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CIRCULATION OF MULTI DRUG RESISTANT PHAGE GROUP III /TYPE 47 HOSPITAL ACQUIRED METHICILIN RESISTANT *STAPHYLOCOCCUS AUREUS* (HA-MRSA) IN TAMILNADU

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ABSTRACT

Background: The epidemiology of MRSA is constantly changing, which results in variation in its drug-resistance patterns throughout regions and countries. Therefore, to support clinicians in preventing and treating infection, epidemiologic surveillance is essential. Phage typing had been reported to be valuable in the identification of known epidemic strains among endemic strains and is preferred as a first line approach in epidemiological investigation of MRSA strains. **Aim:** 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**: Study was undertaken on 136 *S aureus* strains isolated from clinical samples. Strains were phage typed and their resistance to antibiotics was determined by standard microbiological procedures. Molecular detection of MRSA was done using Triplex PCR. **Results:** Among phage typed *S aureus,* phage group III 53(39%) was found to be predominant and percentage typeability among the MRSA and MSSA strains was found to be 71(63%) and 17(71%) respectively. Out of 88 phage typed *S. aureus* isolates, 33 phage type patterns were identified with respect to each phage groups. **Conclusion:** Our study strongly suggests that a multi drug resistant phage group III with predominant phage type pattern 47 of HA-MRSA strains carrying *pvl* gene was found to be established hospital pathogen in our study area.

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INTRODUCTION

Recent studies document the emergence of MRSA isolates typical of Community acquired (CA) genotypes in patients with HA-MRSA in an Indian hospital (Dhawan, 2014). An increased prevalence of PVL-producing CA-MRSA strains in hospitals may increase the virulence of nosocomial MRSA infections. Bacteriophage considered as a major risk factor for *S. aureus* to acquire new virulence genetic elements (Al Khulaifi Manal, 2009). Available data on epidemiology of S *aureus* shows that epidemic methicillin resistant S*taphylococcus aureus* (MRSA) strains of certain phage types are more virulent and spread rapidly in hospitals (Kareiviene, 2006).

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Phage typing had been reported to be valuable in the identification of known epidemic strains among endemic strains and is preferred as a first line approach in epidemiological investigation of MRSA strains (Mehandritta,, 2012). Apart from phage typing, genotypic methods *viz.* SCC*mec* typing, *spa* typing, PFGE and MLST were also employed for strain differentiation. However, they are not readily available in resource-limited settings (Kali, 2013). 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 total of 136 *S. aureus* isolates were collected during 2012-2014, 50 isolates from Govt. Dharmapuri Medical College Hospital, Dharmapuri and 86 isolates from Govt. Theni Medical College Hospital, Theni. Most isolates were clinical samples and originated from pus culture.

Table 1.List of primers used in this study

Target genes	Primer sequence	Amplicon size
femA	F: 5' – AAAAAAGCACATAACAAGCG – 3' R: 5' – GATAAAGAAGAAACCAGCAG – 3'	132bp
mecA	F: 5'-TGCTATCCACCCTCAAACAGG-3' R: 5'-AACGTTGTAACCACCCCAAGA-3'	286bp
pvl	F:5'–ATCATTAGGTAAAATGTCTGGACATGATCCA–3' R: 5'– GCATCAASTGTATTGGATAGCAAAAGC– 3'	441bp

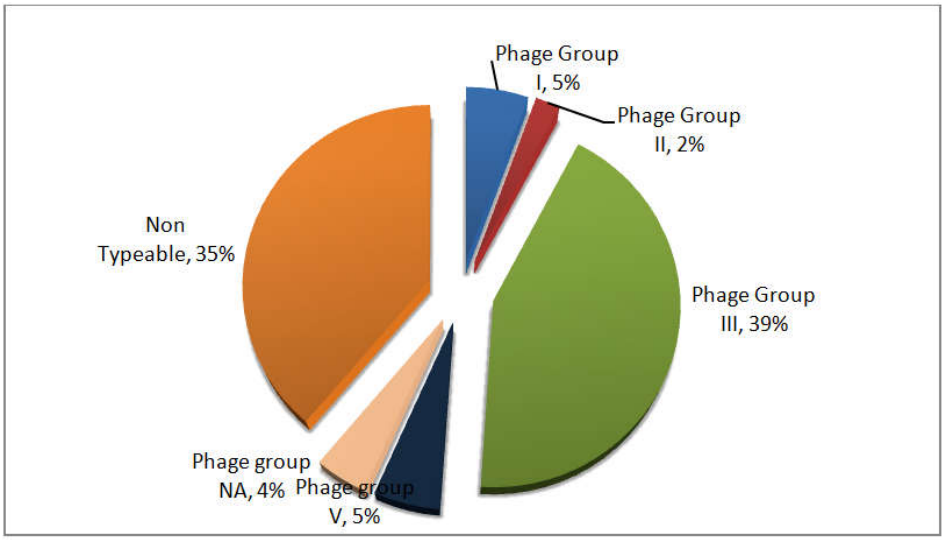

Figure 1. Prevalence of Phage groups among the *S aureus* isolates

Of the 136 isolates, 121(89%) were from hospitalized settings (inpatients) and 15(11%) from community settings (outpatients). The isolates were identified to the species level using standard biochemical techniques. The antibiotic susceptibility tests were interpreted in accordance with the CLSI guidelines (Clinical and Laboratory Standard Institute, 2013). Methicillin resistance was detected by taking cefoxitin disc (30µg) as a surrogate marker. The antibiotics included were amikacin (AK) 30µg, ceftriaxone (CTR) 30µg, ciprofloxacin (CIP) 5µg, clindamycin (CD) 2µg, cotrimoxazole (COT) 25µg, erythromycin (E) 15µg, fusidic acid (FC) 10 µg, gentamicin (GEN) 10µg, linezolid (LZ) 15µg, penicillin G (P) 10U, pristinomycin (RP) 15µg, rifampicin (RIF) 5µg, teicoplanin (TEI) 30 µg, tetracycline(TE) 30µg and mupirocin (MU) 5µg. *S. aureus* ATCC 25923 strain was used as quality control. Vancomycin resistance was screened by BHI vancomycin screen Agar (6µg/ml). Patient data were collected to distinguish CA-MRSA strains from HA-MRSA strains by epidemiological criteria, as defined by the Centre for Disease Control and Prevention (CDC). Molecular detection of MRSA was done using Triplex PCR by Nagarajan *et.al* (Nagarajan, 2013), to detect *mec* A gene which confers resistance to methicillin, *fem* A gene to differentiate *S. aureus* from CONS and *pvl* gene, which codes for PVL toxin (Table 1). All the isolates were sent to National Staphylococcal phage typing centre, Maulana Azad Medical College, New Delhi for phage typing and the results were analyzed.

RESULTS

A total of 136 *S. aureus* isolates were subjected to phage typing, of which 112(82%) were found to be MRSA and 24(18%) were MSSA by cefoxitin disc diffusion method and further confirmed by Triplex PCR. Out of 136 isolates, 39(29%) were found to carry *pvl* gene, of which 36(92%) were MRSA and 3(8%) were MSSA. Among 136 *S. aureus* isolates, 88(65%) isolates were phage typeable, of which phage group III 53(39%) was found to be predominant followed by, other phage groups and 48 (35%) were non typeable (Figure 1). Percentage typeability among the MRSA and MSSA strains was found to be 71(63%) and 17(71%) respectively. Phage group distribution among the MRSA and MSSA was as follows. Of the seven isolates of phage group I, 6 (8.4%) were MRSA and 1 was (5.8%) MSSA; of the 3 isolates of phage group II 2 (2.8%) were MRSA and 1 (5.8%) was MSSA, of phage group III (53), 48 (67.6%) were MRSA and 5(29.4%) MSSA, of phage group V (6), 4 (5.6%) were MRSA and 2 (11.7%) were MSSA, of NA Group (5), 4(5.6%) were MRSA and 1(5.8%) was MSSA and out of 14 mixed phage groups 7(9.8%) were MRSA and 7(41.1%) were MSSA. Out of 88 phage typeable strains, *pvl* gene was detected in 20 (23%) isolates, of which 19(95%) were found to be MRSA and 1(5%) was MSSA, which belonged to mixed phage groups. Out of 19 PVL MRSA strains, phage group III was predominant 10 (52.6%), followed by mixed groups 3(15.7%), NA group 3(15.7%), group I 2(10.5%), group V 1(5.2%) and group II was not detected in any of the isolates. Among 71 phage typeable MRSA, 63 (88.7%) were from hospitalized settings (HA-MRSA) and 8(11.3%) from community settings (CA-MRSA). Out of 63 HA-MRSA isolates, phage group III was found to be predominant 41(65%), followed by mixed phage groups 7(11.1%), group I 5(7.9%), group V 4(6.3%), NA group 4(6.3%) and group II 2(3.1%). Out of 8 CA-MRSA isolates, phage group III was predominant 7(87.5%), followed by group I 1(12.5%) and other phage groups were absent.

Table 2. Prevalence of various phage groups and their susceptibility to various antimicrobial agents among MRSA

Sl No	Antimicrobial agents	Phage groups					
		Group I n=6	Group II n=2	GroupIII n=48	Group V n=4	Group NA n=4	Mixed group n=7
1	Amikacin	67%	100%	50%	50%	80%	57%
2	Cefoxitin	0%	0%	4%	0%	0%	0%
3	Ceftriaxone	50%	50%	10%	0%	0%	86%
4	Ciprofloxacin	33%	50%	15%	0%	20%	14%
5	Cotrimoxazole	33%	100%	19%	25%	0%	14%
6	Clindamycin	83%	100%	81%	50%	100%	100%
7	Erythromycin	0%	100%	29%	100%	20%	43%
8	Fusidic acid	83%	100%	98%	100%	100%	100%
9	Gentamicin	67%	100%	50%	50%	40%	29%
10	Linezolid	100%	100%	98%	100%	100%	86%
11	Mupirocin	100%	100%	83%	100%	100%	100%
12	Penicillin	0%	0%	0%	0%	0%	0%
13	Pristinamycin	50%	100%	35%	75%	80%	57%
14	Rifampicin	100%	100%	94%	100%	100%	100%
15	Tetracycline	50%	100%	50%	75%	40%	57%
16	Teicoplanin	100%	100%	81%	50%	60%	71%
17	Vancomycin	100%	100%	100%	100%	100%	100%

Table 3. Prevalence of various phage groups and their susceptibility to various antimicrobial agents among MSSA

Sl No	Antimicrobial agents	Phage groups					
		Group I n=1	Group II n=1	GroupIII n=5	Group V n=2	Group NA n=1	Mixed group n=7
1	Amikacin	100%	100%	100%	100%	100%	86%
2	Cefoxitin	100%	100%	100%	100%	100%	100%
3	Ceftriaxone	100%	100%	100%	100%	100%	100%
4	Ciprofloxacin	0%	100%	60%	100%	0%	43%
5	Cotrimoxazole	0%	0%	80%	100%	0%	57%
6	Clindamycin	100%	100%	80%	100%	100%	100%
7	Erythromycin	100%	100%	60%	100%	100%	43%
8	Fusidic acid	100%	100%	100%	100%	100%	100%
9	Gentamicin	100%	100%	100%	50%	100%	71%
10	Linezolid	100%	100%	100%	100%	100%	100%
11	Mupirocin	100%	100%	100%	100%	100%	86%
12	Penicillin	0%	0%	80%	50%	0%	86%
13	Pristinamycin	100%	100%	60%	100%	100%	86%
14	Rifampicin	100%	100%	100%	100%	100%	100%
15	Tetracycline	100%	100%	100%	100%	100%	100%
16	Teicoplanin	100%	100%	100%	100%	100%	100%
17	Vancomycin	100%	100%	100%	100%	100%	100%

Table 4. Antibiotic resistance pattern of *S aureus* strains with common phage types from each phage group

Sl No	Antibiotics	52A (Phage group I)	3C,71 (Phage group II)	47 (Phage group III)	94,96 Phage group V)	81 NA group	47,84,81 Mixed phage group
1	Amikacin	50%	0%	36.8%	33.3%	20%	0%
2	Gentamicin	50%	0%	36.8%	50%	60%	100%
3	Clindamycin	25%	0%	15.7%	33.3%	0%	0%
4	Erythromycin	100%	0%	47.3%	66.6%	80%	0%
5	Pristinamycin	25%	0%	15.7%	16.6%	20%	0%
6	Tetracycline	75%	0%	31.5%	16.6%	60%	0%
7	Cotrimoxazole	50%	100%	68.4%	66.6%	60%	50%
8	Mupirocin	0%	0%	10.5%	0%	0%	0%
9	Fusidic acid	25%	0%	5.2%	0%	0%	0%
10	Ciprofloxacin	100%	50%	68.4%	66.6%	80%	50%
11	Rifampicin	0%	0%	5.2%	0%	0%	05
12	Linezolid	0%	0%	0%	0%	0%	0%
13	Teicoplanin	25%	0%	31.5%	0%	40%	0%
14	Vancomycin	0%	0%	0%	0%	0%	0%
15	Cefoxitin	100%	50%	78.9%	66.6%	80%	50%
16	Ceftriaxone	75%	50%	73.6%	66.6%	60%	50%
17	Penicillin	100%	100%	100%	100%	100%	100%

Out of 88 phage typed *S. aureus* isolates, 33 phage type patterns were identified with respect to each phage groups. Of 33 phage type patterns, phage type pattern 52A was found to be common in phage group I (57%) , 3C,71 pattern in group II (67%), 47 pattern in group III (40%), 94,96 pattern in group V(100%), 81 pattern in NA group(100%) and 47,84,81 pattern in Mixed group (14.28%).

Antibiotic susceptibility of MSSA and MRSA strains to various antibiotics with respect to each phage groups are summarized in Table.2 & Table.3. All the isolates were found to be susceptible to vancomycin by vancomycin screen agar (6µg/ml). Antibiotic susceptibility of *S. aureus* strains with common phage type pattern from each phage group are summarized in Table 4.

DISCUSSION

Phage typing is recommended as first line of approach in epidemiological investigations of MRSA strains. This method is used to determine similarities and dissimilarities among the strains which can be correlated to identify epidemic strains. In this manner, the significance of different strains in their spread can be evaluated (Sharma, 2013). In this present study, 88 (65%) out of 136 isolates of *S. aureus* were successfully phage typed, while 48 (35%) out of 136 isolates were nontypeable. In general, the percentage of non-typeable strains is high, whereas in our study, higher percentage (65%) of strains were phage typed, compare to the previous studies in India (Sharma, 2013 and Mehndiratta, 2010).

The most prevalent phage group among the typed *S. aureus* isolates was found to be phage group III. Among MRSA, phage group III (68%) was found to be predominant and mixed phage group (41%) was found to be predominant in MSSA isolates. The phage group I and III typed MRSA strains were found to be multidrug resistant. Phage group I typed MRSA isolates showed resistance to ciprofloxacin (67%), cotrimoxazole (67%), tetracycline (50%), amikacin (33%) and gentamicin (33%). Phage group III MRSA strains were resistant to ciprofloxacin (85%), cotrimoxazole (81%), erythromycin (71%), amikacin (50%), gentamicin (50%) and tetracycline (50%). Both HA-MRSA and CA-MRSA predominantly belonged to phage group III and all the CA-MRSA strains were only confined to phage group I (12.5%) and III (87.5%). Among MRSA which carried *pvl* gene (17%), 79% of strains belonged to phage group III typed HA-MRSA. The distribution of prevalent phage types in our study were consistent with other Indian studies which identified majority of MRSA isolates to belong to phage group III (Kali, 2013; Vazhavandal, 2013; Samant, 2012).

Although several workers reported most MSSA to belong to phage group II and phage type 81 of the non-allocated group as the predominant phage type (Sharma, 2013 and Mehndiratta, 2010). In our study, mixed phage group was predominant among MSSA. Interestingly, 57% of MSSA isolates which belonged to mixed phage groups were found to be resistant to erythromycin and ciprofloxacin. Among phage group I typed *S. aureus* strains, phage type 57A was found to be predominant and showed (100%) resistance to erythromycin and ciprofloxacin similar to recent report from Puducherry, TamilNadu (Velayudham, 2016). Our study also reveals 70 % of the isolates typed phage group III with predominant phage type pattern 47. Majority of phage type 47 were found to be MDR strains. Predominant phage type pattern 47, 84, 81 of mixed phage group typed *S. aureus* were found to be resistant to gentamicin (100%), cotrimoxazole (50%) and ciprofloxacin (50%). All the MRSA and MSSA strains were susceptible to vancomycin.

Presently, MRSA has become endemic in Indian hospitals. It is alarming that the present study reports a very high prevalence (82%) of MRSA infection, whereas it was 42.8% in Maharashtra (Kareiviene, 2006), 48% in Andhra Pradesh and 55.4% in TamilNadu (Vazhavandal, 2017). The highest prevalence rate was observed in Indore (80.8%) (Verma, 2000). However, 31.1% and 23.6% of MRSA prevalence has also been reported in Coimbatore, TamilNadu (Rajaduraipandi, 2006), which is comparatively very low than that reported in the current study.

Conclusion

Our study revealed that phage group III was predominant amongst MRSA strains and mixed phage group was most predominant amongst MSSA strains. We strongly suggest that a multi drug resistant phage group III with predominant phage type pattern 47 of HA-MRSA strains carrying *pvl* gene was found to be strong and stable colonizer in the study sites. Though bacteriophage typing is cumbersome, time consuming typing technique, this method is widely used even today since it is considered a useful and accepted conventional method of typing.

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