A STUDY ON THE ANTIBIOTIC RESISTANCE PATTERNS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM MARKET MILK IN AND AROUND TIRUPATI, ANDHRA PRADESH

Suma P., Swetha C.S., Sudhanthiramani., Soma Sekhar Goud., Annie Supriya and Jagadeesh Babu A

Research Article

A STUDY ON THE ANTIBIOTIC RESISTANCE PATTERNS OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM MARKET MILK IN AND AROUND TIRUPATI, ANDHRA PRADESH

Suma P[1]*., Swetha C.S[2]., Sudhanthiramani[3]., Soma Sekhar Goud[4]., Annie Supriya[2] and Jagadeesh Babu A[5]

[1,2,3,4,5]Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati

ARTICLE INFO

ABSTRACT

A total of 90 market milk samples collected from Tirupati and its surrounding areas were used for isolation of *Staphylococcus aureus*. Out of 90 milk samples, 48 isolates were found as purple coloured cocci in clusters on gram staining. All the 48 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 positive isolates produced haemolysis. A panel of 14 antibiotic discs were tested by using the standard disc diffusion method. Among the 48 isolates all of them were not resistant to Vancomycin (0%). Maximum resistance was observed for cefotoxim (77.50%), followed by, ampicillin (48.24%), penicillin (43.96%), gentamycin (43.00%), streptomycin (38.80%), ciprofloxacin (31.50%), tetracycline (31.20%), cefoperazone (25.60%), erythromycin (23.86%), cephoxitin (23.11%), oxacillin (17.83%) Azithromycin (15.70%) and cephalothin (14.82%).

INTRODUCTION

Staphylococcus aureus is a common human pathogen which can be capable of producing a wide variety of diseases starting from skin and soft tissue infection to life threatening endocarditis, bacteraemia and necrotizing pneumonia (Gordon and Lowy, 2008). Infections caused by *Staphylococcus aureus* have assumed new public health significance due to development of multi drug resistant strains particularly MRSA and its epidemic clones that are increasingly being found in hospitals and communities like Hospital associated - MRSA (HA-MRSA)and community associated –MRSA(CA-MRSA). Detection of MRSA in animals has been reported by various scientists in the previous studies. The prevalence of MRSA in farm or domestic animals like goat, sheep, cattle, horses and further in different companion animals such as dogs and cats (Walther *et al.*, 2008; Saleha *et al.*, 2010) were reported and revealing the fact that MRSA has emerged as a potential zoonotic pathogen. These studies of MRSA among the domestic and companion animals have raised the curtains for extensive further studies to address the issue of MRSA colonization and transmission to human beings particularly those who are in close contact with the animals (Khanna *et al.*, 2010).

Methicillin is grouped under narrow spectrum beta lactamase resistant penicillin. The mechanism of action is by interfering primarily with the synthesis of bacterial cell wall and will be responsible for binding of methicillin to penicillin binding proteins (PBPs) (Walther *et al.*, 2008). *Staphylococcus aureus* has the ability to develop resistance to any antibiotic that comes under clinical use (Pantosti *et al.*, 2007). Methicillin resistance to *Staphylococcus aureus* is due to the acquisition of the *mec* A gene that encodes a new protein designated as PBP 2a which belongs to a family of enzymes in building the bacterial cell wall.PBP 2a has very low affinity for beta lactamases (Pantosti *et al.*, 2007). The *mec* A gene is placed on a mobile genetic element, which is called as Staphylococcal cassette chromosome *mec* (ssc *mec*) inserted in the Staphylococcal chromosome upstream orf x (Katayama *et al.*, 2000). Different type of ssc *mec* can be distinguished on the basis of different key elements present, that are the *mec* gene complex, comprising *mec* A and its regulatory gene *mec* I and *mec* R1

Corresponding author: **Suma P**
Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati

and the ccr genes complex comprising to different ccr recombinases that are responsible for the mobility of the element (Ito *et al.*, 2001.Ma *et al.*, 2002).

There have been a number of reports stating that animals may serve as reservoirs for MRSA infection of human's .In the last two decades, new generations of MRSA have emerged with the ability to transfer to human beings and food producing animals. There is a transferring MRSA from food animals to human beings. The potential of MRSA to become a dangerous zoonotic pathogen could affect the epidemiology of MRSA in humans. As the prevalence of MRSA in animals is continuous to rise, there is an inherent risk for new MRSA colonies to evolve secondary to horizontal gene transfer and host selection pressure and then spread to human hosts. Thus the presence of MRSA in animals is a concern not only to a veterinarians and animal health care workers but also to public health.

MATERIALS AND METHODS

The specimens selected for this study were milk samples. These samples were collected by using sterile test tubes. Test tubes were sterilized in hot air oven at a temperature of 160^0C for 1 hour. Milk samples were collected from the local vendors by using a sterile dipper.. A total of 90 milk samples were collected aseptically in sterile test tubes. The collected specimens were processed within 2 to 24 hours of collection.

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*. 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 hrs and appearance of haemolytic zone around the colony was recorded.

DNase test

About 42 grams of DNase 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 14 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$_2$O) 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 equivalent 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 $2-8^0$C 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 1.

Table.1 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	Azithromycin	15µg	18	14-17	13
3	cefotaxime	30µg	23	15-22	14
4	cefoperazone	75µg	21	16-20	15
5	Cephoxitin	30 µg	22	-	21
6	Cephalothin	30 µg	18	15-17	14
7	Ciprofloxacin	05 µg	21	16-20	15
8	Erythromycin	15 µg	23	14-22	13
9	Gentamycin	10 µg	15	13-14	12
10	Oxacillin	01 µg	13	11-12	10
11	Penicillin-G	10 units	26	-	28
12	Streptomycin	10 µg	22	-	14
13	Tetracycline	30 µg	19	15-18	14
14	Vancomycin	10 µg	21	-	17

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 milk samples collected from the local vendors 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 48 isolates were subjected to Gram's staining and found purple coloured cocci in clusters **(Fig. 2).** All the isolates were

confirmed as pathogenic *Staphylococcus aureus* by a positive coagulase test as shown in **Fig. 7.**
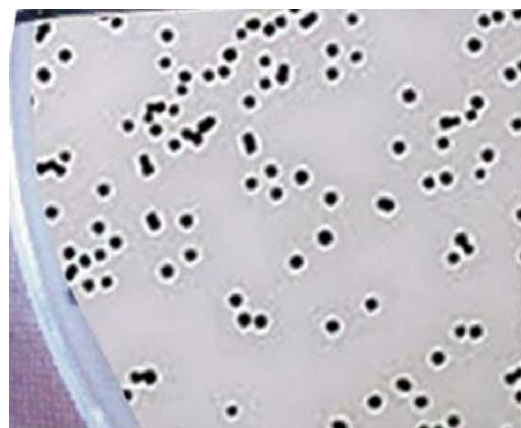
Fig. 1 Plate showing the growth of black and shiny colonies surrounded by clear zone of Staphylococcus *aureus* on Baird Parker Agar
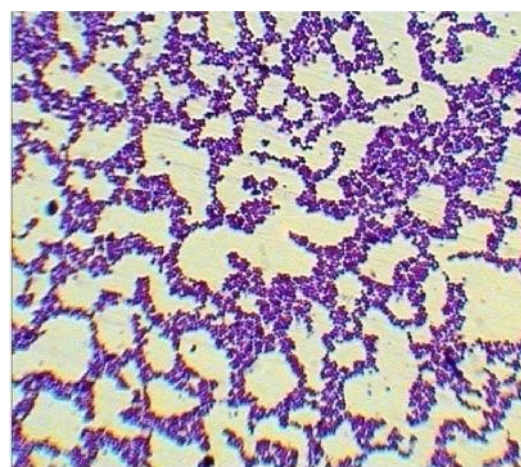
Fig. 2 Gram's staining of *Staphylococcus aureus* exhibited characteristic coccal clusters

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

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.

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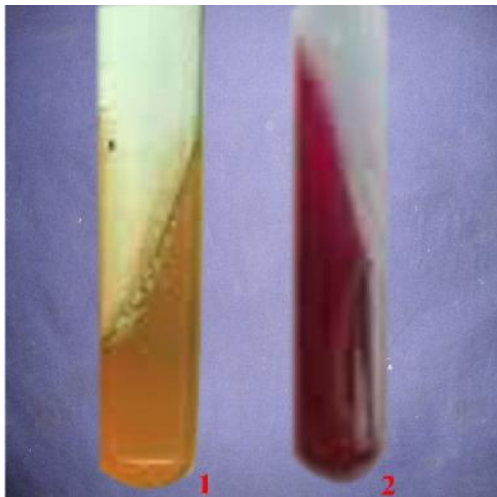
Fig. 4 Tests showing the results of urease test
Tube 1: urease negative
Tube 2: urease positive

Fig 5 Tests showing results of nitrate reduction test
Positive
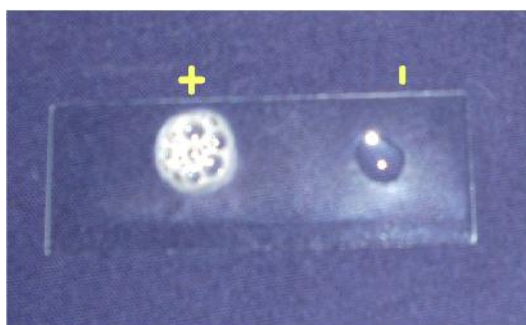
Fig. 6   Test showing result of catalase test

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°C for 18-24 hrs and observed

for blue to purple coloured colonies with clear zones around the colonies **(Fig.9)**.

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

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

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

Blood agar plate test

For further confirmation of *Staphylococcus aureus*, all the isolates were streaked on blood agar plates and incubated at 37°C / 24 hours. The results revealed that all the isolates produced   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 14 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.**1 and **Figure 11 &12.**
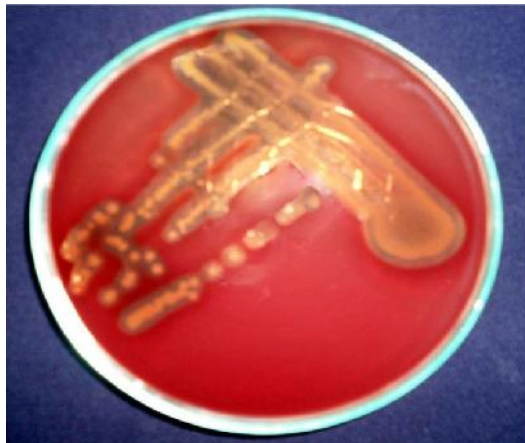
Fig. 10 Plate showing beta haemolysis of *Staphylococcus aureus* on blood agar
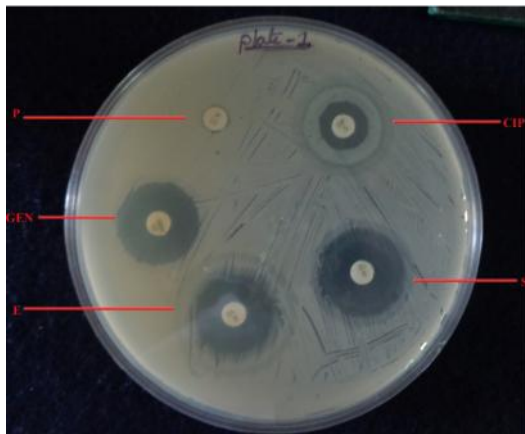
Fig. 11 & 12 Plates showing antibiotic resistance of *Staphylococcus aureus* on Muller-Hinton agar

Antimicrobial sensitivity of isolates from samples

A total of 48 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**.2. Among the 48 isolates all of them were not resistant to vancomycin (0%). Maximum resistance was observed for cefotoxim (77.50%), followed by, ampicillin (48.24%), pencillin (43.96%),

gentamycin (43.00%), streptomycin (38.80%), ciprofloxacin (31.50%), tetracycline (31.20%), cefoperazone (25.60%), erythromycin (23.86%), cephoxitin (23.11%), oxacillin (17.83%) Azithromycin (15.70%) and cephalothin (14.82%).

Table.2. Antimicrobial sensitivity/intermediate/resistant-pattern of *S. aureus* from different sources

S.No	Antimicrobial agent	Pattern of antibiogram		
		Sensitive (%)	Intermediate (%)	Resistant (%)
1	Cefotaxim	8(16.66)	2(41.66)	37(77.50)
2	Ampicillin	20(41.66)	5(10.41)	23(48.24)
3	Penicillin	21(43.75)	6(12.50)	21(43.96)
4	Gentamycin	19(39.58)	9(18.75)	20(43.00)
5	Streptomycin	19(39.58)	10(20.83)	19(38.80)
6	ciprofloxacin	30(62.5)	3(6.25)	15(31.50)
7	Tetracycline	17(35.41)	16(33.33)	15(31.20)
8	cefoperazone	15(31.25)	21(43.75)	12(25.60)
9	Erythromycin	22(45.83)	15(31.25)	11(23.86)
10	Cephoxitin	23(47.91)	14(29.16)	11(23.11)
11	Oxacillin	29(60.41)	10(20.83)	9(17.83)
12	Azithromycin	15(31.25)	25(52.08)	8(15.70)
13	Cephalothin	32(66.66)	9(18.75)	7(14.82)
14	Vancomycin	48(100.00)	0(0)	0(0)

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 - 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).

The resistance to cefotaxim was 77.50% in this study .Similar type of results were obtained by Vidhani S *et al* (2001) where they could found 78.50 % resistance to cefotaxim. 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) who 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) who 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 43% 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) who 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), whereas Kitare *et al* (2011) observed no resistance to gentamycin.

In the present study 38.80% of resistance was observed for streptomycin by the isolates. A little higher than the present results obtained in this study of Nwankwo Emmanuel Onwubiko and Nasiru Magaji Sadiq (2011) who reported 44.2% resistance to streptomycin. And further Adekunle Odunayo Adejuwon *et al* (2010) reported 23%resistance to streptomycin by the *S.aureus* isolates.

S.aureus isolated in this study was highly resistant to ciprofloxacin (31.50%), whereas results obtained from the work carried out by Syed Zahid Bukhari *et al* (2011) who found 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 ciprofloxacin..

The resistance to tetracycline was 31.20% in this study. similar 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. In the present study 25.60% resistance was observed for cefoperazone by the isolates. Similar type of results were obtained by Edward Malinowski (2002) were they observed 27.7% resistance.

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 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 cephoxitin

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

The staphylococcus aureus isolates in this study exhibited 15.7% resistance to Azithromycin. Similar type of results were obtained by Antonia poiata and Cristina Tuchilus (2011).

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.

Acknowledgment

References

Abdul Rahman H. Baidani., Waigh A. Shouny., and Toha M. Shawa. 2011. Antibiotic susce ptibility pattern of methicillin resistant *Staphylococcus aureus* in three hospitals at Hodeidah city, Yemen. Global Journal of Pharmacology, 5(2): 106-111.

Adenkunle Odunayo Adejuwon, Adesola Adetutu Ajayl, Akintunde, and Patrick Ojo Olutiolo. 2010. Antibiotic resistance and susceptibility pattern of a strain of *Staphylococcus aureus* associated with acne. Int. J. Medicine and Med Sciences, 2(4): 277-280.

Antonia poiata and Cristina Tuchilus (2011). Analele ùtiin ifice ale Universit ii „ Alexandru Ioan Cuza", Sec iunea Genetic úi Biologie Molecular, TOM XII.

Barrow G. I., and Feltham, R. K. (editors). 1993**.** *Cowan and Steel's Manual for the Identification of Medical Bacteria.*, 3rd edn. London: Cambridge University Press.

Bergey's Manual of Systematic Bacteriology Volume One.1984. The Archaea and the Deeply Branching and Phototrophic Bacteria. Volume package: Bergey's Manual of Systematic Bacteriology. Editor-in-chief: Garrity., George Boone., David, R., Castenholz, Richard W. (Eds.)Originally published by Williams & Wilkins, 2nd ed., 2001, XXII, 722 p.

Bilal Ahmed Mir, and Srikanth. 2013. Prevalence and antimicrobial susceptibility of methicillin resistant *Staphylococcus aureus* and Coagulase Negative

Staphylococci in a tertiary care hospital. Asian Journal of Pharmaceutical and Clinical Research, 6(3): 231-234.

Dachen C. Tsering., Ranabir Pal, and Sumit Kar. 2011. Methicillin resistant *Staphylococcus aureus* prevalence and current susceptibility pattern in Sikkim. J. Global Infectious Diseases., 3 (1): 9-13.

Edward Malinowski., Anna Klossowska., *et al.*, 2002. Antimicrobial susceptibility of Staphylococci isolated from affected with mastitis cows. Department of pathophysiology of reproduction and mammary gland National veterinary Research Institute. 85-090 Bydgoszcz, Poland

Gordon, R.J, Lowy, F.D. 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clinical Infect Dis., 46 Suppl 5:S350-S359.

Hoerlle Jairo L., and Adriano Brandelli. 2009. Antimicrobial resistance of *Staphylococcus aureus* isolated from the intensive care unit of a general hospital in South Brazil. J. Infect. Dev. Ctries., 3(7): 504-510

Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group India. 2013. Methicillin resistance *Staphylococcus aureus* in India: Prevalence and Susceptibility patterns. Indian J. Med Res., 137: 363-369.

Ito, T., Katayama, Y., Asada, K., Mori, N., Tsutsumimoto, K., Tiensasitorn, C., Hiramatsu, K. 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy., 45, 1323–133

Jayatilleke, K., and Bandara, P. 2012. Antibiotic sensitivity pattern of *Staphylococcus aureus* in tertiary care hospital of Srilanka. *Srilankan Journal of Infectious Diseases*., 2(2): 13-17.

Kandle, S.K., Ghatole, M.P., Takpere, A.Y., Hittinhalli, V.B., and Yemul, V.L. 2003. Bacteriophage typing and antibiotic sensitivity patterns of *Staphylococcus aureus* from clinical specimens in and around Solapur. J.Commun. Dis., 35:.17-23

Kitara, L.D., Anywar, A.D., Acullu, D., Odongo-Aginya, E., Aloyo, J., and Fendu, M.2011. Antibiotic susceptibility of S.aureus in suppurative lesions in Lacor hospital, Uganda. Afr.Health.Sci 11(1): 534-539.

Khanna T., Friendship R., Dewey C., Weese J. 2010. Methicillin-resistant *Staphylococcus aureus* colonization in pigs and Pig farmers. Vet. Microbiol., 128: 298-303.

Kloos, W.E. and Bannerman, T.L. 1995. Staphylococcus and Micrococcus. In: Manual of Clinical Microbiology. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H., (Ed.), 282-298, ASM press, ISBN 13: 978-1555811266, Washington DC.

Mandell, G., Douglas, J., and Bennett, R. (ed.). 1995. Principles and practices of infectious diseases 4th ed. Churchill Livingstone, Ltd., Edinburgh, United Kingdom.

Narasinga Rao, B. and Prabhakar, T. 2011. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* in and around Visakhapatnam, Andhra Pradesh, India. Indian J. Pharmaceutical and Biomedical Sciences, 4(4): 1-5

National Committee for Clinical Laboratory Standards 1990. Approved standard M7-A2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova.

Nwankwo Emmanuel Onwubiko and Nasiru Magaji Sadiq. 2011. Antibiotic sensitivity patterns of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, North Western Nigeria. The Pan American Medical Journal, 8:4

Pandey, S., Raza, M.S., and Bhatta, C.P. 2012 . Prevalence and antibiotic sensitivity patterns of methicillin resistant *Staphylococcus aureus* in Khathmandu medical college-teaching hospital. *Journal of Institute of Medicine*, 34(1): 13-17.

Pantosti, A., Sanchini, A., Monaco, M. 2007. Mechanisms of antibiotic resistance in *Staphylococcus aureus*. Future Microbiol.; 2(3):323-34.

Saleha. A. and Zunita. Z. 2010. Methicillin Resistant Staphylococcus aureus (MRSA): An Emerging Veterinary and Zoonotic Pathogen of Public Health Concern and Some Studies in Malaysia. J. Anim. Vet. Adv. 9(7): 1094-1098.

Saravanan, M., Anima Nanda, and Tewelde Tesfaye. 2013. Antibiotic susceptibility patterns of methicillin resistant *Staphylococcus aureus* from septicaemia suspected children in tertiary hospital in Hosur, South India. *American Journal of Microbiological Research*, 1(2): 21-24.

Shanthi, M., and Umasekar. 2009. Antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* at Sri Ramachandra Medical Centre. *Sri Ramachandra Journal of Medicine*, 2 (2): 1-4

Syed Zahid Bukhari, Safia Ahmed, and Naheed Zia. 2011. Antimicrobial susceptibility patterns of *Staphylococcus aureus* clinical isolates and efficacy of laboratory tests to diagnose Methicillin resistant *Staphylococcus aureus*: A multi centre study. J. Ayub. Med. Colle. Abboltabad, 23(1): 139-142.

Tekalign Kejela, and Ketema Bacha. 2013. Prevalence and antibiotic susceptibility pattern of methicillin resistant *Staphylococcus aureus* among primary school children and prisoners in Jimma town, Soth West Ethiopia. Annals of Clinical Microbiology and Antimicrobials, 12:11

Vidhani S, Mehndiratta PL., Mathur M D., 2001. Study of methicillin resistant S.aureus (MRSA) isolates from high risk patients.Department of Microbiology,Maulana Azad Medical College,New Delhi,India.

Walther, B., Wieler, L.H., Friedrich, A.W., Hanssen, A.M., Kohn, B., Brunnberg, L. and Lubke- Becker, A. 2008. Methicillin- resistant *Staphylococcus aureus* isolated from small and exotic animals at a university hospital during routine microbiological examinations. *Vet Microbiol.,* 127 (1-2), 171-178.