Emergence of Methicillin-Resistant Staphylococcus Aureus from Subclinical Mastitis of Cholistani Sheep Posing a Great Threat to the Development of Antibiotic Resistance

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Abstract

Mastitis is a serious illness that affects dairy sheep. All around the world, dairy farms have been shown to harbour methicillin-resistant S. aureus (MRSA). The study investigated the subclinical mastitis in sheep, associated risk factors, the presence of MRSA its susceptibility against different antibiotics. The positive milk samples were cultured for identification of Staphylococcus aureus on mannitol salt agar and confirmed through biochemical tests and Gram staining. MRSA was identified and confirmed by phenotypic method using oxacillin antibiotic discs through the disc diffusion test. A data capture form was used for recording various assumed determinants related to subclinical mastitis in sheep. The study revealed that 12% (48/400) samples were positive for subclinical mastitis, and among these, a majority of the samples (88%, 42/48) were positive for S. aureus. Prevalence of MRSA based on disc diffusion test was 21.42% (9/42). Poor milker care during milking, unhygienic milking, high milk yield, use of teat dips, and mixed type of grazing were significantly ($p<0.05$) associated with subclinical mastitis in sheep. Results of in-vitro antibiotic susceptibility trials showed that MRSA was 100% resistance to Cefoxitin (30µg), 11.11% to Oxytetracycline (30µg) and Tylosin (30µg), 33.33% to Fusidic acid (10µg) and Vancomycin (30µg), 11.11% to ciprofloxacin (5µg) while it was sensitive to Moxifloxacin (5µg), Trimethoprim + Sulfamethoxazole (1.2µg, 23.75µg) and Linezolid (30µg). The study concluded that subclinical mastitis is an important issue in sheep; the presence of resistant isolate MRSA needs effective treatment to prevent its spread in lambs and humans to reduce antibiotic resistance issues.

Keywords: Subclinical, Mastitis, Prevalence, Sheep, Antibiotic, Methicillin

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Introduction

Pakistan is an agricultural country having two major sectors, including agriculture and livestock, employing almost 45% and contributing 21% of the GDP. While livestock alone contribute 12 % to GDP and 56% by the agricultural sector, both have a remarkable impact in reducing poverty and improving the economy (Shahzad, 2022).

Pakistan has a wide range of genetic resources for animals. This region is thought to have been an important hub for domesticating animals (Iqbal *et al.,* 2020). There is a huge cattle population in Pakistan, and it has adapted well to the region's climate (Aqib *et al.,* 2017). As of 2020, Pakistan was home to around 84.7 million goats, 32.3 million sheep, 5.8 million donkeys, 45 million buffaloes, and 55.5 million cattle (Pakistan Economic Survey 2022-23).

Mastitis is the most common and economically important disease of dairy animals. Mastitis is a major problem for sheep; ewes suffering from this disease have their well-being compromised with reduced milk quality and quantity (Alba *et al.,* 2019). Many animals come with significant symptoms, including apparent pain and systemic indications

The damage to the proteins, cells, and tissues caused by the mastitis's inflammatory process results in the formation of free radicals that cause lesions via lipid peroxidation (Alba *et al.,* 2019). To improve udder health on a herd, regional, or national scale, it is important to get an awareness of its common bacterial infections and risk factors (Jabbar *et al.,* 2020). A decrease in milk production, as well as physical, chemical, pathological, and microbiological changes in the milk composition and quality, an increase in somatic cells (especially leukocytes), and other negative changes, transitory to permanent, have all been linked to mastitis.

Clinical mastitis (CM) and subclinical mastitis (SCM) are the two main categories (Maréchal and Loir, 2011). Signs and symptoms of clinical mastitis include udder swelling, warmth to the touch, and altered milk flavour and aroma, while the diagnosis of subclinical mastitis is a challenge to livestock owners. The number of procedures, including the Surf Field Mastitis Test (SFMT) and the California Mastitis Test (CMT), is carried out in the field conditions for the diagnosis of subclinical mastitis (Bachaya *et al.,* 2005). SCM is mainly responsible for almost two-thirds of economic losses in the dairy industry of Pakistan.

SCM is mainly caused by *Staphylococcus aureus* (*S. aureus*), but these days development of resistance of *S. aureus* against antimicrobials is a growing problem that makes the treatment of this harmful bacterium more difficult (Vasileiou *et al.,* 2018). Resistance development in pathogenic bacteria against commonly used antibiotics is a global challenge in the 21st century (Stastkova *et al.,* 2009). *S. aureus* in this aspect is an important pathogenic bacterium that has developed resistance against different antibiotics commonly used for the treatment of mastitis. This bacterium is quite ubiquitous in the environment, which makes its role even more important in transferring antibiotic resistance to other animals and humans. The bacteria can cause food poisoning, endocarditis, pneumonia, abscess formation in the brain, meningitis, osteomyelitis, and toxic shock syndrome in humans and animals (Chambers and DeLeo, 2009; Papadopoulos *et al.,* 2018).

S. aureus has a property that may develop resistance against antibiotics that interfere with the treatment of its infections (Lowy, 2003; Pantosti *et al.,* 2007). Resistance against methicillin shows resistance to all beta-lactams; therefore, such isolates may also be called multidrug resistant (MDR). Methicillin-resistant *S. aureus* (MRSA) is responsible for serious health issues and is of public health importance as it can grow and infect both humans and animals (Papadopoulos *et al.*, 2018). Subclinical mastitis caused by MRSA will not only increase the cost of its treatment, but also the resistance problem will be transferred to other animals and humans, leading to the emerging issue of resistance development.

Antimicrobial resistance (AMR) has been identified by the WHO as a major threat to human and animal health on a worldwide scale (Minarini *et al.,* 2020). AMR is expected to cost society and the economy a total of $100 trillion by 2050, with infections occurring due to resistant bacteria potentially becoming the major cause of mortality worldwide (Garcia *et al.*, 2022).

The primary goal of this research was to study the prevalence of subclinical mastitis in Cholistani sheep, associated risk factors, and to identify the presence of MRSA in subclinical mastitis cases of sheep along with its *in-vitro* antibiotic susceptibility of MRRSA in District R.Y.K, Punjab, Pakistan.

Materials and Methods

Sampling and Screening for Subclinical Mastitis

A total of 400 milk samples (n=400 sheep) were collected during 2022–2023. The samples were collected from various herds in the area surrounding the district of Rahim Yar Khan and evaluated for subclinical mastitis using the surf field mastitis test (SFMT) (Ali *et al.,* 2011). Following the recommendations of Thrusfield (2007), the sample size was determined by assuming a 50% prevalence of MRSA at a 95% confidence interval. Milk samples that were positive for subclinical mastitis by SFMT were carefully packaged in sterilised Falcon tubes and sent to the medicine lab in the department of clinical medicine and surgery, the Islamia University, Bahawalpur. Milking-related information, such as parity, physiological status, number of milkings, milkers' care during milking, milk hygiene, milk production, usage of teat dips, tick presence, animal health, feed and water, feeding system, grazing type, and veterinary services, was recorded.

Laboratory confirmation of *S. aureus*

The Samples that were positive for subclinical mastitis were further cultured for *S. aureus*. Three millilitre samples of subclinical mastitis-positive milk were centrifuged at 2000 rpm for 10 minutes, and the resulting sediments were swabbed aseptically onto 5% sheep blood agar (TM Media, Titan Biotech Ltd, India) and incubated at 37 degrees Celsius for 24 hours. Mannitol salt agar (OXOID CM0085), a selective medium for *S. aureus*, was used to streak the colonies of *S aureus*. The colonies (Fig. 1) of *S. aureus* were confirmed by phenotypic appearance, Gram's staining, and subsequent biochemical tests, including the catalase test and coagulase test (Altaf *et al.,* 2020).

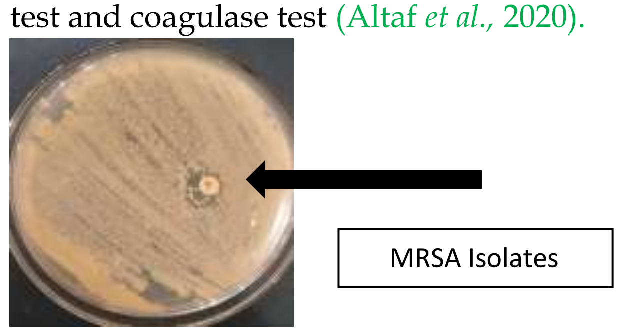

Fig 1: Phenotypic isolation of MRSA from Subclinical mastitis of Sheep

Confirmation of MRSA

Oxacillin antibiotic discs (1µg, Bioanalyse Turkey) were applied to activated growth of *S. aureus* (0.5

McFarland) to detect MRSA on Muller Hinton agar plates (OXOID, UK) and incubated at 37 degrees Celsius for 24 hours. The zones of inhibition around the disc were measured by vernier callipers and compared with a standard zone of inhibition provided by the Clinical and Laboratory Standards Institute (CLSI, 2015). The plates with oxacillin discs having a zone of inhibition <18mm were recorded as MRSA, while those having a zone of inhibition >18mm were recorded as methicillin-sensitive *S. aureus.* These isolates of MRSA, based on the zone of inhibition around oxacillin, were confirmed phenotypically by measuring zones of inhibition (Javed *et al*., 2021).

Antibiotic sensitivity of MRSA

Susceptibility of MRSA to various antibiotics such as Oxytetracycline (30µg), Tylosin (30µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Moxifloxacin (5µg), Linezolid (30µg), Trimethoprim +Sulphamethoxazole (1.25µg, 23.75µg), Cefoxitin (30µg), Fusidic acid (10µg) and Vancomycin (30µg) was determined by using antibiotic discs of Bioanalyse® (Turkey). Activated growth of MRSA ($1x10^8$ CFU/ml) was swabbed on Muller Hinton agar (OXOID, UK), and the antibiotic discs were applied and incubated the plates at 37°C for 24 hours. Zones of inhibition were evaluated using vernier callipers and compared using criteria established by the Clinical and Laboratory Standards Institute (CLSI, 2015) to determine whether MRSA is resistant or sensitive to which of the above tested antibiotics.

Statistical analysis

Univariate analysis was done to find out the association of all determinants in subclinical mastitis of sheep recorded on the data capture form. Selected determinants or the variables having a p-value <0.05 were further analysed by the multivariate logistic regression model. The statistical analysis was done using the software "Minitab".

Results

Screening of samples

S. aureus was confirmed by typical morphological characteristics of colonies (Colonies of a bright yellow colour were seen on mannitol salt agar), Gram staining, and biochemical tests like the catalase test and coagulase test in 48 of the 400 samples that were positive for subclinical mastitis

Prevalence of subclinical mastitis in Sheep

Overall, 12% (48/400) of sheep in this study were positive for subclinical mastitis (SCM) by the Surf field mastitis test (SFMT). These positive samples were further processed for *S. aureus* 42 Of 48 SCM-positive milk samples were found positive for *S. aureus,* with a prevalence of 88%. *S. aureus* in subclinical mastitis of sheep. These *S. aureus-positive* samples were further processed to find out methicillin-resistant *S. aureus* (MRSA). Oxacillin (1µg, Bioanalyse Turkey) was applied to the activated growth of *S. aureus* and incubated at 37 degrees Celsius for 24 hours. The zones of inhibition around the disc were measured by vernier callipers and compared with a standard zone of inhibition provided by the Clinical and Laboratory Standards Institute (CLSI, 2015). Phenotypically assessing zones of inhibition around an oxacillin disc, the prevalence of MRSA was found to be 21.42% (9/42), as shown in Table 1.

Table 1: Screening and Prevalence

Category	No	Prevalence
Total number of samples	400	
SCM (SFMT +ve)	48/400	12%

S. aureus	42/48	88%
MRSA	9/42	21.42%

Risk Factors Association

Disease occurrence was statistically correlated with the following variables: animal parity, physiological status, milking frequency, milker care during milking were almost five higher risks of developing subclinical mastitis than those of with proper milking care, milking hygiene, milk production level, antiseptic teat dip application, tick presence on body, body condition, feed and water, feeding system, grazing type, and veterinary services which has been shown in table 2 as well.

Table 2: Contribution of risk factors

Variables	Variable levels	Positive (+ve)	Negative	P value
District	R.Y.K	48 (12%)	352 (88%)	
Parity	1st	45	33	<0.001
	2nd	32	75	
	3rd	35	80	
	>3rd	49	51	
Physiological status	Lactating	140	223	0.039
	Dry	21	16	
No of milking	Once	140	210	0.69
	Twice	21	29	
Milker's care during milking	Poor	140	97	<0.001
	Good	21	142	
Hygiene during milking	Yes	30	129	<0.001
	No	132	109	
Milk yield	Low	130	213	0.006
	High	31	26	
Use of teat dips	Yes	10	28	<0.001
	No	151	211	
Presence of ticks	Yes	40	12	<0.001
	No	122	226	
Body health	Normal	145	218	0.17
	Thin	16	15	
	Emaciated	1	5	
Feed and water	Well fed	150	233	0.006
	Under fed	12	5	
Feeding system	Stall feeding	68	97	0.39
	Grazing	26	51	
	Grazing +stall feeding	67	91	
Grazing type	Mixed	90	30	<0.001
	separate	71	209	
Veterinary services	Veterinary officer	50	123	<0.001
	Veterinary assistant	86	106	
	Self	25	10	

Multivariate logistic regression was used to examine the correlation between risk factors and subclinical mastitis occurrence in sheep. The initial univariate analysis included 12 variables with P 0.2 (Table 2), all of which were included in the final multivariable logistic regression model. Seven table-level variables were included in the final model, as shown in Table 3.

Table 3: Association or Contribution of Selected Determinants

Variables	Response	Odd ratio	P value
Milker's care during milking	Poor	4.99	<0.001
	Good	1	
Hygiene during milking	No	6.53	<0.001
	Yes	0.98	
Milk yield	High	3.19	0.008
	Low	1	
Use of teat dips	No	7.98	0.014
	Yes	1	
Presence of ticks	Yes	3.69	0.011
	No	1	
Grazing types	Mixed	14.95	<0.001
	Separate	1	
Veterinary services	Self	3.79	0.015
	Veterinary assistant	3.29	<0.001
	Veterinary officer	1	

Subclinical mastitis was shown to be substantially ($p<0.05$) related to poor milker care during milking. Sheep whose milkers neglected proper care during milking had a risk of subclinical mastitis 4.99 times higher than those whose milkers adopted proper care during milking. Animals exposed to dirty and unclean milking settings had 5.98 times the risk of developing SCM, similar to the results obtained for proper cleanliness during milking. Sheep with a high milk yield were 3.19 times more likely to develop subclinical mastitis than those with a low milk yield. Subclinical mastitis in sheep risk was found to be 7.98 times higher where teat dips were not applied as compared to those where teat dips were properly practised, and 14.95 times higher in animals that were grazed in a mixed grazing system as compared to the animals that were grazed separately. Subclinical mastitis in sheep was also found to be substantially linked with the presence of ticks and disease management by veterinary services. While univariate analysis showed a significant relationship ($p<0.05$) between disease dynamics and factors including animal parity and physiological status as well as access to food and water, multivariate analysis showed no such relationship.

Antibiotic susceptibility

The susceptibilities of different antibiotics were evaluated through the disc diffusion test against MRSA from sheep subclinical mastitic milk samples. The results of susceptibility revealed that MRSA isolates were 100% (9/9) resistant to cefoxitin, 25% (2/8) resistant to Gentamicin and Amikacin while 11.11% (1/9) to Oxytetracycline, Tylosin and Fusidic acid while 33.33% (3/9) toVancomycin Similalry MRSA isolates were 11.11% (1/9) resistant to Ciprofloxacin while the antibiotics that were found 100% sensitive against MRSA isolates included Moxifloxacin, Trimethoprim + Sulfamethoxazole and Linezolid.

Discussion

The prevalence of subclinical mastitis varies from less than 10% to 50% or more, although the incidence of clinical mastitis during lactation (or annually) in the sheep is normally less than 5%. From 20 to at least 60 per cent of cases of clinical mastitis are caused by *Staphylococcus aureus* (Bergonier & Berthelot, 2003). The present study revealed a 12% prevalence of subclinical mastitis in cholistani sheep that was much higher than that reported by Vasileiou *et al.* (2018) in who reported 0.260% overall prevalence of subclinical mastitis in sheep. The epidemiology, bacteriology, and clinical symptoms of mastitis in sheep may vary depending on factors like environment, production form, and managemental practices. Similarly, the prevalence of subclinical mastitis in sheep in the present study is in line with Arsenault *et al.* (2008), in which they reported a 14.9% prevalence. A variable range of subclinical mastitis prevalence has been shown in different studies in ewes, ranging from 1% to 92.5% (Queiroga, 2017). Hygienic practices, biosafety precautions, correct milking techniques, regular monitoring and screening of animals, and the proper administration of antibiotics to mastitis-positive animals are all essential parts of effective flock health management. Therefore, screening of animals for subclinical mastitis and further bacterial isolation is the only reliable method for diagnosing intramammary infections (IMI). However, IMI may be correlated with somatic cell count (Fragkou *et al.*, 2014).

It can be challenging to judge economic losses caused by SCM because it is difficult for sheep owners to readily diagnose the condition (Knuth *et al.*, 2019). Stress, trauma, and bacterial invasion of the mammary glands are all potential triggers. *Streptococcus, Staphylococcus, Pasteurella*, and coliforms like *E. coli* are the bacteria that are known to cause mastitis in cattle, sheep, and goats. It is common to see cases of mastitis from right after lambing through the weaning process and an unhygienic milking environment.

Losses linked with subclinical mastitis in ewes and the risk of spreading multidrug-resistant isolates in lambs and humans can be reduced by recognising and reducing the determinants of the mastitis in dairy animals (Aqib *et al.*, 2017). The study revealed that milking without proper care, an unhygienic milking environment, cleanliness of the milker and teats during milking, higher milk yield, mixed type of grazing, presence of vectors including flies and ticks, and disease management by the sheep owners are found important risk factors associated with subclinical mastitis in ewes. These findings of risk factors are in agreement with Megersa *et al*. (2010), Koop *et al*. (2013), Vasileiou *et al*. (2018), Aqib *et al*. (2019) and Altaf *et al*. (2020).

Ovine mastitis can be caused by several different bacteria, although mainly staphylococci seem to be responsible for the vast majority of cases (Bergonier & Berthelot, 2003). When ewes are suffering from subclinical mastitis with an MRSA isolate of *S. aureus,* not only does its treatment become difficult, but also the resistant isolate may be transferred to the lambs and humans through milk, leading to the development of resistance to majority of antibiotics in lambs and humans (Altaf *et al.*, 2020)

S. aureus was isolated from 88% of the total milk samples processed for culturing on mannitol salt agar, while MRSA was found to be 21.42% by using oxacillin discs phenotypically, as there no such data is available on the prevalence of MRSA in subclinical mastitis cases of Cholistani sheep, the results of *S. aureus* and MRSA prevalence are in line with Altaf *et al*. (2020) with prevalence of *S. aureus* 80% in which they isolated MRSA isolates by disc diffusion test from dairy goats with prevalence of 18.8%. These findings of *S. aureus* prevalence are also comparable with Watkins *et al*. (1991) and de Almeida *et al*. (2011), with prevalence of 11.7% and 29%, respectively. The MRSA prevalence of 21.42% in the present study is less than revealed by Obaidat *et al*. (2018), in which they observed 29.8% of MRSA from sheep. Similarly, MRSA prevalence in the present research is higher, as 12.25% was reported by Ahmed & Yousif (2021) from subclinical mastitis of ewes. Such variation in MRSA prevalence may be due to the higher liberation of beta-lactamase enzymes or reduced improper working and expression of resistance gene meca in different ewes, depending on environmental and farm conditions of the animals (Turutoglu *et al.*, 2009; Altaf *et al.*, 2019).

The treatment of mastitis caused by *S. aureus* is usually complicated due to the issue of development of resistance against antibiotics (Altaf *et al.*, 2019; Wang *et al.*, 2015). The treatment of such resistant isolates has become an important issue throughout the world. Antibiotic susceptibility testing showed that MRSA isolates were 100% (9/9) resistant to cefoxitin, 25% (2/8) resistant to Gentamicin and Amikacin while 11.11% (1/9) to Oxytetracycline, Tylosin and Fusidic acid while 33.33% (3/9) to Vancomycin, similarly MRSA isolates were 11.11% (1/9) resistant Ciprofloxacin while the antibiotics that were found 100% sensitive against MRSA isolates included Moxifloxacin, Trimethoprim + Sulfamethoxazole and Linezolid. The susceptibility results are comparable with Vanco Andrade *et al*.

(2021), Andrade *et al*. (2021) and Altaf *et al*. (2019). The results of MRSA antibiotic susceptibilities are also in line with research on antibiotic susceptibilities of MRSA isolates of bovines and caprines (Aqib *et al.*, 2017). The result of ciprofloxacin and linezolid susceptibilities against MRSA isolates of sheep subclinical mastitis are also in agreement with Nemeghaire *et al*. (2014). The study concluded that subclinical mastitis is an important issue in sheep; the presence of resistant isolates of *S. aureus* MRSA needs effective treatment to prevent its spread in lambs and humans and reduce antibiotic resistance issues. Moreover, Linezolid, Moxifloxacin, and the combination of Trimethoprim with Sulfamethoxazole were found to be effective and sensitive antibiotics for the treatment of MRSA subclinical mastitis.

Conclusion

The study concluded the emergence of highly resistant bacteria MRSA in subclinical mastitis of sheep, which not only complicates the treatment of mastitis but also resistant isolates may be transferred to humans, leading zoonotic issue of livestock-associated MRSA in humans. Poor milker care during milking, unhygienic milking, high milk yield, use of teat dips, and mixed type of grazing were significantly ($p<0.05$) associated with subclinical mastitis in sheep. The antibiotics, including moxifloxacin, Linezolid, and a combination of Trimethoprim + Sulfamethoxazole, were found effective for the treatment of MRSA mastitis in sheep.

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Conflict of Interest

The authors have no conflict of interest.

References

Ahmed, Z.A., Yousif, A.A. (2021). Molecular and phylogenetic analysis of methicillin-resistant Staphylococcus aureus isolated from subclinical mastitis in lactating ewes. Iraqi Journal of Veterinary Sciences 35, 121-126.

Alba, D. F., da Rosa, G., Hanauer, D., Saldanha, T. F., Souza, C. F., Baldissera, M. D., and Da Silva, A. S. (2019). Subclinical mastitis in Lacaune sheep: Causative agents, impacts on milk production, milk quality, oxidative profiles and treatment efficacy of ceftiofur. Microbial pathogenesis, 137, 103732.

Ali, M., M. Ahmad, K. Muhammad, A. Anjum (2011). Prevalence of subclinical mastitis in dairy buffaloes of Punjab, Pakistan. Journal of Animal and Plant Science, 21(3): 477-480.

Altaf, M., Ijaz, M., Ghaffar, A., Rehman, A., and Avais, M. (2019). Antibiotic susceptibility profile and synergistic effect of non-steroidal anti-inflammatory drugs on antibacterial activity of resistant antibiotics (Oxytetracycline and Gentamicin) against methicillin-resistant Staphylococcus aureus (MRSA). Microbial Pathogenesis, 137, 103755.

Altaf, M., Ijaz, M., Iqbal, M. K., Rehman, A., Avais, M., Ghaffar, A., and Ayyub, R. M. (2020). Molecular Characterization of Methicillin-Resistant Staphylococcus aureus (MRSA) and Associated Risk Factors with the Occurrence of Goat Mastitis. Pakistan Veterinary Journal, 40(1).

Andrade, N. C., Laranjo, M., Costa, M. M., and Queiroga, M. C. (2021). Virulence factors in Staphylococcus associated with small ruminant mastitis: Biofilm production and antimicrobial resistance genes. Antibiotics, 10(6), 633.

Aqib, A. I., Ijaz, M., Anjum, A. A., Malik, M. A. R., Mehmood, K., Farooqi, S. H., and Hussain, K. (2017). Antibiotic susceptibilities and prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) isolated from bovine milk in Pakistan. Acta tropica, 176, 168-172.

Aqib, A. I., Nighat, S., Ahmed, R., Sana, S., Jamal, M. A., Kulyar, M. F. E. A., and Rahman, A. (2019). Drug Susceptibility Profile of Staphylococcus aureus Isolated from Mastitic Milk of Goats and Risk Factors Associated with Goat Mastitis in Pakistan. Pakistan Journal of Zoology, 51(1).

Arsenault, J., Dubreuil, P., Higgins, R., and Bélanger, D. (2008). Risk factors and impacts of clinical and subclinical mastitis in commercial meat-producing sheep flocks in Quebec, Canada. Preventive Veterinary Medicine, 87(3-4), 373-393.

Bachaya, H. A., Iqbal, Z., Muhammad, G., Yousaf, A., and Ali, H. M. (2005). Subclinical mastitis in buffaloes in the Attock district of Punjab (Pakistan). Pakistan Veterinary Journal, 25(3), 134.

Bergonier, D., and Berthelot, X. (2003). New advances in epizootiology and control of ewe mastitis. Livestock Production Science, 79(1), 1-16.

Chambers, H. F., and DeLeo, F. R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature Reviews Microbiology, 7(9), 629-641.

Clinical Laboratory Standards Institute (2015). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Standard M31-A3.

de Almeida, L. M., de Almeida, M. Z. P., de Mendonça, C. L., and Mamizuka, E. M. (2011). Novel sequence types (STS) of Staphylococcus aureus isolates causing clinical and subclinical mastitis in flocks of sheep in the northeast of Brazil. Journal of Dairy Research, 78(3), 373-378.

Fragkou, I. A., Boscos, C. M., & Fthenakis, G. C. (2014). Diagnosis of clinical or subclinical mastitis in ewes. Small Ruminant Research, 118(1-3), 86-92.

Garcia, E. R., Vergara, A., Aziz, F., Narváez, S., Cuesta, G., Hernández, M., and Casals-Pascual, C. (2022). Changes in the gut microbiota and risk of colonisation by multidrug-resistant bacteria, infection, and death in critical care patients. Clinical Microbiology and Infection, 28(7), 975-982.

Iqbal, N., Aslam, S., Hussain, N., Luqman, Z., and Jawad, H. (2020). Dystocia handling by cesarean section in beetal goat in Pakistan: a surgical approach. Journal of Animal Health and Production, 8(3), 134-137.

Jabbar, A., Saleem, M. H., Iqbal, M. Z., Qasim, M., Ashraf, M., Tolba, M. M., and Ahmad, I. (2020). Epidemiology and antibiogram of common mastitis-causing bacteria in Beetal goats. Veterinary world, 13(12), 2596.

Javed, M. U., Ijaz, M., Fatima, Z., Anjum, A. A., Aqib, A. I., Ali, M. M., and Ghaffar, A. (2021). Frequency and antimicrobial susceptibility of methicillin and vancomycin-resistant Staphylococcus aureus from bovine milk. Pakistan Veterinary Journal, 41(4).

Knuth, R. M., Stewart, W. C., Taylor, J. B., Yeoman, C. J., Bisha, B., Page, C. M., and Murphy, T. W. (2019). Subclinical mastitis in sheep: aetiology and association with milk somatic cell count and ewe productivity in three research flocks in the Western United States. Translational Animal Science, 3(Supplement_1), 1739-1743.

Koop, G., Collar, C. A., Toft, N., Nielen, M., van Werven, T., Bacon, D., and Gardner, I. A. (2013). Risk factors for subclinical intramammary infection in dairy goats in two longitudinal field studies were evaluated by Bayesian logistic regression. Preventive Veterinary Medicine, 108(4), 304-312.

Lowy, F. D. (2003). Antimicrobial resistance: the example of Staphylococcus aureus. The Journal of Clinical Investigation, 111(9), 1265-1273.

Maréchal, L., and Loir, L. (2011). Mastitis impact on technological properties of milk and quality of milk products--a review. Dairy Science and Technology, 91(3), 247-282.

Megersa, B., Tadesse, C., Abunna, F., Regassa, A., Mekibib, B., and Debela, E. (2010). Occurrence of mastitis and associated risk factors in lactating goats under pastoral management in Borana, Southern Ethiopia. Tropical animal health and production, 42, 1249-1255.

Minarini, L. A. D. R., Andrade, L. N. D., De Gregorio, E., Grosso, F., Naas, T., Zarrilli, R., and Camargo, I. L. (2020). antimicrobial resistance as a global public health problem: how can we address it?. Frontiers in Public Health, 8, 612844.

Mørk, T., Waage, S., Tollersrud, T., Kvitle, B., and Sviland, S. (2007). Clinical mastitis in ewes: bacteriology, epidemiology, and clinical features. Acta Veterinaria Scandinavica, 49, 1-8.

Nemeghaire, S., Argudín, M. A., Haesebrouck, F., and Butaye, P. (2014). Epidemiology and molecular characterization of methicillin-resistant Staphylococcus aureus nasal carriage isolates from bovines. BMC Veterinary Research, 10, 1-9.

Obaidat, M. M., Bani Salman, A. E., and Roess, A. A. (2018). High prevalence and antimicrobial resistance of meca Staphylococcus aureus in dairy cattle, sheep, and goat bulk tank milk in Jordan. Tropical animal health and production, 50, 405-412.

Pakistan Economic Survey (2022-23) Economic Advisor's wing, Finance Division: Government of Pakistan.

Pantosti, A., Sanchini, A., and Monaco, M. (2007). Mechanisms of antibiotic resistance in Staphylococcus aureus. Future microbiology, 2(3), 323-334.

Papadopoulos, P., Papadopoulos, T., Angelidis, A. S., Boukouvala, E., Zdragas, A., Papa, A., and Sergelidis, D. (2018). Prevalence of Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) along the production chain of dairy products in north-western Greece. Food microbiology, 69, 43-50.

Queiroga, M. C. (2017). Prevalence and aetiology of sheep mastitis in the Alentejo region of Portugal. Small Ruminant Research, 153, 123-130.

Shahzad, M. A. (2022). The need for national livestock surveillance in Pakistan. Journal of Dairy Research, 89(1), 13-18.

Stastkova, Z., Karpiskova, S., and Karpiskova, R. (2009). Occurrence of methicillin-resistant strains of Staphylococcus aureus at a goat breeding farm. Veterinární medicína, 54(9), 419-426.

Thrusfield, M. (2013). Veterinary epidemiology. John Wiley and Sons.

Turutoglu, H., Hasoksuz, M., Ozturk, D., Yildirim, M., and Sagnak, S. (2009). Methicillin and aminoglycoside resistance in Staphylococcus aureus isolates from bovine mastitis and sequence analysis of their meca genes. Veterinary research communications, 33, 945-956.

Vasileiou, N. G. C., Cripps, P. J., Ioannidi, K. S., Chatzopoulos, D. C., Gougoulis, D. A., Sarrou, S., and Fthenakis, G. C. (2018). Extensive countrywide field investigation of subclinical mastitis in sheep in Greece. Journal of Dairy Science, 101(8), 7297-7310.

Wang, D., Wang, Z., Yan, Z., Wu, J., Ali, T., Li, J., and Han, B. (2015). Bovine mastitis Staphylococcus aureus: Antibiotic susceptibility profile, resistance genes and molecular typing of methicillin-resistant and methicillin-sensitive strains in China. Infection, Genetics and Evolution, 31, 9-16.

Watkins, G. H., Burriel, A. R., and Jones, J. E. T. (1991). A field investigation of subclinical mastitis in sheep in southern England. British Veterinary Journal, 147(5), 413-420.