treated, successfully during lactation. While the intramammary ampicillin + dicloxacillin treatment had the best treatment rates, meanwhile the combination of intramuscular amoxicillin + clavulanic acid was also become successful. Whereas intramuscular amoxicillin + clavulanic acid was observed least effective as intramammary therapy.

#### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Citrobacter* SPECIES

The *Citrobacter* species showed 100% susceptibility to ceftriaxone while the second most highly effective drugs noted sulphamethoxazole/Trimethoprim and their effects against *Citrobacter* species are measured as 80% (Table 1). From the present study, one could conclude that *Citrobacter* species had exhibited its resistance to majority of the drugs but was highly susceptible to ceftriaxone while quite susceptible to amikacin. Papadopoulos et al. (1997) examined antimicrobial sensitivity against *Citrobacter freundii*. On testing the sensitivity, the organism was found highly sensitive to ceftriaxone, ampicillin, amoxicillin plus, clavulanic acid, cephalosporin and co-trimoxazole. Whereas, Baloch et al. (2011) reported that *Citrobacter* species showed 100% susceptibility to amikacin while the second most highly effective drugs noted were ofloxacin, ampicillin and kanamycin (10µg) and their effects against *Citrobacter* species were measured as 94.73, 84.21 and 52.63% respectively. *Citrobacter* species had exhibited its resistance to majority of the drugs but was highly susceptible to amikacin, while quite susceptible to ofloxacin, ampicillin and cefixime.

#### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Escherichia coli* ISOLATES

The antimicrobial susceptibility against *Escherichia coli* recovered from the cases of sub-clinical mastitis in goats was carried-out and results are summarized in Table 1. *Escherichia coli* was observed highly sensitive to ceftriaxone and chloramphenicol and showed 100% sensitivity while second most highly effective drug demonstrated during investigation was amoxicillin/clavulanic acid (73.33% sensitivity). Whereas the organism was also found quite sensitive to doxycycline and its sensitivity was measured 60%.

Khan and Rind (2001) found *Escherichia coli* resistant to tetracycline, ampicillin and sulphamethoxazole-trimethoprim and it was recorded susceptible to gentamycin, kanamycin, neomycin and chloramphenicol drugs. A similar report was given by Khan et al. (2016), who reported that *Escherichia coli* recovered from table eggs were complete

resistant to oxytetracycline, while fully susceptible to second-generation fluoroquinolones including enrofloxacin and ciprofloxacin. These findings are in consistent to our present results for *E.coli* isolates of sub-clinical mastitis.

#### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Micrococcus luteus* ISOLATES

During present survey, oxytetracycline was found 100% effective drug while Norfloxacin were measured the second most highly active (89.65%) drug against *Micrococcus luteus* species. Baloch et al. (2011) reported that rifampicin was found 100% effective drug while ampicillin, erythromycin (10 units), furazolidone, gentamycin, penicillin G (5units) and enrofloxacin were measured the second most highly active drugs against *Micrococcus luteus* and their action recorded were 88.23, 88.23, 88.23, 82.35, 82.35 and 76.47% respectively. The drugs found highly active against the species during present survey are in line to the susceptibility of the organism to antimicrobials recorded by the above author.

#### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Proteus vulgaris* SPECIES

*Proteus vulgaris* was found highly sensitive (100%) to amikacin and sulphamethoxazole/trimethoprim, colistin sulphate and erythromycin drugs, and the second most highly active drug (90%) was observed as enrofloxacin. The efficacy of the other drugs tested against the *Proteus vulgaris* is also given in the same table (Table 1). Baloch et al. (2011) reported that *Proteus vulgaris* was found highly sensitive to enrofloxacin, this drug showed 100% efficacy against *Proteus vulgaris* and the second most highly active drugs determined were chloramphenicol, amikacin and enrofloxacin and showed 93.33, 80.0 and 86.66% action against the species respectively. The species *Proteus vulgaris* was found quite sensitive to gentamycin, doxycycline, kanamycin, norfloxacin, flumequin and neomycin and their efficacy was recorded as 66.66, 73.33, 66.66, 53.33, 66.66 and 66.66% respectively. The results regarding the susceptibility of *Proteus vulgaris* to different antimicrobials recorded in this study were in line to the susceptibility recorded by the above author.

#### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Staphylococcus epidermidis* ISOLATES

The results about drug sensitivity of *Staphylococcus epidermidis* species isolated from sub-clinical mastitis of goats are presented in Table 1. During present survey, only doxycycline and Penicillin G were measured as highly effective drug against the species. Further that, the other antimicrobials found quite effective against *Staphylococcus epidermidis* were enrofloxacin, cefpirome and amikacin and their efficacy was recorded as 75, 68.75 and 68.75% respectively. However, the results regarding the other drugs whether

were effective or not at all noted in the present survey are presented in Table 1.

Virdis et al. (2010) conducted a study about antimicrobial resistance patterns for methicillin resistance *Staphylococcus aureus* and Coagulase Negative *Staphylococci* (CNS) strains isolated from udder milk samples collected from goats with sub-clinical mastitis. Only 14 (56.0%) *Staphylococcus aureus* and 31 (41.3%) CNS isolates were found resistant to one or more antimicrobial agents. *Staphylococcus aureus* showed the highest resistance rate against doxycycline (28.0%), oxytetracycline (16.0%), and ampicillin (12.0%). The CNS isolates tested were found more frequently resistant to ampicillin (36.0%) and kanamycin (6.7%). The results about antimicrobial action against the species recorded in this survey were not in agreement to the findings regarding the action of the antimicrobials against the species observed by the above workers. We recorded some higher action of the antimicrobial against the species while they recorded higher resistance against the antimicrobials.

#### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Streptococcus agalactiae* SPECIES

On conducting the antimicrobial sensitivity test against the bacterial species during present investigation recovered from sub-clinical mastitis of goats, *Streptococcus agalactiae* was found highly susceptible to doxycycline and amoxicillin/clavulanic acid (100%) while quite sensitive to erythromycin (15 units), neomycin (10 ug) and amikacin (50%). The results regarding the efficacy of other antibiotics are given in Table 1. Hameed et al. (2008) carried-out an investigation on lactating cattle and buffaloes in Tehsil Burewala, district Vehari, Pakistan. The bacterial isolates were found sensitive in descending order to enrofloxacin, chloramphenicol, gentamycin, oxytetracycline, doxycycline and amoxicillin.

#### ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Bacillus subtilis* SPECIES

*Bacillus subtilis* isolates of sub-clinical mastitis showed 100% sensitivity to neomycin (Table 1). Weber et al. (2002) conduct a study to demonstrate the antimicrobial susceptibility of *Bacillus subtilis* to 18 antimicrobials. Microdilution MIC susceptibility test showed that all *Bacillus subtilis* strains were susceptible to imipenem, vancomycin, chloramphenicol, gentamicin and ciprofloxacin while non-*Bacillus subtilis* strains were found most susceptible to neomycin, imipenem, vancomycin, LY146032 and ciprofloxacin. Furthermore, a Disk susceptibility test was conducted to record the susceptibility; the bacterial species was found susceptible to penicillin, semisynthetic penicillins, or cephalosporins with the exception of mezlocillin. Therefore, the present results are somehow in the line to the results about drug susceptibility of the species observed by above workers.

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## CONFLICT OF INTERESTS

There is no conflict of interest.

## AUTHORS' CONTRIBUTION

Murk Pirzada and Faiza Habib conducted the research work, Kanwar Kumar Malhi and Ambreen Leghari wrote the manuscript, Muhammad Ismail Memon, Riaz Ahmed Leghari and Rahmatullah Rind guide during research and write up, Shahid Hussain Abro and Hasina Baloch done the proofreading of manuscript.

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