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ARTICLE

Epidemiology and Antibiotic Succeptibility Profile of Methicillin Sensitive *Staphylococcus aureus* among Livestock and Pet Animals

Muhammad Aamir Naseer[1] Amjad Islam Aqib[2*] Muhammad Shoaib[3] Iqra Muzammil[1] Zeeshan Ahmad Bhutta[4] Iqra Gulzar[5]

1. Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad-38000, Pakistan
2. Department of Medicine, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan
3. Institute of Microbiology, University of Agriculture, Faisalabad, 38000, Pakistan
4. Royal (Dick) School of Veterinary Studies, The University of Edinburgh, United Kingdom
5. Department of Zoology and Fisheries, University of Agriculture, Faisalabad-38000, Pakistan

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ABSTRACT

Staphylococcus aureus is an important zoonotic pathogen that is responsible for a variety of infectious diseases in humans and animals. The present study was designed to check the prevalence and antimicrobial resistance of MSSA from three different animal origins (bovine, caprine and pet). A total of n= 450 samples (150 each source) were collected from bovine, caprine and pets. Collected samples were subjected to *S. aurues* identification by microbiological examination and confirmed *S. aurues* isolates were put to oxacillin disk diffusion test to declare them MSSA. The MSSA confirmed isolates were subjected to various antibiotics for susceptibility profiling using Kirby Baur Disk Diffusion test. The present study found higher prevalence of MSSA from caprine origin (goat 83.33%) as compared to pet (cat 69.33%; dog 65.33%) and bovine origin (buffalo 26.66%; cattle 31.66%). The *in-vitro* findings of current study revealed oxytetracycline and gentamicin presented 100% efficacy against MSSA of all origins while the vancomycin presented >35%, >40% and > 65% resistance against MSSA isolated from bovine, caprine and pet origin respectively. However, ciprofloxacin was equally effective (50%) against MSSA from buffalo and cattle while >80% efficacy was noted against MSSA from cat and dogs. Linezolid and amoxicillin+ clavulanic acid were 77.78% and 66.67% sensitive to MSSA isolates from caprine milk. The present study found higher prevalence of MSSA from bovine, caprine and pet isolates with diversified pattern of susceptibility of different antibiotics from all sources.

1. Introduction

Animal human bond has very primitive history as this interaction helps the psychological and physical wellbeing of the person [1]. Animals have a powerful impact in human history as they had served as cavalry horses, sentry dogs, carrier pigeons, and unit mascots, or unofficially as a Soldier's battle companion [2]. Animals can be used as a powerful tool to cop psychological challenges and as a therapeutic modality or as an adap-

**Corresponding Author:*
Amjad Islam Aqib,
Department of Medicine, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan;
Email: amjadwaseer@cuvas.edu.pk

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tive intervention to help facilitate positive rehabilitation outcomes [3]. Pet introduction in human life as a natural extension helps to cop psychological challenges, unleash overburdened healthy life activities, least visits to doctors and part of recreative leisure [4]. A goat is generally entitled as "cow of poor man" [5].

Staphylococcus aureus is an important zoonotic pathogen that is responsible for a variety of infectious diseases of both cadre [6]. *S. aureus* has emerged as superbug of animal and human by compromising health and economy [7,8]. Studies report it to be second most, common etiology accounting to 17 million annual human deaths [9]. About 25-40% of healthy people have *S. aureus* on their skin and nasal cavity [10]. *S. aureus* is a commensal bacteria as well as opportunistic pathogen and capable of colonizing at different sites in a variety of animals species and humans [11]. *S. aureus* has been screened from various sites of animals including the skin, ear, nasal cavity and anal region [12]. Almost 25 % of humans also harbor *S. aureus* in the nasal cavity [13]. Dog nasal cavity is the most frequently known site for colonization when cultures from various sites were processed [14]. *S. aureus* strains have been isolated from animal origin foods like poultry, pork, beef, milk and dairy products [15]especially those expressing a multidrug resistance (MDR.

Public health is exposed to a bitter challenge of antibiotic resistance by the pathogens which results in treatment failure, longer disease course, increased costs of treatment, more morbidity and mortality. [16]. Resistance is the means by which organism responds to changing environment for survival. [17]. *S. aureus* has been assigned to be multidrug resistant. Four resistance mechanisms can be observed in *S. aureus* including trapping of drug, alteration in drug target, drug inactivation by enzymatic pathways and transmembrane efflux pump activation [18]. Methicillin resistant *S. aureus* strains have been designated as emerging pathogen in livestock and companion animals. Hospital acquired MRSA and community associated MRSA are limited to humans only, no cross-infection chances are there. But livestock occupational personals may have infections with animal originated MRSA. [19]40 (8.3%. Devastating resistance pattern of MRSA of human as well as animal origin against commonly used antibiotics has been reported [20-22]successful strategies to combat MRSA need strong and coordinated efforts from both, the human and the veterinary field according to the "One Health" concept. Hence, to identify potential risk factors related to MRSA infections in dogs, cats and horses, a case–control study was conducted, including data on 106 MRSA-infected animal patients as cases and 102 MSSA-infected animals as controls, originating from 155 different veterinary settings within Germany. Demographic data on animal patients, patient history and administration of antibiotics as well as practice/clinic specific parameters were assessed as putative risk factors. Multivariable logistic regression identified the following variables as risk factors for MRSA infection compared to MSSA infection: number of employees working at the veterinary setting ($n>10$; $p<0.001$. Not only MRSA strains are point of concern for such resistive behavior, methicillin sensitive *S. aureus* (MSSA) strains are also on the way to adopt the same resistance mechanism against commonly used antibiotics [23-26]presenting a major and constantly changing clinical challenge. We sequenced the approximately 2.8-Mbp genomes of two disease-causing *S. aureus* strains isolated from distinct clinical settings: a recent hospital-acquired representative of the epidemic methicillin-resistant *S. aureus* EMRSA-16 clone (MRSA252. Thus, this study was designed to check the prevalence and antibiotic resistance pattern of methicillin sensitive *S. aureus* of pets, caprine, and bovine origin.

2. Materials and Methods

2.1 Sample Collection

The sampling was done from pets (dogs, cats) brought to the clinic and dairy farms, located in and around district Faisalabad, Punjab, Pakistan. Total of n=450 samples were collected from all sources having n= 150 from each source using convenient sampling technique [27]. A total of n= 150 were collected from pets (n=75 dog, n=75 cat), n= 150 from bovine (n=90 buffalo, n= 60 cattle) and n= 150 from caprine (goat). Sterile swabs dipped in phosphate buffered saline (PBS) were used for sampling from nose and ear of dogs and cats while milk samples were collected after cleaning the teats, discarding a few streams of milk and scrubbing the teat ends with cotton balls moistened with 70% alcohol. The collected samples were shifted to the laboratory of Institute of Microbiology, University of Agriculture Faisalabad maintaining cold chain (4˚C) for further processing.

2.2 Identification and Confirmation of *Staphylococcus aureus*

Collected samples were cultured on blood agar and overnight incubation was done at 37˚C, for 24 hours for best possible retrieval of *S. aureus* and further culturing was done on Mannitol Salt Agar (MSA) following same incubation conditions. The confirmation of *S. aureus* based on pooled information from culture characteristics, microscopic evaluation and biochemical tests following guidelines of Bergey's Manual of Determinative Bacteriology [28].

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2.3 Identification of Methicillin Sensitive *S. aureus* (MSSA)

S. aureus confirmed isolates from all sources were put to oxacillin disk diffusion test following the guidelines of Clinical Laboratory Institute [29]. Briefly, fresh cultures of *S. aureus* adjusted at 1.5×10^8 CFU/ml were swabbed on Muller Hinton Agar (MHA) plates whereas antibiotic discs were aseptically placed at equal distances from each other. Incubation was given at 37°C for 24hours and zone of inhibitions were measured and compared with standards of CLSI to declare resistant, sensitive or intermediate strains.

2.4 *In-vitro* Efficacy of Various Antibiotics against Methicillin Sensitive *Staphylococcus aureus*

Methicillin sensitive *S. aureus* isolates from all sources were put to in-vitro antibiotic susceptibility testing against various antibiotics vancomycin (30µg), ampicillin (10µg), chloramphenicol (10µg), enoxacin (10µg), amoxicillin (10µg), fusidic acid (10µg), Amoxicillin + Calvulinic acid (20µg) ciprofloxacin (10µg), oxytetracycline (30µg), gentamicin (30µg), amikacin (30µg), and trimethoprim-sulfamethoxazole (25µg) using Kirby Bauer disc diffusion test [30]. Fresh culture adjusted at 1.5×10^8 CFU were swabbed on Muller Hinton Agar whereas antibiotic discs were aseptically placed at equal distances from each other following the guidelines of Clinical Laboratory Institute [29]. Incubation was given at 37°C for 24hours and zone of inhibitions were measured by Vernier callipers in millimetres [30] and compared with standards of CLSI to declare resistant, sensitive or intermediate strains [29].

2.5 Statistical Analysis

Prevalence was determined by using formula described by [27].

$$\text{Prevalence}(\%) = \frac{\text{No. of infected Animal(n)}}{\text{Total no. of sampled Animals(N)}} \times 100$$

The descriptive statistics was applied for estimation of antibacterial assays.

3. Results

3.1 Prevalence of Methicillin Sensitive *Staphylococcus aureus* (MSSA) Isolated from Bovine, Caprine and Pet Origins

The present study found 59.78% (269/450) overall prevalence of MSSA isolated from bovine, caprine, and pets. However, higher prevalence of MSSA was found from caprine origin (goat 83.33%) as compared to pet (cat 69.33%; dog 65.33%) and bovine origin (buffalo 26.66%; cattle 31.66%) (Table 1). The prevalence of MSSA was noted to be higher 69.33% from cats as compared to dogs 65.33%. Similarly, MSSA percentage was noted higher 31.33% as compared to 26.66% from cattle and buffalo origin respectively. The study found significant difference ($p< 0.05$) among all cadre of MSSA origin.

Table 1. Prevalence of methicillin sensitive *Staphylococcus aureus* isolated from bovine, caprine and pet origins

Sample origin	Species	Total	Positive	Percentage	Negative	Percentage	C.I	*p-value*
Bovine	Buffalo	90	24	26.66%	66	73.34%	0.1863-0.3662	
	Cattle	60	19	31.66%	41	68.34%	0.2131-0.4424	
Caprine	Goat	150	125	83.33%	25	16.67%	0.7655-0.8845	
Pet	Dog	75	49	65.33%	26	34.67%	0.5405-0.7511	
	Cat	75	52	69.33%	23	30.67%	0.5817-0.7861	
	Total	450	164	36.44%	286	63.56%	0.3213-0.4098	-

3.2 *In-vitro* Therapeutics Efficacy of Various Antibiotics against Methicillin Sensitive *Staphylococcus aureus* Isolated from Bovine Milk

The findings of present study revealed Oxytetracycline and Gentamicin presented 100%, Ciprofloxacin showed 50% efficacies against MSSA isolated from both cattle and buffalo milk. However, Trimethoprim-Sulphmethoxazole and Vancomycin showed 30% and 23.08% efficacy against MSSA obtained from buffalo while no efficacy was noted against MSSA of cattle origin. The present study found 100% resistance and intermediate variants of fusidic acid and enoxacin against MSSA of buffalo and cattle origin respectively. Amikacin efficacy was increased from 33.33% to 100% against MSSA isolated from buffalo milk as compared to cattle milk. Antibiotic susceptibility profile of various antibiotics against MSSA of bovine origin was observed during current study (Table 2).

Table 2. *In-vitro* therapeutics efficacy of various antibiotics against methicillin sensitive *Staphylococcus aureus* isolated from bovine milk

Antibiotic Name	Potency	Buffalo			Cattle		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Enoxacin		0.000	100	0.000	0.000	100	0.000
Amikacin	30ug	66.67	0.000	33.33	0.000	0.000	100
Fusidic acid	10ug	100	0.000	0.000	100	0.000	0.000
Ciprofloxacin	5ug	50.00	0.000	50.00	50.00	0.000	50.00

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Vancomycin	30ug	38.45	38.47	23.08	66.67	33.33	0.000
Oxytetracycline	30ug	0.000	0.000	100	0.000	0.000	100
Trimethoprim-Sulphmethoxazole	25ug	70.00	20.00	30.00	50.00	50.00	0.000
Gentamicin	30ug	0.000	0.000	100	0.000	0.000	100

Note: R= Resistant, I= Intermediate, S= Sensitive

3.3 *In-vitro* Therapeutics Efficacy of Various Antibiotics against Methicillin Sensitive *Staphylococcus aureus* Isolated from Goat Milk

The *in-vitro* findings of current study reported that MSSA isolates were 100% sensitive to Gentamicin and Oxytetracycline followed by Trimethoprim + Sulphamethoxazole and Cefoxitin (88.89%), Linezolid 77.78%, Chloramphenicol and Amoxicillin+Clavulanic acid 66.67%, Amoxicillin 44.44%, and Vancomycin 22.22%. However, it presented higher reistanace to Vancomycin and Amoxicillin 44.44%, followed by 22.22% to Amoxicillin+ Clavulanic acid, 11.11% to Chloramphenicol. However, intermediate type of response was shown against Vancomycin 33.33%, Chloramphenicol and Linezolid 22.22%, followed by Amoxicillin, Cefoxitin, Amoxicillin, Trimethoprim + Sulphamethoxazole and Amoxicillin+Clavulanic acid 11.11%. Antibiotic susceptibility profile of various antibiotics against MSSA of caprine origin was observed during this study (Table 3).

Table 3. *In-vitro* therapeutics efficacy of various antibiotics against methicillin sensitive *Staphylococcus aureus* isolated from caprine (goat) milk

Antibiotic Name	Potency	Goat		
		R (%)	I (%)	S (%)
Amoxicillin	10µg	44.44	11.11	44.44
Cefoxitin	30µg	0.000	11.11	88.89
Linezolid	30µg	0.000	22.22	77.78
Amoxicillin+ Clavulanic acid	20µg	22.22	11.11	66.67
Vancomycin	30µg	44.44	33.33	22.22
Oxytetracycline	30µg	0.000	0.000	100
Chloramphenicol	10µg	11.11	22.22	66.67
Trimethoprim-Sulphmethoxazole	25µg	0.000	11.11	88.89
Gentamicin	10µg	0.000	0.000	100

Note: R= Resistant, I= Intermediate, S= Sensitive

3.4 *In-vitro* Therapeutics Efficacy of Various Antibiotics against Methicillin Sensitive *Staphylococcus aureus* Isolated from Pets

The *In-vitro* findings of current study revealed Oxytetracycline, Amikacin, and Gentamicin presenting 100%, Chloramphenicol, Ciprofloxacin, and Trimethoprim-Sulphmethoxazole presented >80% efficacies against MSSA isolated from cat and dog. However, vancomycin and ampicillin presented 70% and 90% resistance against MSSA obtained from cat while 85.71% resistance was noted from both antibiotics against MSSA of dog origin. Fusidic acid showed 30% and 57.14% resistance against MSSA isolated from cat and dog respectively. Varying degree of sensitivity of antibiotics against MSSA isolated from pets (cat, dog) was observed during the study as mentioned in (Table 4)

Table 4. *In-vitro* therapeutics efficacy of various antibiotics against methicillin sensitive *Staphylococcus aureus* isolated from pets

Antibiotic Name	Potency	Cat			Dog		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Vancomycin	30ug	70.00	20.00	10.00	85.71	0.000	14.29
Ampicillin	10ug	90.00	10.00	0.000	85.71	14.29	0.000
Chloramphenicol	30ug	0.000	20.00	80.00	0.000	0.000	100
Fusidic acid	10ug	30.00	50.00	20.00	57.14	42.86	0.000
Ciprofloxacin	5ug	0.000	20.00	80.00	0.000	14.29	85.71
Oxytetracycline	30ug	0.000	0.000	100	0.000	0.000	100
Trimethoprim-Sulphmethoxazole	25ug	10.00	0.000	90.00	14.29	0.000	85.71
Amikacin	30ug	0.000	0.000	100	0.000	0.000	100
Gentamicin	30ug	0.000	0.000	100	0.000	0.000	100

Note: R= Resistant, I= Intermediate, S= Sensitive

4. Discussion

The present study found 26.66% and 31.66% prevalence of MSSA from buffalo and cattle milk respectively. A study conducted by [31] on methicillin resistant and susceptible staphylococci from bovine milk in China found 52.80% (113/214) prevalence of MSSA that is higher than the findings of current study. The present study found 65.33% and 69.33% prevalence of MSSA from dogs and cats respectively. A study conducted by [32] on prevalence of MRSA and MSSA among the staff and pets in a small animal referral hospital UK. [32] found 6.66% and 33.33% prevalence of coagulase positive MSSA from dogs and cats respectively. Another study conducted by [33] found 7.85% (46/586) prevalence of MSSA from pets that is lower than the findings of current study. Another study conducted by [34] found 70% of *S. aureus* cat isolates were sensitive to methicillin (MSSA) that is similar with the findings of current study. The higher prevalence of MSSA

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in this area could have been because of less use of beta-lactam antibiotics, geographical variation, influence of genetic and environmental factors (Shoaib *et al.* 2020) In current study, Methicillin susceptible *S. aureus* was found 83.3% which is in order with the previous results 90.8% as discussed by [36], 80% published by Bochev & Russenova (2005), 84% by [38]herd prevalence of *S. aureus*, including MRSA, was estimated from bulk tank milk (BTM and 98% described by [39]. Another study conducted by [40] on MRSA and MSSA from caprine (sheep) milk found 53.5% prevalence of MSSA that is lower than the findings of current study (83.33%).

The findings of present study revealed Oxytetracycline and Gentamicin presented 100%, and Ciprofloxacin showed 50% efficacies against MSSA isolated from both cattle and buffalo milk. These results are in line with the previous research showing more than 85% sensitivity of MSSA isolates against tetracyclines reported by [41]and 60 canine Staphylococcus pseudintermedius isolated from 1986 through 2000 at the Western College of Veterinary Medicine (WCVM and 100% susceptibility to oxytetracycline by [42]CC9, and CC49. The resistance to tetracycline and macrolides (clarithromycin. The excellent response to gentamicin observed during this study is supported by [43] which may be linked with limited use of gentamicin in late 1990's and apparent shift in MSSA isolates. However, Trimethoprim-Sulphmethoxazole and Vancomycin showed 30% and 23.08% efficacy against MSSA obtained from buffalo while no efficacy was noted against MSSA of cattle origin. These results are comparable to results reported by [44]which encodes a two-component signaling pathway whose activating ligand is an agr-encoded autoinducing peptide (AIP in which higher percentages of intermediate or sensitive strains to trimethoprim + sulfamethoxazole was noted. Some studies reported very lower percentages of resistant isolates as conducted by [45] isolated from 54 samples of raw milk and dairy products of bovine, ovine, caprine and bubaline origin were tested for the presence of genes coding for staphylococcal enterotoxins (SEs/SEls which found 1.3% of resistant isolates. Vancomycin resistance shown in the MSSA isolates is in line with previous studies because it is an emerging issue in MSSA isolates which may be due to the acquired resistance just like methicillin [46]. The present study found 100% resistance and intermediate variants of fusidic acid and enoxacin against MSSA of buffalo and cattle origin respectively. Amikacin efficacy was increased from 33.33% to 100% against MSSA isolated from buffalo milk as compared to cattle milk. High resistance to Fusidic acid in MSSA isolates is similar to results reported by [47]"ISBN":"1460-2091\n0305-7453","PMID":"29253168","abstract":"BACKGROUND: *Staphylococcus aureus* skin colonization is common in patients with atopic dermatitis (AD. Remarkable resistive response of MSSA to Fusidic acid is due to mutations in *fus* gene islands resulting in amino acid substitutions of protein encoded [48]isolates, 38 (84%. Deceasing multidrug resistance in community clinical isolates especially in MRSA is due to successful identification and treatment protocol, frequent multidrug therapy, specificity for control, contact precautions, active surveillance and adjunctive control measures adoption [7,49].

The current study *In-vitro* antibiotic trial against MSSA isolated form cats presented 90%, 30%, 0.0%, 0.0% and 10% resistance by ampicillin, fusidic acid, ciprofloxacin, oxytetracycline and trimethoprim-sulphamethoxazole respectively with almost similar resistance pattern was noted against MSSA of dog origin. The similar type of findings were also reported by [32]. Our study indicated that MSSA isolates were 100% sensitive to Gentamicin and Oxytetracycline, and 88.89% to Trimethoprim + Sulphamethoxazole and Cefoxitin. These results were similar to results reported by [50,51] who reported 80-100% sensitivity of *S. aureus* against these antibiotics except cefoxitin sensitivity which is in line with the results reported by [52-54]. The general trend of sensitivity shown by Chloramphenicol, Amoxicillin+Clavulanic acid, and Amoxicillin was in the range of 40-70% in current study. These results are comparable to (Aqib *et al.*, 2019; Befekadu *et al.*, 2016). In our study, MSSA isolates exhibited 77.78% sensitivity to Linezolid, which is comparable to results reported by [7]. However, MSSA showed lesser sensitivity to Vancomycin 22.22%, which may be developed due to use of Vancomycin as last choice in the treatment of *S. aureus* infections [52]. The development of antibiotic resistance in *S. aureus* strains is an alarming situation in dairy goats. Although the antibiotic sensitivity results described here are comparable to earlier studies in which *S. aureus* were mostly sensitive to Vancomycin, Chloramphenicol, and Cefoxitin [55]. Because these drugs are not commonly used in veterinary medicine to treat *S. aureus* infections in goats [56] increased cost and culling. Early and specific antibiotic based treatment reduces the severity of the disease. Over the years the extensive use of antimicrobials has led to increase antimicrobial resistance. The present study was designed to investigate the prevalence of microorganisms responsible for mastitis and their antimicrobial resistance pattern. A total of 282 milk samples were collected from different animal species (sheep, cows and goats, which may be justified by the results reported in this study.

5. Conclusion

The present study found overall higher prevalence of

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MSSA isolated from bovine (buffalo 26.66%; cattle 31.66%), caprine (83.33%), and pets (cat 69.33%; dog 65.33). The higher percentage of MSSA was found from caprine as compared to bovine and pets. *In-vitro* antibiotic therapeutic efficacy indicated amikacin, oxytetracycline, and gentamicin presented higher sensitivity to MSSA isolates from all origins while vancomycin, ampicillin exhibited higher resistance against MSSA isolates from all sources with fusidic acid, amoxicillin and ampicillin resistance against MSSA isoaltes from bovine, cprine and pets respectively. The study found variable response to antibiotic susceptibilities in addition to higher prevalence of MSSA bovine, caprine and pet interface.

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