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Bacteriophage typing and plasmid profiling of Methicillin resistant of *Staphylococcus aureus* strains isolated from human clinical cases in Himachal Pradesh

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ABSTRACT

The present study aims at bacteriophage typing and plasmid profiling of methicillin resistant *Staphylococcus aureus* 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 *S.aurues* strains of Himachal Pradesh. Out of 38 isolates of methicillin resistant *S. aureus*, 32 (84.21%) strains were typable and remaining 5 (13.15%) were non-typable and one was contaminated. The most common group was mixed phage group and phage group III, 37.50% each followed by phage group I and II, 12.5% each. Phage groups NA and V however, did not lysed any strain. Phage type 77 was most predominant which lysed 27 MRSA strains followed by type 84 (19 strains), type 47 (16 strains), 52A (12 strains) and 42 E (5 strains) in the present study. Out of 40 MRSA isolates examined, 36 (82%) harboured a single type of plasmid of M_r less than 2 kDa.

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen affecting immuno-compromised as well as immuno-competent individuals frequently, resulting in high morbidity and thus, presenting problems in treating infections due to *S.aureus* in healthcare facilities [1]. This organism is associated with a variety of clinical conditions such as septicemia, pneumonia, wound sepsis, septic arthritis, post-surgical toxic shock syndrome and scalded skin syndrome in humans [2]. Antibiotic resistances in *S. aureus* develops very quickly as a result of which it become very difficult to treat *S.aureus* infections with the therapeutic agents which have been devolved during past 50 years [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 resistances which required constant surveillance of circulating strains[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 also resistant to a number of antibiotics. Such MDR strains often lead to treatment failures. In order to control the spread of such strains , a number of epidemiologic typing methods are in use: antimicrobial susceptibility testing (AST), biotyping, plasmid analysis, genomic restriction fragment length polymorphism analysis using pulsed-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 as it is considered as ideal method of typing [6, 7]. Typing of staphylococci is important in epidemiology , when it is needed to find the similarities and differences of the strains obtained from different sources, to determine epidemic strains of *S. aureus,* and to evaluate the importance of different strains for human infectious pathology [8].

Plasmids allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera. Resistance of *Staphylococcus aureus* strains to different antibiotics has been linked to antibiotics resistance genes such as (*mec-A, Van-A* and *Pvl* genes). The resistance is mediated by several mechanisms such as production of drug inactivating enzymes such as β-lactamase [9], production of toxins [10] etc. Determination of plasmid profiles is among the DNA-based methods for detecting certain strains with possible variations in plasmid content which is very important in epidemiological studies [11]. The present paper describes the prevalence of different phage groups of methicillin resistant *Staphylococcus aureus* strains in Himachal Pradesh and to determine plasmid profiles of these strains.

MATERIALS AND METHODS

Isolates of *Staphylococcus aureus* obtained from the Dept. of

Microbiology, IGMC Shimla. Were analyzed at the Department of Microbiology of Shoolini University Solan. Out of a total of 150 isolate screened, 135 were confirmed as Staphylococcus *aureus*. Of these, 44 (32%) were found to be methicillin resistant. These isolates were resistant to several other antibiotics also as reported by us earlier [5].

Isolation of Plasmid DNA

Plasmid DNA from forty Methicillin resistant *Staphylococcus aureus* isolates was extracted DNA by a standard alkaline method [12]. For this, few purified colonies of each isolate were inoculated in Lactose broth, incubated at 37^{0}C for 24 hrs, after which the bacterial cultures were harvested. Each culture was suspended in 200µl of solution A (100 mM glucose, 50 mM Tris hydrochloride (pH 8), 10mM EDTA) containing 10 mg of lysozyme per ml and incubated for 30 min at 37^{0}C. Freshly prepared 1% sodium dodecyl sulfate (SDS) 400 µl in volume was then added to 0.2 N NaOH, the samples were mixed by inverting the tubes a number of times, incubated further at 20^{0}C for 10 min. and 30% Sodium acetate solution (pH 4.8) in a volume of 300µl was then added and the samples were again mixed thoroughly by inverting the tubes, a number of a times, The tubes were placed on ice for 5 minutes, and the debris was removed by centrifugation at 10.000 x g for 5-min. Precipitation of plasmid DNA in the supernatant was done by adding an equal volume of isopropanol to the supernatant . The precipitate was then dissolved in 50µl of TE buffer (containing RNaseA), incubated at 37^{0}C for 30 min, followed by overnight incubation at 4°C. The plasmid DNA of each isolate was electrophoresed in 0.8% agarose containing ethidium bromide and observed under UV transilluminator for its presence.

Bacteriophage Typing

Multidrug resistant isolates of *Staphylococcus aureus* (40 in number) were bacteriophage typed 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. Out of 38 isolates of methicillin resistant *S. aureus* examined, 32 (84.21%) strains were typable and remaining 5 (13.15%) were non-typable and one was contaminated. The most common group was mixed phage group (46.87%) followed by phage group I (3.13%), phage group II

Table 1: Prevalence of phage groups affecting Methicillin resistant *Staphylococcus aureus* strains in Himachal Pradesh.

Sr. No.	Phage Group	Number Isolates (%)	Phage type (number isolates) at R.T.D. X 100
1	I	4 (12.50)	29/52/52A (4)
2	II	4 (12.50)	3C/55/71 (4)
3	III	12 (37.50)	47/77/84/85 (1), 77 (2), 77/84 (3), 47/77 (3), 47/77/84 (3)
4	NA	0 (0.0)	-
5	V	0 (0.0)	-
6	Mixed Phage	12 (37.50)	3C/55/77 (1), 52A/79/42E/47/77/48 (1), 53A/42E/77/84 (1), 52A/42E/47/77/84 (1), 52A/79/6/42E/47/77/84 (1), 29/52/77/84/85/81 (1), 29/52/77/84 (1), 29/52/79/80/47/54/77/84 (1), 52A/47/77/84 (2), 29/52/52A/79/80/47/77/84 (2)

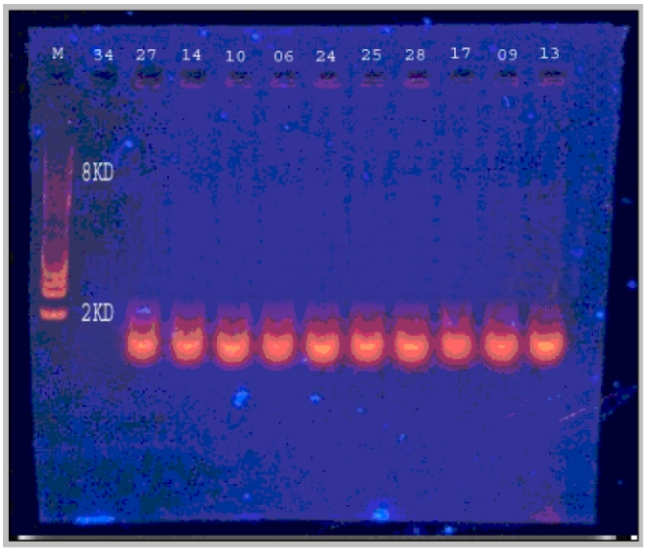

Fig. 2: Gel electrophoresis of plasmid DNA isolated from methicillin resistant *Staphylococcus aureus* isolates. A band of less than 2kDa of single plasmid DNA is visible in all the samples loaded except sample no.34 in which no plasmid DNA is demonstrable; molecular marker ranging from 2kDa to 8 kDa is loaded in Lane-M.

(12.5%), phage group III (37.5%) and phage group NA and V (0%). The results of phage typing are presented in Table.1. Phage type 77 was most prevalent and observed in (27 strains) followed by type 84 (19 strains), type 47 (16 strains), 52A (12 strains) and 42 E (5 strains) in the present study. Table.1

Plasmid Profiling

Out of 40 isolates 36 (82%) isolates harbored a single type of plasmid with M_r less than 2 kDa using a ladder of M_r of 2kDa-8KDa marker. Fig.1

DISCUSSION

Phage typing is one of the conventional typing methods applied for detecting epidemic MRSA strains. This method is an integral part of epidemiological surveillance and infection control in hospitals. For over 24 years, the National Staphylococcal Phage Typing Center in India has been using bacteriophage typing as its *S. aureus* strain typing method. Using international set of bacteriophage, in the present study, 32/37 isolates (86.48%) of MRSA strains were typed. However, only four isolates were non typable while one sample could not be typed because of contamination. Witte and co-workers 1979 have also found about 20% *S.aureus* strains as non-typable using international set of phages [13]. Non-typablility of *S. aureus* strains is a major problem employing the available sets of bacteriophages in India and other developing countries [14]. In our study only 4 (12.5%) strains were non-typable. The value of phage typing has been impaired by the poor type ability of MRSA strains and thus necessitated the implementation of more efficient and advanced methods for strain typing. It has been suggested that non-typability can be reduced by using routine test dilution strength and heat shock treatment before phage typing of *S. aureus*. Bacterium can alter its phage restricting activity, which is unlikely to occur at 37^{0}C [15]. In the present study, the most prevalent phage groups were; mixed type as well as group III each affecting 37.5% MRSA strains, followed by Phage group I and II 12.5% each. Phage group I strains are associated with hospital acquired and endemic infections; however, we did record 12.5% strains of this type. Antibiotic resistant strains usually belong to phage group III. In our study, 37.50% methicillin resistant strains belonged to phage group III. Similar findings have been noted by other workers in case of tetracycline and streptomycin resistance [16]. In our study, none of the strains were typed as groups NA and V. Wide variation in incidence has been reported by various reports published at different places and at different periods of study by many workers [17]**.** The bacteriophage typing is more sensitive technique as compared to serotyping of bacteria [18]. Phage typing is recommended as first line approach in epidemiological investigation of MRSA strains. The limitations of the bacteriphage typing are: the typing technique is cumbersome, time consuming and requires intense efforts in propagation, standardization and maintenance of the phage [19]**.** The treatment of bacterial infections with bacteriophages is termed phage therapy [20].The experimental phage therapy in animals and humans suggest that phage therapy could offer alternative to antibiotics for the treatment of infections caused by MDR strains. Ryszard and his co workers in 2007 have described a case of the successful eradication of MRSA carrier status in a healthcare worker [21]. However, further studies in this regards may lead to more valid conclusions, which requires further epidemiological studies on phage typing of MRSA strains.

Despite the introduction of antimicrobial therapy and the recent improvements of medical services, multiple drug resistance *Staphylococcus aureus* is recognized as a major cause of nosocomial infections which result in significant morbidity and mortality rates [22]. Resistance to high levels of antibiotics has been associated in most instances with plasmids [23, 24,] which play a significant role in the biology of enterococci. They represent an immense reservoir of genetic variability and contribute to genetic exchange between bacteria [25].The ability to detect and classify plasmids based on their phylogenetic relationship would provide an essential tool for investigating their distribution among bacteria and to elucidate their significance in the host cell, such as their role in dissemination of antimicrobial resistance. A simple method for plasmid detection is a very useful tool to trace resistance plasmids in a clinical setting, such as the hospital. We encountered a single plasmid, of M_r less than 2kDa in 36/40 (90%) of the MRSA strains. This finding is consistent with the reports of others [9, 26, and 27]. This plasmid appears to be associated with the antibiotic resistance. However, further studies such as nucleotide sequencing are required in order to establish the exact nature and function of this plasmid in the MRSA strains used in the study. Plasmid profile analysis has been shown to be a good epidemiological tool in investigating epidemics or outbreaks of bacterial resistance [28].

CONCLUSION

In conclusion, our study would provide baseline information with regard to bacteriophge types and plasmid profile of MRSA strains prevalent in the state of Himachal Pradesh (India). However, this is a short duration study in which the isolates were obtained in batches during the period January 2011 to April 2012.

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