Comparison of Capsular Typing of *Staphylococcus aureus* with Bacteriophage Typing: A Study in Gulbarga, India

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Abstract *Staphylococcus aureus* (*S. aureus*) is important both as a nosocomial and community acquired pathogen causing various degrees of infections. Typing *S. aureus* has been a question that is still being addressed. Bacteriophage typing is still used as a golden standard for typing though molecular methods are investigated. In developing countries where neither molecular typing nor the bacteriophage typing methods can be routinely used, the recently developed capsular typing method can be considered as screening method. We compared capsular typing with bacteriophage typing of the strains isolated in Gulbarga, India. We observed that the typeability of capsular typing was significantly higher (96%) among the phage typed strains, and the predominant capsular type in the region was type-8. The data so generated can be used to group *S. aureus* based on capsules both as a screening prior to bacteriophage typing, and to identify capsular candidate for developing prophylactic and therapeutic alternatives.

Keywords Capsular typing · Phage typing · *S. aureus* · Gulbarga · India

Introduction

Typing of *S. aureus* has long been a question that has been verified and tested to develop a reliable method to be used

routinely. Bacteriophage typing is still used as a golden standard for typing though molecular methods are investigated [1]. Among those factors considered for typing, capsular polysaccharides expressed by *S. aureus* are one of them, since they are also important in the pathogenesis of staphylococcal infections. They enhance staphylococcal virulence by impeding phagocytosis, resulting in bacterial persistence in the bloodstream of infected hosts [2–6]. Most *S. aureus* isolates are encapsulated and so far eleven capsular serotypes have been described. Of these, types 5 and 8 predominate in approximately 75% of the clinical isolates [3]. Though the method for capsular typing was described two decades ago [7–9], during the recent years typing of *S. aureus*, based on its capsular polysaccharide types is been increasingly adopted. However, since no such reports were available from the Indian context, we recently reported an epidemiological typing by this method [10]. In this present study we compared the capsular typing method as a screening method prior to subjecting the strains to bacteriophage typing, the golden standard. Capsular typing resulted in two groups with higher frequency of typeability among bacteriophage typed strains. We propose capsular typing in developing countries be incorporated in routine diagnostic testing to group *S. aureus* based on the capsules and use the data for both screening the strains prior to bacteriophage typing, and to identify capsular candidates for exploring prophylactic and therapeutic measures.

Materials and Methods

One hundred *S. aureus* strains from our previous study preserved at -70°C were subcultured, re-selected on Mannitol salt agar and used for the study [10]. The origin of the strains and their characterization are detailed in our

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previous study [10]. The strains, confirmed as *S. aureus* by the standard methods of Baird-Parker [11, 12], were tested for their typeability based on bacteriophage and capsular typing.

Bacteriophage Typing

Bacteriophage typing was conducted at the National Staphylococcal Phage Typing Centre (NSPTC), Maulana Azad Medical College (MAMC), New Delhi. The protocol was essentially as per Blair [13] and Parker [14]. Briefly, the strains of *S. aureus* were selectively isolated on mannitol salt agar (BBL, UK) and the mannitol-fermenting colonies were subcultured into 1 mL Oxoid Nutrient Broth (BBL, UK). The tubes were incubated at 37°C until the turbidity reached McFarland's standard of 0.5. This inoculum was spread on the Oxoid Nutrient Agar and allowed to air dry. Bacteriophages (Tables 1, 2) approved by the International Subcommittee for *Staphylococcus* Phage-Typing were supplied by the Central Public Health Laboratory, Colindale, UK. The conventional set of 23 phages (Table 1) were filled in the labeled wells of the Perspex block of the Lidwell's phage-typing apparatus and spotted at $\times 1$ RTD. Next day the plates were examined against a dark background by indirectly transmitted light with the aid of a $5\times$ hand-lens. If the results were negative, then the same set of phages except 83A, 84, and 85 were spotted at RTD $\times 100$. If the results were still negative, only then the strains were tested with MRSA set of phages (Table 2) at RTD. The experiments were controlled with *S. aureus* strains ATCC 25923 and W57.

Capsular Typing

Capsular typing was performed for the expression of capsular polysaccharide by the agglutination assay, as recently described [8, 10]. Briefly, capsular typing was performed by adding a single drop of the specific antiserum to one drop of bacterial suspension (one overnight colony from

TSA suspended in 1 mL saline). Clumping occurred within 10 s for positive reactions. For every isolate both the type-8 and -5 antisera were used to exclude cross-reactivity. The prototype strains for the capsular types 5 and 8, their respective mutants, along with the necessary protocols were kindly provided by Dr. Jean-Claire Lee, Harvard Medical School, Boston, USA [15, 16].

Results

Bacteriophage typing: In interpreting the results, the generally accepted 'two strong differences rule' was adopted [13], according which, with two or more phages if there is a ++ reaction with one culture and no lysis of the other, then the two cultures are said to be unrelated. Ten patterns of phage typing results (Table 3) were observed and assigned the alphabets A to J. In phage group-I, four patterns distributed among ten strains was observed (A, B, C, and D). In phage group-II, one pattern (E) was expressed by four strains. In phage group-III, two patterns (F and G) distributed among three strains, and in phage group-IV, two patterns (H and I) distributed among seven strains were observed. Clinical strains that were non-typeable even at $\times 100$ RTD were typed with the set of MRSA phages, and one pattern (J) was observed by only one strain. Thus with phage typing, twenty-four strains were typeable with the basic set of International phages (20 strains at RTD, and 4 strains at $\times 100$ RTD). None of the clinical strains were typeable even at $\times 100$ RTD with the basic set of phages. On testing with the MRSA set of phages, only one strain became typeable. Thus, twenty-five out of one hundred strains were phage typeable. Most of the strains belonged to Group-I (40%), followed by Group-IV (28%), Group-II (20%), Group-III (12%), and the Group-MRSA Phage (4%).

Seventy-one strains were capsular typeable, 18 for capsular polysaccharide type-5, and 53 for type-8. Since type-5 and type-8 are the most prevalent types, the remaining 29 strains were grouped as 'non-typeable by type-5 and -8 antisera'. Further comparing the data with phage-typed strains, we observed that 96% (24/25) of phage typeable strains were capsular typeable (19 were phage typed at RTD, 4 at RTD $\times 100$, and 1 with MRSA phage). Among the 25-phage typeable strains, 4 strains belonged to capsular polysaccharide type-5 (4/25; 16%), 20 strains to type-8 (20/25; 80%), and 1 strain was capsular polysaccharide non-typeable (1/25; 4%).

Discussion

The purpose of our study was to use a rapid testing method which could type *S. aureus* based on capsular

Table 1 The International basic set of phages

Lytic group	Phage numbers
I	29 52 52A 79 80
II	3A 3C 55 71
III	6 42E 47 53 54 75 77 83A 84 85
IV	81 94 95 96

Phages 83A, 84, and 85 are used only at RTD

Table 2 The International MRSA set of phages

M3	M5	MR8	MR12	MR25	622	30	33	38	56B
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Table 3 Patterns of Phage typing results observed

Pattern observed	Phage group	Observation	Phage type	No. of strains
A	I	29++	29	6
B		29++/52+/52A+/80++	29/80/+	2
C		29++/52++/52A+/79+/80++	29/52/80+	1
D		29++/52+/79++	29/79/+	1
E	II	71++	71	4
F	III	47++/54++	47/54	2
G		54++	54	1
H	IV	94++	94	6
I		95++	95	1
J	MRSA	MR25++	MR25	1

polysaccharide as its virulence factor, generate data for prophylactic measures, and to screen strains before subjecting for epidemiological (bacteriophage) typing. For this, one hundred strains of *S. aureus* were subjected to bacteriophage and capsular typing. Twenty-five strains were phage-typeable after performing the test with three different sets (basic set at RTD & RTD $\times$ 100; and MRSA phage set). We observed ten phage typing patterns and among the phage typeable strains, 40% belonged to lytic group-I, 28% to lytic group-IV, 16% to lytic group-II and 12% to lytic group-III, and 4% to MRSA Phages. The typeability findings of other researchers from India range from 18 to 71%, and our findings are well within that range (25%) [17–22]. None of the above researchers reported the typeability by MRSA phages, since the MRSA phages set was not available at that time. Recently, Mathur et al. [23–25] have conducted extensive MRSA phage typing on the strains received at the National Staphylococcal Phage Typing Center, New Delhi from across Asia and observed that among the typeable isolates, there was a 16% increase in typeability.

On performing capsular typing on these strains, we observed that 71% strains were capsular typeable (25% type-5 [18/71], 75% type-8 [53/71]). This observation of the predominance of *S. aureus* strains with capsular polysaccharide type-8 in this region is consistent with data published by previous researchers [26, 27].

Various researchers have confirmed the reliability, reproducibility, and specificity of capsular typing method [8, 27–34]. Thus we analyzed capsular typing as a tool for screening *S. aureus* prior to bacteriophage typing. We observed that of the 100 strains tested, 25% were phage typeable and 71% were capsular typeable. Comparison of both the methods tested revealed that among the capsular typeable strains, 34% (24/71) were phage typeable, and among the phage typeable strains, 96% (24/25) were capsular typeable (Table 4). χ^2 analysis confirmed this difference as highly significant ($P < 0.001$). Though bacteriophage typing is still the golden standard for

Table 4 Comparison of phage typeability with capsular typing

No. of capsulated Strains of <i>S. aureus</i>	Phage typeability			
	RTD	RTD $\times$ 100	MRSA ϕ	
Type-5	18	3	1	0
Type-8	53	16	3	1
Non-Typeable	29	1	0	0
Total	100	20	4	1

epidemiological typing of *S. aureus*, it has several constraints if it has to be done routinely such as, it is a very cumbersome, time consuming and sensitive method for propagation, standardization and maintenance of the phage types. In addition, the test has to be performed with four lytic groups including basic phages and the newer MRSA phages, and to be tested in three stages- RTD, $\times$ 100 RTD, and with MRSA set of phages. In contrast, the capsular typing method is easy to perform, and has the advantage of having only two clinically predominant capsular types, and that most of them been phage-typeable. This feature of capsular typing can be taken advantage of as a screening method prior to phage typing. Once the capsular typing antisera are made available commercially, the testing procedure is very simple and rapid. Thus if capsular typing test is incorporated in routine diagnostic procedures, it will type *S. aureus* into two groups will narrow the number of strains that would be bacteriophage typed for epidemiological purposes, and also generate data to determine the candidates for the development of antimicrobials and vaccines [35–37]. The capsular typing method is still being explored as a quick method for the detection of *S. aureus* based on the pathogenicity factor—capsular polysaccharide. Thus in view of these observations, we propose the use of capsular typing of *S. aureus* as a routine screening technique in situations where molecular and bacteriophage typing cannot be used routinely especially in developing countries. We finally report that the predominant capsular type identified in the Gulbarga region of India is type-8.

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References

- Pantůček R, Doskar J, Růžicková V, Kaspárek P, Oráčová E, Kvardová V, Rosypal S (2004) Identification of bacteriophage types and their carriage in *Staphylococcus aureus*. Arch Virol 149:1689–1703
- Luong TT, Lee CY (2002) Overproduction of type 8 capsular polysaccharide augments *Staphylococcus aureus* virulence. Infect Immun 70:3389–3395
- O’Riordan K, Lee JC (2004) *Staphylococcus aureus* capsular polysaccharides. Clin Microbiol Rev 17:218–234
- Robbins JB, Schneerson R, Horwith G, Naso R, Fattom A (2004) *Staphylococcus aureus* types 5 and 8 capsular polysaccharide-protein conjugate vaccines. Am Heart J 147:593–598
- Kampen AH, Tollersrud T, Lund A (2005) *Staphylococcus aureus* capsular polysaccharide types 5 and 8 reduce killing by bovine neutrophils in vitro. Infect Immun 73:1578–1583
- Roghamann M, Taylor KL, Gupte A, Zhan M, Johnson JA, Cross A, Edelman R, Fattom AI (2005) Epidemiology of capsular and surface polysaccharide in *Staphylococcus aureus* infections complicated by bacteraemia. J Hosp Infect 59:27–32
- Karakawa WW, Vann WF (1982) Capsular polysaccharides of *Staphylococcus aureus*. Semin Infect Disease 4:285–293
- Karakawa WW, Fournier JM, Vann WF, Arbeit R, Schneerson RS, Robbins JB (1985) Method for the serological typing of the capsular polysaccharides of *Staphylococcus aureus*. J Clin Microbiol 22:445–447
- Arbeit RD, Karakawa WW, Vann WF, Robbins JB (1984) Predominance of two newly described capsular polysaccharide types among clinical isolates of *Staphylococcus aureus*. Diagn Microbiol Infect Dis 2:85–91
- Paul-Satyaseela M, van Belkum A, Shivannavar CT, Gaddad SM (2004) Carriage of capsulated strains of *Staphylococcus aureus*: a population-based study performed in Gulbarga, South India. Epidemiol Infect 132:831–838
- Baird-Parker AC (1974) The basis for the present classification of staphylococci and micrococci. Ann NY Acad Sci 236:7–14
- Baird-Parker AC (1990) The staphylococci: an introduction. Soc Appl Bacteriol Symp Ser 19:1S–8S
- Blair JE, Williams REO (1961) Phage typing of staphylococci. Bull W H O 24:771–778
- Parker MT (1972) Phage typing of staphylococci in methods in microbiology. Academic Press 7:1
- Albus A, Arbeit RD, Lee JC (1991) Virulence of *Staphylococcus aureus* mutants altered in type 5 capsule production. Infect Immun 59:1008–1014
- Sau S, Bhasin N, Wann ER, Lee JC, Foster TJ, Lee CY (1997) The *Staphylococcus aureus* allelic genetic loci for serotype 5 and 8 capsule expression contain the type-specific genes flanked by common genes. Microbiology 143(Pt 7):2395–2405
- Bhujwala RA, Mohapatra LN (1972) Bacteriophage pattern and antibiotic resistance of *Staphylococcus aureus*. Indian J Pathol Bacteriol 15:66–72
- Ray K, Chowdhuri AN, Agarwal DS (1980) Antibiotic sensitivity and phage typing of *Staphylococcus aureus* from skin lesions at a rural dispensary. Indian J Med Res 72:802–806
- Somasekhar G, Sharma KB (1981) A complementary phage typing scheme for Vi-negative strains of *Salmonella typhi*. Indian J Med Res 74:337–344
- Ichhpujani RL, Bhatia R, Anand BR, Raichowdhuri AN (1982) Differentiation of phage untypable strains of *Staphylococcus aureus*. J Commun Dis 14:256–258
- Bhat SD, Deshmukh AB, Damle AS (1991) Bacteriophage typing and antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical samples. Ind J Med Microbiol 9:173–178
- Kandle SK, Ghatole MP, Takpere AY, Hittinalli VB, Yemul VL (2003) Bacteriophage typing and antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical specimen in and around Solapur (South Maharashtra). J Commun Dis 35:17–23
- Mathur MD, Mehndiratta PL (1997) Reverse phage typing for the characterization of methicillin resistant *Staphylococcus aureus* [correspondence]. Indian J Med Microbiol 15:47
- Mathur MD, Mehndiratta PL (2000) Characterization of methicillin resistant *Staphylococcus aureus* strains by a set of MRSA phages. Indian J Med Res 111:77–80
- Mehndiratta PL, Vidhani S, Mathur MD (2001) A study on *Staphylococcus aureus* strains submitted to a reference laboratory. Indian J Med Res 114:90–94
- Wanger AR, Morris SL, Ericsson C, Singh KV, LaRocco MT (1992) Latex agglutination-negative methicillin-resistant *Staphylococcus aureus* recovered from neonates: epidemiologic features and comparison of typing methods. J Clin Microbiol 30:2583–2588
- Na’was T, Hawwari A, Hendrix E, Hebden J, Edelman R, Martin M, Campbell W, Naso R, Schwalbe R, Fattom AI (1998) Phenotypic and genotypic characterization of nosocomial *Staphylococcus aureus* isolates from trauma patients. J Clin Microbiol 36:414–420
- Sompolinsky D, Samra Z, Karakawa WW, Vann WF, Schneerson R, Malik Z (1985) Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. J Clin Microbiol 22:828–834
- Hochkeppel HK, Braun DG, Vischer W, Imm A, Sutter S, Staebli U, Guggenheim R, Kaplan EL, Boutonnier A, Fournier JM (1987) Serotyping and electron microscopy studies of *Staphylococcus aureus* clinical isolates with monoclonal antibodies to capsular polysaccharide types 5 and 8. J Clin Microbiol 25:526–530
- Boutonnier A, Nato F, Bouvet A, Lebrun L, Audurier A, Mazie JC, Fournier JM (1989) Direct testing of blood culture for detection of the serotype 5 and 8 capsular polysaccharides of *Staphylococcus aureus*. J Clin Microbiol 27:989–993
- Christensson B, Boutonnier A, Ryding U, Fournier JM (1991) Diagnosing *Staphylococcus aureus* endocarditis by detecting antibodies against *S. aureus* capsular polysaccharide types 5 and 8. J Infect Dis 163:530–533
- Salamah AA (1995) Antibiotic resistance, plasmid content, phage type, and capsule type of *Staphylococcus aureus* isolates at a children’s hospital. New Microbiol 18:41–51
- Essawi T, Na’was T, Hawwari A, Wadi S, Doudin A, Fattom AI (1998) Molecular, antibiogram and serological typing of *Staphylococcus aureus* isolates recovered from Al-Makased Hospital in East Jerusalem. Trop Med Int Health 3:576–583
- Cocchiari JL, Gomez MI, Risley A, Solinga R, Sordelli DO, Lee JC (2006) Molecular characterization of the capsule locus from non-typeable *Staphylococcus aureus*. Mol Microbiol 59:948–960
- Jones T (2002) StaphVAX (Nabi). Curr Opin Investig Drugs 3:48–50
- Fattom AI, Horwith G, Fuller S, Propst M, Naso R (2004) Development of StaphVAX, a polysaccharide conjugate vaccine against *S. aureus* infection: from the lab bench to phase III clinical trials. Vaccine 22:880–887
- Fattom A, Fuller S, Propst M, Winston S, Muenz L, He D, Naso R, Horwith G (2004) Safety and immunogenicity of a booster dose of *Staphylococcus aureus* types 5 and 8 capsular polysaccharide conjugate vaccine (StaphVAX) in hemodialysis patients. Vaccine 23:656–663