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Bacteriophage Typing of Methicillin Resistant *Staphylococcus aureus* and Changing Trend in their Antibiotic Profile.

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ABSTRACT

Background: Increasing prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA), a major pathogen in both health care facilities and community, is an escalating public health concern. Knowledge of prevalence of MRSA and their current antimicrobial profile becomes necessary in the selection of appropriate empirical treatment of these infections. **Aims & Objectives:** To estimate the prevalence of MRSA & to evaluate its antibiotic sensitivity pattern and to determine Bacteriophage typing for epidemiological purposes. **Methods:** A total of 182 isolates of *Staphylococcus aureus*, collected over a period of two years, were screened by various phenotypic assays. Randomly selected 50 MRSA isolates were sent to Maulana Azad Medical College, New Delhi, for Bacteriophage typing. Antibiotic sensitivity test was done by Kirby Bauer's disc diffusion method. **Results:** Among the 182 isolates, 120(65.93 %) were identified as MRSA & 62(34.07 %) were identified as MSSA by various phenotypic methods. 50 isolates was sent for PhageTyping. 42(84%) strains were typable. 35(70%) strains belonged to phage III, 1(2%) to phage group II, 6(12%) to more than one phage groups. More than70% strains were resistant to Co-Trimoxazole, Erythromycin, Ciprofloxacin and Gentamicin, but were highly sensitive to Chloramphenicol and Clindamycin. 100% sensitivity was observed with Vancomycin, Teicoplanin and Linezolid. **Conclusion:** It is alarming that the present study reports a high prevalence (65.94%) of MRSA infection. In our study, 70% of the MRSA isolates belong to group III Phage, especially phage no.47. Despite the glycopeptides, Chloramphenicol, and preferably Clindamycin can be used to treat Staphylococcus infections.

Keywords: Bacteriophage typing, Clindamycin, Methicillin Resistant *Staphylococcus aureus*.

INTRODUCTION

The incidence of nosocomial infections, caused by Methicillin Resistant *Staphylococcus aureus*, continues to increase worldwide and it leads to a renewed emphasis on the prevention of MRSA infections. In India, various studies shows a gradual increase in the prevalence of MRSA infections ranging from 6.9 % to as high as 87%.[1,7,8] There has been a steady increase in the prevalence of MRSA, isolated in hospitals. It has triumph over most of the therapeutic drugs that have been developed in recent decades. Hence, this rising prevalence also poses a threat of drug resistance. Our study was aimed at determining the prevalence of MRSA and their changing drug resistance pattern. In addition, we also studied the Bacteriophage typing of MRSA for epidemiological purposes.

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MATERIALS AND METHODS

The present study was a prospective study, carried out from December 2010 to Nov 2011, in the Department of Microbiology, in AarupadaiVeedu Medical College & Hospital, Puducherry. Total number of 3218 samples was sent to the department of Microbiology for culture & sensitivity, of which 2132 were culture positive. Various clinical samples, which yielded *Staphylococcus aureus* (n = 180), taken from patients attending medical, surgical and allied surgical departments as outpatients and in

patients were included in the study. Demographic information like, history of prior hospitalization and presence of major conditions including Diabetes mellitus, renal dysfunctions, post surgical status, malignancy, trauma or burn injury were recorded.

The samples were inoculated on 5% Sheep Blood Agar and MacConkey Agar plates. Blood samples sent in brain heart infusion broths were incubated for 24 hour and then sub cultured onto Blood Agar and MacConkey Agar plates. These plates were then incubated at 37oC for 24 to 48 hours. All the suspected colonies were gram stained and subjected to various biochemical tests to identify and characterize them. *Staphylococcus aureus* was identified by standard microbiological methods.[2] Antimicrobial susceptibility testing was done on Muller Hinton Agar and their susceptibilities to *Staphylococcus aureus* panel of antimicrobials as per CLSI guidelines.[4] Methicillin resistance of the Staphylococcus isolates were determined by Cefoxitin Disk Diffusion method.

Figure 1: Phenotypic identification of *Staphylococcus aureus*.

Table 1: Bacteriophage groups

Phage Group I	29,52,52A,79,80
Phage Group II	3A,3C,55,71
Phage Group III	6,42E,47,53,54,75,77,83A,84, 85
Phage Group NA	81, 95
Phage Group V	94,96

MRSA isolates were termed as Hospital acquired Methicillin resistant *Staphylococcus aureus* if the source patient had history of hospitalization, surgery within one year prior to date of specimen collection, growth of MRSA after 48 hr or more after the admission in the hospital, Presence of a permanent indwelling catheter or percutaneous device at the time of culture or prior to this study. If none of above risk factors were present, the isolate was considered as Community acquired MRSA.[3] Randomly selected fifty MRSA isolates were sent for Bacteriophage typing to the Department of Microbiology, Maulana Azad Medical College, New Delhi. An international basic set of 23 phages were used [Table 1].

RESULTS

Total number of 3218 samples was sent to the Department of Microbiology for culture & sensitivity, of which 2132 were culture positive. A total of 182 *Staphylococcus aureus* were isolated during the study period. Among the 182 isolates, 120(65.93 %) were identified as MRSA & 62(34.07 %) were identified as MSSA by various phenotypic methods [Figure 2].

Maximum number of *Staphylococcus aureus* was isolated from soft tissue infections (33.52%), followed by surgical infections (29.67%) and urinary tract infections (15.38%). Septicaemias' constituted 7.14% of all *Staphylococcus aureus* infections. MRSA was predominant among most infections except in septicaemia, bone infections, etc. [Table 2].

There were 120 cases of MRSA, of which 72 (60%) were community-associated and 48 (40%) were healthcare-associated. Randomly selected fifty MRSA strains were sent for Bacteriophage typing, in which 42 (84%) strains were typable. Among the typable strains, 35 (70%) belonged to phage III, 1(2%) belonged to phage group II, 6 (12%) strains belonged to more than one phage groups. 8 (16%) strains were non typable.

All 120 strains were confirmed to be MRSA based on the phenotypic method, Cefoxitin disc diffusion method. Among MRSA strains, the resistant patterns were observed as follows, Ampicillin (97.5%), Amoxyclav (95.8%), Co-Trimoxazole (78.3%), Erythromycin (72.5%), Ciprofloxacin (60%), and Gentamicin (48.3%). However the strains were highly sensitive to Chloramphenicol (77.5%) and Clindamycin (82.5%). 100% sensitivity was observed with Vancomycin, Teicoplanin and Linezolid [Figure 4].

DISCUSSION

Staphylococcus aureus is responsible for majority of human infections, which can be from minor skin diseases to life-threatening infections. *Staphylococcus aureus* are ubiquitous organisms and among the most commonly encountered isolates in clinical practice. *Staphylococcus aureus* has been reported as a major cause of community and hospital acquired infections.[5] In this study, 219 (10.27%) isolates were Staphylococci, of which 182 were *Staphylococcus aureus* and 37 were CONS.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were identified as early as 1961 soon after the introduction of Methicillin in clinical settings.[11] Subsequently, MRSA have emerged world wide as a major cause of nosocomial infections. A remarkable rise in the prevalence of MRSA has been observed during the last decade. Increasing prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) worldwide is a growing public health concern. It is alarming that the present study reports a very high prevalence (65.94 %) of MRSA infection. Other studies have also shown such a high MRSA prevalence in various parts of the country ranging from 40.6%, 54.85% to 59.3%.[6,8,9] However, 31.1 and 23.6% MRSA prevalence has also been reported [7,10] which is comparatively very low than that reported in the current study. This variation might be because of various factors like infection control policies & practices in healthcare facilities and antibiotic usage that vary from hospital to hospital.

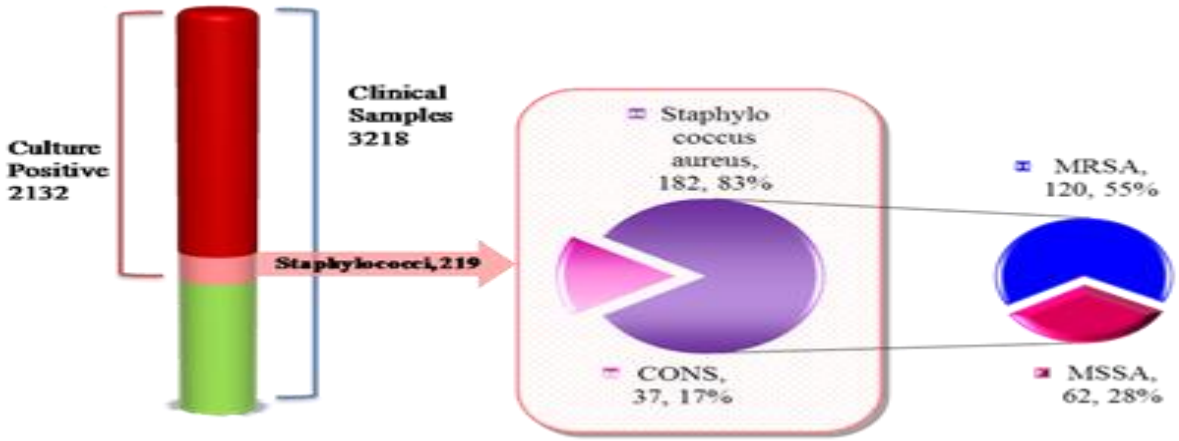

Figure 2: Prevalence of *Staphylococcus aureus.*

We should also note that MRSA is one of the few pathogens routinely involved in all type of nosocomial infection, ranging from pneumonia to bacteremia. Skin and soft-tissue infections (SSTIs) are the most frequent forms of the disease caused by Methicillin-resistant *Staphylococcus aureus* (MRSA). Most cases of MRSA in our hospital were contributed by Soft tissue infections (33.52%). Following SSTI, surgical site wound infections (29.67%) are the most commonly encountered infections in our study. It was also found that surgical units accounted for 80% of the MRSA isolates and postoperative infections in orthopaedic surgery accounted for 28%.[12] There are many studies in support of the same in India[12,13] and abroad.[14]

Diabetics are at a higher risk of acquiring MRSA infections. Although in our study only 6.59% of MRSA was seen among diabetic patients, a slightly higher rate was reported in other studies.[15] Diabetic foot infections should be treated aggressively, since the host is immune-suppressed. Maintaining good glycemic control, monitoring cuts, abrasions, rashes, and other breaks in the skin, for infection, will prevent a MRSA infection in diabetics.

Community acquired MRSA strains are emerging as a major cause of illness in healthcare settings. This changing epidemiology of MRSA poses a challenge to public health and infection control in hospital settings. In accordance with Park et al,[21] the incidence of Community acquired MRSA is 72 (60%) and Hospital acquired MRSA 48 (40%) and it was statistically significant (p value <0.05).

Table 2: *Staphylococcus aureus* in various type of infection.

Type of infection	MRSA		MSSA		Total	
	No.	%	No.	%	No.	%
Soft tissue infection	42	35.00	19	30.65	61	33.52
Surgical infections	28	23.33	26	41.94	54	29.67
UTI	24	20.00	4	6.45	28	15.38
Diabetic wound	9	7.50	3	4.84	12	6.59
Septicaemia	6	5.00	7	11.29	13	7.14
Burns	3	2.50	0	0.00	3	1.65
Bone infections	3	2.50	2	3.23	5	2.75
Respiratory tract infections	2	1.67	0	0.00	2	1.10
Carcinoma	2	1.67	0	0.00	2	1.10
Peritonitis	1	0.83	1	1.61	2	1.10
Total	**120**		**62**		**182**	

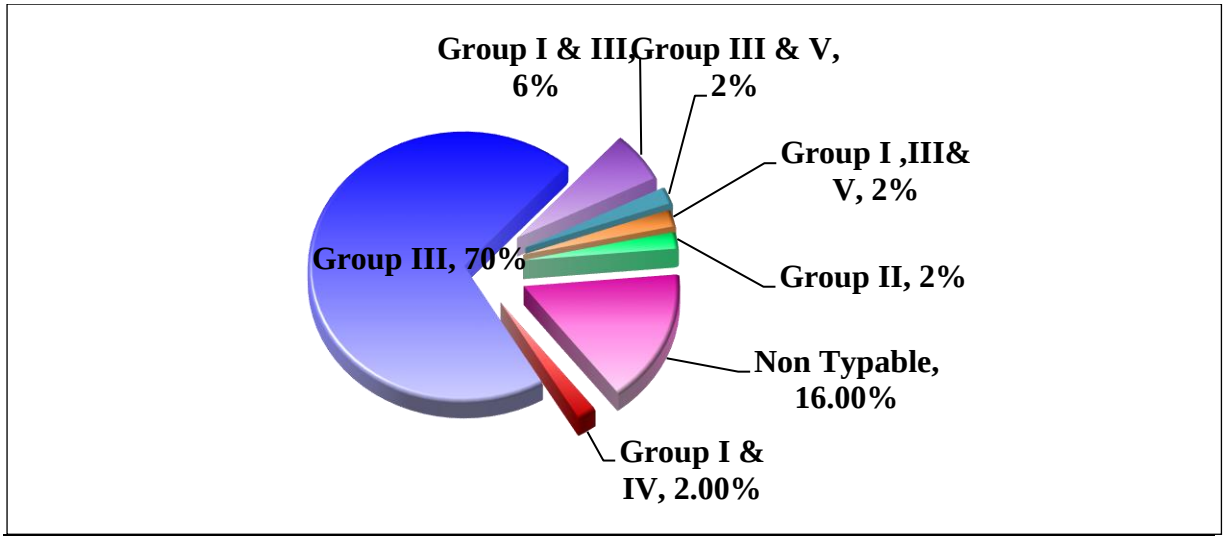

Figure 3: Pattern of Bacteriophage typing in MRSA (n=50)

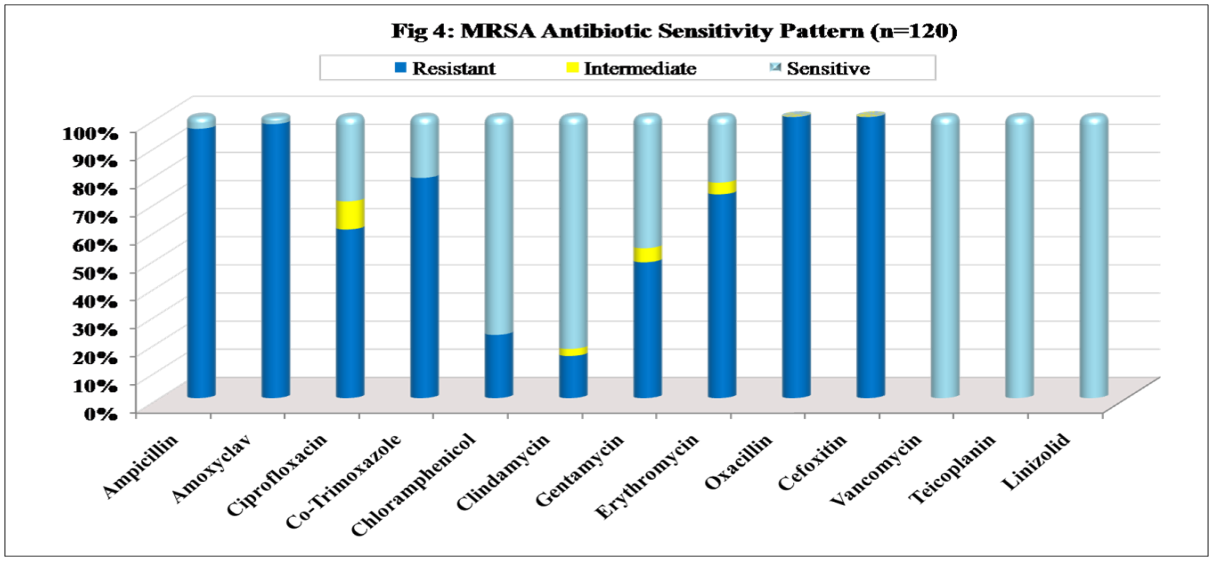

Figure 4: MRSA Antibiotic sensitivity pattern (n=120).

In a hospital environment, accurate strain typing is essential for the epidemiological monitoring & identification of outbreak strains of the MRSA isolates and for the control of transmission, accordingly. Other than molecular methods, the widely used method for the typing of *Staphylococcus aureus* was Bacteriophage typing, employing the international phage typing set (IPS) and additional regional phages. In our study, 70% of the MRSA isolates belong to group III Phages, especially phage no.47. This is in accordance with Mathew et al, who also reported predominance of phage type no.47.[16] Predominance of phage Group III in MRSA strains has been reported by several studies.[22-24] The strains belonging to Phage III were found to be multi drug resistant. Phage typing has been reported to be valuable in the identification of known epidemic strains among endemic strains and is preferred as first line approach in epidemiological investigation of MRSA strains.[17] Only 50 strains were typed in this study, which is the limitation to analyse the typing pattern in large scale.

In this study, prevalence of resistance to Cotrimaxazole (78.33%), Erythromycin (72.50%), Ciprofloxacin (60.00%), Gentamicin (48.33%) has been high among MRSA. In India, the prevalence of resistance for these drugs was high as reported in many other studies.[18,19] Further, it was interesting to note in the present study that Chloramphenicol, which is not being used commonly for quite some time to treat staphylococcal infection, had only 22.50% resistance prevalence. Likewise Clindamycin also shows a (15.00%) lower resistance pattern among MRSA. Hence Clindamycin can be preferred over Chloramphenicol, to avoid the bone marrow suppression, in treating MRSA infections.

All MRSA strains were sensitive to Vancomycin in the present study. This is in accordance with other studies.[7,8] However, Vancomycin-intermediate and Vancomycin-resistant *Staphylococcus aureus* (VISA

and VRSA) strains have been reported recently from various parts of the country.[20,9]

CONCLUSION

It is alarming that the present study reports a high prevalence (65.94 %) of MRSA infection and 70% of the MRSA isolates belong to group III Phage, especially phage no.47. MRSA strains are very difficult to treat as they are multidrug-resistant leaving glycopeptides as the drugs of choice. Resistance has been reported to these drugs also from various parts of the world.[20,9] The knowledge of prevalence of MRSA and their antimicrobial-susceptibility pattern is a must for appropriate treatment of these infections. Proper monitoring, surveillance & management of MRSA infections in hospitals are essential to control the problem. Accurate and timely identification of the MRSA isolates, identification of their antibiotic sensitivity pattern and epidemiological typing could curb the future expansion of these infections.

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