PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATES FROM DIFFERENT CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL IN IMPHAL

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ABSTRACT

BACKGROUND

The prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing worldwide and is a growing public health concern. Serious infections due to *Staphylococcus aureus* and especially due to Methicillin-resistant *Staphylococcus aureus* has become a major clinical challenge.

The present study was planned to determine the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimens and to determine their antibiotic susceptibility pattern.

MATERIALS AND METHODS

The study was carried out in the Department of Microbiology, JNIMS, Manipur, from the year 2016 to 2017. A total of 770 *Staphylococcus aureus* strains were isolated from clinical specimens like urine, pus, blood, sputum, etc. All strains were identified by standard microbiological techniques. *S. aureus* strains were subsequently tested for Methicillin resistance by using Cefoxitin discs. The antibiotic susceptibility pattern of all the MRSA strains identified was determined by Kirby-Bauer disc diffusion method.

RESULTS

A total number of 770 *S. aureus* was isolated, of which 560 were MRSA. The prevalence of MRSA was different among various clinical specimens with maximum seen in urinary isolates (88.4%) followed by pus (79.6%). All the 560 MRSA strains were found to be resistant to Penicillin (100%); 76.9% to Gentamicin, 73.5% to Amikacin, 74.1% to Erythromycin, 69.2% to Cotrimoxazole, 77.7% to Ciprofloxacin and 60.9% to Tetracycline. MRSA strains were resistant to ≥ 8.0 drugs, multidrug resistance. Sensitivity to Linezolid and Vancomycin was seen. A few (3/560) Vancomycin intermediate *Staphylococcus aureus* (VISA) strains were identified by E-test.

CONCLUSION

High rate of prevalence of MRSA with multidrug resistance of MRSA towards commonly used antibiotics was observed. Linezolid was the only antibiotic found to give uniform sensitivity (100%). A few VISA strains were identified. The findings highlight the importance of in vitro susceptibility testing of every isolate of MRSA in clinical laboratories.

KEYWORDS

Staphylococcus aureus, Methicillin, Resistance, Vancomycin.

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BACKGROUND

Staphylococcus aureus bacteria like all forms of bacteria are constantly mutating. In order to survive, bacteria evolve producing newer and stronger strains. Although antibiotics are necessary to fight bacterial infections, exposure to the drugs encourages the development of antibiotic-resistant bacteria. Subsequent to the introduction of Penicillin in clinical settings, *Staphylococcus aureus* strains resistant to beta-lactams including Methicillin, Methicillin-resistant

Staphylococcus aureus (MRSA) came to be recognised as early as 1961 in the UK.¹ These were seen to be resistant to fifteen to thirty different antibiotics, making the availability of effective antibiotics difficult. Making matters worse, MRSA like all forms of bacteria is constantly changing. As a result, new strains of MRSA keep appearing that are resistant to even more antibiotics. The control of the spread of MRSA is important keeping in view the implications of mortality and morbidity. Apart from an understanding of the organism's epidemiology, the knowledge of prevalence and the antibiotic pattern of MRSA help the treating physicians in determining the first line of treatment in the hospitals and thus in the effective control of spread.² The present study was planned with an aim to determine the prevalence of MRSA from different clinical specimens viz. urine, pus, blood, sputum/throat swabs, miscellaneous (ear swabs, body fluids etc.), and to know the current status of response of MRSA to commonly used antibiotics.

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MATERIALS AND METHODS

The study was carried out in the Department of Microbiology, JNIMS, Porompat, Imphal East, during the period from January 2015 to August 2016. A total of 770 *S. aureus* strains were isolated from clinical specimens like urine, pus, blood, sputum, etc. All the strains were identified by colony morphology, Gram stain, Catalase and Coagulase tests and fermentation of Mannitol by conventional methods. The antimicrobial susceptibility pattern of all the recognised strains was determined by Kirby-Bauer Disc Diffusion method on Mueller-Hinton Agar using Clinical and Laboratory Standards Institute (CLSI) guidelines and included Penicillin, Erythromycin (15 µg), Amikacin (30 µg), Gentamicin (10 µg), Cotrimoxazole (25 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Linezolid (30 µg) and Teicoplanin (30 µg). Discs from HiMedia were used and the turbidity of the suspension was adjusted to 0.5 McFarland standard. *S. aureus* ATCC 25923 was used as quality control for disc diffusion. *S. aureus* strains were then tested for Methicillin resistance using Cefoxitin (30 µg).

RESULTS

During the study period, 7200 clinical specimens were received; 770 isolates of *S. aureus* were identified, of which 560 were MRSA [Table 1].

Clinical Specimen	<i>S. aureus</i>	MRSA (n)	Percentage MRSA of <i>S. aureus</i> Isolates
Urine	226	200	88.4
Pus	118	94	79.6
Blood	310	192	61.9
Sputum	44	32	72.7
Throat swab	40	26	65
Body fluids	18	10	55.5
Ear swabs	14	6	42.8
Total	770	560	72.7

Table 1. Prevalence of *S. aureus* and MRSA in various Samples

The antimicrobial susceptibility pattern was found to vary across all samples with maximum observed in urine isolates 200/226 (88.4%).

The antimicrobial susceptibility pattern of the MRSA isolates showed maximum resistance to Penicillin (100%), while all (100%) MRSA isolates were sensitive to Linezolid [Table 2].

Antibiotic	Resistant; n (%)	Sensitive; n (%)
Penicillin (10 units)	560 (100)	0 (0)
Amikacin (30 µg)	412 (73.5)	148 (26.5)
Ciprofloxacin (5 µg)	125 (22.3)	435 (77.7)
Gentamicin (50 µg)	431 (76.9)	129 (23.1)
Cotrimoxazole (25 µg)	388 (69.2)	172 (30.8)
Linezolid (30 µg)	0 (0)	100 (100)
Erythromycin (15 µg)	415 (74.1)	145 (25.9)
Teicoplanin (30 µg)	113 (20.1)	447 (79.9)
Tetracycline (30 µg)	219 (39.1)	341 (60.9)
Vancomycin (30 µg)	3*(0.5)	557 (99.5)
Nitrofurantoin† (30 µg)	50/226 (22.1)	176/226 (77.9)

Table 2. Antibiotics Susceptibility Pattern of MRSA Isolates

*Vancomycin Intermediate *S. aureus* (VISA) by E-test
†only for urine isolates.

DISCUSSION

MRSA has been observed to be one of the major nosocomial agents causing significant morbidity and mortality. Infected patients are important reservoirs of MRSA in Hospitals/Institutions. Healthcare workers by transient hand carriage are a predominant mode of patient to patient transmission.¹

Unlike the Western world, the significance of MRSA came to be recognised late in India and emerged as a problem only in the 80s and 90s.³ The present study identifies and analyses the prevalence and antimicrobial susceptibility pattern of MRSA isolates obtained from different clinical specimens in a specific geographical setting.

Out of 7200 clinical specimens, 770 were identified as *S. aureus*. Out of these 770 isolates, 560 (72.7%) were found to be MRSA, which compares well with the prevalence of MRSA in other parts of India (30 - 80%) in various studies.⁴ Kulkarni et al reported a similar prevalence rate of 70.33%, while D. Majumdar et al reported 52.9% among patients and carriers in their study in Assam.^{1,5} Prevalence rates as low as 6.9% have also been reported by Chakravarthy et al⁶ and 31% by Rajadurai pandi K et al.^{3,6}

In the present study, highest percentage of MRSA strains were seen in urine isolates (88.4%) followed by pus (79.6%); 13.2% MRSA were isolated from miscellaneous samples like throat swab, ear swab, sputum and body fluids. Similar observations were found by Kulkarni et al¹ and Anupurba et al⁶ who reported 82.38% and 76% MRSA from urine and 64.67% and 52.5% from pus respectively.^{1,7} Higher figures have however been reported by Bandaru et al⁸ and Mehta et al⁹ from pus samples, 67.28% and 74.28% respectively.^{8,9} Rajadurai pandi et al observed a higher percentage from sputum/throat swabs (35.7%).³

The drug resistance patterns of MRSA, isolated from different samples were seen to be variable. All the 560 MRSA strains were found to be resistant to Penicillin (100%), Gentamicin (76.9%), Amikacin (73.5%), Erythromycin (74.1%) and Cotrimoxazole (69.2%). Ciprofloxacin and Tetracycline showed a sensitivity of 77.7% and 60.9%, respectively. Good response was also seen against Teicoplanin (79.9%). All the MRSA strains were sensitive to Linezolid (100%).

The high resistance rate against Gentamicin and Amikacin observed in our study is similar to those of Rajadurai pandi et al³ (63.6%), Majumdar et al⁵ (94%), Bandaru et al⁸ (85.80%) and Kulkarni et al¹ (52.57%). However, Pulimood et al¹⁰ observed a low resistance rate of 8%.

Gentamicin resistance has been reported worldwide and has been attributed to drug inactivation by cellular transferase enzyme.⁸

In our study, MRSA strains showed a high resistance to Erythromycin (73.7%) and are similar to the findings of Bandaru et al⁸ (95.67%) and Mukesh Pal et al⁴ (85.71%). Strains resistant to Erythromycin are generally cross resistant to Azithromycin and Clarithromycin.⁸

Various studies have also reported resistance to Ciprofloxacin ranging from 12.8% to 98.9%. Rajadurai pandi et al³ reported 12.8%, Pulimood et al¹⁰ 90% and 88% by Bandaru et al.⁸ Our present study showed 22.4% resistance rate.

The present study shows good sensitivity rate against Linezolid, Teicoplanin and Vancomycin. Linezolid showed good activity against all Staphylococci including strains resistant to Methicillin. It is thus a promising therapeutic option.

A very high prevalence rate of MRSA was observed in the present study (72.7%). Maximum of the MRSA strains were found to be resistant to ≥ 8 antibiotics, and hence were multidrug resistant MDR (MRSA). Even though Vancomycin is the drug of choice for MDR (MRSA), it is considered inferior to beta-lactams for the treatment of MSSA bacteraemia and endocarditis. The first generation Cephalosporins are the drug of choice for treatment of MSSA infections in patients who cannot tolerate anti-Staphylococcal Penicillins.

210 strains (27.3%) in the present study were found to be sensitive to Methicillin (MSSA). They were also seen to be sensitive to most of the antibiotics. Similar observation has been reported by Arora et al.¹¹ It is thus imperative to encourage the de-escalation of the use of Vancomycin to beta-Lactams in all cases of MSSA. MRSA isolates being rampant, it is important that treating physicians de-escalate to beta-Lactams once culture results reveal an MSSA isolate, so as to preserve glycopeptides and Linezolid for use against MRSA.

MIC determination for Vancomycin was done using E-test strips (Bio-Merieux India Pvt. Ltd.) according to manufacturer's instructions. Of the 560 isolates, 557 were found to be sensitive with MIC ≤ 2 mg/L. However, 3 strains were found with MIC of 4 mg/L, and could be identified as Vancomycin Intermediate *S. aureus* (VISA). They were all however sensitive to Linezolid. No Vancomycin resistant *S. aureus* (VRSA) was found by the E-test.

Our study was however limited by the non-inclusion of carriers, which would have helped us to correlate with the high rate of MRSA. Newer drugs like Daptomycin and 4th generation Cephalosporins like Ceftaroline and Tigecycline could have been included to assess their activity against MRSA. Molecular studies could not be done.

CONCLUSION

Our study showed a very high incidence of MRSA in our Hospital. A regular surveillance of Hospital-associated infections including monitoring and strict anti-microbial stewardship and control measures to reduce the high incidence of MRSA infections to prevent the spread of such strains needs to be implemented.

MRSA isolates were more Multidrug Resistant (MDR) as compared to the MSSA isolates. Glycopeptides and Linezolid remains the mainstay for treatment of such MDR-MRSA.

In conclusion, our study has highlighted the high prevalence of MRSA and the emergence of *S. aureus* with intermediate resistance to Vancomycin. There is thus a need to enforce the judicious use of Vancomycin. Alternative anti-MRSA drugs should be used when appropriate in order to balance the antibiotic pressure of Vancomycin on the bacterial population.

One needs to keep in mind that the use of newer anti-bacterial drugs needs to be carefully defined to prevent misuse and subsequent resistance to these drugs.

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