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Prevalence and Antibiotic Resistant Pattern of Methicillin Resistant *Staphylococcus aureus*

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***Abstract*—** *Staphylococcus aureus resistant to methicillin is a major problem that the world is now facing. MRSA is a major nosocomial pathogen causing significant morbidity and mortality. The study was carried out in Tirupur, Karur and Erode districts during January 2014 to March 2015. A total 308 wound samples were collected in this study. The wound samples were screened for MRSA and their antibiotic resistance pattern was performed. Out of 214 isolates of S.aureus 150 (69.2%) were found to MRSA. Both MRSA and MSSA strains showed 100% resistant to Cephotaxime. Resistant of MRSA to Ceftazidime (91.33%), Co-Trimoxazole (75.33%), Amoxycillin (23.33%), and Ampicillin (21.33%) were observed. Our results concluded alternate source of antibiotics can used to treat resistant pathogens and more research also needed for development of novel antibiotics.*

***Keywords*—** *Wound swabs, MRSA, Antibiogram. Antibiotic resistant*.

I. INTRODUCTION

MRSA is well recognized now as a major cause of nosocomial infections worldwide and these infections impose a high burden on health care resource (Boucher *et al*., 2008). A significant concern now is the spreading of MRSA in the community, possibly because of antibiotic pressure outside the hospital and transfer from hospital settings. Recent studies in different countries suggest that the epidemiology of MRSA has changed and that community and healthcare associated reservoir of MRSA have expanded (Hugher *et al*., 2008).

Infection outbreaks have been reported from burn wards, nurseries, intensive care units as well as in clinical and surgical patients and due to misuse of antibiotics, lack of hand washing, irresponsible nursing care and presence of carriers among the hospital staff (Zermina *et al*., 2012). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major hospital-associated as well as a community-associated pathogen causing a wide range of diseases, including endocarditis, osteomyelitis, toxic-shock syndrome, pneumonia, food poisoning and carbuncles.

MRSA is now endemic in India. The incidence of MRSA varies according to the region, 25% in western part of India (Patel *et al*., 2010) to 50% in south India (Gopalakrishnan *et al*., 2010).

II. MATERIALS AND METHODS

Isolation of S. aureus from Clinical Samples

A total of 308 swabs from wound samples were collected over a period from January 2014 to March 2015 in Karur, Tirupur and Erode districts, Tamilnadu. These wound swabs taken from postoperative wound from various diabetic foot ulcer, accidental wound, surgical wound infection, bite wound infection, burn wound infection etc., The samples were collected in sterile vials by using sterilized cotton bud dipped in saline water. Before taking swab samples, both hands were thoroughly washed with soap and disinfected with alcohol. The sterile cotton bud was rotated on to the overall surface area of the wound. The cotton bud after swabbing the wound was again kept in the respective sterile vials.

These collected samples were immediately transported to the microbiology laboratory and inoculated onto nutrient agar and mannitol salt agar plates. These plates were incubated at 37ºC for 24-48 hours. The golden yellow colored colonies of *S. aureus* were noted on the MSA plates (Methew *et al*., 2015). The bacterial isolates were identified by their characteristics appearance on their respective media, gram staining reaction and confirmed by the pattern of biochemical reactions using the standard method (Table I).

Detection of MRSA by Chromogenic Agar

The Hi-Media (India) made HiCrome MeReSa Agar Base (M1674) was used for detection of the MRSA among the clinical isolates of *S. aureus*. The medium was prepared by suspending 41.65 g of the medium into 500 ml of the distilled water and boiling. The medium was cooled to around 45 to 50°C and MeReSa selective supplement (FD229) reconstituted with 5 ml sterile distilled water into each methicillin vials having 2.0 mg of antibiotics (methicillin, cefoxitin) as per the direction of the supplier (HiMedia-India), was added and mixed very well. Soon after, the medium was poured into Petri plates and cooled then checked for sterility by keeping at 37°C overnight. In this study the detection of MRSA was determined by direct culture of each swab on HiCrome medium and by subculture of the identified *S. aureus* strains from mannitol salt agar onto the HiCrome MeReSa agar. Plates were incubated at 35°C for 24 h after which, all cultures showing blue colored growth were taken as MRSA positive strains, while all others are recorded as MSSA strains (HiMedia Labs. Products, India).

Antibiotic Sensitivity Test

Antibiotic Sensitivity Test was screened using Kirby Bauer method. All the isolates were tested for sensitivity against antimicrobial agents such as Amikacin(30mcg), Gentamicin (10mcg), Kanamycin (30mcg), Tetracyclin (30mcg), NalidixicAcid (30mcg), Ceftazidime (30mcg), Cefpodoxime

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(10mcg), Co-Trimoxazole (25mcg), Amoxycillin (10mcg), Ampicillin (10mcg), Neomycin (30mcg), Chloramphenicol (30mcg), by disc diffusion method following CLSI guidelines and the resistance was confirmed on the basis of standard zone of inhibition (CLSI, 2008).

III. Result and Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen, especially in intensive care units in which nasal carriage of MRSA by patients is associated with a high risk of MRSA bacteria (Pujol *et al*., 1996). Skin and soft tissue infections, wound infections, burns, ulcers, pressure sores, lower respiratory and urinary tract infections, septicaemia and infections associated with invasive devices are most frequently reported (Pantosi *et al*., 2009).

The present study was carried out from January 2014 to March 2015. Totally 308 Wound swabs collected from various districts like Tirupur-152 (49.4%), Karur-74 (24%), Erode-82 (26.6%). Out of 308 samples, 214 (69.48%) were identified as *S. aureus*, followed by 106 (34.42%) *Pseudomonas* sp, 95(30.84%) *E.coli*, 66 (21.43%) *Klebsiella* sp, 34 (11.04%) *Streptococcus* sp and 29 (9.42%) *Proteus* sp. Lakshmi *et al*., (2013) also reported the *S.aureus* was found to be the most common organism isolated from pus cultures, Coagulase negative *Staphylococcus* was the next common organism isolated (16.9%) followed by *Pseudomonas* (12.5%), *E.coli* (9.4%) and *Klebsiella* (9.4%) (Figure 1).

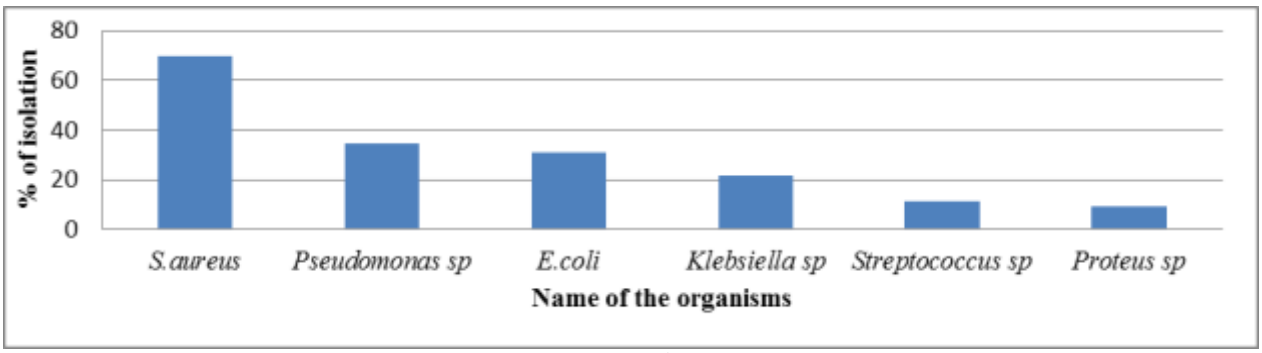

Fig. 1. Microorganisms isolated from the wound samples.

TABLE I. Identification of *Staphylococcus aureus* based on colony morphology and biochemical tests.

Test	Result
Morphology	
Gram staining	Positive
Motility	Non Motile
Structure	Grape like cluster
Colony Character	
Nutrient Agar	Golden Yellow
Mannital Salt Agar medium	Yellow Color growth
Biochemical Characteristics	
Indole	Negative
MR	Positive
VP	Positive
Citrate	Negative
Catalase	Positive
Coagulase	Positive
Gelatin	Positive
Nitrate Reduction	Positive

Out of 214 *Staphylococcus aureus* isolates 150 exhibited resistant to Methicillin and 148 resistant to Cefoxitin antibiotics (Table II). They were considered as MRSA. The prevalence of MRSA in this study was 69.2%.The prevalence of MRSA in India as reported ranges from 20-80% (Mohanasoundaram *et al*., 2008; Mulla *et al*., 2007). Various researchers worldwide have reported the prevalence rate of MRSA to be 68.6 (Sharif *et al*., 2013), 68.25 (Khatoon *et al*., 2010), 57 (Alizargar *et al*., 2013), 48.3 (Al-Baidani *et al*., 2013), 40.14 (Gayathri *et al*., 2013) and 32% (Sadek *et al*., 2013), respectively.

The MRSA and MSSA strains showed the different antibiotic susceptibility pattern. Both of the MRSA and MSSA found to be multidrug resistant strains. Both MRSA and MSSA strains showed 100% resistant to Cephotaxime. Lakshmi *et al*, (2013) reported the MRSA strains show less susceptibility to cefotaxime (39%). Singh *et al*., (2012) showed the resistance for Ceftazidime and Cephotaxime were 72.45% and 62.24% respectively (β-lactam resistance). Resistance to quinolones like Ciprofloxacin and Ofloxacin were 67.35% and 62.24% respectively.

TABLE II. Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA).

S.No	Antibiotics	No of Positive Strains	Percentage	No of Negative Strains	Percentage
1	Cefoxitin	148	69.16	66	30.84
2	Methicillin	150	70.09	64	29.91

In this study MRSA strains found to be high resistant to Co-Trimoxazole, Neomycin, Chloramphenicol compared to the MSSA strains. In MSSA strains resistant for the both Amikacin and Gentamycin were 21.88%. About 50-60% MSSA strains were resistant to Tetracyclin, Nalidixic acid, Co-Trimoxazole, Amoxycillin and Ampicillin (Table III). Mohan kumar *et al*., (2012) reveals that maximum resistant was observed in Ampicillin (89%) followed by Amoxicillin (87%) and least resistance was observed in Gentamycin (21%).

Resistant of MRSA to Ceftazidime (91.33%), Co-Trimoxazole (75.33%), Amoxycillin (23.33%), and Ampicillin (21.33%) were observed in our study (Table IV). Mohan kumar *et al*., (2012) observed Resistance of MRSA to

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Penicillin (72%), Co-trimoxazole (52%), Chloramphenicol (47%) and Erythromycin (73%). Gentamycin is a most commonly used drug, because of its low cost and synergistic activity with beta lactum antibiotics. MRSA strains were more resistant to ampicillin, cefotaxime and clindamycin (Bilal Ahmad Mir *et al*., 2013).

Perwaiz *et al*., (2007) studied the antimicrobial susceptibility pattern of MRSA isolates from tertiary care hospital. They reported that 43% of the isolates were found to be MRSA and they were also resistant to many other anti-staphylococcal antibiotics. Rajaduraipandi *et al*., (2006) reported the prevalence and antibiotic susceptibility pattern of MRSA in major southern districts of Tamilnadu and confirmed that all strains of clinical and carrier strains were sensitive to Vancomycin. The determination of prevalence and antibiotic sensitivity pattern of MRSA will help the clinicians in first line treatment in referral hospitals. Kamat *et al*., (2008) conducted a study among 498 patients from medicine and surgery wards in a tertiary teaching hospital in Goa. The patients were later checked for the occurrence of nosocomial infections. The MRSA were sensitive to 71.4% and *Staphylococcus aureus* were 28.6% were sensitive to Methicillin.

Most common reason for multidrug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing which may be due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or patients poor economic status. (Bilal Ahmad Mir *et al*., 2013). Cefoxitin, a cephamycin is a more potent inducer of thePBP2a and several groups of investigators have reported that the results of cefoxitin disk diffusion test correlate better with the presence of *mec* gene responsible for methicillin resistance (Skov *et al*., 2006).

TABLE III. Antibiotic Sensitive Profile of Methicillin Sensitive *Staphylococcus aureus*.

S. No	Antibiotics	MSSA (n=64)			
		S	%	R	%
1	Amikacin	50	78.13	14	21.88
2	Gentamycin	50	78.13	14	21.88
3	Kanamycin	21	32.81	43	67.19
4	Tetracyclin	27	42.19	37	57.81
5	Nalidixic Acid	31	48.44	33	51.56
6	Ceftazidime	2	3.13	62	96.88
7	Cephotaxime	-	-	64	100
8	Co-Trimoxazole	29	45.31	35	54.69
9	Amoxycillin	30	46.88	34	53.13
10	Ampicillin	30	46.88	34	53.13
11	Neomycin	49	76.56	15	23.44
12	Chloramphenicol	58	90.63	6	9.38

Zermina *et al*., (2012) conducted study in Rawalpindi and found 92% of MRSA were resistant to ampicillin. Emergence of bacterial resistance is promoted by excessive use of antibiotics. The presence of antibiotics residues in livestock products like milk and meat could be another responsible for maintaining resistant strains in environment (Persoons *et al*., 2009). Resistance to quinolones like ciprofloxacin and ofloxacin were 67.35% and 62.24% respectively. The raised isolation of MRSA with the passage of time may be attributed to the transfer of resistance genes between bacterial cell and persistence of bacteria in hospital environment due to antibiotic resistance (Saima *et al*., 2007).

TABLE IV. Antibiotic Resistant Profile of Methicillin Resistant *Staphylococcus aureus*.

S. No	Antibiotics	MRSA (n=64)			
		S	%	R	%
1	Amikacin	105	70	45	30
2	Gentamycin	111	74	39	26
3	Kanamycin	53	35.33	97	64.67
4	Tetracyclin	81	54	69	46
5	Nalidixic Acid	95	63.33	55	36.67
6	Ceftazidime	13	8.67	137	91.33
7	Cephotaxime	-	-	150	100
8	Co-Trimoxazole	37	24.67	113	75.33
9	Amoxycillin	115	76.67	35	23.33
10	Ampicillin	118	78.67	32	21.33
11	Neomycin	71	47.33	79	52.67
12	Chloramphenicol	62	41.33	88	58.67

IV. CONCLUSION

In the past MRSA infected people who had chronic illnesses. But now MRSA has become more common in healthy people. These infections can occur among people who are to have cuts or wounds and who have close contact with one another, such as members of sports teams. Most of the microbes in the hospital environment have developed poly-antimicrobial resistance. The situation presented a challenge in maintaining good quality in patient case and also novel antibiotics must be needed for treat the infection.

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