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Carriage of capsulated strains of *Staphylococcus aureus*: a population-based study performed in Gulbarga, South India

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SUMMARY

Staphylococcus aureus is a common human pathogen in community- and hospital-acquired infections and its capsule is involved in pathogenesis. We report here the identification of type-5 and type-8 capsular antigens of *S. aureus* and the prevalence of such strains among volunteers in various age and population groups from different locations in India. *S. aureus* carriage rates varied between 18 and 50% with the highest values among university students and the lowest in schoolchildren, aged 6–20 years. There was no difference in carriage rates for males *vs.* females ($P=0{\cdot}415$) or in the socioeconomic status of carriers *vs.* non-carriers or age dependence. Among the carriage isolates 21% were type-5, 52% were type-8 and the remaining 27% were non-typable. Among invasive isolates these percentages were 6, 64 and 30 respectively. This implies that type-5 strains may be less invasive than type-8 strains ($P=0{\cdot}0015$).

INTRODUCTION

The first selected target for epidemiological analyses of *Staphylococcus aureus* was the capsular polysaccharide, which was identified in 1939 [1] and the first serotyping system was based on slide agglutination [2]. Independent studies confirmed the reliability, reproducibility, and specificity of the capsular typing method [3, 4]. It was later shown that the capsule of *S. aureus* plays an important role in the pathogenesis of staphylococcal infection and the term aggressin was introduced to indicate its putative virulence capacity [5]. The function of the capsule in infections appears to be primarily anti-phagocytic [6, 7]. Hanessian and Haskell [8] determined the structure of the staphylococcal capsular polysaccharide as consisting of equimolar portions of 2-acetamido-2-deoxy-D-glucuronic acid and 2-[(*N*-acetyl) amino]-2-deoxy-D-glucuronic acid linked by $\beta(1\rightarrow4)$ linkages. Subsequently, it was found that there are at least two serologically distinct capsular types of *S. aureus*, one is virulent to laboratory mice, and one is not [9]. These two types of capsule were first purified and characterized in the mid-1980s [10] and the polysaccharides were found to be cell associated and structurally similar to teichuronic acids. Both contain *O*-acetylated repeat units of manNAcA–fucNAc–fucNAc.

Karakawa et al. [3] described an improved analytical method for typing *S. aureus* on the basis of its capsular polysaccharides utilizing cellular agglutination and immuno-precipitation with mono-specific polyclonal antisera. The capsular typing scheme included 11 types but two capsular types (5 and 8) accounted for approximately 70% of all clinical strains [11].

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Table 1. *Distribution of staphylococcal isolates among different groups*

Location	Population group	Age group (years)	Staphylococcal isolates		
			Total	Male	Female
I. School (284)*	(1) Upper economic group	3 (6–20)	76	52	24
		5 (>40)	1	1	0
	(2) Middle economic group	3 (6–20)	110	74	36
	(3) Lower economic group	3 (6–20)	97	62	35
II. Medical college (25)	(4) Doctors	4 (20–40)	25	10	15
III. Nursing college (80)	(5) Nurses	3 (6–20)	36	28	8
		4 (20–40)	44	38	6
IV. University (165)	(1) Upper economic group	4 (20–40)	159	88	71
	(2) Middle economic group	4 (20–40)	5	3	2
	(3) Lower economic group	4 (20–40)	1	0	1
V. Hospital (127)	(5) Nurses	3 (6–20)	13	3	10
		4 (20–40)	55	18	37
	(6) In-patients	3 (6–20)	5	3	2
		4 (20–40)	24	12	12
		5 (>40)	30	20	10
VI. Private clinic (309)	(1) Upper economic group	2 (1–5)	8	4	4
		3 (6–20)	24	12	12
		4 (20–40)	39	23	16
		5 (>40)	10	2	8
	(2) Middle economic group	1 (Birth–1)	7	5	2
		2 (1–5)	7	5	2
		3 (6–20)	25	21	4
		4 (20–40)	54	27	27
		5 (>40)	23	12	11
VII. Residences (10)	(1) Upper economic group	1 (Birth–1)	1	—	1
		2 (1–5)	1	1	—
		3 (6–20)	1	1	—
		4 (20–40)	5	2	3
	(2) Middle economic group	3 (6–20)	2	2	—
Total (1000)			888	529	359

* No. of individuals screened.

In addition, strains bearing these two capsule types caused the majority of episodes of staphylococcal bacteraemia. A comparison of pulsed-field gel electrophoresis, zymotyping, capsular typing and phage typing, showed that although capsular typing was less discriminatory it gave results concordant with zymotyping and genome profiling typing [12]. Capsular typing is, therefore, a simple, rapid, high throughput procedure for distinguishing among clinically relevant subsets of *S. aureus* strains.

The present study describes a large cohort analysis of staphylococcal carriage among humans living in Gulbarga, India. Strains were collected by culture and typed for capsular characteristics. These characteristics were linked to demographic data collected for carrying and non-carrying individuals.

MATERIALS AND METHODS

Carriage studies and isolation of *S. aureus*

One thousand individuals were included in the study, 606 males, and 394 females. While collecting specimens for bacteriological analyses, the individual's background data were collected enabling grouping according to demographic characteristics. Included were data on the geographical area where the volunteers lived, worked, or paid frequent visits. Volunteers were recruited from seven different locations or institutions and were stratified according to socio-economic status or profession (Table 1). They were all in good health and the cohort was divided into five age groups. Group 1 included birth–1 year (5 boys, 3 girls); group 2 were pre-schoolchildren of >1–5 years

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(17 boys, 11 girls); group 3 comprised adolescents >5–20 years (281 boys, 128 girls). Adults were ranked in groups 4 and 5. Group 4 adults were >20–40 years (241 men, 209 women), and group 5 adults were >40 years (62 men, 43 women). The samples were collected by the 'washing method' for collection of the resident flora from the anterior nares [13], and transient flora from the forearm and the dorsum of the palm [14]. In addition, clinical *S. aureus* isolates from blood ($n=27$) and wound exudates ($n=23$) collected randomly from different individuals from a general hospital were included for capsular typing. After swabbing the site, the cotton swabs were transported in peptone water and inoculated on mannitol salt agar (MSA) plates that were incubated overnight at 37 °C in a candle jar. *S. aureus* was identified on the basis of the methods proposed by Baird-Parker [15].

Capsular typing of *S. aureus*

For capsular typing, specific antisera against the capsular serotype strains of type-5 and type-8 were raised. The prototype strains for these capsular polysaccharides, their respective isogenic knockout mutants and the immunization protocol were obtained from the Channing Laboratory (Boston, MA, USA). Strain codes were *S. aureus* Reynolds (type-5, mutant JL243) and *S. aureus* Becker (type-8, mutant JL252). Bacteria were collected in PBS and the suspension was heat inactivated according to established protocols [3, 16]. Cells were tested for non-viability and used for immunizing female New Zealand White rabbits. Preimmune sera were obtained and the rabbits were inoculated through the peripheral ear vein. Serum pools were absorbed with the respective mutant strains and the serum was stored with 0·02% sodium azide in 0·5 ml aliquots at 4 °C. Serum was confirmed for type-specificity by positive agglutination reactions with the prototype strains and negative reactions with their mutants, several strains of coagulase-negative staphylococci, micrococci, other Gram-positive bacteria including streptococci, lactobacilli, *Bacillus subtilis*, and Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*.

Capsular typing of *S. aureus* strains 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 type-5 antisera were used to exclude cross-reactivity. The reference strains were used as controls during each experimental run.

RESULTS

Assessment of staphylococcal carriage

Among the 1000 individuals screened for staphylococcal carriage at three different anatomical sites, 888 individuals yielded 1312 isolates. A single colony from each site yielding growth from each individual was selected for further studies. However, only 271 strains produced coagulase and were considered to be genuine *S. aureus*. Only one isolate of *S. aureus* was included per individual. The remaining 1041 isolates were considered to be coagulase-negative staphylococci and excluded from the study. Of the 271 *S. aureus* isolates, 221 were obtained from the nasal cavity and 50 were cultured from the forearm or dorsum of the palm. Overall, 221 individuals produced positive nasal cultures amounting for a nasal carriage rate of 24·9%. A small fraction of the individuals, both male and females, had *S. aureus* on their forearm (2·4%) or hand (3·3%).

The locations of individuals with *S. aureus* are given in Table 2. Most isolates originated from individuals at the university campus, where out of 165 individuals screened, 86 proved to be carrying *S. aureus* (52·1%). Colonization was least in private clinics (67 strains from 309 individuals; 21·7%). The overall rate of isolation of *S. aureus* from the anterior nares was highest from individuals in residences (40%) and university (37·5%), and least from schoolchildren (14·7%). There was no notable difference in carriages rates when males were compared to females (167/529 *vs.* 104/359; $P=0·415$). Depending on the location where the volunteers came from the carriage rates differed between 18 and 70%. These differences were most notable when carriage rates in schoolchildren were compared to those in residences (51/284 *vs.* 7/10; $P=0·006$) or university students (51/284 *vs.* 86/165; $P<0·001$).

On assessing the distribution of *S. aureus* in different population groups (Table 3), the highest incidence was observed in individuals belonging to the upper economic group (42%) and least among the lower economic group (10·7%). Anterior nares were the main site of *S. aureus* colonization in all the population groups irrespective of economic and professional variations. The anterior nares of individuals from the upper economic group exhibited the highest

Table 2. *Number of S. aureus distributed among different locations*

	Anterior nares		Forearm		Dorsum of palm		Total		
Location	M	F	M	F	M	F	M	F	Total (%)
I. School (284)*	30	12	3	1	3	2	36	15	51 (18)
II. Medical college (25)	2	5	0	0	0	1	2	6	8 (32)
III. Nursing college (80)	14	2	1	0	0	1	15	3	18 (22·5)
IV. University (165)	32	30	6	5	11	2	49	37	86 (52·1)
V. Hospital (127)	18	11	0	0	1	4	19	15	34 (26·7)
VI. Private clinic (309)	39	22	2	2	1	1	42	25	67 (21·7)
VII. Residences (10)	3	1	0	1	1	1	4	3	7 (70)
Total (1000)	138	83	12	9	17	12	167	104	271

* No. of individuals screened.

Table 3. *Number of S. aureus distributed among different population groups*

	Anterior nares		Forearm		Dorsum of palm		Total		
Population group (*n*)	M	F	M	F	M	F	M	F	Total (%)
(1) Upper economic group (330)*	67	42	7	8	13	4	87	54	141 (42)
(2) Middle economic group (233)	24	14	4	1	3	2	31	17	48 (20·6)
(3) Lower economic group (205)	13	9	0	0	0	0	13	9	22 (10·7)
(4) Doctors (25)	2	5	0	0	0	1	2	6	8 (32)
(5) Nurses (148)	22	8	1	0	0	1	23	9	32 (21·6)
(6) In-patients (59)	10	5	0	0	1	4	11	9	20 (33·9)
Total (1000)	138	83	12	9	17	12	167	104	271

* No. of individuals screened.

Table 4. *Number of S. aureus distributed among different age groups*

	Anterior nares		Forearm		Dorsum of palm		Total		
Age group (years)	M	F	M	F	M	F	M	F	Total (%)
(1) Birth–1 (8)*	1	1	1	1	0	0	2	2	4 (50)
(2) 1–5 (28)	2	5	0	0	0	0	2	5	7 (25)
(3) 6–20 (409)	53	16	3	1	5	4	61	21	82 (20)
(4) 21–40 (450)	70	54	8	6	11	7	89	67	156 (34·6)
(5) >40 (105)	12	7	0	1	1	1	13	9	22 (21)
Total (1000)	138	83	12	9	17	12	167	104	271

* No. of individuals screened.

incidence (33%) of *S. aureus* colonization while the lowest incidence was exhibited by individuals from the lower socioeconomic group (10·7%). Doctors (28%), in-patients (25·4%) and nurses (20%) also demonstrated a relatively high incidence of *S. aureus* in their anterior nares. On a per-individual basis, the carriage rates differed from 33·9 to 20·6. The incidence was highest in upper-economic-group individuals and schoolchildren.

The age-wise distribution of carriage at different body sites is shown in Table 4. The rate of isolation of *S. aureus* was highest in infants (50%), but fell to 20%

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Table 5. *Distribution of S. aureus with capsular polysaccharide types among different body sites and specimens*

	Anterior nares		Forearm		Dorsum of palm		Blood culture		Exudates culture	
	M	F	M	F	M	F	M	F	M	F
Total no. of strains	138	83	12	9	17	12	14	13	10	13
Capsular typing										
Type-5										
No.	33	16	4	3	—	—	2	—	—	1
%	23·91	19·28	33·33	33·33	—	—	14·29	—	—	7·69
Type-8										
No.	69	48	6	4	7	8	7	9	8	8
%	50·0	57·83	50·0	44·44	41·18	66·67	50·00	69·23	80·00	61·54
Non-typable										
No.	36	19	2	2	10	4	5	4	2	4
%	26·09	22·89	16·67	22·22	58·82	33·33	35·71	30·77	20·00	30·77

(82/409) in pre-adults. The isolation rate from the anterior nares was nearly the same in infants (25%), pre-schoolchildren (25%) and adults (27·5%), while it was 16·9% (69/409) and 18% (19/105) among the pre-adults and older people. As a consequence of the low number of young volunteers included in the present study, age-related differences in carriage could not be assessed in detail. When the groups were combined into two main clusters (younger than 6 years *vs.* older than 6 years) there was no statistically significant difference in carriage rates (11/24 *vs.* 260/864; $P = 0{\cdot}12$).

Capsular typing

The results of capsular typing of the isolates are presented in Table 5; 72·6% of the *S. aureus* isolates reacted with one of the two antisera. Isolates that were not agglutinated by either serum were grouped as non-typable. Of 233 typable *S. aureus* strains, 74·7% were type-8 and 25·3% were type-5. Capsulated strains were isolated from all types of sites and specimens. Both serotypes were expressed by isolates from the anterior nares, and forearm, but those from the dorsum of the palm expressed only type-8 capsular polysaccharide. The highest rates of seroreactive strains were observed from the forearm (81·0%), followed by dorsum of the palm (51·7%). The distribution of type-8 capsular polysaccharide between male and female individuals was similar except in the dorsum of the palm, where the strains from females exhibited a comparatively higher incidence of seroreactivity. The strains from the females showed a higher incidence of type-5 capsule on the forearm than in the anterior nares.

Of the *S. aureus* isolated from clinical exudates, 73·9% were typable, and 94·1% of these were type-8. The isolates from males were type-8 only but the incidence of type-8 strains was also very high among females (Table 5). The incidence of type-8 strains was significantly higher than for type-5 ($P < 0{\cdot}01$), but there was no significant difference between the incidence of type-8 strains among the carriage (77·6%) *vs.* clinical (64%) strains ($P = 0{\cdot}4$). When type-5 strains were tested, the incidence was significantly higher among the carriage strains ($P = 0{\cdot}0015$) rather than the clinical strains.

While analysing the data for the incidence of *S. aureus* capsular polysaccharide types at various locations [Fig. (*a*)], we observed that the incidence of the capsulated strains was highest among schoolchildren, followed by individuals attending a private clinic, local residents, and hospital personnel (75–86%). Among the isolates from the medical college and dental internees, 75% were seroreactive, and this frequency diminished for isolates from university students (60·5%), and was lowest for those from the nursing college (38·9%) [Fig. (*a*)]. There was a considerable difference in the distribution among the sexes [Fig. (*b*)]. Both capsular types were identified in the strains from individuals from all locations except in the nursing college and residences where only type-8 was found. Among the male dental internees only type-5 strains were isolated.

The frequency and distribution of seroreactive capsulated *S. aureus* among different population groups is presented in Fig. (*c*). Isolates from individuals in the middle economic group showed the highest rate (87·5%) of typability followed by those from the lower economic group (77·3%). While the frequency

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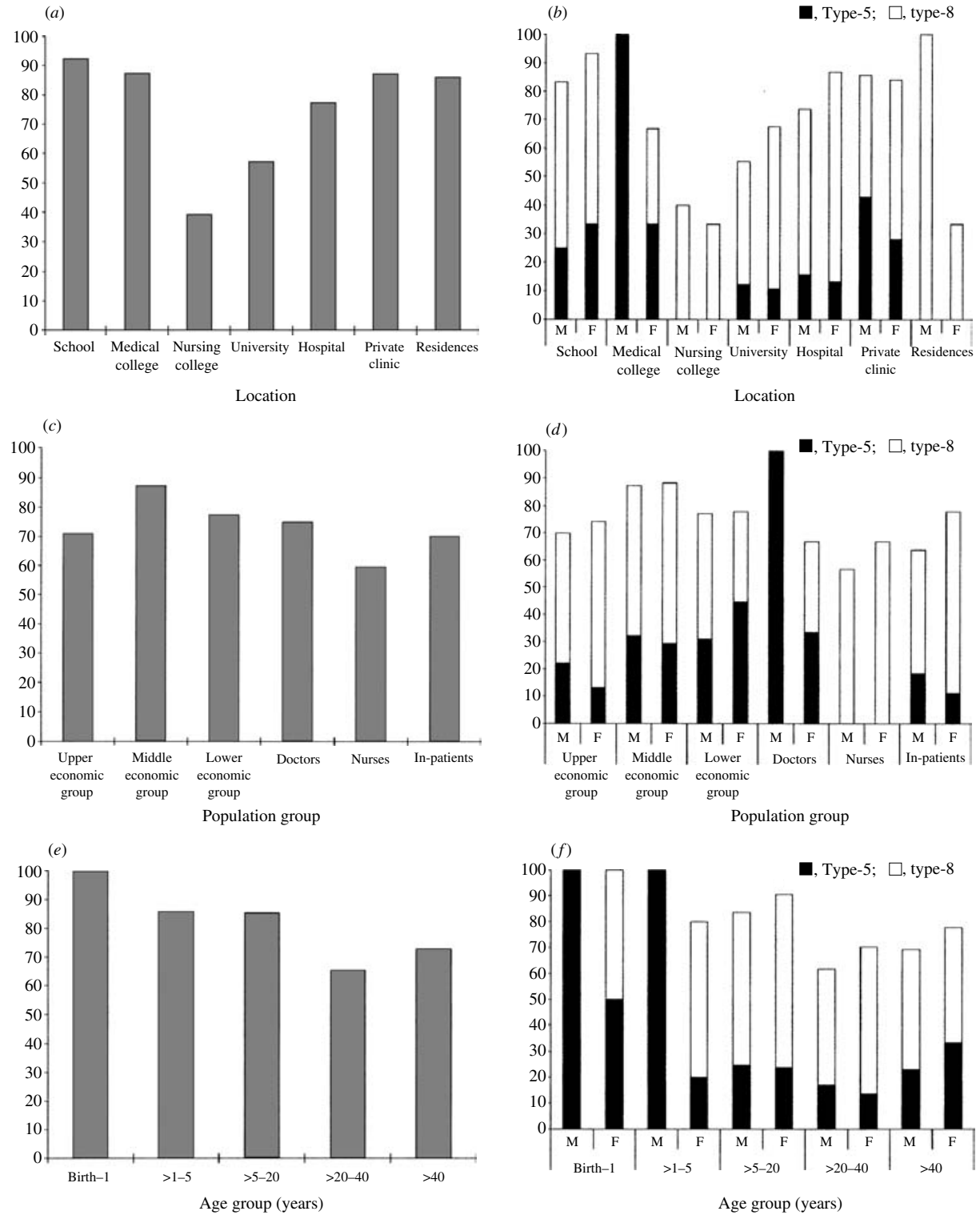

Fig. (*a*) Incidence of capsule seroreactive strains of *S. aureus* from individuals from different locations; (*b*) by gender from different locations; (*c*) from different population groups; (*d*) by gender from different population groups; (*e*) from different age groups; (*f*) by gender from different age groups.

of type-5 and type-8 capsular antigens among isolates from the remaining groups was greater than 70%, isolates from nursing personnel were the least typable (59·4%). All isolates from nursing personnel were of serotype-8 while those from the male doctors were type-5 [Fig. (*d*)]. Isolates from individuals of the other

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population groups expressed both types of capsular polysaccharides, however type-8 predominated. With the exception of group 4 individuals (>20–40 years), the distribution of the capsular types in each group was more than 70%, the highest being from age group 1 (birth–1 year) where it was 100% [Fig. (*e*)]. Most of strains expressed both types of capsular polysaccharide in all the subgroups except in males of age groups 1 and 2 where they were only of capsular polysaccharide type-5 [Fig. (*f*)]. The distribution of capsular polysaccharide-producing strains was almost the same in both sexes of all age groups.

Among the capsule-typed strains from clinical specimens (Table 5), 51·4% were from blood cultures and 48·57% were recovered from exudates. For the blood culture isolates, capsule typability was 66·7%. There were no type-5 strains among the females, but the incidence of type-8 strains among them was higher than in the males; 88·9% of seroreactive strains were type-8. Interestingly, type-5 strains were significantly more prevalent among carriage than clinical isolates ($P = 0{\cdot}0015$).

DISCUSSION

Worldwide approximately half the population is colonized by *S. aureus* [13]. Although carriage predisposes to the development of a variety of staphylococcal infections the importance of bacterial virulence factors in the transition from colonization to infection has been scarcely addressed. We try here to define the importance of the *S. aureus* capsule type in this process. We conducted capsular typing of isolates from various human sources and our findings correlated well with those reported across the world. Although we observed that 72·6% of all *S. aureus* isolates reacted with two capsule type-specific antisera, the frequency of these types among isolates from the three body sites sampled and clinical isolates was remarkably similar (73% *vs.* 70%) suggesting that carriage and infection isolates are derived from the same population as far as capsule types are concerned. This observation is similar to that reported by Sompolinsky et al. [4]. In all sites, including clinical isolates, the number of strains with type-8 capsular polysaccharide was significantly higher, indicating the predominance of this type of *S. aureus* in this geographical region. These findings are consistent with previous data [17, 18].

Among the individuals from various population groups, we observed that isolates from individuals in the middle economic group were more often seroreactive followed by the lower economic group, doctors, upper economic group, in-patients and nurses. However, it is noteworthy that serotypable isolates from subjects in the lower economic group and those from doctors were all recovered from the anterior nares only.

In conclusion, colonization with *S. aureus* of type-5 and type-8 capsules was most frequent among health-care workers and hospitalized patients. No obvious difference in the socioeconomic status of carriers *vs.* non-carriers was recorded and age dependence of carriage was not substantiated. Most importantly, capsule type-5 strains were significantly more prevalent among the carriage isolates than clinical isolates. This is the first epidemiological *S. aureus* carriage study conducted in India based on capsular typing. In the future these studies will need extension, with overall genetic characterization and general virulence gene profiling of the type-5 and type-8 encapsulated strains to further identify factors associated with staphylococcal epidemicity and virulence.

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