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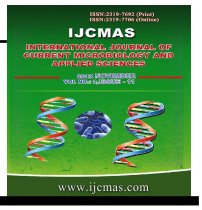

Original Research Article

Prevalence rate and Antibiotic susceptibility test (AST) pattern of Methicillin resistant *Staphylococcus aureus* (MRSA) isolates from different clinical specimens of Teerthankar Mahaveer Hospital, Moradabad, India

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A B S T R A C T

Microorganism's evolution towards resistance to antimicrobial drugs (Antibiotics), including multi-drug resistance, is unavoidable because it shows a particular aspect of the general evolution of microbes that is unstoppable. Methicillin Resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial infections worldwide. Between November; 2012 to August; 2013, 110 clinical specimens from various patients and 110 nasal swabs from healthcare personnel of Teerthanker Mahaveer Hospital, Moradabad, Uttar pradesh were screened for the presence of Methicillin Resistant *Staphylococcus aureus* (MRSA) & their antibiotic susceptibility test (AST) pattern. The overall prevalence rate of MRSA from clinical specimens is 16.27%. The MRSA prevalence rate in the healthcare personnel as carriage is 36.84%. Thus the prevalence rate of MRSA in the carriers is high as compared to the clinical specimen. The AST pattern of isolated MRSA strain from patients and carriers shows highly resistant pattern to different dosage of antibiotics but the MRSA strain isolated from clinical specimens are sensitive to Co-trimoxazole (CT/25 mcg) & the MRSA strain isolated from carriers are sensitive to Tetracyclin (TE/30 mcg). The high incidence of MRSA can be prevented by identifying and screening MRSA carrier inside high-risk wards and healthy health care personnel.

K e y w o r d s

Methicillin Resistant *Staphylococcus aureus* (MRSA); Antibiotic Susceptibility Test (AST) Pattern; Phenol Red Mannitol Salt Agar; Prevalence rate.

Introduction

Staphylococcus aureus is one of the most significant human pathogen that causes both nosocomial and community-acquired infection (Diekema *et al*., 2001). *Staphylococcus aureus* mainly cause opportunistic infections acquired from different sources like patients, hospital staff mainly through their hands and also

from their normal flora. The common types of disease caused by *Staphylococcus aureus* are various types of skin infections including; Staphylococcal Scalded Skin Syndrome (SSSS), Osteomyelitis, Meningitis, Pneumonia, Septicemia, Gastroenteritis etc. Strains of S.aureus that are resistant to methicillin (and oxacillin) have spread worldwide from the last four decades (Ambramson and Sexton, 1992). Infection with MRSA strains, which are resistant to wide range of antibiotics, is associated with considerable morbidity and mortality (Capitano *et al*., 2003; Cosgrove *et al*., 2003). Infection with MRSA is likely to be more severe and requires longer hospitalization. The spread of MRSA may indicate that recommended preventive strategies are either inadequate or improperly implemented (Jean-Christophe Lucet *et al*., 2005). The incidence of methicillin resistant S.aureus (MRSA) in India ranges from 30-70% (Rajaduraipandi *et al*., 2006; Verma*et al*., 2000). *Staphylococcus aureus*, whether methicillin resistant (MRSA) or methicillin susceptible, exhibits a propensity to asymptomatically colonize human hosts. Common anatomic locations of asymptomatic MRSA carriage include anterior nares, throat, groin region, perineal region, mammary folds, axilla, umbilicus and the sites where the skin integrity has been breached, such as wounds (Evans *et al*., 2008). The carrier rate of *Staphylococcus aureus* in the nasal canal among the healthy people range from 20-30%. From the healthy carriers among the hospital health care personnel, there are more chances of spreading from their hands, nose or throat by way of touching, sneezing, talking, coughing etc.

The present study is been conducted to evaluate the comparison of MRSA strains obtained by AST pattern from clinical samples and healthy hospital staff members who remain persistently in contact with patients.

Materials and Methods

Study design

Total 110 clinical specimens from patients and 110 nasal swab from healthcare personnel were collected at Teerthanker Mahaveer Hospital, Moradabad, Uttar pradesh. Sterile dry cotton swab were used for the collection of pus specimen and nasal swab. For collection of nasal swab, the swabs were rubbed very well by rotating 5-7 times over the inner wall of ala and nasal septum and immediately processed for culture and isolation.

Culture of clinical specimen and nasal swab

The specimens were cultured on the Phenol Red Mannitol Salt Agar (a selective medium for *Staphylococcus aureus*) by streaking & the specimens collected in swabs were processed within one hour after collection as per the conventional techniques. The culture plate incubated at 37°C for 24-48 hours in incubator**.**

Isolation and Identification of *Staphylococcus aureus*

Mannitol fermenting yellow colored colony is selected and subject to Gram stain and subcultured on the 5% Blood Agar to observe the β-hemolysis. The isolates showing gram-positive cocci in clusters and β-hemolysis were subjected to catalase and coagulase test by slide and test tube technique using undiluted and 1:6 diluted human plasma respectively.

Screen test for MRSA

A suspension equivalent to 0.5 Mac Farland was prepared from each strain. A swab was dipped and streaked over an area of approximately 2x2.5 Cm. on the surface of a Mueller-Hinton agar supplemented with 4% NaCl and 6 mcg/ml Methicillin (Sigma-Aldrich). Plates were incubated overnight at 37ºC. A growth indicates that the strain is methicillin resistant.

Sensitivity to other antibiotics

AST (Antibiotic susceptibility testing) pattern were studied by Kirby Bauer Disc diffusion techniques as per CLSI (Clinical Laboratory Standards) Guidelines formerly National Committee for Clinical Laboratory Standards. The inoculums of the isolates equivalent to 0.5 Mc Farlands unit were swabbed onto the Muller-Hinton Agar Plate and then the antibiotic disc were placed on it and incubated overnight at 37°C. The zone of inhibition is interpreted acording to CLSI Guidelines. The antibiotics used for testing were Penicillin (PG/10mcg), Amoxicilin (AX/10mcg), Amoxicilin-clavulinicacid (AC/10mcg), Co-trimoxazole (CT/25mcg), Cephalexin (CP/25mcg), Cefazolin (CF/30mcg) , Cefuroxime (CR/30mcg), Erythromycin (ER/15mcg), Chloramphenicol (CK/30mcg), Iprofloxacin (CI/5mcg), Ofloxacin (OF/5mcg), Piperacillin (PC/100mcg), Azithromycin (AZ/15mcg), Tetracycline (TE/30mcg), Methicillin (ME/1mcg), Methicillin (ME/5mcg).

Result and Discussion

Out of 110 clinical specimens, 47 (42.73%) Staphylococcus spp. was isolated. Among which 43 (91.49%) were S.aureus and 7 (16.27%) MRSA strains were isolated. From 110 nasal swabs of healthcare staff, 65 (59.09%) Staphylococcus spp. was obtained. Out of which 19 (29.23%) were S. aureus and the prevalence rate of MRSA strains from nasal swabs of carriers were 7 (36.84%) (Table-3). The prevalence rate of MRSA from pus specimen is 12.90% while from blood specimen is 42.85% (Table-1). The prevalence rate of MRSA in carriers were high in nurses (71.42%) followed by Doctors (50%) (Table-2). The resistivity pattern of the isolated MRSA strains from patients and carriers shows highly resistance pattern. The MRSA strains isolated from clinical specimen are highly sensitive to Co-trimoxazole (CT/25 mcg) while the MRSA strains isolated from carriers are highly sensitive to Tetracycline (TE/30 mcg) (Table-4).

For the past 50 years, S. aureus has been a dynamic human pathogen that has gained the deepest respect of clinician since the report of MRSA infection in US at a Boston city hospital in 1961. Since, then MRSA has become wide spread all over the world (Anupurba *et al*., 2003; Fernandez *et al*., 2005). The incidence of Methicillin resistant S. aureus (MRSA) in India ranges from 30-70%. Anila A. Mathew has reported a prevalence rate of MRSA of about 34% in clinical specimen (Anila). Prevalence rate of MRSA in Eastern U.P. and AIIMS in New Delhi is 54.85% and 44% respectively (Anupurba *et al*., 2003; Arti Tyagi *et al*., 2008). My result shows the prevalence rate of MRSA is about 16.27% in Moradabad District, Uttar pradesh. The anterior nares are considered to be primary colonization site and approximately 30% of healthy people carry the bacteria in the anterior nares (Peter Nilsson and Torvald Ripa,2006).

Table.1 Prevalence rate of Methicillin Resistant *Staphylococcus aureus* (MRSA) from various clinical specimens

Sample	Total no. of specimen (n=110)	Staphylococcus Spp. (n=47)	*S. aureus* (n=43)	MRSA (n=7)
Pus	43	33/43 (76.74%)	31/33 (93.93%)	04/31 (12.90%)
Blood	12	07/12 (58.33%)	07/07 (100%)	03/07 (42.85%)
Urine	32	01/32 (3.12%)	0/01 (0%)	-
Stool	04	01/04 (25%)	01/01 (100%)	-
Wound Swab	12	04/12 (33.33%)	03/04 (75%)	-
Sputum	03	0/03 (0%)	0/0 (0%)	-
Vaginal Swab	02	01/02 (50%)	01/01 (100%)	-
Throat Swab	02	0/02 (0%)	0 (0%)	-

Table.2 Prevalence rate of MRSA from nasal swabs of healthy hospital staff members

Personals	Total no. of sample (n= 110)	Staphylococcal spp. (n= 65)	*S. aureus* (n= 19)	MRSA (n= 7)
Doctor	18	14/18 (77.77%)	04/14 (28.57%)	02/04 (50%)
Nurses	27	17/27 (62.96%)	07/12 (58.33%)	05/07 (71.42%)
Receptionist	04	02/04 (50%)	01/02 (50%)	-
Visitors	04	02/04 (50%)	02/02 (100%)	-
Ward Boy	04	01/04 (25%)	0/01 (0%)	-
Sweeper	26	12/26 (46.15%)	03/08 (37.5%)	-
Lab-technician	27	17/27 (62.96%)	02/12 (16.66%)	-

Table.3 Overall prevalence rate of Methicillin Resistant *Staphylococcus aureus* (MRSA) from clinical specimens and nasal swabs of healthy staff members as carrier

Micro-organism	Clinical Specimens Total (n=110)	Nasal Swabs of carriers Total (n=110)
Staphylococcal species	47/110 (42.73%)	65/110 (59.09%)
Staphylococcus aureus	43/47 (91.49%)	19/65 (29.23%)
MRSA	07/43 (16.27%)	07/17 (36.84%)

Table.4 Antibiotic Sensitive Test (AST) pattern of MRSA strains of clinical specimens and carriers

Antibiotic	Resistivity pattern of MRSA from clinical specimen	Resistivity pattern from carrier
Penicillin (PG/10mcg)	100%	100%
Amoxicillin (AX/10mcg)	100%	100%
Anoxicillin-Clavulanic acid (AC/10mcg)	100%	100%
Co-trimoxazole (CT/25mcg)*	57.14%	80%
Cephalexin (CP/30mcg)	100%	100%
Cefazolin (CF/30mcg)	100%	100%
Cefuroxime (CR/30mcg)	85.71%	100%
Erythromycin (ER/15mcg)	85.71%	40%
Chloramphenicol (CK/30mcg)	85.71%	60%
Ciprofloxacin (Cl/5mcg)	100%	80%
Ofloxacin (OF/5mcg)	100%	100%
Piperacillin (PC/100mcg)	100%	100%
Azithromycin (AZ/15mcg)	85.71%	40%
Tetracyclin (TE/30mcg)*	71.42%	20%
Methicillin (ME/1mcg)	100%	100%
Methicillin (ME/5mcg)	100%	100%

*: Highly sensitive

This nasal carriage can be a potential source of Staphylococcus aureus Bacteremia (Christof *et al*., 2001). Nasal swabs were found to be colonized with at least one staphylococcal species in 92.8% of the cases reported by Karsten Becker (2006). His results shows overall, 52 S. aureus isolates encompassing 47 MRSA and 5 (9.61%) MRSA isolates. Rezvan Moniri has presented the prevalence rate of MRSA in nasal carriage as 52.6% (Rezvan Moniri, *et al*., 2009). In our current study, frequency of Staphylococcal species, S. aureus and MRSA is 59.09%, 29.23% and 36.84% respectively which is nearly analogous to Rezvan's study.

The prevalence rate of various MRSA isolates obtained from different clinical and carrier subjects were determined. The prevalence rate of MRSA is 31.1% in clinical specimens and 37.9% MRSA in carriers respectively; by Rajaduraipandi *et al*. (2006). MRSA carriage is far more prevalent than MRSA-positive clinical specimen (Jean-Christophe Lucet *et al*., 2005). My result shows 36.84% MRSA isolates from carriage and 16.27% from clinical specimen. Thus my study also shows high prevalence rate of MRSA in carriers compared to clinical specimen.

Maximum isolation of MRSA was from pus , reported by Tiwari *et al* and also by S. Anupurba (Anupurba *et al*., 2003; Hare Krishna Tiwari *et al*., 2009). As high as 35.7% of MRSA strains were obtained from throat swabs and 33.6% of strains

were obtained from pus among clinical isolates, reported by Rajaduraipandi *et al*. (2006). Similar observation was made by Mehta, who in his study on control of MRSA in a tertiary care center, had reported an isolation rate of 33% from pus and wound swabs (Mehta *et al*., 1998). However, Qureshi from Pakistan reported a high isolation rate of up to 83% MRSA from pus (Qureshi *et al*., 2004). In our study maximum isolates of MRSA is found from blood specimen compared to pus specimen which co-relates with the study of Anbumani N. at Chennai (Anbumani *et al*., 2006).

The highest level of resistance of S. aureus strain has been observed with penicillin (100%), amoxicillin (91.9%) and Cefalexin (55.5%), which is in accordance with the reports of Tiwari *et al*. (2009). The level of resistance observed in reports of Nwankwo EOK as Amoxycillin-clavulinic acid (51%), Ciprofloxacin (36%) and Oxacillin (10%) (Nwankwo *et al*., 2010). Our result also showed the highest level of resistance pattern compared to other studies i.e. Penicillin (100 %), Amoxicillin (100%), Amoxycillin-clavulinic acid (100%), Ciprofloxacin (100%) and Cefalexin (100%), Methicillin (100%).

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