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Research Article

GROWING CONCERN OF METHICILLIN RESISTANCE *Staphylococcus aureus* FROM A TERTIARY CARE HOSPITAL, INDIA

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Abstract- Background: Staphylococcus aureus (S. aureus) remains, to date, one of the major causes of both health-care associated (HA) and community-associated (CA) infections. S. aureus causes a variety of infections, ranging from skin and soft tissue infections [SSTI] to life threatening endocarditis. The present study was conducted to characterize Methicillin resistant S. aureus (MRSA) infections with reference to associated risk factors, clinical syndrome and its development of antimicrobial resistance.
Methods: 400MRSA were isolated by standard conventional methods from various clinical samples received in the department of microbiology. Antimicrobial susceptibility was determined by CLSI guidelines. Inducible clindamycin were detected by as per CLSI guidelines by D-zone test. Demographic and clinical history was collected from medical record.
Results: Total of 400 MRSA were collected from various clinical samples received from various wards and intensive care units (ICUs). 107 (26.75%) MRSA were from blood stream infections (BSIs) and endocarditis, 81(20.25%) were from osteomyelitis and septic arthritis, 97(24.25%) were from skin and soft tissue infections, 62(15.5%) were from pneumonia, 45(11.25%) were from urinary tract infection (UTI). Of the total 400 MRSA strains; 183(45.75%) strains were isolated from pediatric and neonatal age group. 41 % MRSA strains were isolated from various intensive care Unit. 35.75% strains were determined as a inducible clindamycin phenotype while all MRSA strains were susceptible for vancomycin and tigecycline. 99.5% strains were susceptible for linezolid. 75.25% and 42.75% MRSA strains were defined as CA-MRSA and HA-MRSA according to CDC epidemiologic definitions by clinical criteria;
Conclusion: Local surveillance data to identify prevalent pathogens, detect bacterial resistance and to identify disseminated strains is decisive to the selection of best possible treatment regimens.

Keywords- MRSA, CA-MRSA, HA-MRSA, Inducible clindamycin resistance

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Introduction

Staphylococcus aureus (*S. aureus*) remains, to date, one of the major causes of both health-care associated (HA) and community-associated (CA) infections. *S. aureus* causes a variety of infections, ranging from skin and soft tissue infections [SSTI] to life threatening endocarditis. The frequency of methicillin- resistant *S. aureus* (MRSA) infections continues to grow in hospitals-associated settings and more recently in community settings globally [1, 2]. The 2004 National Nosocomial Infection Surveillance (NNIS) system data demonstrate a steady increase in the incidence of nosocomial infections caused by MRSA among intensive care unit [ICU] patients over time. MRSA now accounts for ≥ 60% of *S. aureus* isolates in US hospital ICUs [3]. This represented an 11% increase in resistance compared with rates for the period 1998 to 2002. Proportion of Blood stream infection (BSI) by MRSA in hospitalized pediatric patients increased from 10% to 29% in 2001 [3, 4]. The [Surveillance and Control of Pathogens of Epidemiologic Importance] SCOPE project United States (2004) report showed a significant increase in the proportion of MRSA infections among ICUs from 1995 to 2001 (22% vs. 57%; $P < 0.001$) [5]. Consequently a growing concern is the emergence of MRSA infections in patients with no evident risk factors. Significant morbidity and mortality have been reported in association with MRSA infections in community settings. Increasing frequency of MRSA infections and changing patterns in antimicrobial resistance have led to concern in the use of macrolide lincosamide – streptogramin B (MLSB) antibiotics to treat such infections. However, their widespread use has led to an increase in the number of *S. aureus* strains resistant to MLSB antibiotics. Data describing MLSB - prevalence or clinical predictors of the presence of MLSB among MRSA isolates are quite limited in India. The epidemiological and microbiological characteristics of pathogenic organisms have been rapidly shifting because of selection pressure. Multidrug-resistant strains are rapidly evolving, including the more serious glycopeptides resistant strains and leaving the clinicians with very few therapeutic options. A therapeutic decision is not possible without the relevant clinical and microbiological data. Hence, we have investigated the incidence of prevalent *S. aureus* infection in relevance of clinical infections with associated risk factors. Antimicrobial susceptibility profiles were also analyzed to improve antibiotic policy in support to reduce frequency of MRSA infection.

Materials and Methods

The present study was conducted in department of Microbiology, Dr. D.Y. Patil Medical College, Hospital and Research Centre Pimpri -Pune 411018. It is tertiary care hospital. The study was conducted from January 2013 to December 2014. The study was approved by the Institutional ethics committee.

Inclusion criteria

All Isolates of MRSA from the various clinical samples received in the Microbiology department

Clinical samples

Blood, pus, wound swab, urine, CSF and body fluids.

Sample processing

S. aureus isolates were identified by the standard conventional methods from various clinical specimens. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method for co-trimoxazole (25µg), Gentamycin (30µg), erythromycin (15µg), linezolid (30µg), tetracycline (30µg), and vancomycin (30µg) as per guidelines from Clinical and Laboratory Standards Institute (CLSI). Screening for Oxacillin resistance using Oxacillin (1µg) on (Muller-Hinton) M-H agar supplemented with 2% NaCl followed by overnight incubation at 35°C [6, 7].

Phenotypic detection of inducible resistance to Clindamycin by D-zone test

The inducible Clindamycin resistance was performed by D- zone test using erythromycin (15µg) and clindamycin (2 µg) discs as per CLSI (Clinical Laboratory Standard Institute) guidelines. Three different phenotypes were interpreted as MS phenotype, Inducible MLSB phenotype and Constitutive MLSB phenotype [8, 9].

Quality control

S. aureus ATCC 25923 were used as the quality control strain.

Medical records for the source patients were reviewed for the demographic information, history of prior hospitalization, presence of major comorbid conditions (e.g. Diabetes mellitus, renal dysfunction, post-surgical status, malignancy, solid organ or stem cell transplantation, neutropenia, trauma or burn injury) and antibiotic exposure within the preceding year.

MRSA isolates were designated as HA - MRSA if the source patient had any of the following risk factors: a history of hospitalization, residence in a long term care facility (e.g. nursing home), dialysis, or surgery within one year to the date of specimen collection; growth of MRSA within 48 h after admission to a hospital, the presence of permanent indwelling catheter or percutaneous device at the time of culture; or prior positive MRSA culture report. If none of the above risk factors were present, the isolates were considered CA – MRSA [10-12].

Results and Discussion:

A total of 400 MRSA were collected from various clinical samples received from various wards and ICUs. 107 (26.75%) MRSA were from Blood sample of BSI and endocarditis 81 (20.25%) were from osteomyelitis and septic arthritis, 97 (24.25%) were from skin and soft tissue infections (SSTI), 62 (15.5%) were from pneumonia, 45 (11.25%) were from UTI. Of the total 400 MRSA strains; 183 (45.75%) strains were isolated from pediatric and neonatal age group, 52.72% MRSA infections were detected in female and predominance were also detected in the clinical syndrome like SSTI and osteomyelitis and UTI where as 47.25% MRSA infections were from male predominantly detected in bacteremia, endocarditis and pneumonia. 75.25% and 42.75% MRSA strains were defined as CA-MRSA and HA-MRSA by clinical criteria; CDC epidemiologic definitions [Table-1]

Table-1 *Demographic and clinical characteristics of patients with MRSA infections. (n=400)*

Clinical syndromes	
Bacteremia, endocarditis or sepsis	107 (26.75%)
Osteomyelitis or septic arthritis	81 (20.25%)
Pneumonia	62 (15.5%)
Skin and soft tissue infection [SSTI]	97 (24.25%)
Urinary tract infection	45 (11.25%)
Other	08 (2%)
Age group	
Pediatric	183 (45.75%)
Adult-	217(54.25%)
Gender	
Male	189(47.25%)
Female	211 (52.75%)
Presence of risk factors for HA MRSA(n=229)	**50.5%**
Inpatient culture obtained >48 hrs after admission -	84 (21%)
Hospital stay, past year	27 (6.75%)
Surgery ,Past 6 months	21 (5.25%)
Haemodialysis, past year	19 (4.75%)
Indwelling catheter	63 (15.75%)
Stay in long care facility, past year	15 (3.75%)
Location of care	
Intensive care units	164 (41%)
Various wards	231 (57.75%)
Emergency department	4 (1%)
Outpatient	23 (5.75%)
CDC criteria for infection type	
CA-MRSA	229 (57.25%)
HA-MRSA	171 (42.75%)

Table-2 *Distribution of the antibiotic resistance pattern among the isolates*

Antibiotics	n=400	
	Sensitive	Resistant
Oxacillin	00(0%)	400(100%)
Gentamycin	278(69.5%)	122(30.5%)
Tetracycline	152(31.25%)	248(62%)
TMP-SMX	305(76.25%)	97(24.25%)
Linezolid	398(99.5%)	2 (0.5%)
Erythromycin	139(34.75%)	261(65.25%)
Clindamycin	162(40.5%)	238(59.5%)
Vancomycin	400 (100%)	00(0%)
Cefotaxime	189(47.25%)	211(52.75%)
Tigecycline	400(100%)	00(0%)
Ciprofloxacin	232(58%)	168(42%)

All MRSA strains were susceptible to tigecycline and vancomycin. 99.5% strains were susceptible to linezolid. Of the total MRSA; 40.5% strains were susceptible for clindamycin and all strains isolated from SSTIs were susceptible clindamycin. 248 (62%) MRSA strains were resistant to tetracycline. Of the total 81 MRSA strains isolated from osteomyelitis; 44 strains were resistant to tetracycline. 69.5% MRSA strains showed susceptibility for gentamycin, 305(76.25 %) strains were susceptible for TMP-SMX [Table-2].

Table-3 *Distribution of MRSA Among Different Wards and ICUs*

Ward	No. of MRSA isolation (n=400)
NICU[Neonatal ICU]	46 (11.5%)
PICU [Pediatric ICU]	26 (6.5%)
SICU [Surgical ICU]	34 (8.5%)
MICU [Medicine ICU]	58 (14.5%)
OBGY[Obstetrics and gynecology]	26 (6.5%)
Surgery	62 (15.5%)
Medicine	44 (11.5%)
Orthopedic	35 (8.75%)
Pediatrics	26 (6.55)
Skin and VD	12 (3%)
Opthalm	04 (1%)
Emergency department	04 (1%)
OPD	23 (5.75%)
TOTAL	

Of the total 400 MRSA strains; 183(45.75%) strains were isolated from pediatric and neonatal age group. 41 % MRSA strains were isolated from various intensive care Unit. Of the total MRSA isolated from various ICUs; maximum strains were from MICU (35.35%) and NICU 28.04%) [Table-3].

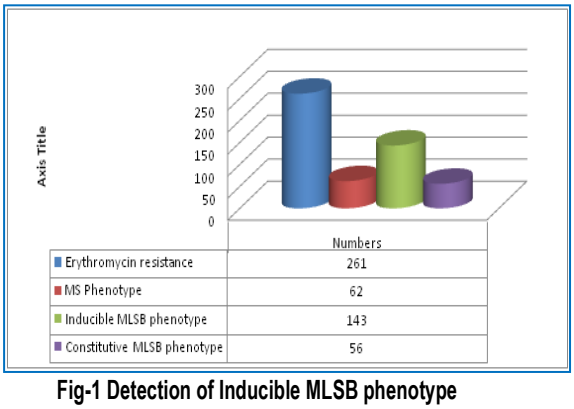

Fig-1 Detection of Inducible MLSB phenotype

Of the total 400 MRSA strains 261 (65.25%) strains were erythromycin resistance and further detected for production of inducible clindamycin. 143 (35.75%) strains were positive for D-zone test i.e. Inducible MLSB phenotypes of which 44 (11%) strains were isolated from various ICUs. 56 (14%) strains were constitutive MLSB phenotype [Fig-1]. Two linezolid resistant strains were positive for Inducible clindamycin resistant and were isolated from MICU and SICU.

Discussion

The treatment of serious MRSA infections presents a great challenge to clinicians, particularly bacteremias and infective endocarditis, for which bactericidal therapy is essential to improve clinical outcome. *S. aureus* infective endocarditis represents nearly 30% of definite cases of infective endocarditis [1-3], in present study 107 (26.755) MRSA strains were from bacteremia and endocarditis patients. 81 (20.25%) strains were from osteomyelitis and septic arthritis, 97 (24.25%) strains were from skin and soft tissue infection, 62 (15.55%) strains were from pneumonia, 45 (11.25%) strains were from urinary tract infection [Table-1]. Of the total MRSA; 46 (11.5%) strains were from NICU and predominantly isolated from sepsis while 58 (14.5%) strains were from MICU [Table-3]. 57.25% patients with MRSA infections had one or more established risk factors for HA-MRSA and these includes; 6.75% patients who had been hospitalized within the past year, 5.25% patients who had been operated in last six months, 4.75% patients were on haemodialysis, 15.75% patients were hospitalized with indwelling catheter while 3.75% were taken long care facility while 21% strains was from inpatient culture obtained > 48 hrs. after admission. Although there are no established risk factors for CA-MRSA infections; person to person transmission has been reported and numerous risk factors have been recognized to predict disease of which necrotizing fasciitis, empyema, septic thrombophlebitis, an influenza-pneumonia, pyomyositis with or without osteomyelitis, bacteremia, septicemia were recorded in the present study. Prevalence of SSTI was reported by Gregory et. al.(2006) from 11 U.S. centers from emergency department [13]. Glycopeptides, particularly Vancomycin have been the preferred antimicrobial agent to treat such MRSA infections; however, many investigators reported MRSA strains with reduced susceptibility i.e. vancomycin-intermediate *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) [14-16]. In the present study, all MRSA strains were susceptible to vancomycin and tigecycline. The first documented infection caused by VRSA in the United States was reported by the Michigan department of Community health in 2002 [17, 18]. Since then, 8 additional cases have been confirmed by CDC [14-18]. Doxycycline is FDA-approved for the treatment of SSTI due to *S. aureus*. Although tetracycline has *in-vitro* activity; data on the use of tetracycline for the treatment of MRSA infections are limited. Tetracycline appears to be effective in the treatment of SSTI, but data are lacking to support their use in more-invasive infections. In the present study overall 62% resistance were determined in tetracycline while of 97 MRSA strains isolated from SSTI patients 44 (45.36%) strains were resistant to tetracycline [Table-2]. Franz JS et. al. (2001) reported susceptibility to tetracycline 89.7% in the MSSA isolates and 42.9% in the MRSA isolates [19]. Clindamycin is approved by the US Food and Drug Administration (FDA) for the treatment of serious infections due to *S. aureus*. Although not specifically approved for treatment of MRSA infection, it has become widely used for treatment of SSTI and has been successfully used for treatment of invasive susceptible CA-MRSA infections in children, including osteomyelitis, septic arthritis, pneumonia, and lymphadenitis [18-20]. In the present study, 59.5% MRSA strains were resistant clindamycin and 40.5% MRSA strains were susceptible to clindamycin. The D-zone test was recommended for erythromycin-resistant, clindamycin-susceptible isolates to detect inducible clindamycin resistance. Of the total 261 erythromycin resistant MRSA strains 143 (54.78%) strains were MLSB phenotype and 62 (23.75%) MS phenotypes [Fig-1]. Clindamycin has excellent tissue penetration, particularly in bone and abscesses, although penetration into the CSF is limited [21]. In the present study; 75.25% and 42.75% MRSA strains were defined as CA-MRSA and HA-MRSA by clinical criteria; CDC epidemiologic definitions. *In vitro* rates of susceptibility to clindamycin were higher among CA-MRSA than they were among HA-MRSA. Although there is variation by geographic region; Martine A.G. et. al 92001) and Franz J.S. et. al (2009) detected similar findings [19,20]. Jadhav et. al (2011) reported 9.19% MLSB phenotype strains from the present institute [9]. The clinical significance of inducible clindamycin resistance is unclear because the drug may still be effective for some patients with mild infections; however, its presence should preclude the use of clindamycin for more-serious infections.

Linezolid is FDA-approved for adults and children for the treatment of SSTI and nosocomial pneumonia due to MRSA. Linezolid resistance is rare, although an outbreak of linezolid-resistant MRSA infection has been described. The increases in vancomycin resistance among MRSA and excessive use of antimicrobial agents have worsened the sensitivity [22-25]. In present study; 2 (0.5%) strains were linezolid resistant from MICU and SICU while all are susceptible to linezolid. TMP-SMX is not FDA-approved for the treatment of any staphylococcal infections. However, because 95%–100% of CA-MRSA strains are susceptible *in vitro* it has become an important option for the outpatient treatment of SSTI [22-23]. In the present study; 76.25% MRSA strains were TMP-SMX susceptibility TMP-SMX were effective for the treatment of purulent SSTI in children. Evidence-based guidelines for the management of patients with MRSA infections were prepared by Expert Panel of the Infectious Diseases Society of America. The guidelines are intended for use by health care providers who care for adult and pediatric patients with MRSA infections [26].

Conclusion

The result of this study showed occurrence and characterization of MRSA in a tertiary care hospital in India. Early detection, isolation and or decolonization of infected and colonized persons are needed. More data necessitate to be done in various geographical regions of the country. Stringent approach to prevention and control of antimicrobial resistance is needed.

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