

ANTIMICROBIAL SENSITIVITY OF BACTERIAL SPECIES ISOLATED FROM SUB-CLINICAL MASTITIS IN SHEEP

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ABSTRACT *Microorganism resistance against antibiotic is a major worldwide problem. Antibiotic sensitivity of the identified organisms was investigated in the present study. Twenty different antibiotics were applied and check their sensitivity pattern. The specie which was recorded highly sensitive (100%) to gentamycin was Staphylococcus aureus. Whereas, Bacillus cereus was noted 100% susceptible to chloramphenicol and oxytetracycline. While, Escherichia coli were detected as 100% sensitive to neomycin and van comic in. Micrococcus luteus was noted 100% susceptible to ampicillin. Corynebacterium pyogenes was noted 100% susceptible to kanamycin and cefaclor. Micrococcus luteus, Citrobacter and Proteus Vulgaris were also observed highly susceptible (100%) to ampicillin, neomycin and of loxacin respectively. Streptococcus uberis was noted 100% susceptible to penicillin, streptomycin, and oxytetracycline. It is suggested from present the study that proper treatment with antibiotics can control subclinical mastitis in sheep.*

Keyword: Antimicrobial sensitivity, Bacterial species, Sheep, Sub-clinical mastitis.

INTRODUCTION

Mastitis is classified into clinical and subclinical mastitis. Clinical mastitis is diagnosed clinically by observations, whereas, subclinical mastitis is diagnosed by bacterial culture as well as somatic cell count. In general mastitis is usually caused by bacterial infection and lack of management and hygienic practices during milking of animals [1-3]. Mastitis is the inflammation of the mammary gland that can cause economical losses in sheep all around the world including Pakistan [4-7]. Economic losses in terms of decreased milk production, alteration in milk composition, treatment cost, mortality of lambs and potentially premature culling of ewes [8-11]. Similarly [12] studied clinical mastitis in sheep after inoculation of *S. aureus* in the mammary gland of sheep. Experimental infection revealed decrease in milk production and fat content without altering physicochemical properties of the milk. After treatment sheep were recovered from disease condition but mammary glands was completely nonfunctional. Staphylococcal infection is usually results in chronic infection having low rate of cure that results in low milk production [13] because several virulent factors possessed in *Staphylococcus aureus* that leads to its persistence in mammary tissue [14]. Mastitis can be treated by antibiotics after identification of the infectious agents. Antibiotic susceptibility test can be effective tool for the treatment against various pathogenic microorganism of mastitis. In sheep, treatment with antibiotics by using a combination of penicillin, nafcillin, streptomycin are seems to be effective in reducing bacterial content of mastitis pathogens after lambing. The purpose of the present study was to investigate the relative prevalence, incidence and to the biochemical profiles of bacterial species from sheep suffering from subclinical mastitis and to determine *In-vitro* susceptibility of bacterial species through different chemotherapeutic agents commonly available in the market for the treatment of subclinical mastitis.

MATERIAL AND METHODS

Two hundred milk samples of sheep, from surrounding of Tandojam were collected in sterilized specimen bottles and sent to the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam and Central Veterinary Diagnostic Laboratory, Tandojam. Before conducting experiment glassware were washed in 1% HCL solution for 24 hours, washed with distilled water and dried at 65°C in hot air oven. The clean dried glassware was sterilized at 165°C for 3h in hot air oven. Samples were cultured on Brain Heart Infusion agar, blood agar and MacConkey's agar etc. After culture bacterial species were identified by morphological, culture, biochemical i.e Coagulase, Catalase, Oxidase and TSI and sugar fermentation characteristics [15]. The antibiotic sensitivity test was performed by method described by [16]. Following antibiotics were used: amikacin 30µg, amoxicillin 10µg, ampicillin 25µg, bacitracin 10µg, cefuroxime sodium 30µg, cefixime 30µg, colistinsulphate 10µg, cefaclor 30µg, cefoxitin 30µg, cloxacillin 5µg, chloramphenicol 30µg, enrofloxacin 5µg, erythromycin 5µg, erythromycin 10µg, furazolidone, gentamicin 10µg, kanamycin 5µg, kanamycin 30µg, lincomycin 10µg, metronidazole 25µg, norfloxacin, neomycin 30µg, ofloxacin, oxytetracycline 30µg, penicillin G 5µg, penicillin G 10µg, rifampicin 5µg, streptomycin 30µg, sulphamethoxazole trimethoprim 25µg, vancomycin 30µg. Muller Hinton agar was used for the sensitivity tests. Pure colonies were picked and dispensed in barium chloride. The cotton swab were sterilized and dipped in the bacterial solution and rolled over entire surface of agar medium. Antibiotic discs were placed on the surface of Muller Hinton agar plate by disc dispenser and slightly pressed with sterile forceps to make it adhere to the surface of the medium. The plates were closed, inverted and incubated at 37°C for 24 h. Zones of inhibition were recorded by following parameters:

RESULTS AND DISCUSSION

Description	Significance	Sensitivity level
Absence of clear zone	No sensitivity	-
Clear zone with 0.9-3mm	Weakly sensitive	+
Clear zone with 3-7 mm	Moderately sensitive	++
Clear zone with 7-11mm	Quite sensitive	+++
Clear zone with 11-18mm	Highly sensitive	++++

Eight bacterial species i.e *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Micrococcus Luteus*, *Corynebacterium pyogenes*, *Citrobacter species*, *Proteus vulgaris*, *Streptococcus uberis* were recognized. The antibiotic susceptibility was carried out against bacterial species recovered from the cases of subclinical mastitis in sheep. The results regarding the efficacy of antibiotics against Bacterial species are recorded and summarized in the Table 1.

Sensitivity test against *Staphylococcus aureus* was performed and found highly sensitive to gentamicin (100%). While the quite effective antibiotics against the organism were erythromycin, oxytetracycline, rifampicin and their efficacy was recorded as 68.75, 62.5 and 87.5% respectively. Similar results regarding the susceptibility of *Staphylococcus aureus* to gentamicin and other drugs were noted [17]. Antimicrobial sensitivity test was performed against *Staphylococcus* isolates. Results showed that cephalothin, sulfamethoxazole-trimethoprim, amoxicillin + clavulanic acid enrofloxacin, gentamicin, and erythromycin were the most effective antibiotics and their sensitivity was recorded as 97.4%, (97.4%, 97.4%, 94.9%, 92.3%, 84.6% respectively [18]. Whereas, [19] stated that gentamycin, kanamycin, chloramphenicol, oxytetracycline were highly effective drugs with sensitivity of 93.68%; 91.43%; 87.20%, 40.90% efficacy against *Staphylococcus aureus*. Our results are not in agreement with a study by Devrajani [20] who reported that gentamycin was resistant but highly effective for tetracycline 96.42% which may be because of isolate from different host species.

Bacillus cereus was recorded as sensitive to chloramphenicol, oxytetracycline and their efficacy was recorded as 100%. While the quite effective antibiotics against the organism were furazolidone and vancomycin, their efficacy were recorded as 93.33, and 86.66% respectively. Antibiotic susceptibility of *Bacillus cereus* carried out by [21] who recovered the species from different animals and used similar antibiotics through disc diffusion technique for *Bacillus cereus* and observed ofloxacin (100%) as highly sensitive drug. Our results are not in agreement with Finnegold and Baron [22] who recorded *Bacillus cereus* as highly sensitive to gentamicin. During present study ampicillin was found 100% effective drug while chloramphenicol were measured the second most highly active drugs against *Micrococcus luteus* and their efficacy was recorded 92.30%. Whereas, cefuroxime sodium was observed as quite sensitive and its sensitivity was measured 78.57 %. Furthermore, [23]

recorded that gentamycin and tetracycline were highly sensitive drugs against *Micrococcus citreus*.

Corynebacterium pyogenes was observed highly sensitive to kanamycin, cefaclor and their efficacy against tested organism was recorded as 100% and the second most highly active drugs was gentamicin and showed 92.30%. Our results are in agreement with some authors [24] who tested the susceptibility of *Micrococcus*, *Streptococcus*, *Staphylococcus aureus*, *Corynebacterium* and other organisms, using Muller and Hinton agar through Disc Diffusion Technique [16]. All above bacterial species showed some susceptibility to ampicillin, streptomycin, gentamicin, penicillin and tetracycline. Similar results were observed by authors [25] who reported that above species were highly susceptible to various antibiotics.

Escherichia coli was observed highly sensitive to neomycin and vancomycin, their efficacy against tested organism was recorded as 100%. While second most highly effective drug demonstrated during investigation was ofloxacin 83.30% sensitivity. Similar results were obtained by [25] conducted *In-vitro* antibiotic sensitivity and reported that *Escherichia coli* was susceptible to gentamicin, chloramphenicol and exhibited resistance to kanamycin, neomycin, tetracycline, ampicillin, polymyxin and sulphamethoxazole. However, the results of the present study revealed that neomycin and vancomycin were highly effective drugs against *Escherichia coli*. Our results are not in agreement with [26] who reported *Escherichia coli* as susceptible to gentamicin, kanamycin and chloramphenicol. Buriro [27] recorded chloramphenicol 58.8% and gentamycin 47.0% were effective drugs against *Escherichia coli*, while ineffective against ampicillin, penicillin and sulphonamide.

Citrobacter species showed 100% susceptibility to neomycin while the second highest effective drugs noted was cefuroxime and their effects against were measured as 90%. While, similar results were observed by [26] observed that the organism was highly sensitive to pipemidic and its sensitivity was recorded as 87.5%. While second highly active drug against the species observed was Cefuroxime and its efficacy noted as 68.75%.

Proteus vulgaris showed that the organism was highly sensitive to ofloxacin (100%) and chloramphenicol and showed (94.44%). While penicillin, streptomycin, oxytetracycline were found 100% effective drug while norfloxacin was measured the second most highly active drug against *Streptococcus uberis* and its action recorded was 94.11%. In study by [28] bactericidal activity of amoxicillin with gentamicin which was found more active than ampicillin+colistin or benzylpenicillin + streptomycin against 15 *Streptococcal* strains isolated from cattle, sheep and goats with pneumonia or mastitis. The results obtained for the antibiogram study for *Proteus vulgaris* and *Streptococcus uberis* in the present study are in agreement with report by above authors because we got similar response from same antibiotics against the bacterial species.

Table No 1. Antibiotic sensitivity of bacterial organisms identified from subclinical mastitic milk samples of sheep

Bacterial species	Antibiotics	Zone of inhibition around discs (mm)	Indication of sensitivity	Sensitivity (%)	Degree of sensitivity
<i>Staphylococcus aureus</i>	Amikacin	6	++	37.5	Moderately sensitive
	Ampicillin	0	-	0	No sensitivity
	Cefuroxime sodium	0	-	0	No sensitivity
	Cefaclor	0	-	0	No sensitivity
	Cloxacillin	6	++	37.5	Moderately sensitive
	Chloramphenicol	9	+++	56.25	Quite sensitive
	Enrofloxacin	8	+++	50	Quite sensitive
	Erythromycin	11	++++	68.75	Highly sensitivity
	Furazolidone,	9	+++	56.25	Quite sensitive
	Gentamycin	16	++++	100	Highly sensitivity
	Kanamycin	0	-	0	No sensitivity
	Lincomycin	2	+	12.5	Weakly sensitive
	Norfloxacin	4	++	25	Moderately sensitive
	Neomycin	5	++	3.25	Moderately sensitive
	Ofloxacin,	6	++	37.5	Moderately sensitive
	Oxytetracycline	10	+++	62.5	Quite sensitive
	Penicillin G	0	-	0	No sensitivity
	Rifampicin	14	++++	87.5	Highly sensitivity
Streptomycin	0	-	0	No sensitivity	
Vancomycin	8	+++	50	Quite sensitive	
<i>Bacillus cereus</i>	Amikacin	0	-	0	No sensitivity
	Ampicillin	6	++	40	Moderately sensitive
	Cefuroxime sodium	5	++	33.33	Moderately sensitive
	Cefaclor	0	-	0	No sensitivity
	Cloxacillin	10	+++	66.66	Quite sensitive
	Chloramphenicol	15	++++	100	Highly sensitivity
	Enrofloxacin	6	++	40	Moderately sensitive
	Erythromycin	5	++	33.33	Moderately sensitive
	Furazolidone	14	++++	93.33	Highly sensitivity
	Gentamycin	6	++	40	Moderately sensitive
	Kanamycin	0	-	0	No sensitivity
	Lincomycin	0	-	0	No sensitivity
	Norfloxacin,	8	+++	53.33	Quite sensitive
	Neomycin	0	-	0	No sensitivity
	Ofloxacin,	0	-	0	No sensitivity
	Oxytetracycline	15	++++	100	Highly sensitivity
	Penicillin G	0	-	0	No sensitivity
	Rifampicin	5	++	33.33	Moderately sensitive
Streptomycin	0	-	0	No sensitivity	
Vancomycin	13	++++	86.66	Highly sensitivity	
<i>Escherichia coli</i>	Amikacin	2	+	16.66	Weakly sensitive
	Ampicillin	4	++	33.33	Moderately sensitive
	Cefuroxime sodium	0	-	0	No sensitivity
	Cefaclor	9	+++	75	Quite sensitive
	Cloxacillin	0	-	0	No sensitivity
	Chloramphenicol	0	-	0	No sensitivity
	Enrofloxacin	2	+	16.66	Weakly sensitive
	Erythromycin	8	+++	66.66	Quite sensitive
	Furazolidone,	5	++	41.66	Moderately sensitive
	Gentamycin	0	-	0	No sensitivity
	Kanamycin	0	-	0	No sensitivity
	Lincomycin	0	-	0	No sensitivity
	Norfloxacin,	0	-	0	No sensitivity
	Neomycin	12	++++	100	Highly sensitive
	Ofloxacin,	10	+++	83.33	Quite sensitive
	Oxytetracycline	2	+	16.66	Weakly sensitive
	Penicillin G	0	-	0	No sensitivity
	Rifampicin	0	-	0	No sensitivity
Streptomycin	2	+	16.66	Weakly sensitive	
Vancomycin	100	++++	100	Highly sensitive	

<i>Micrococcus luteus</i>	Amikacin	4	++	28.57	Moderately sensitive
	Ampicillin	14	++++	100	Highly sensitivity
	Cefuroxime sodium	11	+++	78.57	Quite sensitive
	Cefaclor	0	-	0	No sensitivity
	Cloxacillin	0	-	0	No sensitivity
	Chloramphenicol	13	++++	92.30	Highly sensitivity
	Enrofloxacin	0	-	0	No sensitivity
	Erythromycin	0	-	0	No sensitivity
	Furazolidone,	6	++	42.85	Moderately sensitive
	Gentamycin	0	-	0	No sensitivity
	Kanamycin	0	-	0	No sensitivity
	Lincomycin	3	+++	21.57	Moderately sensitive
	Norfloxacin,	4	+++	28.71	Moderately sensitive
	Neomycin	0	-	0	No sensitivity
	Ofloxacin,	8	+++	57.14	Quite sensitive
	Oxytetracycline	5	++	35.71	Moderately sensitive
	Penicillin G 10	0	-	0	No sensitivity
	Rifampicin	8	+++	57.41	Quite sensitive
Streptomycin	0	-	0	No sensitivity	
Vancomycin	0	-	0	No sensitivity	
<i>Corynebacterium pyogenes</i>	Amikacin	3	++	23.07	Moderately sensitive
	Ampicillin	0	-	0	No sensitivity
	Cefuroxime sodium	0	-	0	No sensitivity
	Cefaclor	13	++++	100	Highly sensitivity
	Cloxacillin	6	++	46.15	Moderately sensitive
	Chloramphenicol	0	-	0	No sensitivity
	Enrofloxacin	8	+++	61.53	Quite sensitive
	Erythromycin	0	-	0	No sensitivity
	furazolidone	9	+++	69.23	Quite sensitive
	Gentamycin	12	++++	92.30	Highly sensitivity
	Kanamycin	13	++++	100	Highly sensitivity
	Lincomycin	4	++	30.76	Moderately sensitive
	Norfloxacin,	0	-	0	No sensitivity
	Neomycin	0	-	0	No sensitivity
	Ofloxacin,	0	-	0	No sensitivity
	Oxytetracycline	5	++	38.46	Moderately sensitive
	Penicillin G	0	-	0	No sensitivity
	Rifampicin	6	++	46.15	Moderately sensitive
Streptomycin	2	+	15.38	weakly sensitivity	
Vancomycin	0	-	0	No sensitivity	
<i>Citrobacter species</i>	Amikacin	0	-	0	No sensitivity
	Ampicillin	4	+	36.36	Moderately sensitive
	Cefuroxime sodium	10	++++	90.90	Quite sensitive
	Cefaclor	0	-	0	No sensitivity
	Cloxacillin	8	+++	72.72	Quite sensitive
	Chloramphenicol	0	-	0	No sensitivity
	Enrofloxacin	5	++	45.45	Moderately sensitive
	Erythromycin	4	++	33.33	Moderately sensitive
	Furazolidone,	9	+++	81.81	Quite sensitive
	Gentamycin	0	-	0	No sensitivity
	Kanamycin	0	-	0	No sensitivity
	Lincomycin	6	++++	54.54	Moderately sensitive
	Norfloxacin,	0	-	0	No sensitivity
	Neomycin	11	++++	100	Highly sensitivity
	Ofloxacin,	0	-	0	No sensitivity
	Oxytetracycline	2	+	18.18	weakly sensitivity
	Penicillin G	0	-	0	No sensitivity
	Rifampicin	9	+++	81.81	Quite sensitive
Streptomycin	3	++	27.27	Moderately sensitive	
Vancomycin	0	-	0	No sensitivity	

<i>Proteus vulgaris</i>	Amikacin	0	-	0	No sensitivity
	Ampicillin	4	++	22.22	Moderately sensitive
	Cefuroxime sodium	0	-	0	No sensitivity
	Cefaclor	10	+++	55.55	Quite sensitive
	Cloxacillin	0	-	0	No sensitivity
	Chloramphenicol	17	++++	94.44	Highly sensitivity
	Enrofloxacin	2	+	11.11	Weakly sensitive
	Erythromycin	4	+++	22.22	Moderately sensitive
	Furazolidone,	5	++	27.77	Moderately sensitive
	Gentamycin	6	++	33.33	Moderately sensitive
	Kanamycin	0	-	0	No sensitivity
	Lincomycin	8	+++	44.44	Quite sensitive
	Norfloxacin,	0	-	0	No sensitivity
	Neomycin	4	++	22.22	Moderately sensitive
	Ofloxacin,	18	++++	100	Highly sensitivity
	Oxytetracycline	2	+	11.11	Weakly sensitive
	Penicillin G	0	-	0	No sensitivity
	Rifampicin	12	+++	0	No sensitivity
Streptomycin	0	-	0	No sensitivity	
Vancomycin	4	+++	22.22	Moderately sensitive	
<i>Streptococcus uberis</i>	Amikacin	3	++	17.64	Moderately sensitive
	Ampicillin	2	-	11.76	weakly sensitivity
	Cefuroxime sodium	9	+++	52.94	Quite sensitive
	Cefaclor	0	-	0	No sensitivity
	Cloxacillin	0	-	0	No sensitivity
	Chloramphenicol	0	-	0	No sensitivity
	Enrofloxacin	10	+++	58.82	Quite sensitive
	Erythromycin	0	-	0	No sensitivity
	Furazolidone,	4	++	23.52	Moderately sensitive
	Gentamycin	5	++	29.41	Moderately sensitive
	Kanamycin	0	-	0	No sensitivity
	Lincomycin	4	++	23.52	Moderately sensitive
	Norfloxacin,	16	++++	94.11	Highly sensitivity
	Neomycin	0	-	0	No sensitivity
	Ofloxacin,	8	+++	47.05	Quite sensitive
	Oxytetracycline	17	++++	100	Highly sensitivity
	Penicillin G	17	++++	100	Highly sensitivity
	Rifampicin	8	+++	47.05	Quite sensitive
Streptomycin	17	++++	100	Highly sensitivity	
Vancomycin	0	-	0	No sensitivity	

CONCLUSION

In vitro antibiotic sensitivity of the identified organisms were carried out on twenty different antibiotics. *Staphylococcus aureus* was highly sensitive to gentamicin whereas, *Bacillus cereus* was observed highly sensitive to chloramphenicol, oxytetracycline and their action against the species was measured as 100%. While *Escherichia coli* were detected as 100% sensitive to neomycin and vancomycin. *Micrococcus luteus* was noted 100% susceptible to ampicillin. *Corynebacterium pyogenes* were detected as 100% sensitive to kanamycin and cefaclor. *Citrobacter* species were also observed highly susceptible to neomycin the action of the drugs was noted as 100% respectively. Moreover, present survey also showed the other bacterial species such as *Proteus vulgaris* were susceptible to ofloxacin, whereas, *Streptococcus uberis* was found 100% susceptible to penicillin, streptomycin and oxytetracycline respectively.

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