Original article:

Bacteriophage typing of methicillin sensitive *Staphylococcus aureus* (MSSA) strains recovered from human clinical cases in Himachal Pradesh and their *in vitro* susceptibility to different antibiotics.

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Abstract

The present study aims at bacteriophage typing of methicillin sensitive *Staphylococcus aureus* (MSSA) strains recovered from patients with different clinical conditions at Indira Gandhi Medical College, Shimla Himachal Pradesh. We have previously reported the emergence of such resistance among 66% *S. aureus* strains of Himachal Pradesh. Of these 42 MSSA strains were submitted at National Centre for bacteriophage typing, Maulana Azad Medical College, Delhi. 20 strains (54.05%) were typable and 17 (45.94%) were non-typable by the available typing tools. The strains were assigned to group I (45%), group II and group III 10(% each), group NA (20%) and mixed phage group (15%). None of the MSSA strain was lyses by the phages of group V. Phage type 52 and 79 were the most predominant which lyses 9 MSSA strains followed by phage type 81 (5 strains), phage type 84, type 80 and 29 (4 strains each), type 47 (2 strains), 55 (2 strains).

Key words : *Staphylococcus aureus* , Bacteriophage Typing.

Introduction

Staphylococcus aureus has emerged as a prime pathogen of nosocomial and community acquired infections world over. *Staphylococcus aureus* is an opportunistic pathogen affecting immuno-compromised as well as immuno-competent individuals frequently, resulting in high morbidity although the very less mortality rate is very low. *S .aureus* is involved in several clinical conditions such as septicemia, pneumonia, wound sepsis, septic arthritis, post-surgical toxic shock syndrome and scalded skin syndrome in humans. [1] This organism not only to methicillin but also to others such as clindamycin, erythromycin and tetracycline.[2]

The organism presents problems in treating infections.[3] Keeping in view, the increasing trend of antimicrobial resistance among *S. aureus* strains, the clinicians and researcher have focused on the measures to combat antimicrobial resistance. This requires constant surveillance of strains circulating in the communities.[4] We have reported the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) strains in the state of Himachal Pradesh.[5] In fact, these strains were resistant to a several antibiotics belonging to different groups of antibiotics and were therefore, regarded as MDR *S. aureus* strains which often lead to treatment failures. In order to control the spread of such strains among hospital patients and community, a number of epidemiologic typing methods are in use: antimicrobial susceptibility testing (AST), biotyping, plasmid profiling, genomic restriction fragment

length polymorphism analysis using pulse -field gel electrophoresis, DNA hybridization methods etc.

Phage typing is a conventional epidemiological tool which was first employed for typing *S. aureus* strains in England as early as 1940. This method is widely used even now days since it is considered as an ideal method of typing. In conjunction with other molecular methods, this method is used to determine similarities and dissimilarities among the strains which can be correlated to epidemic strains. In this manner, the significance of different strains in their spread in the communities and the pathological conditions they cause can be evaluated fully well.[6]

The present paper describes the prevalence of different phage groups of methicillin-sensitive *Staphylococcus aureus* strains in patients outdoor as well as indoor in Govt. medical College, Shimla in Himachal Pradesh.

Materials and Methods

Confirmation of MSSA strains

Isolates of *Staphylococcus aureus* obtained from the Dept. of Microbiology, IGMC Shimla, were processed at the Department of Microbiology of Shoolini University Solan, as described previously by us.[5] In this study 91/135 (66%) isolates were found sensitive to methicillin..

Bacteriophage Typing

A total of 42 strain of MSSA were submitted for typing at the Staphylococcal Phage Typing Centre, Dept. of Microbiology, Maulana Azad Medical College, New Delhi. The typing was done using 23 sets of phages in a routine test dilution (RTD) X 100.

Results

Bacteriophage Typing

The bacteriophage typing is used as a precise identification method of *S. aureus* and provides valuable information in epidemiological studies. The results of typing are presented in Table 1. Out of 37 isolates of MSSA examined, 20 strains were typable (54.05%) and remaining 17(45.94%) were non-typable as five isolates could not be processed because of contamination. As given in Table 1, the most common phage group was group I in which 45.0% strains could be lysed by specific group I phages. This percentage for group II and III was 10% for each group. In the phage group NA 20.08% strains were lysed whereas 15 % strains were lysed by mixed phage group. Phages in group V did not lyse any strain. Phages types 52 and 79 were the predominant phages which lysed 9 MSSA strains followed by phage type 81 (5 strains), phage types 84, 80 and 29 (4 strains each). Type 47 and 55 lysed 2 strains each.

In vitro susceptibility of MSSA strains to different antibiotics and their correlation to phage groups.

The results of the antibiotic sensitivity to different phage groups are presented in Table 2.

All the 9 MSSA strains in phage group I was sensitive to methicillin, oxacillin, novoboicin and tetracycline. Most strains in this group showed resistance to teicoplanin chloramphenicol and vancomycin, penicillin, ampicillin, azithromycin, linezolid. The percentage of sensitive strains against these antibiotics ranged from 11.11% to 44.44%. In other words, majority of strains were resistant to these antibiotics. Strains in phage group II were resistant to penicillin. In the mixed phage group, all the three strains tested were resistant to penicillin,

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erythromycin ampicillin and amoxycillin. However, resistance was also seen against other antibiotics also but to a lesser extent. In the non-typable group, all the 17 strains besides methicillin were susceptible to ceprofloxicin but the percentage of susceptibility of these strains ranged from 47.05% to 88.23% in this group.

Discussion: Bacteriophage typing is one of the oldest techniques for studying the epidemiology of infectious agents. Compared to serotyping of bacteria, this technique has been considered as more sensitive.[3] Therefore, phage typing is recommended as first line of approach in epidemiological investigations of MRSA and MSSA strains. During past few decades the epidemiology of *S. aureus* has been continuously changing. In order to control the spread of these strains, it becomes essential to understand their epidemiology. Using international set of bacteriophages, in the present study, 20/37 isolates (54.05%) of MSSA strains were successfully typed. However, 17/32 (45.94%) isolates were non-typable. Another five isolates could not be typed because of contamination. In general, the percentage of non-typable strains is high, which is 45.94% in the present study. Witte *et al.*, 1979 recorded only 20% strains as non-typable.[7] In a recent study, Mehndiratta *et al.*, 2010 have reported a higher percentage of 39.0% among MRSA as non-typable.[8] Non-typability of *S. aureus* strains is a major problem with the available sets of bacteriophages in India and other developing countries.[4] On analysis of the phage typing data, we observed that most of the MSSA (45.0%) strains from hospital setting in Himachal Pradesh belonged to phage group I. The MSSA strains in these group I are generally associated with hospital acquired and endemic infections.[3,9] Similarly Usman *et al.*, 1996 have also reported the predominance of MSSA strains in hospital settings.[10,11] We observed two strains in phage group III. In general, MSSA strains do not fall in Phage group III. The phage group III consists of multidrug resistant strains particularly MRSA.[12] We did not record any strain in phage group V. It is interesting to note that the phage type 81 in phage group NA lysed the MSSA strains which were resistant to penicillin but sensitive to number of antibiotics. This observation is consistent with others.[2]

The phage group I all the nine MSSA strains typed in were sensitive to methicillin oxacillin novoboicin and tetracycline, similar observations have been made by Mahndiratta *et al.*, 2010.[8] Most strains in phage group I showed resistance to teicoplanin chloramphenicol and vancomycin, penicillin, ampicillin azithromycin and linezolid, observations are consistant with the findings of others.[13] The percentage of sensitive strains against antibiotics specifies above ranged from 11.11% to 44.44%. In other words, more number of strains was resistant to these antibiotics.

Strains in group II were resistant to penicillin. In the mixed phage group, all the three strains tested were resistant to penicillin, erythromycin ampicillin and amoxycillin. However, resistance was also seen against other antibiotics also but to a lesser extent. In the non-typable group, all the 17 strains besides methicillin were susceptible to cephalothin but the percentage of sensitive strains ranged from 47.05% to 88.23%. Jones *et al.*, 1999 reported that MSSA strains were more susceptible to antibiotics as compared to MRSA.[14]

Although bacteriophage typing is very good technique but it has certain limitations such as; the

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typing technique is cumbersome, time consuming and requires intense efforts in propogation, standardization and maintenance of phgaes.[15] The data indicated that phage type 52 and 79 were most predominant in MSSA strains. In the present study demonstrates that the community acquired as well as was prevalent in the state of Himachal Pradesh which could be differentiated on the basis of bacteriophage typing. However, further studies are required to be conducted on large number of MSSA strains recovered during different time frames before arriving at definitive conclusions. Further, we have characterized these isolates at molecular level. This might help in better correlation.

Conclusions: In conclusion our study would provide base line information with regards to bacteriophage types of MSSA strains prevalent in Himachal Pradesh, India.

Table 1: Bacteriophage typing result Methicillin sensitive *Staphylococcus aureus* (MSSA).

Sr. No	Phage group	Number of isolates (%)	Phage type (number isolates) at R.T.D X100
1	I	9 (45.0%)	29/52/52A/79/80 (2), 29/52/79/80 (1), 52/52A/79 (2), 52/79 (1), 79 (2), 52(1)
2	II	2 (10.0%)	3A/3C/55/71 (1), 3A/3C/55/71 (1)
3	III	2 (10.0%)	47/54/84 (1), 84 (1)
4	NA	4 (20.0%)	81 (4)
5	V	0 (0.0%)	(0)
6	Mixed Phage	3 (15.0[%)	29/52/52A/79/80/47/54/83A/84/81 (1), 29/52/80/54/75 (1), 79/83A/84(1)

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Correlation of antibiotic sensitivity pattern of MSSA to different phage groups.

Antibiotics	Phage Group					
	Group I n=9	Group II n=2	Group III n=2	Group NA n=4	Group Mixed Phage n=3	Non-typable n=17
CEP	88.88%	100%	100%	100%	66.66%	100%
CD	77.77%	50%	100%	100%	66.66%	82.35%
COT	77.77%	50%	50%	75%	33.33%	64.70%
E	55.55%	50%	50%	100%	0.0%	52.94%
GEN	88.88%	50%	100%	100%	33.33%	88.23%
OF	77.77%	100%	50%	100%	100%	76.47%
P	44.44%	0.0%	50%	75%	0.0%	47.05%
VA	22.22%	100%	50%	100%	33.33%	47.05%
AMP	44.44%	50%	50%	100%	0.0%	70.58%
C	11.11%	50%	100%	100%	33.33%	64.70%
OX	100%	50%	100%	100%	66.66%	88.23%
LZ	44.44%	100%	50%	75%	33.33%	58.82%
AZM	44.44%	50%	50%	100%	33.33%	41.17%
AK	100%	50%	100%	100%	100%	76.47%
CLR	33.33%	50%	50%	100%	33.33%	52.94%
TEI	11.11%	100%	100%	100%	66.66%	88.23%
MET	100%	100%	100%	100%	100%	100%
AMC	33.33%	50%	50%	75%	0.0%	64.70%
NV	100%	50%	100%	100%	33.33%	76.47%
TE	100%	50%	100%	100%	33.33%	64.70%

Percent refers to the percentage of strains sensitive to an antibiotic.

n- refers to no. of MSSA strains tested.

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