

Research Article

Prevalence and Antimicrobial Susceptibility Pattern of Different Clinical Isolates of HA-MRSA and CA-MRSA in a Tertiary Care Rural Hospital, Bankura, West Bengal, India

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is the major causative bacterial pathogen responsible for hospital and community associated infections. Currently, MRSA is divided into two subgroups: the healthcare associated MRSA (HA-MRSA) and community associated MRSA (CA-MRSA). HA-MRSA is the major problem in nosocomial infections. For instance, patients in hospital with open wounds, invasive devices or under immune compromised conditions are at much higher risk of getting HA-MRSA infection. On the other hand, CA-MRSA has recently risen as a major public health concern. The study was conducted to find the prevalence and antibiotic susceptibility pattern of HA-MRSA & CA-MRSA in a tertiary care hospital of rural West Bengal. In this hospital based prospective study, 940 samples collected over a three months period were analyzed phenotypically using conventional microbiological methods. Subsequently, the antibiotic sensitivity tests were performed for the confirmed MRSA isolates. Of the 940 clinical specimens included in the present study, only 431 were growth positive out of which 122 were identified as *S.aureus*. Among the 122 *S. aureus* isolated, 23 were MRSA. Out of the 23 MRSA isolates 15 were HA-MRSA and 8 were CA-MRSA. The study revealed that the prevalence of HA-MRSA (65.21%) infections is higher than CA-MRSA (34.78%) in our hospital. The resistance to different antibiotics of HA-MRSA is not significantly different to that of CA-MRSA. While the incidence of MRSA in this study is lower than other parts of India, HA-MRSA contributes a larger percentage in the total.

Keywords: MRSA, HA-MRSA, CA-MRSA, *Staphylococcus aureus*.

INTRODUCTION

The genus *Staphylococcus* includes pathogenic organisms in which *Staphylococcus aureus* is the most important. *S. aureus* is the most prevalent pathogen causing hospital infection throughout the world [1] and the incidence is still increasing that ranges from minor skin infections to fatal necrotizing pneumonia [2]. It has overcome most of the therapeutic agents that have been developed in the recent years [3]. The most notable example of this phenomenon was the emergence of methicillin resistant *Staphylococcus aureus* (MRSA). It was reported just one year after the launch of methicillin [4].

MRSA is a bacterium responsible for hospital and community-associated infections [5]. MRSA are a type of staphylococcus or "staph" bacteria, resistant to many antibiotics. Staph bacteria normally live on the skin and in the nose, usually without causing problems.

MRSA is different from other types as it cannot be treated with certain antibiotics such as methicillin. They are bacteria more likely to develop when antibiotics are used too often or incorrectly used. Given enough time, bacteria can change and the antibiotics no longer work well [6]. Thus, MRSA and other antibiotic-resistant bacteria are sometimes called "super bugs" [7].

MRSA strains have acquired a gene that makes them resistant to all beta-lactam antibiotics. Until the development of penicillin for use as an antibiotic in the 1940s, up to 50% of serious *S. aureus* infections resulted in death. Unfortunately, shortly after the introduction of penicillin, *S. aureus* strains resistant to penicillin were isolated [8]. MRSA were first reported in the early 1960's and are now regarded as a major hospital acquired pathogen worldwide. The term methicillin resistant is historically used in order to describe the resistance to any of this class of

antimicrobials [5, 7, 9]. The drugs of choice for treatment of staphylococcal infections are the beta-lactam antibiotics, such as penicillins, cephalosporins, monobactam and carbapenems. However, through the years, the bacterium has evolved several mechanisms that render it to be resistant to the antimicrobials. The most common mechanism is the production of β -lactamase that inactivates many of the β -lactam antibiotics [10]. Currently, MRSA is divided into two subgroups: the healthcare associated MRSA (HA-MRSA) and community associated MRSA (CA-MRSA), CA-MRSA strains are genetically different from HA-MRSA strains [11-13].

These divisions were originally based on epidemiological features and microbiological characteristics. Later it become an important character for molecular typing, antimicrobial susceptibility testing, and identification of methicillin resistance besides the presence of special toxin genes [14]. HA-MRSA is the major problem in nosocomial infections in hospitals, where patients with open wounds, invasive devices or under immune compromise conditions are at much higher risk of getting HA-MRSA infection [15].

On the other hand, CA-MRSA has recently risen as a major public health concern. CA-MRSA is defined as an MRSA infection by individuals in an outpatient setting or by inpatients discharged within 48 hours of hospital admission [16]. Although the border between HA-MRSA and CA-MRSA is not clearly distinguishable, CA-MRSA infections generally differ from the HA-MRSA both phenotypically and genotypically [17]. CA-MRSA is usually resistant to the β -lactam antibiotics and usually susceptible in vitro to Fluoroquinolones, Trimethoprim/sulfamethoxazole, Clindamycin and Chloramphenicol. This is in contradistinction to HA-MRSA, which is usually resistant to Fluoroquinolones, Clindamycin, and Chloramphenicol, and is less sensitive to Trimethoprim/sulfamethoxazole [18]. While HA-MRSA isolates are typically multi-drug-resistant, CA-MRSA isolates are susceptible to more classes of antibiotics [18, 19]. Given the complex epidemiology of CA-MRSA strains in health care settings and the circulation of HA-MRSA strains that occurs in the community, establishing a clear delineation between CA-MRSA and HA-MRSA strains has not been possible [20].

In addition, the Pantone-Valentine leukocidin (PVL) gene encodes a pore-forming cytotoxin that acts preferentially against leukocytes and erythrocytes, and this is commonly found in CA-MRSA and only rarely in HA-MRSA [21]. CA-MRSA differs in several other ways from HA-MRSA and these differences are summarized (chart-1).

Resistance to methicillin in staphylococci is mediated by an altered penicillin-binding protein (PBP2a), which

is encoded by the *mecA* gene and confers resistance to most of the current β -lactam antimicrobial agents [22].

S. aureus can cause a wide range of infections from non-invasive skin and soft tissue infections to invasive infections of the bone, joint, and blood; but it can also colonize the human body without causing disease. Up to 30% of the population at any point in time is colonized with *S. aureus*, most often in the anterior nares [23].

A surveillance conducted in ICUs of hospitals in seven Indian cities reported that 87.5% of all *S. aureus* HCAs was caused by MRSA strains [24]. Also isolation of CA MRSA from skin and soft tissue infections [25] and even blood stream infections have been reported [26].

Thus, in order to combat the significant problem of this drug resistant bacterium, further surveillance is needed, so that the data obtained may be analysed and utilized for Infection control measures in the hospital as well as in the community.

MATERIALS AND METHODS

All samples submitted for bacteriological culture from Outpatient Department as well as Wards were included in the study. For isolation and identification, samples were inoculated on a sterile MacConkey's agar, Blood agar, Nutrient agar plates and the plates were incubated at 37°C for 18 to 24 hours. Plates were observed for growth and Gram staining and biochemical tests were performed from isolated colonies. *MRSA Susceptibility Testing*: The isolates were tested by the modified Kirby-Bauer disk diffusion method on Muller Hinton agar (Hi-Media) and interpreted according to CLSI guidelines. The antibiotics included in the study were ampicillin, levofloxacin, azithromycin, vancomycin, cefoxitin and linezolid. Methicillin resistance was determined by the disk diffusion method using Cefoxitin disc (30 μ g) on Mueller-Hinton agar supplemented with 2% sodium chloride [27].

RESULTS

The characteristics of *S. aureus* include golden yellow colour colonies on Nutrient agar, lactose fermentation on MacConkey agar, gram positive cocci arranged in clusters seen in gram staining and positive catalase test, tube coagulase and mannitol fermentation test (Table 2 and 3). The study was also conducted to find the incidence and antibiotic susceptibility pattern of MRSA in a period of three months from 15th Nov 2014-15th Feb 2015. Of the 940 clinical specimens included in the present study, only 431 were growth positive out of which 122 were identified as *S. aureus*. Among the 122 *S. aureus* isolated, 23 were MRSA. Out of the 23 MRSA isolates 15 were HA-MRSA and 8 were CA-MRSA (Table 4). There was no significant difference in the resistance pattern between the CA-

MRSA and the HA-MRSA to different antibiotics tested in this study (Table 5).

Table 1: Characters that are used to distinguish between HA-MRSA and CA-MRSA [21]

Factor	HA-MRSA	CA-MRSA
Risk factors and at-risk populations	Previous contact with healthcare settings	Team-sport participants, incarcerated persons, military, and children
Antibiotic resistance pattern	Multiply resistant	Sensitive to many except β -lactams
Associated clinical syndromes	Bacteraemia, pneumonia	Skin and soft tissue infections
Mean age at infection	Older	Younger

Table 2: Biochemical characteristics of *S. aureus*

No.	Biochemical Test	Reaction (+/-)
1.	Catalase	+
2.	Oxidase	+
3.	Indole production	-
4.	Nirtate reduction	+
5.	Methyl Red	+
6.	Voges-Proskauer	+
7.	Tube coagulase	+
8.	Mannitol fermentation	+
9.	Hemolysis	+
10.	Phosphatase	+

Table 2: Detection and identification of colony of *S. aureus*

Identification media	Testing feature
Nutrient Agar	Colonies are 2-4mm in diameter, circular, smooth, convex, opaque and easily emulsifiable and most of the strains produce golden yellow pigment.
Blood Agar	Colonies are 2-4mm in diameter, circular, smooth, convex, opaque and easily emulsifiable and a beta type of hemolysis is seen.
MacConkey's Agar	Colonies are very small and pink due to lactose fermentation.
In liquid media	Uniform turbidity is produce.

Table 3: Table showing isolated *S aureus* with breakup of MRSA types

Clinical Samples	940	
<i>S. aureus</i>	122	
	23 (18.85% of <i>S aureus</i>)	
MRSA	HA-MRSA	15
	CA-MRSA	8

Table 4: Antimicrobial sensitivity of CA-MRSA and HA-MRSA to various antibiotics

Antimicrobial agent	No.(%) of CA-MRSA Resistance out of 8 strains	No.(%) of HA-MRSA Resistance. out of 15 strains
Linezolid	6(75%)	11(73.33%)
Levofloxacin	5(62.5%)	9(60%)
Vancomycin	6(75%)	10(66.66%)
Azithromycin	7(87.5%)	11(73.33%)

DISCUSSION

The prevalence of MRSA ranges from 23.6% in Assam [28], 54.85% in from Uttar-Pradesh [29]. The 1980s shows a prevalence of the growing problem in the Indian scenario is that MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999 [30, 31] and later studies in 2007-2008 shows 35%, 26.14% and 35% reported from Tamilnadu [32], Nepal [33] and China [34] respectively, showing a rising trend. In this study, 18.85% of MRSA were isolated in a three months period. This low figure may be due to the unexposed rural population that this

tertiary care hospital serves. Phenotypically differentiation of HA-MRSA and CA-MRSA classified by source of the samples and drug resistance pattern shows higher numbers of HA-MRSA in this study, although genetic markers of CA-MRSA and HA-MRSA have to be studied in order to confirm the epidemiology of the MRSA in this area. Studies in India show higher number of CA-MRSA [35] being isolated rather than HA-MRSA. But in this study HA-MRSA was found in higher numbers than CA-MRSA. A larger sample size through a longer time period will verify this finding.

CONCLUSION

The major features that emerge in this initial study is that the incidence of MRSA infections is lower than in other Indian studies, probably because of rural background of population and lower population density. But unlike other Indian studies incidence of HA MRSA is higher than CA-MRSA in our study. The resistance to different antibiotics of HA-MRSA is not significantly different to that of CA-MRSA. This reflects the complex epidemiology of MRSA in the hospital and community with probable dissemination of hospital strains causing infection in the community. The establishment of an Infection control program with documented Antibiotic policy will help in keeping rates of emergence of resistant organisms low in this region and may also help in arresting the spread of infections in this part of India.

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