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ORIGINAL ARTICLE

Antimicrobial Resistance Pattern among Isolated Staphylococcus Aureus Causing Bovine Mastitis in District Khairpur, Sindh, Pakistan

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ABSTRACT

The objective of this study was to determine the incidence of bovine mastitis caused by Staphylococcus aureus and to evaluate antibiotic resistant pattern among isolated S. aureus causing bovine mastitis. A total of 400 cattle milk samples from 3 dairy farms of district Khairpur located in Thehri, Gambat and Khairpur were collected in sterilized bottles. Clinical mastitis was noted based on signs of inflammation in the udder and obvious changes in milk. Subclinical mastitis was evaluated using the California mastitis test (CMT). Bacteria were isolated and identified from milk samples based on cultural, microscopic and biochemical tests. The isolated strains were then tested for their antibiotic sensitivity profile by Kiry-Baur disc diffusion assay. Out of 400 milk samples tested, 255 samples (63.55%) were positive for mastitis as indicated by CMT. S. aureus was isolated from 109 samples (26.25%) as confirmed by yellow colored colonies on Mannitol salt agar, Gram positive cocci in bunches and positive for catalase, coagulase, DNase and beta hemolysis. Area wise prevalence of S. aureus was noted as 31.34%, 28.57% and 21.8% in Khairpur, Thehri and Gambat respectively. S. aureus strains were highly resistant to the antibiotic Meropenem (81.6%) followed by Cefixime (61.4%) Cefotaxime (60.55%), Oxycillin (55%), Erythromycin (54%), Ampicillin (51.3%), Ciprofloxacin (38%), Cefradine (37.6%), Sulphametho-Trim (21.1%), Vancomycin (21.1%), Doxycycline (21.1%) and Amoxicillin-clavulanate (5.5%). This study suggests that clinical and subclinical form of S. aureus associated mastitis is highly prevalent among dairy cattle of district Khairpur and S. aureus has become resistant to most of the antibiotics. Hygiene status of animal is very much essential to control mastitis and to prevent the spread of resistant bacteria to other animals.

Keywords: Mastitis; Staphylococcus aureus; Antibiotic Resistance

INTRODUCTION

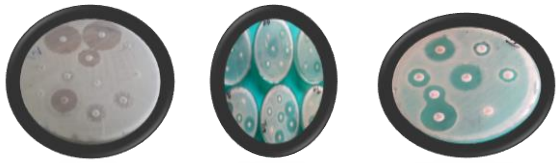
Milk is considered as an excellent medium for growing of many microorganisms. Milk can be contaminated with several bacteria during milking process from the milking personnel, utensils used for milking (Tanzin et al., 2016). Besides, microorganisms may enter the udder through teat canal, and the bacteria may come out through milk (Smith et al., 2007). Staphylococcus aureus is a major contaminant of milk. The presence of pathogen in surrounding environment mainly Staphylococcus aureusis the main pathogen that causes bovine mastitis, it is prevalent in all around the world, due to poor hygiene and Long-term antibiotic usage in cattle enhances in the development of resistance against methicillin and other beta-lactam antibiotic that are being injected by the owners. Despite all strict control measure the pathogen is not being eradicated from the local environment. Antibiotic susceptibilities of S. aureus may help veterinarian for Antimicrobial therapy, the measures can be taken in order to control of staphylococcal mastitis, Detection of antibiotic susceptibilities of clinical isolates is necessary not only for treatment but also for preventing spread of resistant isolates.(Aarestrup et, al., 19952).The currently observed S.aureus are known to be having different genotypic and phenotypic characteristics ,few are about geographical distribution of those isolates and types of the pathogen in the herd. Different methods have been adapted to diagnose physical changes in milk and isolation of effecting agents for subclinical Mastitis (Raza et, al., 2003) the research work was done on Prevalence and risk factors associated with sub-clinical mastitis by (Baloch et al., 2013) in the past the work has been done on taxonomic method specially phage typed had been applied to humans and cattle originated S.aureus isolates(Zadoks et al., 2002) researchers also find out plasmids analysis ,pulsed-field gel electrophoresis ,ribotyping, PCR-based fingerprinting ,genes which are being amplified on specific regions, and binary typing technique ware started to be applied(. Myllys et al., l998). This study helps To observe the antimicrobial resistance pattern among isolated S.aureus recovered from cattles infected with mastitis in khairpur district also performing molecular typing on coagulase gene polymorphism.

MATERIAL AND METHODS

The sample of milk were taken during the mid-lactation period from the different forms of Khairpur district ,only positive sample which are observed by performing California Mastitis Test (CMT) were taken to laboratory .400 sample ware taken from these samples 109 ware mastitis positive .The samples were inoculated onto Nutrient agar supplemented with 7% sheep blood, incubated at 37°C for 24-48 h,(32)thirty two S. aureus has been isolated also identified by the conventional tests such catalase and coagulase positive (slide and tube), hemolysis, pigment Mannitol Salt Agar (MSA), DNase Agar (Bisping et al., 1998 : Quinn et al., 1998).

Gram positive cocci were further identified with conventional biochemical test, the isolates were kept at -70°C preserved 16 % glycerin Brath. Isolated S.aureuswas father checked for Antimicrobial susceptibility test according to with National Committee for Clinical Laboratory Standards-NCCL (NCCLS document M2-A8, Wayne, PA 2003.). The isolates were tested against to the following antibiotics: S. aureus ATCC 25923 was used as control strain (Quinn et al., 998) To evaluate the

significance between antimicrobial sensitivities or resistances of S. aureus isolates Chi square test was performed.



All the S. aureus isolates were subjected to antibiotic sensitivity testing by standard disc diffusion method on Muller-Hinton agar (Oxoid) according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. Sensitivity pattern of isolates to Ampicillin (10µg), Ciprofloxacin (5 µg), Cephradine, (5 µg) Erythromycin (15µg), Doxycycline (30 µg), Cefixime (5 µg), Sulphamethoxazole-Trimethoprim(10µg), Vancomycin (30µg), Meropenem (30 µg), Amoxycillin clavulanate (30µg) Cefotaxime (10 µg)and Oxacillin (5 µg) was determined.

Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc: susceptible, intermediately susceptible, and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016).

RESULTS

Out of 400 milk samples tested, 255 samples (63.55%) were positive for mastitis as indicated by CMT. S. aureus was isolated from 109 samples (26.25%) as confirmed by yellow colored colonies on Mannitol salt agar, Gram positive cocci in bunches and positive for catalase, coagulase, DNase and beta hemolysis. Area wise prevalence of S. aureus was noted as 31.34%, 28.57% and 21.8% in Khairpur, Thehri and Gambat respectively. S. aureus strains were highly resistant to the antibiotic Meropenem (81.6%) followed by Cefixime (61.4%) Cefotaxime (60.55%), Oxycillin (55%), Erythromycin (54%), Ampicillin (51.3%), Ciprofloxacin (38%), Cefradine (37.6%), Sulphametho-Trim (21.1%), Vancomycin (21.1%), Doxycycline (21.1%) and Amoxicillin-clavulanate (5.5%).

Table 1: Antimicrobial susceptibilities of S. aureus isolates

Table no 01Antibiotics and their standard zone size suggested by CLSI

S/No	Antibiotics	Abb. Disc potency	Standard		
			R	I	S
1	Meropenem	MEM 30	<19	20 -22	>23
2	Oxacillin	OX1	<10	11-15	>22
3	sulfamethoxazole-Trimethoprim	SXT 10	<10	11-15	>16
4	Ciprofloxacin	CIP 5	<15	16-20	>21
5	Vancomycin	VA 30	<2	4-8	>16
6	Cefixime	CFM 5	<13	14-18	>19
7	Amoxicillin/clavulanate	AMC 30	<13	14-17	>18
8	Ertrhroymycin	E 15	<11	12 -14	>15
9	Ampicilin	AMP 10	<13	14 -16	>17
10	Doxycycline	DO 30	<9	10-12	>18
11	Cefradine	CEF 5	<0	10-12	>18
12	Ceftaxime	CTX 10	<22	23-25	>26

Table no 02 Antibiotic Susceptibility of *S. aureus* Test result of Khairpur

S/No	Antibiotics	Abb. Disc potency	± Zone measurement in mm (Average)									
			K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
1	Meropenem	MEM 30	0	0	0	0	0	0	0	0	0	0
2	Oxacillin	OX1	22	0	0	14	0	3	0	10	0	0
3	Sulfametho- Trim	SXT 10	23	0	10	30	0	22	0	35	25	20
4	Ciprofloxacin	CIP 5	0	16	0	0	25	0	3	0	0	0
5	Vancomycin	VA 30	12	12	0	17	13	0	16	29	0	0
6	Cefixime	CFM 5	0	0	0	0	0	0	0	0	0	0
7	Amoxicillin/Calvunate	AMC 30	19	10	0	10	8	12	18	0	0	13
8	Ertrhroymycin	E 15	0	15	0	15	10	6	0	6	28	18
9	Ampicilin	AMP 10	14	0	0	0	10	16	0	26	10	0
10	Doxycycline	DO 30	20	10	12	24	25	16	15	26	10	0
11	Cefradine	CEF 5	0	0	5	7	0	2	5	9	0	12
12	Ceftaxime	CTX 10	0	0	28	0	27	0	26	0	0	0

Table no 03 Antibiotic Susceptibility of *S. aureus* Test result of Khairpur

S/No	Antibiotics	Abb. Disc potency	Zone measurement in mm (Average)									
			K11	K12	K13	K14	K15	K16	K17	K18	K19±	K20
1	Meropenem	MEM 30	8	0	11	0	17	0	0	9	0	0
2	Oxacillin	OX1	0	10	15	0	14	0	0	8	17	0
3	Sulfametho- Trim	SXT 10	30	20	17	28	20	11	28	0	32	0
4	Ciprofloxacin	CIP 5	10	0	7	16	35	11	33	16	27	18
5	Vancomycin	VA 30	0	16	0	19	21	23	18	28	12	21
6	Cefixime	CFM 5	12	16	13	12	18	28	0	7	21	8
7	Amoxicillin/clavulanate	AMC 30	0	0	15	0	0	12	29	22	18	0
8	Ertrhroymycin	E 15	16	35	11	33	15	0	0	0	12	0
9	Ampicilin	AMP 10	19	21	23	18	28	16	18	14	18	0
10	Doxycycline	DO 30	12	18	28	0	7	10	0	7	16	37
11	Cefradine	CEF 5	0	0	12	29	22	0	16	0	19	0
12	Ceftaxime	CTX 10	9	23	21	30	22	9	0	11	0	26

Table no 04 Antibiotic Susceptibility of *S. aureus* Test result of Khairpur

S/No	Antibiotics	Abb. Disc potency	Zone measurement in mm (Average)									
			K12	K22	K23	K24	K25	K26	K27	K28	K29	K30
1	Meropenem	MEM 30	0	0	20	20	0	0	0	23	26	20
2	Oxacillin	OX1	22	0	0	0	10	10	22	10	29	0
3	Sulfametho- Trim	SXT 10	23	0	18	30	13	22	0	35	22	0
4	Ciprofloxacin	CIP5	0	16	0	25	25	0	40	40	0	22
5	Vancomycin	VA30	13	12	0	13	17	16	15	19	29	0
6	Cefixime	CFM 5	0	0	19	0	0	5	0	0	18	21
7	Amoxicillin/clavulanate	AMC 30	19	10	12	10	8	12	0	18	19	14
\8	Ertrhroymycin	E 15	0	15	15	15	11	0	0	28	25	13
9	Ampicilin	AMP10	12	12	0	0	14	17	16	0	20	18
10	Doxycycline	DO 30	20	10	10	20	21	16	15	6	17	13
11	Cefradine	CEF 5	0	0	0	18	0	0	8	0	0	0
12	Ceftaxime	CTX 10	0	0	22	18	0	0	0	23	18	26

Table no 05 Antibiotic Susceptibility of S. aureus Test result of Khairpur

S/No0	Antibiotics	Abb. Disc potency	Zone measurement in mm (Average)								
			K31	K32	K33	K34	K35	K36	K37	K38	K39	K40
1	Meropenem	MEM 30	0	0	20	0	0	18	23	0	0	11
2	Oxacillin	OX1	0	10	22	0	15	0	27	0	17	27
3	Sulfametho- Trim	SXT 10	18	20	35	29	35	11	33	15	22	33
4	Ciprofloxacin	CIP 5	16	25	0	0	21	23	18	28	20	18
5	Vancomycin	VA 30	10	10	16	12	16	28	0	7	22	0
6	Cefixime	CFM 5	0	0	0	0	0	12	29	29	0	29
7	Amoxicillin/Calvunate	AMC 30	0	14	13	18	14	18	32	0	19	32
8	Ertrhroymycin	E 15	0	0	10	0	7	19	20	24	23	20
9	Ampicilin	AMP 10	0	18	0	16	0	19	0	12	22	0
10	Doxycycline	DO 30	27	11	13	15	22	10	28	10	20	28
11	Cefradine	CEF 5	0	0	0	0	10	0	11	0	23	11
12	Ceftaxime	CTX 10	26	0	0	13	28	30	23	0	4	23

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We found some intermediate resistant isolates to like **Erythromycin**. The possession of such factors by the S.aureusisolates signifies the fact that the intermediate

resistance organisms may gain resistance property due to the indiscriminate use of antibiotics **Amoxicillin/clavulanate** is most resistant and **Meropenemi**s most suspectable against this pathogen. The S.aureus should be considered as hazardous to health and advocate the preventing risk factors. However, in the present study **Cefotaxime** were proved to be the best antibiotics to treat. S.aureus infection/mastitis in cattle since they were highly effective.

Table no 06 Overall resistance of isolates to the different antibiotics

S.No	Antibiotics	% resistant
1	Meropenem	81.6
2	Oxacillin	55.0
3	sulfamethoxazole-Trimethoprim	21.1
4	Ciprofloxacin	38.53
5	Vancomycin	21.1
6	Cefixime	61.4
7	Amoxicillin/clavulanate	5.50
8	Ertrhroymycin	54.12
9	Ampicilin	51.3
10	Doxycycline	21.1
11	Cefradine	37.6
12	Cefotaxime	60.55

Prevalence of S. aureus in Khairpur city

Samples collected	No. of S. aureus isolates	% Prevalence
400	109	26.25

DISCUSSION

This approach mainly focused on contagious udder pathogen such as S. areurs which is spread primarily from one cattle to another during milking S arures infection remains the largest mastitis problem of dairy animals. Cure rate with antimicrobial therapy during lactations is very low due to this infection many animals become chronic. the antimicrobial susceptibility was conducted on randomly selected S.arures isolates and isolates ware tested for eight antimicrobials using the Kirby Bauer disc diffusion method (Quinn et al.,1994 : NCCLS,1997).

Antibiotic susceptibility of staphylococcal isolates was determined by disk diffusion method, on Muller-Hinton agar (Merck). Antibiotic disks (PadtanTeb, Tehran, Iran) including methicillin (5 µg), streptomycin (10 µg), penicillin (10 U), amoxicillin (25 µg), tetracycline (30 µg), ampicillin (10 µg), neomycin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), and vancomycin (30 µg) were used for antibiotic susceptibility test. These antibiotics are used in the treatment of mastitis in Iran. The results of antibiotic susceptibility test were interpreted according to the Clinical and Laboratory Standards Institute. The antimicrobial susceptibility test was conducted on randomly selected S.aurerus isolates (n=32)isolated during the study.

Antimicrobial susceptibility test of all bacterial isolates was assayed on Muller-Hinton agar (Oxoid Ltd., UK) using disc diffusion method, as described by (Scrascia et al., 2003) and. Proper antibiotic discs were used to find out antimicrobial resistance profile of isolated bacteria antibiotics names ampicillin (10µg), amoxicillin-clavulanic acid (30µg), ceftazidime (30µg), cefotaxime (30µg),), ciprofloxacin (5µg), (30µg), Erythromycin (15µg), cefoxitin (30µg), Doxycycline (30µg), erythromycin (15µg)), Sulfa/trimethoprim (25µg) ofloxacin (30µg), and oxacillin (1µg) Cefradine (5µg) resistant, Intermediate ,susceptible it is according to the guidelines of NCCLS (1997).Antibiotic treatment may not be the hundred percent effective in certain cases, but it shorten the duration of infection even treatment decreases as the cow become older as cure rate were 34 present when 89 caws in 10 Dutch herds were treated for subclinical S.aureus of mastitis (sol et al.,1997).In Chhattisgarh state of India out of 300 ,164 isolates were positive of S.aureus . All 164 isolates of Staphylococcus aureus, were resistant to one or more antimicrobial agents tested by this method Resistance was detected highest against penicillin (83.5%), which agrees with the reports of (Abera et al., 2010: Shiferaw et al., 2009.) .Also in India, high resistance against penicillin reported by (Chandrashekaran et al., 2014).the s, aureurs was also resistant against other antibiotics like gentamicin 17% tetracycline 12.8%, cefepime ,linezolid 3.04% in their research ,whereas, S.aureus was less resistant against erythromycin and tetracycline.(Sori et al., 2011)reported almost similar results to the present work including clindamycin (4.8%) showing very less resistance, and (only) single isolate was found resistant to vancomycin which is in conference with work reported by (Pati and Mukherji 2016). in which all the isolates were susceptible to vancomycin. There is no such report available regarding

the significant amount of vancomycin resistant Staphylococcus aureus in dairy animals in India. The present study also revealed the presence of vancomycin intermediate Staphylococcus aureus, which is an indication of future vancomycin resistant strains of Staphylococcus aureus major human pathogen that causes a wide variety of diseases ranging in severity from food poisoning (Le Loir et al., 2003) and life-threatening toxic shock syndrome (Proft and Fraser 2003) to lesser infections, e.g boils (Stulberg et al., 2002). S. aureus can also cause several infections in animals, such as tick-associated pyaemia in lambs (Webster and Mitchell, 1989), staphylococci in rabbits (Hermans et al., 2003), oedematous and necrotic dermatitis, septicaemia, abcesses and chondronecrosis in chickens (McCullagh et al., 1998: McNamee et al., 1998: Takeuchi et al., 2002) and pneumonia and osteomyelitis complex in turkeys (Linares and Wigle 2001).

CONCLUSION

Damage Caused by Staphylococcus aureus in Mastitis produce toxins that destroy cell membranes and can directly damage milk-producing tissue. White blood cells (leukocytes) are attracted to the area of inflammation, where they attempt to fight the infection. Initially, the bacteria damage the tissues lining the teats and gland cisterns within the quarter, an antibiotic use during infection is common and can help to cure the infection caused by the pathogen.

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