Research Article

Prevalence of *Staphylococcus aureus* Phage Types and their Correlation to Antimicrobial Resistance in a Tertiary Care Hospital

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ABSTRACT

Staphylococcus aureus is a major cause of nosocomial and community acquired infections. The increasing prevalence of multi drug resistant strains cause failure of treatment of patients resulting in prolonged hospital stay. The objective of the study is to identify the prevalence of phage types of *S.aureus* and antibiotics resistance. A total of 106 *S.aureus* strains identified, their resistance to antibiotics was determined and strains were phage typed. Prevalence of MRSA and MSSA strains was found to be 55.4% and 44.6% respectively**.** Only 46.5% strains were typable and the percentage of typability among MSSA and MRSA strains was found to be 23.76% and 22.77% respectively. The predominant phage group among MRSA strains was III-47 and among MSSA was mixed group**.** All typable strains (47) were resistant to penicillin (100%). From this study, it was identified that phage typing may act as a diagnostic and epidemiological tool for future studies.

Keywords: *Staphylococcus aureus,* MRSA, MSSA, Phage grouping, Phage typing, antimicrobial resistance.

INTRODUCTION

Staphylococcus aureus has emerged as a prime pathogen of nosocomial and community acquired infections around the world. It causes variety of infections, ranging from skin infections to life-threatening endocarditis.[1] Infections caused by *S.aureus* used to respond to β-lactam and related group of antibiotics. The spread of methicillin resistant *Staphylococcus aureus* (MRSA) in hospitals has been the major problem in India. A lot of investigations of MRSA by phage typing have been reported since MRSA was first identified in 1961. The international phage typing set (IPS) has been used for phage typing of MRSA, but many strains (33-75%) are non-typable (NT) and even the typable ones give only restricted number of phage reactions with the available phages.[2]Phage typing for tracing the source of an epidemic caused by *S.aureus* is a reliable epidemiological way for controlling the epidemics caused by such microorganisms.

However, due to the development of MRSA isolates, treatment of these infections has become problematic.[3] Available data on epidemiology of *S.aureus* shows that MRSA strains of certain phage types are more virulent and spread rapidly in hospitals.[4] These phage types responsible for infections may vary from time to time and hospital to hospital in their antibiotic resistance pattern.[5] The present study was carried out to identify the epidemiology of *S. aureus* strains with reference to phage types, its prevalence and antimicrobial resistance in relation to phage groups.

MATERIALS AND METHODS

A prospective study was carried out at Department of Microbiology, Chennai Medical College Hospital and Research Centre, Irungalur, Tiruchirapalli, Tamilnadu, India after obtained approval from Institutional ethical committee. One hundred and six strains of *S.aureus* isolated from clinical samples of 91 from pus (85.8%), 4 from blood (3.8%), 6 from sputum (5.7%) and 5 from urine (4.7%) collected over a period of consecutive 12 months commencing from April 2013 were studied**.** All these strains were confirmed as *S. aureus* by standard techniques based on colony morphology, gram staining, catalase, slide and tube coagulase tests and mannitol fermentation.[6]

The antimicrobial susceptibility pattern was determined by the Kirby Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute guidelines.[7]The antimicrobials included were cefoxitin (30µg), ciprofloxacin (5µg), clindamycin (2µg), co-trimoxazole (1.25+23.75µg), doxycycline (30µg), erythromycin (15µg), gentamicin (10µg), linezolid(30µg), penicillin (10U), rifampicin (5µg), teicoplanin (30µg)and vancomycin (30µg). *S. aureus* strain ATCC 25923 was used as quality control. All isolates showing an inhibition zone of ≤ 21mm and ≥ 22mm surrounding the cefoxitin disk were considered as MRSA and MSSA respectively[7]. All the isolates were sent to National Staphylococcal phage typing center, Maulana Azad Medical College (MAMC), New Delhi for phage typing and the results were statistically analyzed.

RESULTS AND DISCUSSION

Of the total 101 strains, 56 (55.4%) were found to be resistant and 45 (44.6%) were sensitive to methicillin. Susceptibility of MSSA and MRSA strains to various antibiotics is summarized in table 1. Out of 106 strains studied, 47 (46.5%) strains were typable and remaining 54(53.5%) were nontypable (NT). Distribution of phage patterns of *S.aureus* isolated from heterogeneous clinical samples is shown in table 2. The percentage of typability among the MSSA and MRSA strains was found to be 24(23.76%) and 23 (22.77%) respectively. Phage group distribution among MSSA and MRSA strain is shown in figure 1. Phage group distribution among MSSA and MRSA strain is found as Group II [6(13.3%) MSSA and 1(1.7%) MRSA], Group III [6(13.3%) MSSA and 18(32.1%) MRSA], Mixed group [11(24.4%) MSSA and 3(5.35%) MRSA] and non-allocated (NA) [1(2.2%) MSSA and 1(1.79%) MRSA]. No strains were found in group I and V. Correlation of different phage groups of *S.aureus* to antibiotic susceptibility is shown in table 3.

Table 1: Antimicrobial resistance pattern of 101 *Staphylococcus aureus* strains

Antibiotics	MSSA (n=45)		MRSA (n=56)	
	Sensitive	Resistant	Sensitive	Resistant
Penicillin	2 (4.44)	43 (95.6)	0	56 (100)
Doxycycline	41 (91.1)	4 (8.9)	44 (78.6)	12 (21.4)
Erythromycin	30 (68.2)	14 (31.8)	34 (65.4)	18 (34.6)
Clindamycin	40 (90.9)	4 (9.1)	40 (77)	12 (23)
Gentamicin	35 (77.8)	10 (22.2)	17 (30.4)	39 (69.6)
Cotrimoxazole	38 (84.4)	7 (15.6)	34 (60.7)	22 (39.3)
Ciprofloxacin	22 (48.9)	23 (51.1)	13 (23.2)	43 (76.8)
Linezolid	45 (100)	0	56 (100)	0
Rifampicin	45 (100)	0	56 (100)	0
Vancomycin	45 (100)	0	56 (100)	0
Teicoplanin	45 (100)	0	56 (100)	0
Nitrofuantoin	1 (100)	0	3 (75)	1 (25)
Norfloxacin	0	1 (100)	0	4 (100)

[Figure in parenthesis denotes percentages]

Table 2: Distribution of Phage patterns of *S. aureus* isolates

Phage group	Number of isolates (n=101)	Phage type (number of isolates)
I	Nil	-
II	7 (6.9)	3C/55/71 (2), 3A/55/71(1), 55/71(1), 3C/55(3)
III	24 (23.8)	47/77 (4), 47/84 (3), 47/77/84 (3), 85 (3), 47 (5), 77 (1), 84 (1), 84/85 (1), 47/85 (1), 77/84/85 (1), 47/77/84/85 (1)
V	Nil	-
Non Allocated (NA)	2 (2)	81 (2)
Mixed (MIX)	14 (13.9)	I/ III - 52/79/47/84 (1), 29/52/80/47/54/77/84 (1), 29/52A/79/77/84/85 (1) III/NA - 47/81 (2), 47/77/81 (2), 77/84/81 (1), 47/54/84/81 (1) I/ II - 52A/3C/55 (1), I/III/NA - 52/79/47/84/81 (1), 29/52/79/47/84/81 (1) III/NA/V - 47/81/96 (1) NA/V - 81/96 (1)
Non– typable (NT)	54 (53.5)	-

[Figure in parenthesis denotes percentages]

Table 3: Susceptibility pattern of *S. aureus* phage groups to antimicrobial agents

Phage groups	Number	CX (MSSA)	DOX	ERY	CD	Gent	COT	CIP	NIT	NOR	L/Rif/Va/ Tei
II (n=7)	0	6 (86)	6 (86)	5 (71.4)	5 (71.4)	6 (86)	6 (86)	6 (86)	-	-	7 (100)
III (n=24)	0	6 (25)	18 (75)	11 (45.8)	15 (62.5)	9 (37.5)	13 (54.2)	5 (20.8)	2 (40)	0	24 (100)
NA (n=2)	0	1 (50)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	-	-	2 (100)
MIX (14)	0	11 (78.6)	12 (85.7)	9 (64.3)	11 (78.6)	10 (71.4)	13 (92.9)	3 (21.4)	1 (7.1)	-	14 (100)
NT (n=54)	2 (3.7)	21 (38.8)	47 (87)	38 (70.4)	47 (87)	25 (46.3)	38 (70.4)	19 (35.2)	1 (20)	-	54 (100)
Total (n=101)	2 (1.98)	45 (44.6)	85 (84.2)	64 (63.4)	80 (79.2)	52 (51.5)	72 (71.3)	35 (34.7)	4 (80)	0	101 (100)

[Figure in parenthesis denotes percentages]

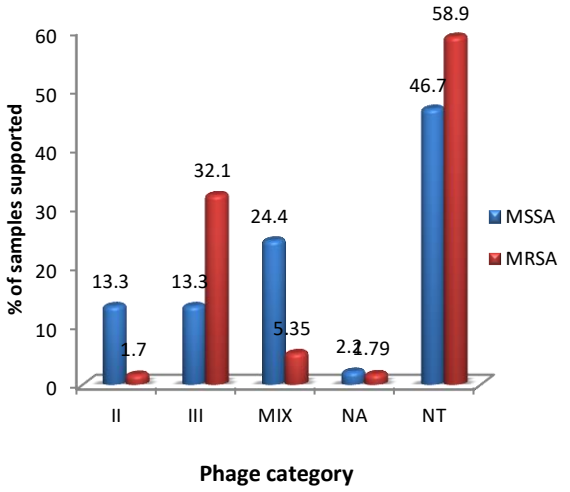

Figure 1: Phage group distribution among MSSA and MRSA

The burden of methicillin-resistant *S.aureus* (MRSA) has been rising in the past years in many parts of the world.[8,9] MRSA accounts for substantial morbidity, mortality, and socioeconomic costs.[10] The bacteriophage typing of *S.aureus* strains revealed that, 46.5% (n=47) of the total isolates were typable (23.76% in MSSA and 22.77% in MRSA strains) and 53.5%(n=54) isolates (20.8% in MSSA and 32.7% in MRSA) were non typable. In our study, the most common pattern is group III (17.8%) in MRSA & mixed group (10.9%) in MSSA. Among the phage types in group III, 47 was most common (n=5), followed by 47/77(n=4), 47/84(n=3), 47/77/84(n=3) and 85(n=3). When compared these results with a study from the same Centre earlier[11] reported a predominance of group III-47/84 among MRSA and MSSA and a lower incidence (32.7%) of non-typability.

In accordance with this study, other reports in the country also showed that majority of MRSA strains were non typable (64.4%, 68.4%, 39% respectively) and most of the typable ones belonged to Phage group III.[4,12,13] The reports from India indicated that group III phages are frequently found among the hospital strains.[14] In a recent study, have reported a higher percentage (45.94%) of MSSA as non-typable and among the typable MSSA, 45% belonged to Phage I - 52 / 79.[15] A study reported higher incidence of phage group II- 3C among MSSA.[5] On the contrary, in another study at Riyadh reported that only 30.7% strains were non typable and among the typable strains, Mixed group (64.8%) followed by group III (13%) were the predominant phage groups.[16] Some study highlighted 27.3% as non-typable and among the typable, Phage group III as the most predominant (54.54%).[17,18]

In our study 55.4% of the total isolates of the *S.aureus* were MRSA whereas it was32.8% in Riyadh, [16] 39.2% in Iran,[19] 20.3% in Germany.[20] A study from Europe reported a wide prevalence of MRSA ranging from<1% to >20%[21] and the changing trends were noted from 2009 to 2014. When compared with these results, lower prevalence rates of MRSA were recorded in Maharashtra 42.8% and 24.67%, [13, 22] New Delhi 29.36%[23] and Andhra Pradesh 48%.[24]Over a period of time, the occurrence of MRSA in India varied from 25% in western part of India to 50% in Southern India.[25]

Maximum resistance in MRSA was seen to penicillin (100%) followed by ciprofloxacin (76.8%), and gentamicin (69.6%). Among the MSSA, maximum resistance was seen to penicillin (95.6%) followed by ciprofloxacin (51.1%). High resistance to these drugs has also been reported in other studies from India. Similar results of over 90% ciprofloxacin resistance was reported from India.[22]

In our study, out of 101 strains, 5 were from urine samples. All the 5 urinary strains were 100% resistant to norfloxacin and only 20% were resistant to nitrofurantoin. Among the remaining 96 strains, 35.4% MRSA and 31.3% MSSA were sensitive to both clindamycin and erythromycin. In concordance with these results, a study from the same institute revealed doxycycline and co-trimoxazole as the most effective first line drugs against MRSA.[11]A study from Maharashtra in 2012 observed gentamicin (91.5%) and erythromycin (69.5%) as the most effective drugs.[23]

In this study, all typable strains (n-47) were resistant to penicillin (100%). Strains in phage group III/Mixed were highly sensitive to doxycycline (75%/85.7%), co-trimoxazole (54%/93%), and clindamycin (62.5%/78.6%) and least sensitive to ciprofloxacin (20.8%/21.4%). Among the non-typable (n-54) strains, 87% were sensitive to clindamycin and doxycycline, 70.4% were to erythromycin and co-trimoxazole, and only 35.2% were sensitive to ciprofloxacin. Literatures revealed that all Phage group II strains were resistant to penicillin[17] whereas only 40.2% penicillin resistant strains belonged to phage group III.[23] In our study, no strains were found in Phage group I and V and this phenomenon was reported elsewhere.[11, 15]

CONCLUSION

Therefore, regular surveillance of hospital-associated infections including monitoring of antimicrobial susceptibility (especially vancomycin, linezolid and other newer glycopeptides) pattern of MRSA and formulation of a definite antimicrobial policy may be helpful to reduce the occurrence of these infections in hospitals. Although bacteriophage typing has certain limitations such as; the typing technique is cumbersome, lack of reproducibility, time consuming and requires intense efforts in propagation, standardization, and maintenance of phages, this method is widely used even nowadays since it is considered as an ideal method of typing. Our study helps to facilitate epidemiologists to understand the nature of MRSA isolates in this part of India.

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REFERENCES

1. Chambers HF, The changing epidemiology of *Staphylococcus aureus*, Emerging Infectious Diseases, 7, 2001, 178-182.
2. Midori K, Sadayori H, Katsuhiko M, Phage set for methicillin-resistant *Staphylococcus aureus* (MRSA) strains combined with the international phage typing set and the phages from coagulase-negative staphylococci, Japanese Journal of Clinical Microbiology, 14,2004, 14-20.
3. Kluytmans J, Belkum AV, Verbrugh H, Nasal Carriage of *Staphylococcus aureus*; Epidemiology, underlying mechanisms and associated risk, Clinical Microbiology Reviews, 10, 1997, 505-520.
4. Mehndiratta PL, Gur R, Saini S, Bhalla P, *Staphylococcal aureus* phage types and their correlation to antibiotic resistance, Indian Journal of Pathology and Microbiology, 53, 2010, 738-741.
5. Kareiviene V, Pavilonis A, Sinkute G, Liegiute S, Gailiene G, *Staphylococcus aureus* resistance to antibiotics and spread of phage types, Medicina, 42, 2006, 332-339.
6. Baird D.*Staphylococcus*. Cluster forming gram positive cocci. Chapter 11. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medial Microbiology. New York, Churchill Livingstone, 1996, 127-133.
7. Clinical and Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing: 23rd Document M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA, 2013, 14-19.
8. Tiemersma EW, Bronzwaer SL, Lyytikainen O, Degener JE, Schrijne makers P, Bruinsma N, European Antimicrobial Resistance Surveillance System Participants, Emerging Infectious Diseases,10, 2004, 1627-1634.
9. Shorr AF, Epidemiology of Staphylococcal Resistance, Clinical Infectious Diseases, 45, 2007, 171-176.
10. Gould IM, Costs of hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and its control, International Journal of Antimicrobial Agents, 28, 2006, 379-384.
11. Vazhavandal G, Vallab GBB, Uma A, Chitra RP,Thirumalaikolundu subramanian P, *Staphylococcus aureus* phage groups and their relation to antibiotic resistance pattern in a tertiary care hospital, south Tamil Nadu, Journal of Evolution in Medical and Dental Sciences, 2, 2013, 5366-5374.
12. Vidhani S, Mehndiratta PL, Mathur MD, Study of methicillin resistant *Staphylococcus aureus* (MRSA) isolates from high risk patients, Indian Journal of Medical Microbiology, 19, 2001, 87-90.
13. Asmita TB, Akulwar SL,Chaya AC, Study of methicillin resistant *Staphylococcus aureus* (MRSA) in a tertiary care hospital, Bombay Hospital Journal, 53, 2011, 329-332.
14. Mathew JJ, Shanmugam JJ, Rout DD, Valiathan MS, Predominance of *S. aureus* phage type-47 among the isolates from cardiac and neurosurgical patients, Journal of Postgraduate Medicine,29, 1983, 34-37.
15. Sharma DK, Sharma PC, Bacteriophage typing of methicillin sensitive *Staphylococcus aureus*(MSSA) strains recovered from human clinical cases in Himachal Pradesh and there *in vitro* susceptibility to different antibiotics, Indian Journal of Basic and Applied Medical Research, 3, 2013, 296-302.
16. Manal MAK, Nagwa MAA, Al Salamah AA, Phage typing, PCR amplification for mec A gene, and antibiotic resistance patterns as epidemiologic markers in nosocomial outbreaks of methicillin resistant *Staphylococcus aureus*, Saudi Journal of Biological Sciences, 16, 2009, 37-49.
17. Samant SA, Pai C, Prevalence of *Staphylococcus aureus* phage-types and their correlation to antimicrobial resistance, International Journal of Medical and Pharmaceutical Research,2, 2012, 32-37.
18. Dugid JP, *Staphylococcus*: cluster forming Gram positive bacteria, Chapter 16. In: Mackie &Mc Cartneys Practical Medical Microbiology, UK, Churchill Livingstone, 1989, 65-71.
19. Rajabiani A, Kamrani F, Boroumand MA, Saffar H, mec-A-mediated Resistance in *Staphylococcus aureus* in a Referral Hospital, Tehran, Iran, Jundishapur Journal of Microbiology,7, 2014, 9181-9183.
20. Korczak D, Schoffmann C, Medical and health economic evaluation of prevention- and control measures related to MRSA infections or colonization at hospitals, GMS Health Technology Assess, 6,Doc 04, 2010, 42-49.
21. Dulon M, Haamann F, Peters C, Schablon A, Nienhaus A, MRSA prevalence in European healthcare settings: a review, BMC Infectious Diseases, 11, 2011, 138-151.
22. Gade ND,Qazi MS, Fluoroquinolone therapy in *Staphylococcus aureus* infections: Where do we stand?, Journal of Laboratory Physicians, 5, 2013, 109-112.
23. Sanjay MW, Sarita NK, Mangala PG, Multi drug resistance and Phage pattern of *Staphylococcus aureus* in Pyoderma case,JKIMSU, 1, 2012, 48-54.
24. Shazia PS, Jyothsna K, Methicillin resistance among isolates of *Staphylococcus aureus*: antibiotic sensitivity pattern and phage typing, Annals of Biological Research, 2, 2011, 57-61.
25. Patel AK, Patel KK, Patel KR, Shah S, Dileep P, Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years, Journal of Association of Physicians in India, 58, 2010, 37-40.

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