

Antimicrobial Susceptibility Pattern among Methicillin-Resistant *Staphylococcus Aureus* (MRSA) Isolates in Bihar

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Abstract: *Introduction:* Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare and community associated infection worldwide. Life threatening sepsis, endocarditis and osteomyelitis caused by methicillin resistant *Staphylococcus aureus* (MRSA) have been reported from several parts of the world. Since MRSA strains are also resistant to multiple antibiotics, there is possibility of extensive outbreaks, which may be difficult to control. *Objective:* To study the prevalence of MRSA from a tertiary care hospital in Bihar. *Methods:* Each of the isolated and confirmed *Staphylococcus aureus* strains were tested for methicillin resistance by Kirby-Bauer disc diffusion method in Mueller-Hinton agar using oxacillin discs (1 µg). **RESULT:** From a total of 150 samples of *Staphylococcus aureus*, 87 (58%) samples were MRSA. *Conclusion:* MRSA is emerging to be a significant problem pathogen.

Keywords: MRSA, Oxacillin disc, vancomycin, Kirby-Bauer disc diffusion method, Mec A

1. Introduction

The bacterium *Staphylococcus aureus* is a common cause of human infection, and it is becoming increasingly virulent and resistant to antibiotics. The nosocomial strains of *Staphylococcus aureus* are very prone to develop resistance to various anti-Staphylococcal drugs. The beta-lactamase resistant Penicillins (methicillin, oxacillin, cloxacillin and flucloxacillin) were developed to treat penicillin resistant *Staphylococcus aureus* and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used, but only 2 years later, the first case of Methicillin Resistant *Staphylococcus aureus* (MRSA) was reported in England [1].

S. aureus is well known for its ability to become resistant to antibiotics. Infections that are caused by antibiotic-resistant strains often occur in epidemic waves that are initiated by one or a few successful clones. MRSA features prominently in these epidemics. Epidemic strains of these methicillin-resistant *S. aureus* (MRSA) are usually also resistant to other antibiotics. Methicillin resistance is a complex property, and more than one mechanism is involved. Resistance to methicillin is due to low affinity PBP's substituting the activities of the normal and essential PBP's. This low affinity PBP's are called PBP2a (PBP2), which is encoded for by the Mec A gene. In addition methicillin resistance is dependant on other factors like Methicillin-repressor Mec-1, anti-repressor MecR1 and factors essential for expression of methicillin resistance [2,3]. Historically associated with hospitals and other health care settings, MRSA has now emerged as a widespread cause of community infections. Community or community-associated MRSA (CA-MRSA) can spread rapidly among healthy individuals. Outbreaks of CA-MRSA infections have been reported worldwide [2].

The increase in the incidence of infections due to *S. aureus* is partially a consequence of advances in patient care and also of the pathogen's ability to adapt to a changing environment.

Infection due to *S. aureus* imposes a high and increasing burden on health care resources. A growing concern is the emergence of MRSA infections in patients with no apparent risk factors [4]. Methicillin resistant *S. aureus* (MRSA) is now endemic in India. The incidence of MRSA varies from 25 percent in western part of India [5] to 50 percent in South India [6].

Community acquired MRSA (CA-MRSA) has been increasingly reported from India [7]. The growing problem in the Indian scenario is that MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999 [8]. Nosocomial transmission of MRSA serves as a source of hospital outbreaks, and recent reports of vancomycin resistant *S. aureus* strains in the United States emphasize the need for better control of MRSA and other resistant bacteria within healthcare settings [9].

Aims and Objective

The present study will be undertaken with the aim to establish:

- Detection of methicillin resistant *Staphylococcus aureus* (MRSA) in hospitalized patients and patients attending Outdoor service of Patna Medical College and Hospital, Patna.
- Patterns of antibiotic susceptibility of methicillin resistant *Staphylococcus aureus* isolated from various sources

2. Materials and Methods

The proposed study was carried out in the Department of Microbiology, Patna Medical College and Hospital, Patna, during the period commencing from September 2013 to August 2014.

Source of Sample: A total of one hundred fifty isolates of *Staphylococcus aureus* isolated from various clinical specimens were included in this study. The various clinical

specimens were collected from patients attending Outpatient Department Service and patients admitted in the various wards of Patna Medical College and Hospital, Patna, referred to the Department of Microbiology, Patna Medical college and Hospital, Patna for culture and antibiotic susceptibility testing of clinical specimens.

Clinical Specimen: The clinical specimens were swabs collected from different sites and lesions along with body fluids .

The origins of the specimens were:

- Wound swab
- Pus
- Throat/ Aural/ Nasal Swabs
- Sputum
- Blood
- Urine
- Other Body Fluids (Pleural/ Ascitic/ Cerebrospinal Fluid)

3. Methodology

The specimens were collected onto sterile swabs which were either dry or dipped in sterile physiological saline and then inserted into sterile container tubes to be transported to the bacteriology laboratory. Sputum was collected into sterile, wide-mouthed, screw- capped containers or wide mouthed test tubes which were then stoppered. Blood was collected by aseptic technique and immediately inoculated into glucose broth. Urine samples were collected using the mid-stream, clean catch method into wide-mouthed ,sterile ,screw-capped pots.

Media for Isolation of Organism:

- 5% sheep blood agar medium
- MacConkey's agar
- Nutrient agar medium

Preparation of Media: Media was purchased from Hi Media Laboratoies Pvt. Ltd., A-406, Bhaveshwar Plaza, LBS Marg, Mumbai – 400086, India.

Identification : *Staphylococcus aureus* isolated from samples was identified by standard methods described by Baird D (1996)[10], based on :

- (1) Colony Morphology
- (2) Pigment production
- (3) Gram's Stain
- (4) Catalase Test
- (5) Slide and Tube Coagulase Test
- (6) Modified Hugh and Leifson (O/F) Test
- (7) Fermentation of Mannitol

Quality Control :

- Positive Control : *Staphylococcus aureus* – NCTC 6571
- Negative Control : *Staphylococcus pyogenes*

Each of the isolated and confirmed *Staphylococcus aureus* strains were tested for methicillin resistance by Kirby-Bauer disc diffusion method in Mueller-Hinton agar using oxacillin discs (1 µg) .Oxacillin discs were obtained from Hi-Media Laboratories Pvt. Ltd. Inoculum was prepared to give semi-confluent growth. At least 4-5 representative colonies are touched and suspended in 4 ml sterile saline to adjust

equivalent to 0.5 McFarland Standard. A zone of inhibition less than 10 mm or any discernible growth within zone of inhibition was indicative of methicillin/oxacillin resistance. The antibiotic susceptibility pattern of the MRSA strains were then determined by modified disc diffusion method (Kirby Bauer Technique). Commercially available antibiotic discs were used, which were obtained from Hi Media Laboratories Pvt. Ltd.

Antimicrobial Discs used in the study were:

- Penicillin 10 units
- Gentamicin 10 mcg
- Erythromycin 15mcg
- Cotrimoxazole 25 mcg
- Ciprofloxacin 10 mcg
- Tetracycline 30 mcg
- Cephalotaxime 30mcg
- Vancomycin 30 mcg
- Clindamycin 2mcg

4. Result

The present study consisted of 150 isolates of *Staphylococcus aureus* isolated from various clinical specimens . The identities of these isolates were then confirmed by standard biochemical methods. All the confirmed *Staphylococcus aureus* strains were subsequently tested for methicillin resistance by disc diffusion method using oxacillin discs(1µg).The antibiotic susceptibility pattern of the MRSA strains as well as the MSSA strains were then determined to a battery of nine antibiotics.From a total of 150 samples of *Staphylococcus aureus*, 87 (58.00%) were seen to be resistant to oxacillin (MRSA).

ANALYSIS OF THE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE 87 MRSA ISOLATES AND 63 MSSA ISOLATES

The results of the in-vitro antimicrobial sensitivity test carried out on the 63 Methicillin sensitive *Staphylococcus aureus* (MSSA) strains and 87 Methicillin resistant *Staphylococcus aureus* (MRSA) strains against nine antibiotics are described below.

None of the MRSA isolates were found to be sensitive to penicillin and Cephotaxime whereas 4.76% and 73.01% of MSSA were sensitive to these antibiotics respectively. MSSA isolates also revealed higher susceptibility to Gentamicin (58.73% vs. 43.67%), Erythromycin (65.07% vs. 20.68%), Cotrimoxazole (41.26% vs. 27.58%). Ciprofloxacin (57.14% vs. 43.67%),Tetracycline (69.84% vs. 49.42%) and Clindamycin (92.06% and 40.22%) as compared with MRSA. However all 87 MRSA isolates and 63 MSSA isolates were found to be 100% sensitive to Vancomycin.

5. Discussion

Staphylococcus aureus has been reported as a major cause of community and hospital acquired infections [11]. It has overcome most of the therapeutic agents that have been developed in the recent year and hence the antimicrobial chemotherapy for this species has always been empirical [12].The most notable example of this phenomenon was the

emergence of methicillin resistant *Staphylococcus aureus* (MRSA), which was reported just one year after the launch of methicillin[13].

In the present study, 150 strains of *Staphylococcus aureus* from various clinical samples were isolated, characterized and processed for their sensitivity to methicillin as well as other antibiotics. The study was carried out in the Department of Microbiology, Patna Medical College and Hospital, Patna, BIHAR from September 2013 to August 2014. In the present study, the percentage of *Staphylococcus aureus* isolates which were found to be resistant to methicillin was 58.00%.

Vidhani S et al (2001)[14] reported a 51.6% isolation rate. Anupurba S et al (2003)[15] reported a 54.85% MRSA rate. A study from Indore has shown a rise in MRSA prevalence from 12% in 1992 to 80.89% in 1999[8]. In India, Bhujwala RA et al (1972)[16] reported a 15% resistance rate. Sunderrajan PP et al (1984)[17] reported a 14.5% rate. In 1994, a 32.8% resistance rate was reported by Mathur SK et al (1994)[18] and a 51.6% rate in 2001 by Vidhani et al (2001)[14]. In a study carried out by Arti Tyagi et al (2008) [19] the MRSA prevalence rate was 44% and in another study carried out by Murugan S et al (2008)[19], the MRSA prevalence rate of MRSA to be 46%.

In the present study, samples were collected from ten different sources. Of these the highest isolation rates for MRSA was found to be from wound swabs (65.62%). Isolation rate for MRSA of pus was found to be 63.15%. Anupurba S et al (2003)[15] reported a 52.5% isolation rate of MRSA from pus and wound swabs. Lee KJ et al (2001)[3] reported a 45.6% isolation rate from pus. Anbumani N et al (2006)[26] reported a 64% isolation rate of MRSA from pus and wound swabs. However isolation rates from pus reported by Lalitha MK (1997)[22] were slightly lower at 29.3%.

The isolation of MRSA from blood culture samples was quite high in our study (65.00%). Anupurba S et al (2003)[15] reported a 49.1% isolation rate of MRSA from blood culture. Anbumani N et al (2006)[21] reported a 38.1% isolation rate of MRSA from blood culture.

The next highest isolation of MRSA in our study was from urine (62.50). Anupurba S et al (2003) [15] reported a 76% isolation rate of MRSA from urine. Lee HJ et al (2001)[3] however reported a lower isolation rate of MRSA from urine (13.3%).

In our study, among the 150 isolates of *Staphylococcus aureus* under study, 24 isolates were found to be from urine samples which have been sent for culture and sensitivity with the suspicion of urinary tract infection. Out of 24 isolates of *Staphylococcus aureus*, 15 isolates were found to be MRSA.

As mentioned earlier, one of the disadvantages of MRSA is that apart from Methicillin, they are resistant to multiple antibiotics as well. In view of this it is important to have an idea of the response of MRSA to various other antimicrobials as well. MRSA and MSSA are equally pathogenic and are capable of causing the same spectrum of nosocomial infection. In this study all 87 MRSA isolates

(100%) were uniformly resistant to two antibiotics, Penicillin and Cephalexin. Bhujwala and Mohapatra (1972)[16] reported a 70.6% resistance rate to Penicillin. Kulkarni et al (1979)[23] reported a 92.3% resistance rate. Majumber D et al (2001)[24] reported that 83.3% of their isolates were resistant to Penicillin. This pattern of increasing resistance was uniformly being reported from the country as well as from studies conducted abroad and continued into the present decade, with Anupurba S et al (2003)[15] reported a 100% resistance pattern.

In our observation, 56.33% of the MRSA isolates showed resistance to Gentamicin. K. Rajadurai pandi et al (2006)[25] reported a 62% resistance rate, However Anupurba S et al (2003)[15] reported a 90% resistance rate of the MRSA isolates to Gentamicin. An increase of Gentamicin resistance from 0% before 1996 to 80% after 1996 has been reported (Price MF, 1998)[26]. According to a previous study from the same institute by Mazumber D et al (2001)[24], 70.30% of the MRSA isolates were resistant to Gentamicin. In our study, 79.32% of the MRSA isolates showed resistance to Erythromycin. K. Rajadurai pandi et al (2006)[25] reported a 60% resistance rate. Anbumani S et al (2006)[21] reported a 61.7% resistance rate of MRSA isolates to Erythromycin. According to two previous studies Mazumber D et al (2001)[24] and L. Saikia et al (2009) susceptibility to Erythromycin was found to be 66.10% and 18.75% respectively compared to 65.07% and 20.18% in our study. The resistance rate of Cotrimoxazole in our study was found to be 72.42% which is quite high. K. Rajadurai pandi et al (2006)[25] reported a 63.2% resistance rate whereas Anbumani S et al (2006) [21] reported a 88% resistance rate. Sensitivity of the MRSA isolates to Ciprofloxacin in our study was found to be 43.67%, whereas that of the MSSA isolates was 57.14%. Previous studies by Mazumber D et al (2001)[24] showed the sensitivity rate of MRSA isolates to Ciprofloxacin to be 77.2%. In one study by Anbumani S et al (2006)[21] resistance to Ciprofloxacin was found to be 62% compared to 56.33% in our study. Resistance to quinolone is perhaps due to differential clonal expansion and drug pressure in the community. Resistance to Clindamycin in our study was found to be 59.78%. Higher resistance of MRSA isolates to Clindamycin was also reported by Lee HJ et al (86.7%) [3].

All 87 isolates (100%) of MRSA in our study were found to be susceptible to the glycopeptides Vancomycin. This is similar to many other studies carried out elsewhere. Anupurba S et al (2003)[15] reported a 100% susceptibility to Vancomycin. Mazumber D et al 2001[24] also reported a similar result with K. Rajadurai pandi et al (2006)[25]. Thus Vancomycin seems to be the only antimicrobial agent which showed 100% sensitivity and may be used as the drug of choice for treating multidrug resistant MRSA infections.

6. Conclusion

It may be concluded from study that Methicillin Resistant *Staphylococcus aureus* is emerging to be a significant problem pathogen with Vancomycin probably the only reliable choice for these infections. The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotic before coming to the hospital are

possible predisposing factors of MRSA emergence. Furthermore, unless reduction of the various risk factors specially the indiscriminate use of antibiotic are not checked, the problem may escalate to unmanageable levels.

From this study it may be inferred that isolates of *Staphylococcus aureus* from pus and wound swabs are more likely to be methicillin resistant than isolates from other samples. The first and the most effective way among these are to avoid transmission through hand contamination by the person responsible for caring the infected patients. Injudicious use of antibiotics will lead to development of drug resistance. One should always look for Methicillin resistance in isolates of *Staphylococcus* as the treatment options for Methicillin resistant strains are limited. Timely detection of Methicillin resistant strains will help in prevention of hospital acquired infections.

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