

## Antibiotic Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated from Chronic Wound of Fisherman Community

<sup>1</sup>A. Mohankumar and <sup>2</sup>S. Tamil Selvi

<sup>1</sup>Department of Zoology, Chikkanna Govt. Arts College, Tirupur 641 602, Tamilnadu, India

<sup>2</sup>Department of Microbiology, Sengunthar Arts and Science College, Tiruchengode 637 205, Tamilnadu, India

**Abstract:** About 92 Pus samples were collected from wound infection of fisherman community; hundred Methicillin Resistant *Staphylococcus aureus* (MRSA) were selected from isolates of *Staphylococcus aureus*. The epidemiological data showed that males were more prone to infection and with regard to age group adults were more prone to infection. The antibiotic patterns of these isolates were studied using twenty four antibiotic disks. Out of 100 strains, 72 MRSA isolates were possessing more than 50% resistance and 31 MRSA isolates showed more than 70% resistance, about 97 different types of antibiogram patterns were observed by Kirby-Bauer disk diffusion method. The MRSA strains obtained from wound infection among fisherman community needs bioactive compound for the treatment since it develops resistance to almost all types of antibiotics till date.

**Key words:** *Staphylococcus aureus* • Swab • Wound • Antibiotics • Disk Diffusion • Sensitive

### INTRODUCTION

Open chronic wounds provide a portal of entry for MRSA (Methicillin Resistant *Staphylococcus aureus*) to the underlying tissues, which can lead to local or generalized infection. The presence of wounds in geriatric patients is a risk factor for MRSA colonization [1]. The emergence of multi-drug-resistant strains of bacteria and the indiscriminate use of antibiotics highlight the urgent need for development of novel strategies to treat bacterial infections. Peptides from marine invertebrates are promising candidates as new antibacterial agents [2].

MRSA strains were first identified in the early 1960's. The recent description of clinical strains resistant to glycopeptides will become an impossible to treat patients infected with these epidemic strains. Its prevalence is highest in France among the European Union [3]. MRSA were first reported in 1961 in Europe healthcare setting. Cases of CA-MRSA infections were first reported in the late 1980s and early 1990s. MRSA is neither more infectious nor more virulent than Methicillin susceptible *S. aureus* (MSSA). It is difficult to eradicate and control because of its resistance to commonly used antibiotics

[4]. CA-MRSA strains differ from hospital-acquired strains with regard to genotypes and susceptibility pattern. Community strains are more sensitive than hospital strains to multiple anti-staphylococcal agents such as Clindamycin, Erythromycin, Trimethoprim-sulfamethoxazole, aminoglycosides and Fluoro-quinolones [5].

First report of a Penicillin-resistant strain of *S. aureus* was published in 1945, the  $\beta$ -lactamase-resistant Penicillin (Methicillin, Oxacillin, Cloxacillin and Flucloxacillin) were developed to treat it. Methicillin was the first antibiotic in this class. Worldwide, an estimated 2 billion people carry some form of *S. aureus*; of these, up to 53 million (2.7% carriers) thought to carry MRSA. A 40% increase in resistance was noted in 1999 compared to 1994-1998 data [6].

In the mid-1980s, the  $\beta$ -lactamase-stable penicillin resistant *S. aureus* had emerged. Later use of Oxacillin as an alternative to Methicillin in susceptibility tests resulted in the term 'Oxacillin-resistant *S. aureus*' (ORSA). Methicillin resistance in *S. aureus* is primarily mediated by the *mecA* gene, which codes for the modified Penicillin-binding protein 2a (PBP 2a or PBP 2'). PBP2a is located in the bacterial cell wall and has a low binding affinity for  $\beta$ -lactams. Although all cells in a population of *S. aureus*

may carry the *mecA* gene, often only a few of cells will express the gene [7].

In 1970s MRSA has become a nosocomial problem and sensitive to Clindamycin, Macrolides, Tetracycline, Trimethoprim-Sulfamethoxazole and Quinolones, or it may be resistant to all antibiotic except Vancomycin. Vancomycin remained the only predictable active antibiotic against all strains of *S. aureus* and MRSA in particular. In May 1997, the Center for Disease Control and Prevention (CDC) confirmed that it has documented the first failure of Vancomycin in Japan [8]. Early detection of emerging trends in antimicrobial resistance may facilitate implementation of effective control measures. The antibiotic susceptibility contributes directly to patient care and the expertise of the microbiology laboratory can have powerful influence on antibiotic usage and hence on the pressure that facilitates the emergence of antimicrobial drug resistance [9]. Multidrug resistant (MDR) *Staphylococcus* isolates in hospital have been recognized as one of the major challenges in the hospital infection control [10]. These strains are resistant to multiple antibiotics and act as reservoir for drug resistant gene [11]. The aim of the study was to study the antibiotic resistance pattern among multidrug resistant MRSA strains isolated from pus samples of fisherman community.

## MATERIALS AND METHODS

**Community People:** Prospective study was carried out among coastal area people, in India, for the public health such as east and west coastal area village regions, by approaching them directly.

**Sample Collection and Transportation:** 92 Pus samples were collected from wounds caused by marine organisms, while fishing among coastal community or any type of the prolonged wound among those community people such as diabetic wound, burn wound, bite wound, septic wound etc. The swab was held in contact with the wound for at least 5 seconds before any debridement was done [12]. The sterile swab samples were placed in sterile transport containers: swabs in a tube containing Amies transport medium without charcoal [13] in an ice pack box and transported carefully to the laboratory within 12 hrs and processed.

**Phenotypic Characterization:** Wound swabs were streaked on Mannitol salt agar (MSA) and incubated at

37°C for 24-48 hrs. Growth and fermentation of Mannitol on MSA were examined. Two or three well separated colonies from each medium were picked and subcultured on nutrient agar for further characterization. Preliminary identification of isolates resembling *Staphylococci* was performed on the basis of colonial and cultural morphology and biochemical characterization [14]. *S. aureus* was confirmed by pigment production on nutrient agar, hemolytic activity on blood agar, DNase activity, Lysozyme activity, Gelatinase activity, growth on Barid-Parker agar medium, lipase activity on egg yolk agar medium [15]. The isolates were identified using taxonomic, physiological and biochemical methods based on Bergey's manual of systematic bacteriology [16].

**Detection of Methicillin Resistance:** This was carried out according to NCCLS guidelines using Oxacillin agar screen test whereby all MRSA isolates were spot inoculated onto MHA supplemented with 6 µg/ml oxacillin and 4% NaCl, from a 0.5 McFarland standard suspension. The plates were incubated at 35°C for 24 hrs [17].

**Antibiotic Susceptibility Testing by Disc Diffusion Test:** The antibiotic susceptibility by the method of disc diffusion test was followed according to Kirby-Bauer method [18]. The 24 hrs old cultures of *S. aureus* isolates grown in nutrient broth were compared with 0.5 McFarland standard to check the turbidity of growth and swabbed by sterile cotton swab directly on MHA containing 2-4% NaCl. Twenty four antibiotic (Hi-media) were used which included, Amikacin (Ak)-30 mcg, Amoxicillin (Am)-10 mcg, Ampicillin (A)-10 mcg, Ceftriaxone (Ck)-30 mcg, Ceftazidime (Ca)-30 mcg, Co-Trimoxazole (Co)-25 mcg, Chloramphenicol (C)-30 mcg, Cefazolin (Cz)-30 mcg, Cephoxitin (Cn)-30 mcg, Clindamycin (Cd)-2 mcg, Ciprofloxacin (Cf)-30 mcg, Erythromycin (E)-15 mcg, Gentamicin (G)-10 mcg, Kanamycin (K)-30 mcg, Methicillin (M)-5 mcg, Moxalactam (Mx)-30 mcg, Nalidixic acid (Na)-30 mcg, Netilmicin (Nt)-30 mcg, Norfloxacin (Nx)-10 mcg, Oxacillin (Ox)-5 mcg, Penicillin G (P)-10 mcg, Rifampicin (R)-30 mcg, Tetracycline (T)-30 mcg and Vancomycin (V)-5 mcg. The plates were incubated at 37°C for 24 hrs. The diameters of zone of inhibition were recorded. The organisms which showed different resistant pattern were included, out of which hundred cultures were isolated. The control organism was *Staphylococcus aureus* MTCC 96.

#### **Determination of MIC and MBC by Agar Plate Dilution**

**Method:** Antimicrobial agents' diluents with 10X concentration were added to molten agar. The MIC ranges were followed according to the NCCLS guidelines [19]. Antimicrobial concentration was added to each flask at 10X concentration and antibiotic-containing media was poured on the MHA. Two fold serial dilutions of the antimicrobial were added with agar medium. Inoculum preparation was done by transferring overnight culture into a tube containing 4 to 5 ml of normal saline. The broth culture was incubated at 35°C until matched the turbidity of 0.5 McFarland standards. The MIC was defined as the minimum concentration of the dilutions that inhibited the growth of the test microorganism [20]. MBC was defined as 99.99% reduction of cell viability with respect to that of the initial inoculum [21].

Stock solutions of Ciprofloxacin (Cf)-10 µg/10 ml, Rifampicin (R)-1000 µg/10 ml, Chloramphenicol (C)-1000 µg/10 ml, Tetracycline (T)-100 µg/10 ml and Streptomycin (S)-100 µg/10 ml were prepared. 200 µl and 100 µl of each antibiotic were poured in MHA plates. The inoculums of 32 MRSA strains were incubated [6]. The persistence of antimicrobial activity was checked by subjecting them to the same test 6 months later [22].

Multiple antibiotic resistance (MAR) index: MAR index was calculated as the ratio of number of antibiotics to which isolate is resistant and to the multiplication of number of antibiotics and number of isolates [23]. MAR index for antibiotics was calculated as the ratio of number of isolates to which antibiotic is resistant and to the multiplication of number of antibiotics [24].

## **RESULTS**

#### **Screening of Methicillin Resistant *Staphylococcus aureus* (MRSA):**

Totally 92 wound samples were processed from east and west coast regions. Among positive samples 100 isolates of MRSA were obtained. The confirmations of the strains were done by comparing the results with standard biochemical chart of *S. aureus* and observation of positive growth in MRSA selective media.

**Epidemiological Study of MRSA:** In the fisherman community, compared to that of females (22%), males (78%) were more prone to the infection of MRSA, based on gender. Based on age groups adults were more prone to infection compared to that of children's and old peoples.

#### **Antibiotic Susceptibility Testing by Kirby-Bauer Method:**

The antibiotic susceptibility test was performed for 100 MRSA strains against 24 antibiotics using Kirby-Bauer method. The resistance, intermediate and susceptibility of MRSA were observed and interpreted as per the National Committee for Clinical Laboratory Standards (NCCLS).

#### **Number of Resistant Patterns Observed in MRSA:**

One strain was resistant to 23 antibiotics, 1 to 23 (Pattern # 1), 1 to 22 (Pattern # 2), 1 to 21 (Pattern # 3), 4 to 20 (Pattern # 4 to 7), 2 to 19 (Pattern # 8, 9), 10 to 18 (Pattern # 10 to 19), 12 to 17 (Pattern # 20 to 31), 8 to 16 (Pattern # 32 to 39), 5 to 15 (Pattern # 40 to 44), 6 to 14 (Pattern # 45 to 50), 14 to 13 (Pattern # 51 to 62), 8 to 12 (Pattern # 63 to 69), 7 to 11 (Pattern # 70 to 76), 7 to 10 (Pattern # 77 to 83), 5 to 9 (Pattern # 84 to 88), 6 to 8 (Pattern # 89 to 94), 1 to 7 (Pattern # 95), 2 to 6 (Pattern # 96, 97). Amikacin was resistant to 37% of MRSA isolate, Amoxicillin to 87% MRSA isolate, Ampicillin to 89%, Ceftizoxime to 42%, Ceftazidime to 73%, Co-Trimoxazole to 52%, Chloramphenicol to 47%, Cefazolin to 34%, Cephoxitin to 43%, Clindamycin to 58%, Ciprofloxacin to 65%, Erythromycin to 73%, Gentamicin to 21%, Kanamycin to 58%, Methicillin to 67%, Moxalactam to 51%, Nalidixic acid to 82%, Netilmicin to 40%, Norfloxacin to 61%, Oxacillin to 49%, Penicillin G to 72%, Rifampicin to 45%, Tetracycline to 71% and Vancomycin was resistant to 76% of MRSA isolate. There were 97 different types of patterns among 100 isolates (those isolates with same pattern were from different samples) were observed. Among 100 isolates 72 MRSA isolates showed more than 50% of resistance and 31 MRSA showed more than 70% resistance, whereas only 2 MRSA showed more than 90% resistance pattern. MRSA isolate No. 68 possessed maximum resistance of 95.83% (Table 1).

#### **Minimal Inhibitory Concentration by Agar Dilution**

**Method:** The MIC concentration of MRSA ranged between 2-20 µg/ml. The MBC concentration of MRSA ranged between 0.5-15 µg/ml.

Comparative study of Multiple Antibiotic Resistance (MAR) index for isolates and antibiotics were calculated. In case of resistant isolates the maximum MAR index of 0.9583 was obtained for isolate No. 68 and the minimum value of 0.2500 was obtained for isolate No. 52. In case of resistant antibiotics the maximum MAR index of 0.0370 was obtained for ampicillin and the minimum value of 0.0087 was obtained for gentamicin (Table 2).

**Table 1: Antibiogram patterns of MRSA showing greater than 70% resistance**

S. No	Isolate No.	Pattern of antibiotic phenotype	% of resistance
1	68	Ak-Am-A-Ck-Ca-Co-C-Cz-Cn-Cd-Cf-E-G-K-M-Mx-Na-Nt-Nx-Ox-P-R-T	95.83
2	54	Am-A-Ck-Co-C-Cz-Cn-Cd-Cf-E-G-K-M-Mx-Na-Nt-Nx-Ox-P-R-T-Va	91.66
3	62	Ak-Am-A-Ca-C-Cz-Cd-Cf-E-G-K-M-Mx-Na-Nt-Nx-Ox-P-R-T-Va	87.50
4	50	Am-A-Ck-Ca-Co-C-Cz-Cd-Cf-E-K-M-Mx-Na-Nt-Nx-Ox-P-R-T-Va	83.33
5	53	A-Ca-Co-C-Cz-Cn-Cf-E-G-K-M-Mx-Na-Nt-Nx-Ox-P-R-T-Va	83.33
6	58	Ak-Am-A-Ck-Ca-Co-C-Cz-Cn-Cf-E-G-K-M-Mx-Na-Nx-Ox-T-Va	83.33
7	80	Ak-Am-A-Ck-Ca-Co-C-Cz-Cn-Cd-Cf-E-K-M-Mx-Na-Ox-P-R-T-Va	83.33
8	48	Am-A-Ca-Co-Cz-Cn-Cd-Cf-E-K-M-Mx-Na-Nx-Ox-P-R-T-Va	79.16
9	70	Ak-Am-A-Ca-Co-C-Cz-Cn-Cf-E-G-K-M-Mx-Na-Nx-R-T-Va	79.16
10	6	Am-A-Ck-Ca-Co-C-Cd-Cf-E-G-M-Na-Nx-Ox-P-R-T-Va	75.00
11	20	Am-A-Ck-Ca-Co-C-Cz-Cn-Cd-Cf-G-M-Mx-Na-Ox-P-T-Va	75.00
12	47	A-Ca-Co-C-Cz-Cn-Cd-E-K-Mx-Nt-Na-Nx-Ox-P-R-T-Va	75.00
13	55	Ak-Am-Ca-Co-Cz-Cn-Cd-Cf-E-G-M-Mx-Nt-Ox-P-R-T-Va	75.00
14	61	Ak-Am-A-Ck-Ca-Co-C-Cz-Cn-Cd-Cf-E-G-K-M-Na-Nx-T	75.00
15	72	Ak-Am-A-Ck-Ca-Co-Cd-E-K-M-Mx-Na-Nt-Ox-P-R-T-Va	75.00
16	85	Am-A-Ck-Ca-Co-C-Cz-Cn-Cd-E-M-Mx-Na-Ox-P-R-T-Va	75.00
17	88	Ak-Am-A-Ck-Ca-Co-C-Cz-Cn-Cd-E-K-M-Mx-Nx-P-R-T	75.00
18	93	Ak-Am-A-Ck-Ca-Co-C-Cd-Cf-E-K-M-Mx-Na-Nt-Nx-P-Va	75.00
19	95	Ak-Am-A-Ck-Ca-Co-C-Cf-K-M-Mx-Na-Nt-Nx-Ox-P-T-Va	75.00
20	5	Am-A-Ck-Ca-Co-C-Cd-Cf-E-G-M-Na-Nx-P-R-T-Va	70.83
21	49	Ca-Co-Cn-Cd-Cf-E-K-M-Mx-Na-Nt-Nx-Ox-P-R-T-Va	70.83
22	51	Ak-Am-A-Ck-Ca-Co-Cz-Cn-Cd-Cf-E-K-M-Na-Nx-T-Va	70.83
23	56	Ak-Am-A-Ck-Ca-C-Cf-E-G-K-Mx-Na-Nt-Nx-R-T-Va	70.83
24	60	Ak-Am-A-Ck-Ca-Co-C-Cz-Cn-Cd-Cf-E-K-M-Na-Nx-T	70.83
25	71	Ak-Am-A-Ck-Ca-Cd-E-K-M-Mx-Na-Nt-Nx-Ox-P-R-T	70.83
26	73	Ak-Am-A-Ck-Ca-Co-Cd-E-K-M-Mx-Na-Nt-Ox-P-R-T	70.83
27	74	Ak-Am-A-Ck-Ca-Co-Cd-Cf-E-G-K-Na-Nt-Ox-R-T-Va	70.83
28	76	Ak-Am-A-Ck-Ca-Co-Cn-Cd-Cf-E-G-K-Na-Nt-Ox-R-T	70.83
29	82	Am-A-Ck-Ca-Co-C-Cz-Cn-Cd-Cf-E-K-M-Mx-Ox-P-T	70.83
30	84	Am-A-Ck-Ca-Co-C-Cz-Cd-E-M-Mx-Na-Ox-P-R-T-Va	70.83
31	86	Am-A-Ck-Ca-Co-Cz-Cn-Cd-E-K-M-Mx-Nx-P-R-T-Va	70.83

Key: Ak- Amikacin (30 mcg), Am- Amoxicillin (10 mcg), A- Ampicillin (10 mcg), Ck- Ceftrizoxime (30 mcg), Co- Co- Trimoxazole (25 mcg), C- Chloramphenicol (30 mcg), Cz- Cefazolin (30 mcg), Cn- Cephoxitin (30 mcg), Cd-Clindamycin (2 mcg), Cf- Ciprofloxacin (30 mcg), E- Erythromycin (15 mcg), G- Gentamicin (10 mcg), K- Kanamycin (30 mcg), M- Methicillin (5 mcg), M- Moxalactam (30 mcg), Na- Nalidixic acid (30 mcg), Nt- Netilmicin (30 mcg), Nx- Norfloxacin (10 mcg), Ox- Oxacillin (5 mcg), P- Penicillin (10 mcg), R- Rifampicin (30 mcg), T- Tetracycline (30 mcg), Va- Vancomycin (5 mcg).

**Table 2: Calculation of comparative antibiogram pattern of Antibiotics tested in MRSA isolates from fisherman community**

S. No	Antibiotics	Resistant			Intermediate			Sensitive		
		No. of strains	%	MAR Index	No. of strains	%	MAR Index	No. of strains	%	MAR Index
1.	Amikacin	37	37	0.0154	38	38	0.0158	25	25	0.0104
2.	Amoxicillin	87	87	0.0362	05	05	0.0020	08	08	0.0033
3.	Ampicillin	89	89	0.0370	06	06	0.0025	05	05	0.0020
4.	Ceftizoxime	42	42	0.0175	16	16	0.0066	42	42	0.0175
5.	Ceftazidime	73	73	0.0304	21	21	0.0087	06	06	0.0066
6.	Co- Trimoxazole	52	52	0.0216	13	13	0.0054	35	35	0.0145
7.	Chloramphenicol	47	47	0.0195	08	08	0.0033	45	45	0.0187
8.	Cefazolin	34	34	0.0141	09	09	0.0037	57	57	0.0237
9.	Cephoxitin	43	43	0.0179	09	09	0.0037	48	48	0.0200
10.	Clindamycin	58	58	0.0241	08	08	0.0033	34	34	0.0141
11.	Ciprofloxacin	65	65	0.0270	12	12	0.0050	23	23	0.0095
12.	Erythromycin	73	73	0.0304	17	17	0.0070	10	10	0.0041
13.	Gentamicin	21	21	0.0087	07	07	0.0029	72	72	0.0300
14.	Kanamycin	58	58	0.0241	27	27	0.0112	15	15	0.0062
15.	Methicillin	67	67	0.0279	17	17	0.0070	16	16	0.0066
16.	Moxalactam	51	51	0.0212	32	32	0.0133	17	17	0.0070
17.	Nalidixic acid	82	82	0.0341	15	15	0.0062	03	03	0.0012
18.	Netilmicin	40	40	0.0166	08	08	0.0033	52	52	0.0216
19.	Norfloxacin	61	61	0.0254	10	10	0.0041	29	29	0.0120
20.	Oxacillin	49	49	0.0204	16	16	0.0066	35	35	0.0145
21.	Penicillin	72	72	0.0300	10	10	0.0041	17	17	0.0070
22.	Rifampicin	45	45	0.0187	16	16	0.0066	39	39	0.0162
23.	Tetracycline	71	71	0.0295	14	14	0.0058	15	15	0.0062
24.	Vancomycin	76	76	0.0316	14	14	0.0058	10	10	0.0041

## DISCUSSION

Among 92 wound samples, 75% of sample shows positive growth in which several *Staphylococcus aureus* was obtained. About 100 MRSA isolate showing different antibiotic pattern may or may not be in same sample was obtained in our study. Among 109 clinical MRSA isolates 49 samples were derived from wounds, 41 MRSA from Respiratory tract and 19 MRSA from Urinary tract [25].

With regard to age group adults were prone to infection, since they were involved in fishing. Age has been previously described as a risk factor for community acquired MRSA [26]. The prevalence of MRSA among young age groups indicates that they were community acquired. The risk among younger patients is likely related to increased participation in risky activities rather than physiologic changes due to aging as observed by Jacobus *et al.* [27]. Kamberovic and Sivic, [28] has documented that CA-MRSA is predominant among adults and children.

In case of gender distribution, 22% were females and 78% were recorded from males, which reveal that males were mostly affected. These values are similar to those reported by van Belkum *et al.* [29] from King Faisal Specialist hospital in Saudi Arabia; in which 64.4% were recovered from male patients while 35.6% females. They reported procurement of 66% of male isolates and 34% from females. Madani *et al.* [30] also reported that recovery of MRSA of 65.8% from males and 34.2% from females in Saudi Arabia. Similarly, from the eastern province of Saudi Arabia, Bukharie and Abdelhadi [31] discussed that 63% male and 37% females so this reflects the distribution of MRSA with a male patient predominance. Gender distribution is similar to Tentolouris *et al.* [32] where 60.7% males and 39.3% females are affected.

CA-MRSA strains were identified among coastal area people in our study. According to Japoni [33], in 1980, the first CA-MRSA infection was reported in the United States. There has been a steady increase in the prevalence of the MRSA isolated from hospital in the United States, in 1997. Previous study in Shiraz University affiliated hospitals showed MRSA had risen up to 33%.

In our report 67% and 17% of MRSA isolates were resistant and intermediate to methicillin from fisherman wound. El-Jakee *et al.* stated that 13.3% and 6.7% of the examined isolates were resistant and intermediately resistant to methicillin respectively from 45% of septic wounds. A recent prospective USA cohort study found that clinical and epidemiological risk factors in persons hospitalized for CA-MRSA infection cannot distinguish

reliably between MRSA and MSSA. MRSA isolates detected in animal staphylococci have most been assumed to originate from human sources [15].

MRSA is an important nosocomial problem for the failure of antimicrobial treatments and an increasing problem in community-acquired infections. High occurrence of MRSA from surgical wound infection is documented, especially in the neurosurgical and orthopedics patients [34].

All the isolates of MRSA were sensitive to Vancomycin [35] in contrast to recent reports of MRSA isolates with reduced susceptibility to Vancomycin and of 76% of isolates were fully resistant to Vancomycin. According to Vlack *et al.* [36] who reported that a characteristic feature of CA-MRSA, is the sensitivity to many non- $\beta$ -lactam antibiotics such as Clindamycin and Trimethoprim-sulfamethoxazole.

In Takniwale Supriaya study [37] multidrug resistance was found to be less common among MSSA. Maximum resistance was observed against Co-trimoxazole (55.67%) followed by Penicillin (40.54%). Least resistance was observed against Ciprofloxacin 4.86%. Our study reveals that maximum resistance was observed in Ampicillin (89%) followed by Amoxicillin (87%) and least resistance was observed in Gentamicin (21%).

Resistance of MRSA to Penicillin (72%), Co-trimoxazole (52%), Chloramphenicol (47%) and Erythromycin (73%) were recorded in our study. Resistance of MRSA to Penicillin (100%), Co-trimoxazole (97%), Chloramphenicol (93.33%) and Erythromycin (68.68%) were recorded. MIC values to Oxacillin  $\geq$  to 250  $\mu\text{g}/\text{ml}$  were found only in two strains, while MIC of others were between 2-12  $\mu\text{g}/\text{ml}$ . MIC concentration of MRSA ranges between 2-16  $\mu\text{g}/\text{ml}$  by the isolate of Pettit [38], which slightly differs from our study.

In El-Jakee *et al.* [15] investigation high resistance was recorded to Enrofloxacin (86.7%) among the examined *S. aureus* isolates, followed by Oxy-tetracycline (80%) and Ampicillin (73.3%). Meanwhile 93.3% of *S. aureus* were sensitive to Cefoperazone, 86.7% to Cefotaxime and 80% to Methicillin, in which variations of resistance pattern were observed among different antibiotics in our study.

MIC of MRSA ranges from 2-20  $\mu\text{g}/\text{ml}$ . Waites *et al.* [39] noted that among the *S. aureus* isolates collected, 813 (48.0%) were MSSA and 879 (52.0%) were MRSA. Against both MSSA and MRSA, the lowest MIC50s and MIC90s were for tigecycline and minocycline. Similarly, a low MIC50 and MIC90 were recorded for Imipenem against MSSA isolates (0.25  $\text{ig}/\text{ml}$ ). While the MIC50 of Imipenem remained low (0.5  $\text{ig}/\text{ml}$ ), the MIC90 was 16  $\text{ig}/\text{ml}$  against MRSA.

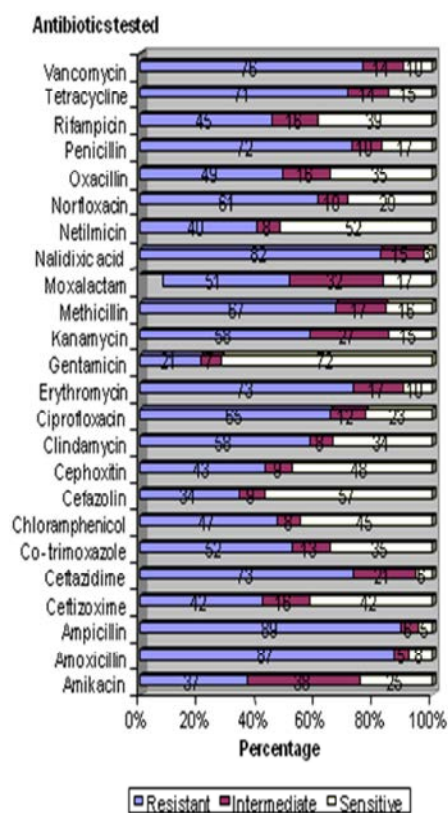


Fig. 1: Antibiogram pattern of antibiotics in MRSA from fisherman community

Vidhani *et al.* [40] finalized that none of the MRSA isolate were sensitive to Penicillin and Amoxicillin, but MSSA were sensitive to 6(5.5%) and 12(11%) respectively, whereas in our report 17% and 8% were sensitive to Penicillin and Amoxicillin. 85(77.9%) of MSSA were sensitive to Cefotaxime while only 17(21.5%) of MRSA were sensitive to this antibiotic. Sensitivity to macrolide group of antibiotics like Erythromycin and Roxithromycin was seen in 77(70.6%) of MSSA in comparison to 14(17.7%) of MRSA. Amongst the Amino glycosides; maximum sensitivity was seen with Amikacin and 74(67.9%) of MSSA were sensitive to this antibiotic while only 21(26.6%) MRSA were sensitive to the same. Fifty three (67%) of MRSA and 76(69.7%) of MSSA were found to be sensitive to fluoroquinolone group i.e. Ofloxacin. All *S. aureus* isolates were found to be uniformly sensitive to Vancomycin which is the drug of choice. Recent study proves that only 10% of MRSA were sensitive to Vancomycin.

The most-sensitive techniques for determining Vancomycin susceptibility are vulnerable to the problems of inoculums size reported by Dunne *et al.* [41]. NCCLS standards for susceptibility testing recommended an

inoculum density of  $5 \times 10^5$  CFU/ml for performance of standardized susceptibility testing, while Vancomycin-intermediate strains can occur in frequencies ranging from 10-6 CFU/ml to 10-7 CFU/ml. This raises the possibility that resistant sub-population may be missed at the time of initial testing due to sample error. Ciprofloxacin, which was proposed to be an alternative therapy to MRSA infection, in which 65% of isolate developed resistance (Fig. 1) were found to be still better in this part of the country (23%), than in Pondicherry [10]. Thus the resistant and sensitive nature of MRSA varies due to different biological factors. This study was designed to highlight the current antimicrobial susceptibility pattern of staphylococcus [42].

## CONCLUSION

MRSA strains were found to be developing resistance day by day to the recent antibiotics, hence an alternative treatment of MRSA are necessary for the treatment of chronic wound. The discovery and the development of new antibiotics are still a high priority in biomedical research.

## REFERENCES

- Guillaume, K., A. Buu-Hoi, E. Herisson, P. Biancardini and C. Debure, 2000. Methicillin-Resistant *Staphylococcus aureus* (MRSA) Nosocomial Acquisition and Carrier State in a Wound Care Center. Arch. Dermatol., 136: 35-739.
- Cueto, M., P.R. Jensen, C. Kauffman, W. Fenical, E. Lobkovsky and J. Clardy, 2001. J. Nat. Prod., 64: 1444-1446.
- Minary-Dohen, P., P. Bailly, X. Bertrand and D. Talon, 2003. Methicillin-Resistant *Staphylococcus aureus* (MRSA) in rehabilitation and chronic-care-facilities: What is the best strategy? BMC Geriatrics, 3: 1-6.
- Ashok, R., K. Anuradha, S.S. Babu, N. Bheerappa, R.A. Sastry and V. Lakshmi, 2004. Postoperative Infection of An Abdominal Mesh due to Methicillin-Resistant *Staphylococcus aureus*-A case Report. Indian Journal of Medical Microbiology, 22(4): 260-262.
- Schlesinger, Y., S. Yahalom, D. Raveh, A.M. Yinnon, R. Segel, M. Erlichman, D. Attias and B. Rudensky, 2003. Methicillin-resistant *Staphylococcus aureus* nasal colonization in children in Jerusalem: community Vs. chronic care institutions. Israel Medical Association Journal, 5(12): 847-851.

6. Islam, M.A., M.M. Alam, M.E. Choudhury, N. Kobayashi and M.U. Ahmed, 2008. Determination of Minimum Inhibitory Concentration (MIC) of Cloxacillin for Selected Isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA) with their Antibiogram. Bang. J. Vet. Med., 6(1): 121-126.
7. Sridhar Rao, P.N., 2009. Methicillin Resistant *Staphylococcus aureus* (MRSA). www.microrao.com
8. Hakim, S.T., S. Arshed, M. Iqbal and S.G. Javaid, 2007. Vancomycin Sensitivity of *Staphylococcus aureus* isolates from Hospital Patients in Karachi, Pakistan. Libyan J. Med., 2(4): 1-6.
9. Sudha, V., A. Prasad, S. Khare and R. Bhatia, 2001. Antimicrobial Susceptibility testing in India-A status survey. Indian Journal of Medical Microbiology, 19(4): 222-223.
10. Majumder, D., J.N.S. Bardoli, A.C. Phukan and J. Mahanta, 2001. Antimicrobial Susceptibility Pattern among Methicillin-Resistant *Staphylococcus aureus* Isolates in Assam. Indian Journal of Medical Microbiology, 19(3): 138-140.
11. Jessen, O., P.R. Bulow, V. Faber and K.R. Erickson, 1996. Changing Streptococci and *Staphylococcus aureus* infection, a ten year study of bacteria and cases of bacteremia. N. Engl. J. Med., 281: 627-632.
12. Slater, R.A., T. Lazarovitch, I. Boldur, Y. Ramot, A. Buchs, M. Weiss, A. Hindi and M.J. Rapoport, 2003. Swab Cultures accurately identify bacterial pathogens in diabetic foot wounds not involving bone. Diabetic Medicine, 21: 705-709.
13. Drews, S.J., B.M. Willey, N. Kreiswirth, Min Wang, Teresa Ianes, J. Mitchell, M. Latchford, A.J. McGeer and K.C. Katz, 2006. Verification of the IDI-MRSA Assay for Detecting Methicillin-Resistant *Staphylococcus aureus* in Diverse Specimen Types in a Core Clinical Laboratory setting. Journal of Clinical Microbiology, 44(10): 3794-3796.
14. Shittu, A., J. Lin, D. Morrison and D. Kolawole, 2004. Isolation and molecular characterization of multi resistant *Staphylococcus sciuri* and *Staphylococcus haemolyticus* associated with skin and soft-tissue infections. Journal of Medical Microbiology, 53: 51-55.
15. EL-Jakee, J., S. Ata Nagwa, M. Bakry, A. Sahar, E.Z. Elgabry and W.A. Gad El-Said, 2008. Characteristics of *Staphylococcus aureus* Strains isolated from Human and animal source. American-Eurasian J. Agric. and Environ. Sci., 4(2): 221-229.
16. Bergey, D.H. and J.G. Holt, 1994. Bergey's Manual of Determinative Bacteriology 9<sup>th</sup> Edition; Lippincott Williams and Wilkins, pp: 1268.
17. Baddour, M.M., M.M. Abuelkheir and J.A. Fatani, 2006. Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia. Annals of Clinical Microbiology and Antimicrobials, 5(30): 1-11.
18. Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45(4): 493-496.
19. NCCLS, 1997. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.
20. Adwan, G. and M. Mhanna, 2008. Synergistic Effects of Plant Extracts and Antibiotics on *Staphylococcus aureus* Strains Isolated from Clinical Specimens. Middle-East Journal of Scientific Research, 3(3): 134-139.
21. El-Shekh, N.A., A.M.A. Ayoub, H.H. El-Hendawy, E.A. Abada and S.Y.E. Khalifa, 2010. *In vitro* Activity of some Antimicrobial Agents against Intact and Disrupted Biofilms of *Staphylococci* in the Indwelling Vascular Catheter Patients. World Applied Sciences Journal, 10(1): 108-120.
22. Fabregas, J., A. Munoz, A. Otero, J.L. Barja and M.L. Romaris, 1991. A Preliminary Study on Antimicrobial Activities of Some Bacteria Isolated from Marine Environment. Nippon Suisan Gakkaishi, 57(7): 1377-1382.
23. Tambekar, D.H., D.V. Dhanorkar, S.R. Gulhane, V.K. Khandelwal and M.N. Dudhane, 2010. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. World Applied Sciences Journal, 10(1): 108-120.
24. Olayinka, B.O. and A.T. Olayinka, 2003. Methicillin resistance in staphylococcal isolates from clinical and asymptomatic bacteriuria specimens: implications for infection control. African journal of clinical and experimental microbiology, 4(2): 79-90.
25. Grisold, A.J., E. Leitner, G. Muhlbauer, E. Marth and H.H. Kessler, 2002. Detection of Methicillin-Resistant *Staphylococcus aureus* and Simultaneous Confirmation by Automated Nucleic Acid Extraction and Real-Time PCR. Journal of Clinical Microbiology, 40(7): 2392-2397.

26. Naimi, T.S., K.H. Ledell, K. Como-Sabetti, S.M. Borchardt, D.J. Boxrud, J. Etienne, S.K. Johnson, F. Vandanesch, S. Fridkin, C. O'Boyle, R.N. Danila and R. Lynfield, 2003. Comparison of Community-and Health Care-Associated Methicillin-Resistant *Staphylococcus aureus* Infection. *JAMA.*, 290: 2976-2984.
27. Jacobus, C.H., J.C. Lindsell, D.S. Leach, J.G. Fermann, A.B. Kressel and E. Laura Rue, 2007. Prevalence and demographics of methicillin resistant *Staphylococcus aureus* in culturable skin and soft tissue infections in an urban emergency department. *BMC Emergency Medicine*, 7(19): 1-7.
28. Kamberovic, S.U. and S. Sivic, 2007. Methicillin-resistant *Staphylococcus aureus* (MRSA) in the community-laboratory based study. *Acta Medica Academica*, 36: 3-9.
29. van Belkum, A., M. Vandenbergh, G. Kessie, H. Qadri, G. Lee, N. vanDen Braak, H. Verbrugh and M.N. Al-Ahdal, 1997. Genetic homogeneity among methicillin-resistant *Staphylococcus aureus* strains from Saudi Arabia. *Microbial Drug Resistance*, 3(4): 365-369.
30. Madani, T.A., N.A. Al-Abdullah, A.A. Al-Sanousi, T.M. Ghabrah, S.Z. Afandi and H.A. Bajunid, 2001. Methicillin-resistant *Staphylococcus aureus* in two tertiary-care centers in Jeddah, Saudi Arabia, *Infect Control Hosp Epidemio*, 22: 211-216.
31. Bukharie, H.A. and M.S. Abdelhadi, 2001. The epidemiology of Methicillin-resistant *Staphylococcus aureus* at a Saudi University Hospital. *Microb. Drug. Resist.*, 7: 413-416.
32. Tentolouris, N., G. Petrikos, N. Vallianou, C. Zachos, G.L. Daikos, P. Tsapogas, G. Markou and N. Katsilambros, 2006. Prevalence of methicillin-resistant *Staphylococcus aureus* in infected and uninfected diabetic foot ulcers. *Clin. Microbiol. Infect*, 12: 186-189.
33. Japoni, A., A. Alborzi, M. Rasouli and Bahman Pourabbas, 2003. Modified DNA Extraction for Rapid PCR Detection of Methicillin-Resistant *Staphylococci*. *Iran Biomed. J.*, 8(3): 161-165.
34. Tyagi, A., A. Kapil and P. Singh, 2008. Incidence of Methicillin Resistance *Staphylococcus aureus* (MRSA) in Pus Samples at a Tertiary Care Hospital, AIIMS, New Delhi. *Indian Academy of Clinical Medicine*, 9(1): 33-35.
35. CDC, 1997. Centres for Disease Control. *Staphylococcus aureus* with reduced susceptibility to Vanamycin, United States. *MMWR.*, 46: 765-776.
36. Vlack, S., L. Cox, Y.A. Peleg, C. Canuto, C. Stewart, A. Conlon, A. Stephens, P. Giffard, F. Huygens, A. Mollinger, R. Vohra and James S. McCarthy, 2006. Carriage of methicillin-resistant *Staphylococcus aureus* in a Queensland Indigenous community. *MJA*, 184(11): 556-559.
37. Supriya, S.T., S. Roy and S.V. Jalgaonkar, 2002. Methicillin resistant among *Staphylococcus aureus*: Antibiotic sensitivity pattern and phage typing. *Indian J. Med. Sci.*, 56: 330-334.
38. Pettit, R.K., R.B. Fakoury, C.J. Knight, A.C. Weber, R.G. Pettit, D.G. Cage and S. Pon, 2004. Antibacterial activity of the marine sponge constituent cribrostatin 6. *Journal of Medical Microbiology*, 53: 61-65.
39. Waites, K.B., B.L. Duffy and J.M. Dowzicky, 2006. Antimicrobial Susceptibility among Pathogens Collected from Hospitalized Patients in the United States and *In vitro* Activity of Tigecycline, a New Glycylcycline Antimicrobial. *Antimicrobial Agents and Chemotherapy*, 50(10): 3479-3484.
40. Vidhani, S., P.L. Mehndiratta and M.D. Mathur, 2001. Study of Methicillin Resistant *S. aureus* (MRSA) Isolates from high risk patients. *Indian Journal of Medical Microbiology*, 19(2): 13-16.
41. Dunne, W.M., Jr, Qureshi, H. Pervez and D.A. Nafziger, 2001. *Staphylococcus epidermidis* with intermediate resistance to vancomycin: elusive phenotype or laboratory artifact. *Clin. Infect. Dis.*, 33: 135-137.
42. Akindele, A.A., I.K. Adewuyi, O.A. Adefioye, S.A. Adedokun and A.O. Olaolu, 2010. Antibiogram and Beta-Lactamase Production of *Staphylococcus aureus* Isolates from Different Human Clinical Specimens in a Tertiary Health Institution in Ile-ife, Nigeria. *American-Eurasian Journal of Scientific Research*, 5(4): 230-233.