Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 1056-1061

Prerit Kumar Singh
Department of Veterinary Microbiology, C.V.Sc & A.H., N.D.U.A & T., Kumarganj, Faizabad, U.P, India

RK Joshi
Department of Veterinary Microbiology, C.V.Sc & A.H., N.D.U.A & T., Kumarganj, Faizabad, U.P, India

RP Diwakar
Department of Veterinary Microbiology, C.V.Sc & A.H., N.D.U.A & T., Kumarganj, Faizabad, U.P, India

Correspondence
Prerit Kumar Singh
Department of Veterinary Microbiology, C.V.Sc & A.H., N.D.U.A & T., Kumarganj, Faizabad, U.P, India

Identification and study of antibiogram pattern in *Methicillin Resistant Staphylococcus aureus* from milk samples of farm animals

Prerit Kumar Singh, RK Joshi and RP Diwakar

Abstract
Isolation and characterization of Methicillin resistant *S. aureus* was attempted from pigs, buffalos, goats and sheep and the antibiotic resistant pattern of *S. aureus* isolates was studied in the present study. Processing of 90 milk samples (buffaloes 30, goats 40 and sheep 20) yielded 121 *S. aureus* isolates. All isolates were catalase positive and majority of isolates gave positive slide caogulase and / or tube coagulase test, nitrate reduction and β haemolysis and fermented mannitol sugar.

Among *S. aureus* isolates from pigs, highest isolates showed resistance to ampicillin followed by cephalexin, methicillin, lincomycin, chloramphenicol, streptomycin and tetracycline. All the isolates were found sensitive to gentamicin and ciprofloxacin. From buffaloes, highest isolates exhibited resistance towards ampicillin and cephalexin followed by chloramphenicol, lincomycin, methicillin, tetracycline, ciprofloxacin streptomycin. All the isolates were sensitive for gentamicin. From goats, resistance for ampicillin was revealed by highest isolates followed by chloramphenicol, lincomycin, tetracycline cephalexin, streptomycin, methicillin and only one isolate was resistant to gentamicin. All the isolates from goats were found sensitive to ciprofloxacin. Similarly in sheep, highest resistant isolates were found for cephalexin followed by chloramphenicol, ampicillin, lincomycin, streptomycin, methicillin and tetracycline respectively. All the isolates from sheep were found sensitive to gentamicin and ciprofloxacin.

Keywords: antibiogram, *Staphylococcus aureus*, farm animals

Introduction
S. aureus are cluster forming, facultative aerobic, Gram-positive cocci with intrinsic ability to ferment carbohydrates, producing white to deep yellow pigment on solid culture media. They also ferment mannitol. The organisms produce deoxyribonuclease (DNase) and catalase enzymes and coagulate proteins, the character which is used for their identification (Bannerman, 2004) [2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a zoonotic and veterinary bacterial pathogen of public health importance in last few decades. It causes nosocomial and community onset infections in human population. MRSA is considered being resistant to virtually all available beta-lactam antibiotics which include the penicillins such as methicillin, dicloxacillin, nafcillin, oxacillin and cephalosporins. In last four decades, it has shown an increasing endemic and epidemic spread (Kuehnert *et al.*, 2005) [10].

The proportion of MRSA among *S. aureus* from nosocomial infections increased considerably from the end of the 1980s until 2000, in nearly all of Europe and worldwide (EARS 2013) [7]. There are a few countries, such as the Netherlands and the Scandinavian countries etc, where the consequent implementation of appropriate infection control measures prevented the spread of MRSA. During the past five years, the rising trend was halted and even reverted in several European countries, which is likely due to the introduction of mandatory surveillance for MRSA bacteremia in some countries, and to the region-wide search and follow-up strategies reported in Germany.

There are three types of MRSA viz. healthcare-associated MRSA, community-associated MRSA and livestock-associated MRSA. (Huijsdens *et al.*, 2006; Layer *et al.*, 2012) [9, 14].

Since the late 1990s, MRSA has emerged in many countries as a cause of invasive skin infections in the community independently from the health care setting in many countries around the world (Salgado *et al*., 2003; Tristan *et al*., 2007) [20, 27]. MRSA associated infections in human patients impose serious burden in terms of treatment costs, and cause significant morbidity and mortality (Bratu *et al.*, 2005) [4].

Material and Method

Collection of milk samples

The composite milk samples were collected from Buffalos (30), Goats (40) and Sheep (20) maintained at the Instructional Livestock Farm Complex (ILFC) of the college of Veterinary Sciences and A.H, and by local farmers around the University Campus. Following proper washing of the udder with tap water and drying the teat ends were swabbed with cotton soaked in 70% ethyl alcohol. Approximately 10 ml of milk was collected aseptically from all quatres, and mixed in sterile universal bottles after discarding the first two to three milk streams. The Samples thus obtained were then transported on ice to microbiology laboratory where they were immediately streaked on standard bacteriological media.

1. Isolation of *S. aureus*

Isolation and identification of *S. aureus* was done as per the recommendations of the National Mastitis Council (NMC, 1990) [17], Quinn *et al.* (1994) [19] and National Committee for Clinical Laboratory Standards (NCCLS, 1997) [16]. Mannitol salt agar was used for primary isolation of *S. aureus* from the nasal swabs samples.

2. Biochemical characterization of *S. aureus*

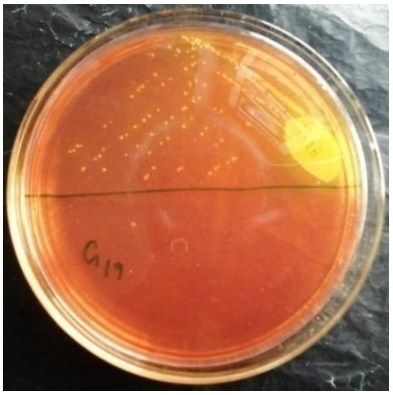
The organism on slants were subjected to gram staining, and biochemical tests *viz.* MR test, VP test, Catalase test, Coagulase test, Nitrate Reduction test and Haemolysis on blood agar were performed as per method described by Barrow and Feltham (1993) [3].

3. Study of Antibiogram of isolates (Methicillin Resistance)

Antimicrobial drug sensitivity of the isolates was tested for 9 antibiotics Ampicillin (A), Streptomycin (S), Chloramphenicol (C), Tetracycline (TE), Lincomycin (L), Gentamicin (GEN), Cephalexin (Cp), Ciprofloxacin (Cf) and Methicillin (MET) using the modified disc diffusion method of Bauer *et al.* (1966).

Result & Discussion

Present study was designed with the aim of isolating and characterizing methicillin resistant *S. aureus* from pigs, buffalos, goats and sheep, and to study their antibiotic resistant pattern in the study area. Bacteriological examination of the 90 samples yielded 121 *S. aureus* isolates, of which 55 isolates were recovered from milk samples, inoculation of the samples on mannitol salt agar (MSA) (Table-1). The appearance of characteristic pink yellow colored colonies on MSA was considered as *S. aureus* (Figure- 1).



Growth of *S. aureus* on mannitaol salt agar

Table 1: Isolation of S. aureus and their methicillin resistance

S. No		Pig	Buffalo		Goat		Sheep		Total
		Nasal swabs	Nasal swabs	Milk samples	Nasal swabs	Milk samples	Nasal swabs	Milk samples	
1.	Total No of samples processed	60	30	30	20	40	40	20	240
2.	Number of *Staph.* Isolates recovered	36	18	24	5	23	7	8	121
3.	Methicillin sensitive	5(13.89)	13(72.22)	17(70.83)	4(80.00)	15(65.22)	6(85.71)	4 (50.00)	64(52.89%)
4.	Methicillin resistant	31(86.11%)	5(27.78%)	7(29.17%)	1(20.00%)	8(34.78%)	1(14.29%)	4(50.00%)	57(47.11%)
5.	*mec A gene*	29	5	7	1	8	0	3	53(92.98%)
6.	*fem A gene*	20	4	7	1	7	0	3	42(73.68%)

Among the 24 *S. aureus* isolates recovered from buffalo milk samples, 20 (83.33%) isolates each were positive for MR and VP tests and 22 (91.67%) and 21 (87.56%) isolates showed slide coagulase and tube coagulase activity, respectively.

Of the 23 *S. aureus* isolates from goat milk samples, 20 (86.75%) isolates each were found positive for MR, VP, slide coagulase and β haemolysis test while all 23 isolates were positive for catalase but only 5 (21.14%) isolates showed nitrite reduction.

From sheep milk, all the 8 *S. aureus* isolates were catalase positive, 6 isolates each (75.00%) showed positive MR and VP tests and appeared β haemolytic while 5 isolates each (62.5%) were tube coagulase positive and reduced nitrate.

Among 24 isolates from buffalo milk, all the isolates fermented mannitol followed by 22 (91.67%) and 21 (87.5) isolates fermenting lactose and glucose, respectively. Similarly, out of 23 isolates recovered from goat milk, highest 20 (86.95%) isolates fermented mannitol followed by 17 (73.91%) isolate fermenting glucose and 9 (39.13%) isolates fermenting lactose. The 7 (87.5%) out of 8 isolates of *S. aureus* from sheep milk fermented lactose as well as mannitol while only 4 (50.00%) isolates fermented glucose (Table-1.2).

Table 1.2: Fermentation of sugars by *S. aureus* isolates from Milk samples.

S. No	Sugars	Buffalo (24)				Goat (23)				Sheep (08)			
		Acid		Gas		Acid		Gas		Acid		Gas	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	Glucose	21	87.5	22	91.67	17	73.91	17	73.91	4	50.00	3	37.5
2	Lactose	22	91.67	16	66.67	9	39.13	15	65.21	7	87.5	1	12.5
3	Mannitol	24	100	20	83.33	20	86.95	15	65.21	7	87.5	3	37.5

Among isolates from milk samples, 83.33%, 86.95% and 75.00% isolates from buffalos, goats and sheep, respectively exhibited β haemolysis. These results are in agreement with those reported by Tyagi *et al.* (2013) and Singh (2015) [23].

The β haemolysis, although related to the virulence of *S. aureus*, represents specific but not very sensitive criteria for identification of *S. aureus* (Larsen *et al.*, 2002) [13]. In present study, β haemolysis and 83.33% isolates from buffalo milk exhibited β haemolysis. In goat milk samples, 86.95% isolate exhibited β haemolysis, while in sheep 75% isolates of *S. aureus* were β haemolysis. The results are in conformity to those reported by Tyagi *et al.*, (2013) and Singh. (2015) [23].

Antibiotic resistance is a major public health concern today as a large number of organisms are becoming resistant to various antibiotics and trend is increasing day by day. On the basis of antibiotic resistance, various group of antibiotic resistant bacteria have been defined, for example, MRSA, ESBLs (Extended Spectrum Beta-Lactamases), VRSA (Vancomycin Resistant *Staphylococcus aureus*) etc. The MRSA has immerged as the most problematic Staphylococcal strains in last few years. After the first report of isolation from mastitic milk (Devries *et al.*, 1972) [6],

24 isolates from milk samples of buffalos, 7 (29.17) isolates exhibited resistance to methicillin.

Similarly, from goat milk samples, 4 (34.78%) isolates were resistant to methicillin and 4 (50%) out of 8 *S. aureus* isolates from milk samples of sheep exhibited resistant to methicillin.

MRSA in Sheep and goat has been reported (Chu *et al.*, 2012) [5]. Stastkova *et al.*, (2009) [25] reported isolation of MRSA from goat milk samples and found that the animals with MRSA infection had experienced a miscarriage and retained placenta. Similarly, Chu *et al.* (2012) [5] also reported MRSA in goats suffering from mastitis.

The MRSA isolates recovered in present study were also found resistant to a Cephalexin (52, 91.22%), Ampicillin (50, 89.47%), Chloramphenicol (31,54.39%), Lincomycin (27,47.37%), Tetracycline (09, 15.79%), Streptomycin (06, 10.53%), Ciprofloxacin (04, 7.01%) and Gentamicin (01, 1.75%) (Table– 1.3). There are many reports of MRSA being resistant to number of other antibiotics of various groups. Widianingrum *et al.* (2016) reported that the 60% MRSA were also resistant to oxacillin (60.00%), ampicillin (66.70%), tetracycline (40.00%), erythromycin (33.3%) and gentamycin (20.00%). Low resistance to ciprofloxacin and gentamycin may be attributed to the infrequent use of these antibiotics for the treatment of animal diseases in this area in last few years.

Table 1.3: Resistance pattern of MRSA isolates.

S. No	Antibiotics	Con. (mg)	Pig(31)		Buffalo(12)		Goat(9)		Sheep(5)		Total(57)	
			R%	S%	R%	S%	R%	S%	R%	S%	R%	S%
1	*Ampicillin*	25	31(100)	-	7(58.33)	5(41.67)	8(88.89)	1(11.11)	4(80.00)	1(20.00)	50(87.72)	07(12.28)
2	*Streptomycin*	25	2(6.45)	29(93.55)	-	12(100)	3(33.33)	6(66.67)	1(20.00)	4(80.00)	6(10.53)	51(89.47)
3	*Chloramphenicol*	30	14(45.16)	17(54.84)	3(25.00)	9(75.00)	9(100)	-	5(100)	-	31(54.39)	26(45.61)
4	*Tetracycline*	10	2(6.45)	29(93.55)	3(25.00)	9(75.00)	4(44.44)	5(55.56)	-	5(100)	9(15.79)	48(84.21)
5	*Lincomycin*	2	14(45.16)	17(54.84)	6(50.00)	6(50.00)	5(55.56)	4(44.44)	2(40.00)	3(60.00)	27(47.37)	30(52.63)
6	*Gentamicin*	50	-	31(100)	-	12(100)	1(11.11)	8(88.89)	-	5(100)	1(1.75)	56(98.25)
7	*Cephalexin*	30	30(96.77)	1(3.22)	10(83.33)	2(16.67)	7(77.78)	2(22.22)	5(100)	-	52(91.22)	5(8.77)
8	*Ciprofloxacin*	5	-	31(100)	4(33.33)	8(66.67)	-	9(100)	-	5(100)	4(7.01)	53(92.98)

All the 121 *S. aureus* isolates recovered from milk samples were tested for methicillin resistance using disc diffusion method.

All isolates, except one from goat milk, found sensitive to gentamicin. Out of 36 *S. aureus* isolates from pigs, highest 33 (91.67%) isolates showed resistance to ampicillin followed by cephalexin (32,88.89%), methicillin (31,86.11%), lincomycin (17,47.22%), chloramphenicol (16,44.44%), streptomycin and tetracycline (2 each 5.56%). All the 36 isolates were found sensitive to gentamicin and ciprofloxacin. Resistant to many antibiotics in clinical use like β lactams, flouroquinolones, amino glycosides, rifampin and mupirocin has been reported time to time (Carbon, 2000). The highest resistance to ampicillin may be a result of production of β lactamase the enzymes that inactivate penicillin and closely related antibiotics (Abera *et al.*, 2010) [1]. Ciprofloxacin has been reported to be most effective antibiotic against *S. aureus* isolates (sudhaker *et al.*, 2009). Kumar *et al.* (2010) [12] also reported 26.2% isolates to be sensitive to ciprofloxacin, 25.2% lincomycin and 84.1% isolates to be susceptible to cephalexcin. From buffaloes, 42 *S. aureus* isolates were recovered, of which highest 24 (57.14%) isolates were resistant to ampicillin and cephalexin followed by 16 (38.09%) isolates resistant to chloramphenicol, 15 (35.71%) isolates for lincomycin, 12 (28.57%) for methicillin, 9 (21.43%) for tetracycline, 7 (16.67%) for ciprofloxacin and 5 (11.90%) for streptomycin. All the isolates were sensitive for gentamicin. The high susceptibility of *S. aureus* isolates from buffalo milk for gentamycin, tetracycline and methicillin may be attributed to less use of these antibiotics for the treatment of mastitis. In a study, Oliveira et al. (2011) [18] found 100% isolates of *S. aureus* from buffalo milk to be sensitive to methicillin and amoxicillin associated with clavulanic acid while all the isolates were resistant to ampicillin, penicillin and oxacillin. Sharma *et al*, (2015) [22] reported that the *S. aureus* isolates recovered from mastitis and subclinical mastitis cases in buffaloes revealed 100 percent sensitivity to amikacin, azithromycin, imipenem and nitrofurantoin and high sensitivity (88.89%) towards cefotaxim, ceftriaxone, chloramphenicol, erythromycin, fusidic acid, piperacillin/tazo, rifampicin and tylosin. All the isolates were reported to be resistant to penicillin (100.00%), followed by vancomycin (88.89%), nalidixic acid (77.78%), cefixime, methicillin, novobiocin (66.67% each), amoxiclav, colistin, pipemidic acid (55.56% each), ofloxacin, streptomycin, sulphamethizole (44.44% each), ampicillin/sulbactam, cefalexin, cefazolin, cefoperazone, enrofloxacin, floxidin, meropenem (33.33% each), cefuroxim, ciprofloxacin, clindamycin, gentamicin, levofloxacin, norfloxacin and tetracycline (22.22% each).

The resistance pattern of *S. aureus* isolates form goats revealed that all the 28 isolates were sensitive to ciprofloxacin while 20 (40.43%) isolates were resistant to ampicillin followed by 19 (67.86%) for chloramphenicol, 16 (57.14%) for lincomycin, 12 each (42.86%) for tetracycline and cephalexin, 9 each (32.14%) for streptomycin and mithicillin and only 1(3.57%) isolate was resistant to gentamicin. Virdis *et al.*, 2010 [29] reported the susceptibility of *S. aureus* from goat mastitis to be 92.00% or greater for seven out of ten antibiotics tested but was lower for kanamycin (60.0%), oxytetracycline (84.0%), and ampicillin (88.0%). The

susceptibility of coagulase negative *S. aureus* was between 94.0%– 100.0% for eight antimicrobials, but was somewhat lower for ampicillin (64.0%) and kanamycin (78.7%). In an another study, Chu *et al.* (2012) [5] reported the *S. aureus* isolates from goats to be more resistant to ampicillin, cephalothin, oxacillin, oxytetracycline, penicillin G, and tetracycline.

Very few studies have reported the prevalence of *S aureus* in sheep. Overall antibiotic use tends to be much lower in sheep than in pigs, poultry, or dairy cows, which may result in a low prevalence of drug resistant *S. aureus* (Gharsa *et al.,* 2011) [8]. However, antibiotics are likely to be used in greater quantities in herds of dairy sheep, particularly for the treatment or prevention of mastitis. In present study, all 15 *S. aureus* isolates from sheep were found sensitive to gentamicin and ciprofloxacin while highest 13 (87.67%) out of 15 isolates were resistant to cephalexin followed by 11 (73.33%), 10 (67.67%), 9 (60.00%), 6 (40.00%), 5 (33.33%) and 1 (6.67%) isolates were found resistant to chloramphenicol, ampicillin, lincomycin, streptomycin, methicillin and tetracycline, respectively. Martins *et al.* (2015) [15] studied the antimicrobial susceptibility of *S. aureus* isolates from sheep milk and found only one isolate was resistant to tetracycline. The remaining isolates were susceptible to all drugs tested. Similar results have also been reported by Vyletelová *et al.* (2011) [30] for *S. aureus* isolates from sheep.

Among the 24 *S. aureus* isolates from buffalos milk, 19 (79.17%) isolates found resistant to cephalexin followed by 12 (50.00%) for ampicillin, 11(45.83%) for chloramphenicol, 8 (33.33%) for lincomycin, 7 (29.17%) for methicillin and 2 each (8.33%) for streptomycin and tetracycline. All 24 isolates from buffaloes milk were found sensitive to gentamicin and ciprofloxacin. Similarly, out of 23 *S. aureus* isolates recovered from goats milk, highest 17 (73.91%) isolates appeared resistant to ampicillin and chloramphenicol followed by 12 (52.17%) each for tetracycline and lincomycin, 9 (39.13%) for streptomycin, 7 (30.43%) cephalexin, 8 (34.78%) for methicillin and1 (4.35%) isolates was found resistance to gentamicin. All the 23 isolates were sensitive to ciprofloxacin. Among 8 isolates from sheep milk samples, 7 (987.50) isolates were resistant to cephalexin and chloramphenical followed by 5 (62.50%) showing resistance to ampicilin, 4 (50.00%) isolates resistant to methicilin, 3 (37.5%) isolates resistant to lincomycin and 1 (12.5%) isolate was resistant to streptomycin. All the 8 isolates recovered form sheep milk were sensitive to tetracycline, gentamicin and ciprofloxacin. Singh (2015) [23] also reported the *S. aureus* isolates from mastitic milk to be sensitive to Amoxycillin, Gentamicin, and Ciprofloxacin. Out of 18 isolates from buffalo nasal samples, 12 (66.67) isolates were found resistant to ampicillin, 7 (38.89%) isolates each were resistant to tetracycline, lincomycin and ciprofloxacin, 5 (27.87%) isolates each were resistant to chloramphenicol, cephalexin and methicillin and 3 isolates showed resistance to streptomycin while all 18 isolates were sensitive to gentamicin. Out of 5 isolates from goat nasal samples, all were sensitive to streptomycin, tetracycline gentamicin and ciprofloxacin while all the isolates were resistant to cephalexin followed by 4 (80%), 3 (60%), 2 (40%) and 1 (20%) isolate exhibited resistance to methicillin, chloramphenicol, ampicillin and lincomycin, respectively. Singh (2015) [23] reported 16.00% isolates to be resistant to streptomycin while 5.33% isolates were resistance to Ampicillin and 1.33% isolate was resistance to Penicillin. The findings of the present study are in the agreement with the finding of Sudhakar *et al.* (2009) [26] who also reported highest sensitivity to Ciprofloxacin. Kumar *et al.* (2011) also observed higher resistance to Streptomycin (36.4% of the isolates), Ampicillin (29.9%) and Penicillin-G (28.9%).

Of the 7 *S. aureus* isolates of nasal swabs from sheep, 6 (85.71%) showed resistance to lincomycin and cephalexin followed by 5 (70.41) for ampicillin, 4 (57.47%) for chloramphenicol and 1 each (14.78%) isolate was found resistance to streptomycin, tetracycline and methicillin while all the 7 isolates were sensitive to gentamicin and ciprofloxacin. The results revealed that all the isolate from goats milk sample except 1, were sensitive to gentamicin. Spanu *et al.* (2010) [29] found none of the tested isolates of *S. aureus* from raw sheep milk cheese showed resistance to Cephalothin (CF), Cefoperazone (CFP), Erythromycin (E), Gentamicin (GM), Kanamycin (K), Novobiocin (NV), Ofloxacin (OFX), Rifampin (RA), Streptomycin (S), and Trimethoprim (TM). Only two isolates showed intermediate levels of resistance to Kanamycin (K) (5.6%), and one to Ofloxacin (OFX) (2.8%).

Multiple drug resistant (MDR) strains are common in bacteria now a day. To study the MDR pattern, data obtained after antibiotic sensitivity test were compiled and analyzed for MDR pattern in *S. aureus* isolates recovered in this study. In 121 *S. aureus* isolates obtained in the present study, the multi drug resistant pattern involving 2 to 7 antibiotics was evident in 100 (82.64%) isolates, of which, 3 (2.48%) isolates each were found resistant to 7 and 6 antibiotics while 2 (18.18%) isolates exhibited resistant to 5 antibiotics and 37 (30.58%) isolates were resistant to 4 antibiotics. Twenty five (20.66%) isolates were resistant to 3 antibiotics and 10 isolates showed resistance to 2 antibiotics. Out of 36 *S. aureus* isolates obtained from the nasal swabs of pigs, 2 (5.56%) isolates exhibited resistance to 6 antibiotics while 6 (16.66%) isolates were found resistant to 5 antibiotics, 14 (38.89%) isolates for 4 antibiotics and 11 (30.56%) isolates exhibited resistance to 3 antibiotics. Similarly the pattern of MDR among 24 isolates recovered from buffalo milk was: 9 (37.5%) isolates resistant to 4 antibiotics, 3 (12.5%) isolates to 3 antibiotics and 8 (33.33%) isolates to 2 antibiotics. Of the 18 *S. aureus* isolates from buffalo nasal swabs, 6 (33.33%) isolates were resistant to 5 antibiotics, 3 (16.67%) isolates for 4 antibiotics and 3 (16.67%) isolates for 3 antibiotics. Among the isolates from the goat milk, 3 (13.04%) out of 23 isolates were found resistant to as much as 7 antibiotics out of 9 antibiotics tested followed by 9 (39.13%) isolates showing resistance to 5 antibiotics, 3 (13.04%) isolates for 4 antibiotics and 1 (4.35%) isolate each exhibited resistance to 3 and 2 antibiotics. Similarly all the 5 (100%) isolates recovered from goat nasal swabs showed resistance to 3 antibiotics. Out of 8 *S. aureus* isolate from sheep milk, 1 (12.5%) isolate was found resistant to 5 antibiotics while the 5 (62.5%) isolates and 1 (12.5%) isolate exhibited resistance to 4 and 2 antibiotics respectively. Among 7 *S. aureus* isolates from sheep nasal samples, 1 (14.28%) isolate was found resistance to 6 antibiotics followed by 3 (42.86%) isolates resistance to 4 antibiotics and 2 (28.57%) isolates exhibited resistance to 3 antibiotics. The presence of multidrug resistance in staphylococcal isolates has been reported by several investigators (Wang *et al.,* 2008; Kumar *et al.,* 2010) [31, 12]. Shana *et al.* (2009) [21] reported 57% isolates of *S. aureus* from bovine mastitis from Rio de Janeiro to be resistant to one or more than one antibiotic of which 38% isolates were resistant to three or more antibiotics. Tyagi *et al.* (2013) studied the multiple drug resistance pattern of *S. aureus* isolates from mastitis and reported that out of 68

isolates tested, 36 isolates were resistant to single antibiotic while remaining 32 isolates exhibited multiple drug resistance involving two and more antibiotics. The authors found that 23.52% isolates were resistant to two antibiotics, 19.11% isolates were resistant to three antibiotics, 2.94% isolates were resistant to four antibiotics and only one isolate exhibited resistance to five antibiotics. The most common group of multiple drug resistance observed in present study was ampicillin + chloramphenicl + cephalixin + methicillin (12 isolates) followed by ampicillin + cephalexin + methicillin (10 isolates), ampicillin + lincomycin + cephalixin + methicillin (8 isolates). Three isolates showed multiple resistances for 6 antibiotics that involved ampicillin + chloramphenicol+ tetracycline + lincomycin + cephalixin + methicillin (1 isolate) and ampicillin + streptomycin + chloramphenicol + lincomycin + cephalixin + methicillin (2 isolates). While 3 isolates were resistant to seven antibiotics with a combination of amplicillin + streptomycin + chloramphanicol + lincomycin + cephalixin + tetracycline + methicillin. Almost similar results were also reported by Singh (2015) [23] in *S. aureus* isolates from cattle mstitic milk and nasal swabs. Shana *et al.* (2009) [21] found the predominant resistance pattern ampicillin/penicillin in 42% isolates, either alone or in combination. WHO report (2002) suggested that overuse and misuse of antibacterial agent could be responsible as the major selective force leading to the development of bacterial resistance. The results very clearly indicate a very high incidence to Multiple Drug Resistance in *S. aureus* isolates from animals that has devastating implications as far as public health consequences are concern.

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