

Study on antibiotic resistance pattern of MRSA among patients in M.G.M. Hospital

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Abstract

Problem statement: The bacterium *S. aureus* is a common cause of human infection, and it is becoming increasingly virulent and resistant to antibiotics. Our understanding of the evolution of this species has been greatly enhanced by the recent sequences of genome of seven strains of *S. aureus*. In this present study we shall try to demonstrate and compare the epidemiological features and antibiotic susceptibility pattern of MRSA infection in patients attending the OPDs and IPDs of MGM Medical College and Hospital, Kishanganj. **Methods:** 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. **Results:** 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 (1 µg). The antibiotic susceptibility pattern of the MRSA strains as well as the MSSA strains were then determined to a battery of nine antibiotics. The isolates of *Staphylococcus aureus* were also grouped into biotypes using accepted phenotypic characters. The following observations were made in the study. **Conclusion:** It may be concluded from study that methicillin resistant *Staphylococcus aureus* (MRA) is emerging to be a significant problem pathogen with vancomycin probably the only reliable choice for these infection. 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.

Key Words: MRSA, MSSA.

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INTRODUCTION

Staphylococcus 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 *Staphylococcus aureus* (MRSA) are usually also resistant to other antibiotics. Methicillin resistance is a complex property, and more than one mechanism is involved.

Three different mechanisms of resistance to β - lactam (penicillins and cephalosporins) antibiotics have been identified for Staphylococci.¹ The inactivation of penicillin and cephalosporins by penicillinase (β - lactamase) mediated hydrolysis of the β -lactam ring of the antibiotics,² A tolerance to the bactericidal effect of β -lactam antibiotics, due to an excess of autolysin inhibitor.³ An intrinsic resistance resulting from a reduction in the affinity or amount of Penicillin Binding (PBPs). Penicillin resistant strains began emerging during the early 1940's after the introduction of penicillin and the percentage today of resistant strains has risen to 75-95%, with the highest percentages among hospital strain. Resistance to methicillin and other β -lactamase resistant penicillins in *Staphylococcus aureus* was first observed in Britain¹. 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 infection have been reported world². Methicillin

resistant *S. aureus* (MRSA) is now endemic in India. The incidence of MRSA varies from 25 per cent in western part of India³ to 50 per cent in South India (Gopalakrishnan R, Sureshkumar D *et al* 2010).⁴ Although prevalence of MRSA strains is reported to be increasing, neither there was any study of MRSA prevalence in this region nor there was any study on antibiotic resistance pattern of MRSA among patients in this region (specially Bihar).

MATERIAL AND METHODS

A total of one hundred fifty isolates of *Staphylococcus aureus* isolated from various clinical specimens were included in this study. The clinical specimens were swabs collected from different sites and lesions along with body fluids. 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. Peritoneal fluids were collected by sterile syringes, using aseptic puncture technique.

Media for Isolation of Organism

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

Sample Processing

The various clinical specimens (urine, pus, throat swab, Wound swab, sputum, body fluids etc.) were inoculated in Blood Agar and MacConkey Agar plates immediately on receipt if possible or kept in refrigerator at 2-8 °C in case of any delay and incubated at 37 °C aerobically for 18-24 hours. If no growth was seen, the plates were re-incubated for another 24 hours and then examined. If there was still no growth, the plates were discarded. Those specimens which yielded pure and abundant colonies of Staphylococcal species were isolated and sub-cultured on nutrient agar. In case of blood cultures the specimens in glucose broth was first incubated aerobically at 37 °C for 18-24 hours. Subcultures were done daily for first 5 days on solid media and the blood culture bottles were re-incubated at 37°C after subculturing. Final subculture was done on the seventh day. Staphylococcal isolates were then identified and characterized.

Morphology

From the isolated colonies, smears were prepared and Gram stained. Morphological characteristics such as size,

shape and typical cluster formation along with their Gram reaction were noted.

RESULTS

The age distribution of the one hundred fifty isolates of *Staphylococcus aureus* included in this study. It is seen from the below Table-1 that the association of *Staphylococcus aureus* is less in the extremes of age. Only 10 strains were isolated from patients below 1 year of age and 5 strains were isolated from patients above 60 years of age. 40 patients (26.67%) belonging to the group 21-30 years, showed the maximum number of *Staphylococcus aureus* isolates.

Table 1:

Age	Patients with <i>Staphylococcus aureus</i>		Patients with MRSA	
	Number	Percentage (%)	Number	Percentage (%)
<1	10	6.67	3	30.00
1-10	15	10.0	7	46.67
11-20	20	13.33	12	60.00
21-30	40	26.67	29	72.50
31-40	25	16.67	17	68.00
41-50	20	13.33	12	60.00
51-60	15	10.00	6	40.00
61-70	5	3.33	1	20.00
Total	150	100	87	

Age wise distribution of 150 isolates of *Staphylococcus aureus* and 87 isolates of MRSA

The above Table-1 also shows the age distribution of the 87 MRSA strains isolated from the 150 isolates of *Staphylococcus aureus* included in the study. It is evident from the Table-1 that MRSA is less common in persons in the extremes of age. Out of 10 strains of *Staphylococcus aureus* isolates from infants only 3 (30.00) were methicillin resistant and out of 5 strains of *Staphylococcus aureus* from patients above 60 years of age, only 1 (20%) was methicillin resistant. It was also seen from the Table-4.1 that from patients above 50 years, the isolation of MRSA decreases with advancing age. The below Table-2 shows the sex distribution of the 150 *Staphylococcus aureus* isolates included in the study. It is seen from the below Table -2 that isolation of *Staphylococcus aureus* is slightly more in males than in females. 80 strains (53.33%) were isolated from males contrast to 70 strains (46.67%) from females.

Table 2:

Sex	Patients with <i>Staphylococcus aureus</i>		Patients with MRSA	
	Number	Percentage (%)	Number	Percentage (%)
Male	80	53.33	53	62.25
Female	70	46.67	34	48.57
Total	150	100	87	

Sex distribution of 150 isolates of *Staphylococcus aureus* and 87 isolates of MRSA

The above Table-2 also shows the sex distribution of the 87 MRSA strains isolated from the 150 isolates of *Staphylococcus aureus* included in the study. It is seen from the Table-2 that males are more likely to get infected by methicillin resistant strains (66.25%) than females (48.57%).

Table 3: Culture characters of 150 *Staphylococcus aureus* isolates

Culture Character	Number of Positive	Percentage (%)
Beta Haemolysis		
Pigment Procedure		
Cream	125	83.33
Buff	4	3.20
Variable	61	48.80
Gold	19	15.20
Gold	41	32.80
GPC in clusters from gram stain in Culture	150	100.00

Table 4: Biochemical characteristics of 150 *staphylococcus aureus* isolates

Biochemical Characteristics	No of Positive	Percentage (%)
Catalase Test	150	100.00
Slide Coagulase	130	86.66
Tube Coagulase	150	100.00
Fermenters in Modified Hugh and Leifson's (O/F) Test	150	100.00
Esterase Producers	60	40.00
Urease Producers	129	86.00

The salient features of the biochemical character of the isolates are:

- All isolates were catalase Positive (100%).
- In the slide coagulase test, 86.66% (130 samples) were found to give a positive result.
- All samples were positive for tube coagulase (100%).
- All samples were found to be Fermentors in the Modified Hugh and Leifson (O/F) test (100%).

Table 5: Results of Biotyping *Staphylococcus aureus* isolates

Number of esterase positive isolated	Number of urease positive isolated	Gentamicin		Pigment produced				
		R	S	C	B	V	G	
60	129	120	30	4	61	19	4	
							1	

S = Sensitive, B = Buff, R = Resistant, V = Variable, C = Cream, G = Gold

Table 6: Percentage of MRSA and MSSA from 150 *staphylococcus aureus* isolates

Total isolates of <i>Staphylococcus aureus</i>	Oxacillin Sensitive	Oxacillin Resistant
150	63 (42.00%)	87 (58.00%)

From a total of 150 samples of *staphylococcus aureus*, 87 (58.00%) were seen to be resistant to oxacillin.

Table 7: Analysis of different clinical samples from which *Staphylococcus aureus* MRSA stains were isolated in the study

Nature of sample	Patients with <i>staphylococcus aureus</i>		MRSA isolates in relation to <i>staphylococcus aureus</i>	
	Number	Percentage	Number	Percentage
Pus				
Wound				
Swab				
Urine	38	25.33	24	63.15
Blood	32	21.33	21	45.62
Blood	24	16.00	15	62.50
Sputum	20	13.34	13	65.00
Throat	12	8.00	4	33.33
Swab	12	8.00	5	41.66
Body Fluid (Pleural, CSF, Ascitic)	9	6.00	4	44.44
Aural Swab	3	2.00	1	33.33
Total	150	100.00	87	

Table 8: Analysis of the antibiotic susceptibility pattern of the 87 MRSA isolates and 63 MSSA isolates

Antimicrobials	MSA (n=63)		MRSA (n=87)	
	Number	Percentage	Number	Percentage
Penicillin	3	4.76	0	0.00
Gentamicin	37	58.73	38	43.67
Erythromycin	41	65.07	18	20.68
Cotrimoxazole	26	41.26	24	27.58
Ciprofloxacin	36	57.14	38	43.67
Tetracycline	44	69.84	43	49.42
Cephotaxime	46	73.01	0	0.00
Clindamycin	58	92.06	35	40.22
Vancomycin	63	100.00	87	100.00

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 and 87 methicillin resistant *staphylococcus aureus* (MRSA) strains against nine antibiotics are shown in the above Table 8: 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.

DISCUSSION

In the present study, one hundred fifty strains of *Staphylococcus aureus* from various clinical samples were isolated, characterized and processed for their sensitivity to methicillin as well as other antibiotics. They were also biotyped. The study was carried out in the Department of Microbiology, MGM Medical College and Hospital, Kishanganj, Bihar, from Nov 2015 to Oct 2016. *Staphylococcus aureus* was isolated from persons of all age groups in our study. It is in agreement with the nature of Staphylococcal infections that Staphylococci are the causative organisms of diseases in all the age groups (Speller David CE. Hospital Associated Infections, 1990)⁽⁵⁾ In this study, comparatively fewer strains were isolated in the extremes of age. This may be due to the fact that comparatively lesser number of patients in extremes of age were included in this study. Analyzing the age of patients from whom MRSA were isolated in the age group of 21-30 years. In the extreme of age (<1 year and > 60 years), the incidence of isolation of Staphylococci is low. This may be due to the fact that people of 21-30 year age groups are most active and hence more prone to exposure to infection by methicillin resistant strains. Regarding the incidence of isolation of methicillin resistant *Staphylococcus aureus* in males and females, it has been observed that both males and females are infected by MRSA, but incidence is little higher in males (66.25%) than females (48.57%). This is in agreement with the report by Thompson (1966)⁽⁶⁾ who found that male carriers suffer more from Staphylococcal lesions than female carriers. 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)⁽⁷⁾ reported a 51.6% isolation rate. Anupurba S *et al* (2003) 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 (Verma S, 1999)⁽⁸⁾. It can be seen that the overall MRSA rates have been steadily increasing over the last decade or two to approach the present levels. In the United States, the National Infections Surveillance System has recorded an increase from 2.4% in 1975 to 29% in 1991 in New York State alone (Lalitha MK, 1997)⁽⁹⁾. In a study carried out by Arti Tyagi *et al* (2008)⁽¹⁰⁾, the MRSA prevalence rate was 44% and in another study carried out by Murugan S *et al* (2008)⁽¹¹⁾, the MRSA prevalence rate of MRSA to be 46%. The isolation of MRSA from blood culture samples was quite high in our study (65.00%). Anupurba S *et al* (2003) reported a 49.1% isolation rate of MRSA from

blood culture Anbumani N *et al* (2006) 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) reported a 76% isolation rate of MRSA from urine. Lee HJ *et al* (2001) 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 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 cephotaxime. In our study, 79.32% of the MRSA isolates showed resistance to Erythromycin. 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. All 87 isolates (100%) of MRSA in our study was found to be susceptible to the glycopeptides vancomycin. This is similar to many other studies carried out elsewhere. Thus vancomycin seems to be the only antimicrobial agents which showed 100% sensitivity and may be used as the drug of choice for treating multidrug resistant MRSA infections.

CONCLUSION

It may be concluded from study that methicillin resistant *Staphylococcus aureus* (MRA) is emerging to be a significant problem pathogen with vancomycin probably the only reliable choice for these infection. 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. Biotyping of these organisms can be carried out to assess the type of strains, which are prevalent in a particular environment, in this case, biotypes type -D. since complete eradication of MRSA may not be possible, control of transmission

seems to be the appropriate goal. 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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