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RESEARCH ARTICLE

A STUDY ON THE ANTIMICROBIAL RESISTANCE PATTERNS OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DOMESTIC ANIMALS AND MAN

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ABSTRACT

All the nasal swabs collected from goat, pigs, dogs and from animal handlers were used for isolation of *Staphylococcus aureus*. From a total of 455 samples, 398 isolates were found as purple coloured cocci in clusters. Among the 398 isolates 122 isolates were confirmed as pathogenic *Staphylococcus aureus* by a positive coagulase test. The biochemical tests like IMViC tests, urease test, oxidase test, nitrate reduction test and catalase tests, confirmed the presence of *Staphylococcus aureus*. DNase test revealed the presence of blue to purple coloured colonies with clear zones around the colonies which are the characteristic colonies on DNase agar. On blood agar plates the isolates produced β haemolysis. A pannel of 11 antibiotic discs were tested by using the standard disc diffusion method. Among the 126 isolates all of them were not resistant to vancomycin (0%). Maximum resistance was observed for ciprofloxacin (56.78%), followed by ampicillin (48.24%), penicillin (43.96%), gentamycin (42.21%), streptomycin (37.68%), tetracycline (28.89%), erythromycin (23.86%), cephoxitin (23.11%), oxacillin (17.83%) and cephalothin (14.82%). For the detection of MRSA all the isolates were streaked on Hi-crome MeReSa agar plates and the results revealed that 4 isolates were grown as bluish-green coloured colonies.

INTRODUCTION

Staphylococcus is a versatile organism with several virulent characteristic and resistance mechanisms and was first identified in pus by the surgeon Sir Alexander Ogston in Aberdeen, Scotland in the year 1883 (Fowler Jr *et al*, 2006). The first observation pointing to the endogenous source of bacterial wound infection was made in 1915 by Sir Almorth Wright. Humans are the natural reservoirs for *Staphylococcus aureus* and asymptomatic colonization is far more common than the infection. Colonization of the nasopharynx, perineum or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may occur any time thereafter.

Fekety Jr (1964) revealed that direct contact transmission involves contact of body surface to physical transfer of *Staphylococcus aureus* to the host from an infected or colonized person. Williams (1959) reported that the nose is regarded as the major site of *Staphylococcus aureus* carriage from where the organism can spread to other parts of the body.

It is estimated that over 60% of the emerging human pathogens come from animals (Cutler *et al*, 2010). To feed the growing human population, there is an increasing demand for intensive animal farming involving large numbers of animals, different species in the same area, and the use of growth promoters and antibiotics. These practices can facilitate the emergence of new pathogens including antibiotic resistant organisms and their transmission to humans. In addition foods of animal origin can be a major vehicle for animal pathogens and their spread can be amplified by the market globalization.

There has been an increased concern throughout the world about the pathogenic micro organisms which are resistant to commonly used antibiotics for their control. Among different multi drug resistant pathogenic micro organisms *Staphylococcus aureus* is one of the most important bacteria, particularly its methicillin resistant strains. The antibiotic methicillin was introduced in to medical practice in 1960s, and now resistant strains of *Staphylococcus aureus* were found in human population throughout the world (Grundmann *et al*, 2006). Now MRSA has become a global health problem and

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there is a significant increase in both morbidity and mortality in humans throughout the world (Van Loo *et al*, 2007).

Staphylococcus aureus is a common human pathogen which can be capable of producing a wide variety of diseases, starting from skin and soft tissue infections to life threatening endocarditis, bacteraemia and necrotizing pneumonia (Gordon, 2008). Infections caused by *Staphylococcus aureus* have assumed a new public health significance due to the development of multi antibiotic resistant strains particularly MRSA and its epidemic clones that are increasingly being found in hospitals and communities. (HA-MRSA, CA-MRSA)

MATERIALS AND METHODS

The specimens selected for this study were nasal secretions. These secretions were collected by using sterile cotton swabs. Cotton swabs were sterilized in hot air oven at a temperature of 160^0C for 1 hour. Cotton tipped dry swab was inserted into the anterior nares of animals and human beings and rubbed gently against the mucosa for approximately 5 seconds and it was placed in normal saline. A total of 455 nasal swabs from anterior nare of animals and human beings were collected aseptically in sterile normal saline tubes. The collected specimens were processed within 2 to 24 hours of collection. The source and number of samples collected in this study are given in Table.1

For the isolation and identification of *Staphylococcus aureus* Tryptic soy broth was used for enrichment of inoculum. Baird Parker agar supplemented with 5% sterile egg yolk tellurite suspension was used for isolation of *Staphylococcus aureus*. Isolation was carried out by inserting the cotton tipped dry swabs into the anterior nares and rubbed gently against the mucosa for approximately 5 seconds and they were placed in normal saline tubes. Loop full of inoculum from the tubes was transferred to tryptic soy broth tubes and the tubes were incubated at 37^0C for 24 hours. After incubation a loop full of inoculum from tryptic soy broth tubes was streaked over Baird Parker agar with egg yolk tellurite plates and the plates were incubated at 37^0C for 48 hours. A smear was prepared from the growth on Baird Parker agar and it was stained with Grams method of staining. Gram positive bacteria were identified up to genus level as staphylococcus based on morphology. All the isolates were identified up to species level based on biochemical and sugar fermentation tests as per the methods described by Barrow and Felthan (1993) and Bergeys manual of systemic bacteriology (1984).

For confirmation of *Staphylococcus aureus*, the biochemical tests conducted were catalase test, DNase test, nitrate reduction test, oxidase test, urease test and IMViC tests, the sugars used for sugar fermentation tests were raffinose, sucrose, maltose, d-mannitol and d-mannose.

Tube coagulase test

A heavy suspension of the bacterial culture under test was inoculated into 0.5 ml of undiluted human plasma in a test tube and incubated in a water bath maintained at 37^0c. The tube was examined for coagulation at 30 min, 2 h, 4 h, 6 h and overnight. A positive reaction was indicated by the conversion of plasma into stiff gel, best recognized by its remaining in place when the tube was tilted or inverted.

Test for haemolysis

Isolates of *S. aureus* grown over night on baird parker agar plates were spot inoculated on tryptose soya agar plates containing 5% of disseminated sheep blood cells. The plates were incubated at 37^0C for 24 hs and appearance of haemolytic zone around the colony was recorded.

DNAase test

About 42 grams of DNAase test agar base was suspended in 1000 ml distilled water and the same was heated with frequent agitation to dissolve the medium completely and 0.1 gm Toluidine Blue (FD051) was added to the medium. The medium was sterilized by autoclaving at 12 to 15 lbs pressure (118°C to 121°C) for 15 minutes. After sterilization the medium was cooled to 45°C and approximately 15 to 20 ml of the medium was added to the sterile petriplates. The plates were incubated at 35-37°c for 18-24 hrs. The plates were observed for blue to purple coloured colonies with clear zones around the colonies.

The biochemically confirmed strains of Staphylococcus aureus were preserved by taking a loop full of the isolated organism and the same was added to the sterile tryptone soya glycerol broth vials and mixed well in vortex mixer. The vials were then labelled and stored at -20^0C.

Antimicrobial susceptibility testing

Antimicrobial sensitivity against 11 antibiotics was done as per the standard disc diffusion method of Bauer *et al* (1966).

Bacterial suspension was made by transferring 4-5 colonies from primary isolated medium i.e. Baird Parker agar with 5% egg yolk tellurite to 5ml of tryptic soya broth by touching the top of the colonies with a flame sterilized and cooled platinum loop. The resulting culture after incubation at 37^0C for 8 hours was compared with the turbidity standard prepared separately for adjustment of bacterial suspension. The turbidity standard was prepared by adding 0.5 ml of (1.17% w/v) Barium chloride dehydrate ($BaCl_2$ $2H_2O$) solution to 1% Sulphuric acid. The turbidity standard was placed in a tube identical to the one used for the broth sample and was stored in the dark at room temperature. The turbidity was equaivalent to 10^8 cfu/ml which is half the density of a Mac Farland 0.5 standard. The standard was agitated on a vortex mixer immediately before use. If the culture was found less turbid than the turbidity standard it was further incubated for 2-8 hours at 37^0C until turbidity was equivalent to the standard. If the turbidity exceeds that of the standard the culture solution was diluted with tryptic soya broth to equitate with the standard.

Commercially available standard antimicrobial discs (Hi-Media) were procured and stored at 2-8^0C in the refrigerator.

Unopened disc containers were removed from the refrigerator 1-2 hours before use, to bring them to room temperature. The antimicrobial discs with known concentrations as noted in micrograms (µg) or International Units (IU) per disc were used to study the antimicrobial susceptibility of the isolates. The antimicrobial discs used in this study are given in Table 2.

Muller–Hinton agar, the recommended medium for disc diffusion test was employed in this study. The prepared medium was autoclaved, when the temperature of medium reached between 45 – 50^{0}C, it was mixed well and approximately 15-20ml was added to the sterile petridishes and incubated overnight at 37^{0}C for sterility testing and the uncontaminated plates were wrapped with aluminium foil and they were stored at 4^{0}C till use. The sterile cotton swab was dipped in the standardized inoculum (turbidity so adjusted) and rotated several times. Then the cotton swab was gently pressed on the upper inside wall of the test tube to remove excess inoculum. The swab was then streaked over the entire surface of the muller-hinton agar plate for three times. The plate was turned at 60^{0}angle between each streak to ensure even distribution of the inoculum. A final sweep of the swab was made around the agar rim. Allowed the inoculums to dry for 5 to 15 minutes. Selected antimicrobial discs were placed at least 24mm apart by using a disc dispenser and gently pressed down on to the agar surface to provide uniform contact. The inoculated plates were inverted and incubated at 37^{0}C for 24-48 hours. Each plate was examined after incubation for the diameter of zones of complete inhibition including the diameter of the disc was measured up to the nearest whole millimetre with ruler in non reflecting background. The zone margin was the area where no obvious growth was visible and the readings were compared with that specified readings in the interpretive chart supplied by the manufacturer of the antibiotic discs and the results were documented as sensitive (S), intermediate (I) and resistant (R).

RESULTS

All the nasal swabs collected from the domestic and pet animals viz: goat, pigs, dogs and from humans who were in close association with the animals were inoculated into tryptic soy broth and incubated at 37^{0}C for 24 hours. After the incubation period a loop full of inoculum from trypticase soy broth tubes was streaked on Baird Parker agar plates by following all the aseptic precautions. The plates were incubated at 37^{0}C for 48 hours. The plates were observed for black and shiny colonies on Baird Parker agar plates (Fig. 1).

All the isolates which have shown characteristic colonies on baird parker agar were streaked on HiChrome Me Re Sa agar for confirmation of methicillin resistance and found characteristic bluish green colonies on the same (**Fig. 8**). The particulars of the samples which were positive for *Staphylococcus aureus* were given in Table 6. All the 398 isolates were subjected to Gram's staining and found purple coloured cocci in clusters **(Fig. 2).** Among the total isolates 122 isolates were confirmed as pathogenic *Staphylococcus aureus* by a positive coagulase test as shown in **Table 3** and **Fig. 7**

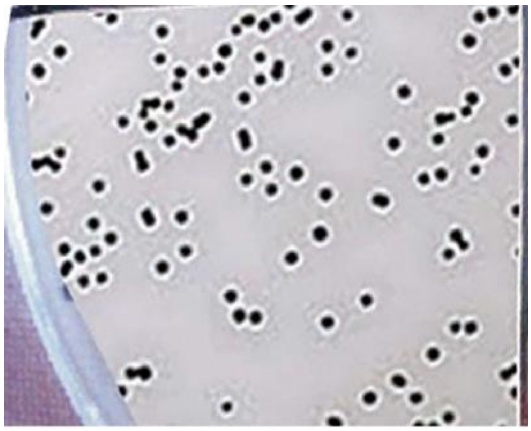

Fig. 1 Plate showing the growth of black and shiny colonies surrounded by clear zone of *Staphylococcus aureus* on Baird Parker Agar

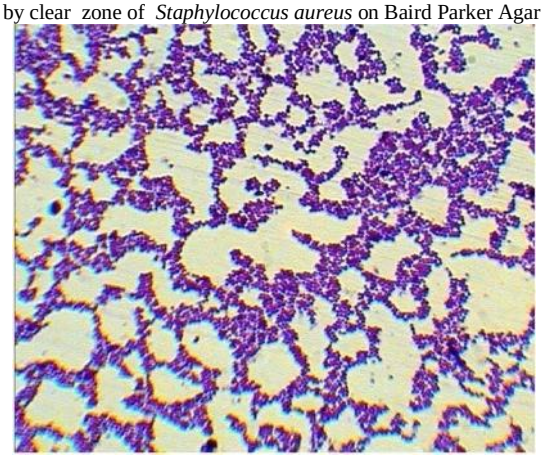

Fig. 2 Gram's staining of *Staphylococcus aureus* exhibited characteristic coccal clusters

The biochemical reactions of all the isolates were given in **Table 4**. The isolates were subjected to the biochemical tests like IMViC tests, urease test, oxidase test, nitrate reduction test, DNase test, blood agar plate test and catalase tests.

Table1 Source and number of samples collected

Species	Source of the samples	Number collected	Total
Goats	Kanigiri Mandal, Prakasam District, Andhra Pradesh	54	151
	Goat farm, College of Veterinary Science, Tirupati	20	
	Private goat farm, Thondawada, Chittoor District,Andhra Pradesh	19	
Pigs	Naidupet Mandal, Nellore District, Andhra Pradesh	58	102
	AICRP on pigs, College of Veterinary Science, Tirupati	54	
	Private pig farm, Tiruchanooru, Chittoor District,Andhra Pradesh	48	
Dogs	Veterinary clinical block, College of Veterinary Science, Tirupati	31	62
	Animal birth control centre, Tiruchanooru, Chittoor District, Andhra Pradesh	31	
Humans	Goat handlers	74	140
	Pig handlers of AICRP on pigs, College of Veterinary Science, Tirupati	12	
	Dog handlers	52	
	Animal handlers of department of Clinical Medicine, College of Veterinary Science, Tirupati	02	
	GRAND TOTAL		**455**

Table2 Antimicrobial discs used to study the antimicrobial susceptibility of the isolates

S.No	Name of the antimicrobial disc	Quantity of antimicrobial substance per disc	Diameter of zone of inhibition in mm (as per the manufacturer guidelines)		
			Sensitive	intermediate	resistant
1	Ampicillin	10 µg	29	-	28
2	Cephoxitin	30 µg	22	-	21
3	Cephalothin	30 µg	18	15-17	14
4	Ciprofloxacin	05 µg	21	16-20	15
5	Erythromycin	15 µg	23	14-22	13
6	Gentamycin	10 µg	15	13-14	12
7	Oxacillin	01 µg	13	11-12	10
8	Penicillin-G	10 units	26	-	28
9	Streptomycin	10 µg	22	-	14
10	Tetracyclin	30 µg	19	15-18	14
11	Vancomycin	10 µg	21	-	17

Table 3 Prevalence of *S. aureus* from various sourses

S.No	Sourse	No. of samples screened	No. of samples positive for *S. aureus*	No. of samples positive for coagulase test
1	Goats	151	115 (76.15%)	61 (40.39%)
2	Pigs	102	96 (94.1%)	23 (22.54%)
3	Dogs	62	58 (93.5%)	21 (33.87%)
4	Humans (Associated with animals)	140	129 (92.1%)	17 (12.14%)
	total	455	398 (87.47%)	122 (26.81%)

Fig. 3 Tests showing results of Voges-Proskauer
Left: Negative Right: Positive

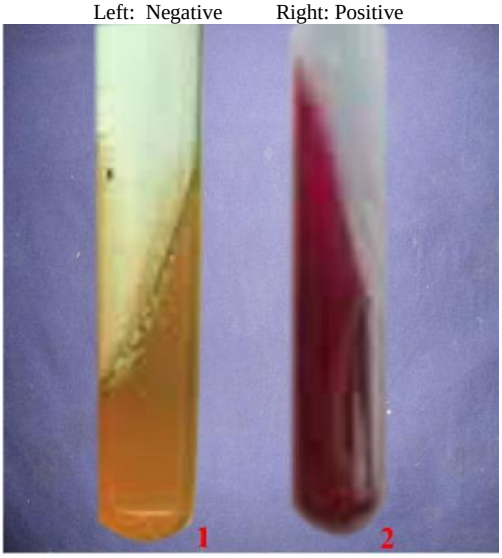

Fig. 4 Tests showing the results of urease test
Tube 1: urease negative
Tube 2: urease positive

Fig. 5 Tests showing results of nitrate reduction test
Left: Negative Right: Positive

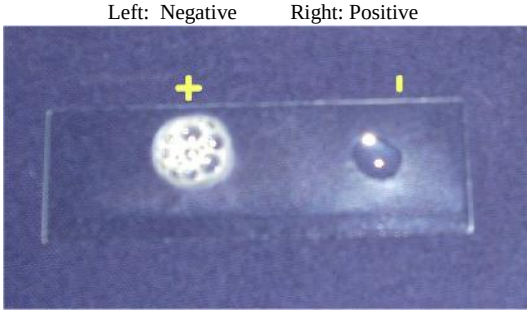

Fig. 6 Test showing result of catalase test

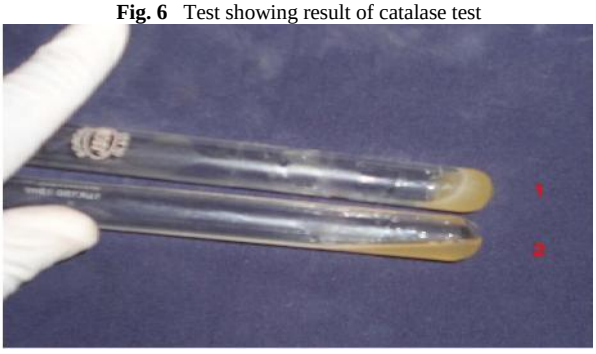

Fig. 7 Test showing result of Coagulase test
Tube 1: Coagulase positive
Tube 2: Coagulase negative

Table 4 Results of the confirmation tests for *S. aureus*

S.No	Name of the bio-chemical test	Number of isolates positive for the bio-chemical and other tests			
		Goats (n-151)	Pigs (n-102)	Dogs (n-62)	Humans (n-140)
1	Gram's staining	115	96	58	129
2	Indole test	0	0	0	0
3	Methyl red test	115	96	58	129
4	Voges-proskauer test	115	96	58	129
5	Citrate utilization test	0	0	0	0
6	Urease test	115	96	58	129
7	Oxidase test	0	0	0	0
8	Nitrate reduction test	115	96	58	129
9	Catalase test	115	96	58	129
10	Coagulase test	61	23	21	17
11	DNase test	62	23	21	17
12	Blood agar plate test	115	96	58	129

Table.5 Antimicrobial sensitivity/intermediate/resistant-pattern of *S. aureus* from different sources

S.No	Antimicrobial agent	Pattern of antibiogram		
		Sensitive (%)	Intermediate (%)	Resistant (%)
1	Ciprofloxacin	168(42.21)	4(1.0)	226(56.8)
2	Ampicillin	124(31.15)	82(20.60)	192(48.24)
3	Penicillin	154(38.69)	69(17.33)	175(43.96)
4	Gentamycin	134(33.60)	96(24.12)	168(42.21)
5	Streptomycin	150(44.17)	71(17.83)	150(37.68)
6	Tetracycline	127(31.90)	156(39.19)	115(28.89)
7	Erythromycin	195(48.99)	108(27.13)	95(23.86)
8	Cephoxitin	155(38.94)	151(37.93)	92(23.11)
9	Oxacillin	225(56.53)	102(25.62)	71(17.83)
10	Cephalothin	275(69.09)	64(16.08)	59(14.82)
11	Vancomycin	398(100.00)	0(0)	0(0)

All the isolates were negative for Indole and Citrate utilization tests. Whereas all the isolates produced bright red colour in methyl red test and red colour in Voges – proskauer test **(Fig.3)**. All the biochemical reactions confirmed the presence of *Staphylococcus aureus.* Further all the isolates were subjected to urease test **(Fig-4),** oxidase test, nitrate reduction test **(Fig-5)** and catalase test **(Fig-6).** The results revealed that all the isolates were positive for urease test, catalase test and nitrate reduction tests, on the other hand all of them were negative for oxidase test.

DNase test

The sterilized plates of DNase test agar base were streaked with the inoculums took from positive Baird Parker agar plates. The plates were incubated at 35-37^{o}C for 18-24 hrs and observed for blue to purple coloured colonies with clear zones around the colonies **(Fig. 9)**.

Fig. 9 Plate showing growth of purple coloured colonies of *Staphylococcus aureus* on DNAase agar.

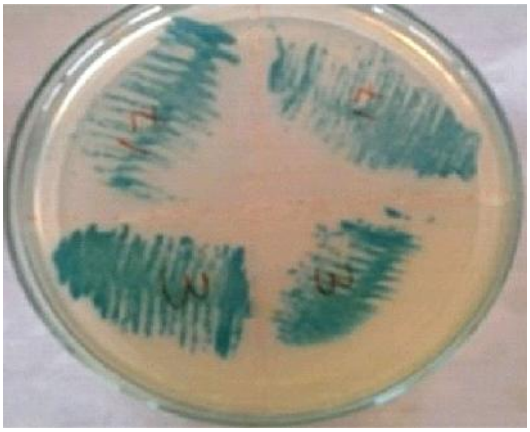

Fig. 8 Plate showing growth of bluish green coloured colonies of *Staphylococcus aureus* on Hichrome MeReSa agar.

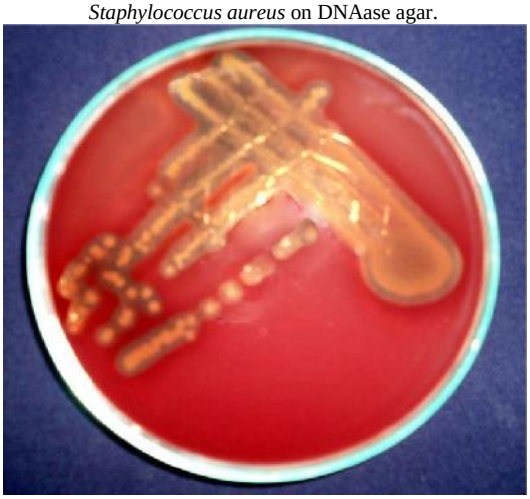

Fig. 10 Plate showing beta haemolysis of *Staphylococcus aureus* on blood agar

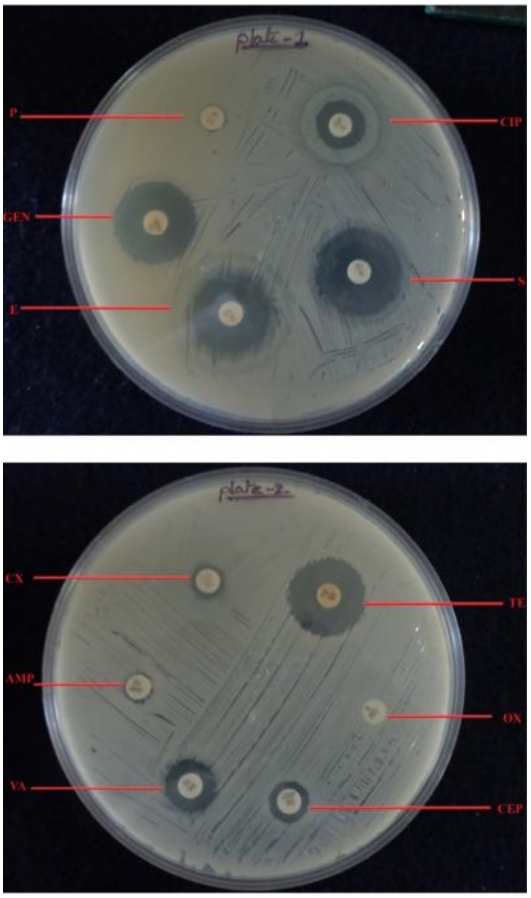

Fig. 11 & 12 Plates showing antibiotic resistance of *Staphylococcus aureus* on Muller-Hinton agar

Blood agar plate test

For further confirmation of *Staphylococcus aureus*, all the isolates were streaked on blood agar plates and incubated at 37^0C / 24 hours. The results revealed that all the isolates produced β haemolysis on blood agar plates (Fig.10).

Antimicrobial sensitivity testing

To detect the resistant/sensitivity pattern of *S. aureus* from different sources, *in-vitro* antibiotic sensitivity was carried out by disc diffusion as per the method of Bauer *et al.,* (1966) using 11 commercially available antibiotic discs. Muller Hinton agar plates showing the sensitivity, intermediate sensitivity and the resistance patterns of various antibiotic discs were shown in Table.8 and Figure. 11 & 12.

Antimicrobial sensitivity of isolates from samples

A total of 398 isolates were resistant to different antibiotic discs used in this study. The sensitivity patterns of the isolates for various antibiotic discs are given in Table 5. Among the 398 isolates all of them were not resistant to vancomycin (0%). Maximum resistance was observed for ciprofloxacin (56.78%), followed by ampicillin (48.24%), penicillin (43.96%), gentamycin (42.21%), streptomycin (37.68%), tetracycline (28.89%), erythromycin (23.86%), cephoxitin (23.11%), oxacillin (17.83%).and cephalothin (14.82%).

DISCUSSION

Staphylococcus aureus causes severe animal diseases such as suppurative diseases, mastitis, arthritis and urinary infections that are associated with various virulent factors such as the production of extracellular toxins and enzymes. For humans this organism is an important cause of food poisoning, pneumonia, post operative wound infections and nosocomial bacteraemia. Human isolates of *S. aureus*, unlike animal isolates, are frequently resistant to the penicillinase resistant penicillins (Kloos WE and Bannerman TL, 1995). An organism exhibiting this type of resistance is referred to as Methicillin (oxacillin) Resistant *Staphylococcus aureus* (MRSA). Such organisms are also frequently resistant to most of the commonly used antimicrobial agents including the aminoglycosides, macrolides, chloromphenicol, tetracyclines and fluroquinolones (Mandell *et al,* 1995). In addition MRSA strains should be considered to be resistant to all cephalosporins, cephems and other β- lactams such as ampicillin, sublactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactum and the carbapenems, regardless of the *in-vitro* test results obtained with those agents (National Committee for Clinical Laboratory Standards, 2001).

S.aureus isolated in this study was highly resistant to ciprofloxacin (56.61%) Similar type of results were obtained from the work carried out by Syed Zahid Bukhari *et al* (2011) where 75.80% resistance to ciprofloxacin was observed, Saravanan *et al* (2013) observed a lower resistance of 12.50%, Nwankwo Emmanuel Onwubiko and Nasiru Magaji Sadiq (2011) found 78.0% resistance, Shanthi and Uma Sekar (2009) observed 60% resistance, Jayatilleke and Bandara (2012) showed 54% resistance to ciprofloxacillin. The resistance to ampicillin was 48.24% in this study and the percentage of resistance can be considered as less when compared to the results of Kandle *et al* (2003) where they reported 90% resistance to ampicillin, Saravanan *et al* (2013) reported 100% resistance, Kitara *et al* (2011) found 75% resistance, Tekalign Kejela and Ketema Bacha (2013) observed 76.3% resistance to ampicillin on the contrary Dachen Tsering *et al* (2011) reported only 4.81% resistance to ampicillin.

The resistance to penicillin was 43.96% in this study. Nearer to these findings were observed in the study made by Nwankwo Emmanuel Onwubiko and Nasiru Magaji Sadiq (2011) where they have reported 31.2% resistance to penicillin where as Tekalign Kejela and Ketema Bacha (2013), Narasinga Rao and Prabhakar (2011), Saravanan (2013), Pandey *et al* (2012) and Hoerle Jairo and Adriano Brandelli (2009) have reported 100% resistance to penicillin whereas Kandle *et al* (2003) observed only 90% resistance.

The *Staphylococcus aureus* isolates in this study exhibited 42.21% resistance to gentamycin. Similar type of results were obtained by Jayatilleke and Bandara (2012) where they could found 44% resistance to gentamycin, whereas a high resistance to gentamycin than in the present study was observed by Saravanan *et al* (2013) found 100% resistance, Bilal Ahmed Mir and Srikanth (2013) observed 91.8% resistance, Nwankwo Emmanuel Onwubiko and Nasiru Magaji Sadiq (2011) reported

92.4% resistance and 97.6% resistance was found by Syed Zahid Bukhan *et al* (2011), where as Kitare *et al* (2011) observed no resistance to gentamycin. In the present study 37.68% of resistance was observed for streptomycin by the isolates. A little higher than the present results obtained in this study, Nwankwo Emmanuel Onwubiko and Nasiru Magaji Sadiq (2011) reported 44.2% resistance to streptomycin. and further Adekunle Odunayo Adejuwon *et al* (2010) reported (23%)resistance to streptomycin by the *S.aureus* isolates.

In the present study 23.86% of resistance was observed for erythromycin by the isolates. Abdul Rehman Baidani (2011) reported 31% resistance to erythromycin which was almost similar to the results obtained in this study, whereas high percentage of resistance was observed by Narasinga Rao and Prabhakar (2011).

where they observed 95.6% resistance, 98.3% resistance was found by Syed Zahid Bukhari *et al* (2011), 90% resistance by Kandle *et al* (2003), 52.4% resistance by Nwankwo Emmanuel Onwubiko and Nasiru Magaji Sadiq (2011), 62.5% resistance by Shanthi and Uma Sekar (2009), on the other hand least resistance of 4.12% and 7.8% was observed by Dachen Tsering *et al* (2011) and Kitare *et al* (2010) respectively.

The resistance to tetracycline was 28.89% in this study. Nearer to these results were obtained by Nwankwo Emmanuel Onwubiko and Nasiru Magaji Sadiq (2011) where he found 31.2% resistance to tetracyclines where as Narasinga Rao and Prabhakar, (2011) found 68.5% resistance and Kitare *et al* (2011) observed 45.3% resistance to tetracyclines.

The resistance of cephoxitin in the present study is 23.11% which is almost similar to the resistance observed by Saravanan *et al* (2013) where he observed 25% resistance to cefoxitin. The resistance to oxacillin in this study is 17.83%. High resistance to oxacillin than in the present study was observed by Abdul Rahman Baidani *et al* (2011) where they identified 86.2% resistance to oxacillin and similar to the results of the present study Bilal Ahmed Mir and Srikanth (2013) observed 10.6% resistance to oxacillin.

Cephalothin has exhibited 14.82% of resistance in this study but a high percentage of resistance than in the present study was observed by Syed Zahid Bukhari *et al* (2011). In the present study all the isolates were sensitive to vancomycin. Similar to the results of the present study Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India (2013), Hoerlle Jairo and Adriano Brandelle (2009), Pandey *et al* (2012), Abdul Rahman Baidani *et al* (2011) reported that they have not observed any resistance to vancomycin by the isolates where as Dacher Tsering *et al* (2011) observed 20.27% resistance and 3% resistance was observed by Telign Kajela and Ketema Bacha (2013).

Although it is extremely difficult to explain these conflicting data with regards to both time and place of study, the variation is probably due to differential clonal expression and drug pressure in community.

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