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Correspondence

Presence of virulence determinants amongst *Staphylococcus aureus* isolates from nasal colonization, superficial & invasive infections

Sir,

Staphylococcus aureus is a highly versatile and adaptable microorganism, existing either as a commensal in the anterior nares of about 30 per cent of the human population, or as a pathogen implicated in skin, soft tissue, respiratory system, bone, joints and endovascular disorders[1]. *S. aureus* infections vary greatly in severity and range from superficial or localized, deep-seated or invasive to toxemic syndromes[1]. The genotypic attributes governing the occurrence of *S. aureus* as a commensal, and its transition to a pathogen involved in both superficial and invasive infections remain unclear. Our previous studies have revealed a dichotomy in *S. aureus* isolates from localized and deep-seated infections[2,3]. We, therefore, sought to study the differences in genetic repertoire of *S. aureus* isolates from nasal colonization, superficial infections and invasive infections, with respect to 11 major virulence determinants.

During 2004-2006, clinical isolates of *S. aureus* (n=69) obtained in bacteriology laboratory, Post-graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India from consecutive cases with invasive (n=35) and superficial (n=34) infections were used in this study. These isolates were selected from 500 consecutive isolates on the basis of pulsed-field gel electrophoresis (PFGE), wherein only one isolate belonging to each PFGE type was included to prevent over-representation of any gene owing to the effect of clonal similarity (data not shown). Invasive cases were defined as cases with clinical evidence of sepsis or deep-seated infection, with isolation of *S. aureus* in blood or an aspirate from a normally sterile site. Cases with evidence of prior trauma or penetrating injury and conditions such as diabetes mellitus, haematological malignancies and immunosuppression were excluded. The cases with superficial infections included infections limited to skin and/or subcutaneous tissue, without any clinical evidence of invasion into deeper tissues. Twenty five *S. aureus* isolates from the anterior nares of healthy volunteers of the institute without any history of an infective episode or antibiotic uptake in the last two weeks were also included. The study protocol was approved by the Institute's Ethics Committee.

All the 94 isolates were screened for the presence of 10 virulence genes [fibronectin-binding protein A (*fnbA*), collagen-binding protein (*cna*), serine-aspartate repeat protein (*sdrE*), staphylococcal enterotoxins (*sea, sem*, *seo* and *sej*), exfoliative toxin A (*eta*), intercellular adhesin (*ica*A) and gamma hemolysin (*hlg*)], and a major virulence regulator in *S. aureus* (accessory gene regulator; *agr* subgroup I-IV). The genes were detected by PCR according to the methods described previously, employing genomic DNA (for all genes except *sej*) or plasmid DNA (for *sej*)[4-6]. To determine the significance of association between various virulence genes and origin type of particular isolate (superficial, invasive or nasal), the data were tested statistically by univariate analysis using odds ratio and Fisher's exact test using SPSS software (SPSS Inc., USA). Further, a multivariate analysis was performed by logistic regression to confirm if the genes which were significantly associated with a group (on univariate analysis) were both independent of each other and also independently associated with the anatomic site of isolation. The statistical significance was calculated with 95% confidence intervals.

Comparison of virulence genes in *S. aureus* isolates from invasive infections with those from nasal colonization (Tables I and II) revealed a significant association of *cna*, *ica*A and *sej* with invasive isolates [*ica*A, $P<0.001$ and *cna* and *sej*, $P<0.01$ (univariate analysis); *ica*A, $P<0.001$ and *sej*, $P<0.01$ (multivariate analysis)]. *cna* encodes for collagen-binding protein which mediates attachment of staphylococci to

Table I. Presence of 11 virulence determinants in *S. aureus* isolates from nasal colonization, superficial and invasive infections

Gene	*n* (%)			
	Total clinical isolates (SC + IC), *n* = 69	SC n = 34	IC n = 35	Nasal colonizers n = 25
*agr*I	20 (28.9)	14 (41.2)¶	6 (17.1)	8 (32.0)
*agr*II	22 (31.9)	8 (23.5)	14 (40.0)	8 (32.0)
*agr*III	7 (10.1)	2 (5.9)	5 (14.3)	7 (28.0)¤
*agr*IV	19 (27.5)	9 (26.5)	10 (28.6)	2 (8.0)
fnbA	59 (85.5)	30 (88.2)	29 (82.9)	19 (76.0)
cna	34 (49.3)	12 (35.3)	22 (62.9)*,§	5 (20.0)
sdrE	29 (42.0)	12 (35.3)	17 (48.6)	7 (28.0)
sej	33 (47.8)	13 (38.2)	20 (57.1)*,**	5 (20.0)
eta	21 (30.4)	11 (32.4)	10 (28.6)	5 (20.0)
hlg	37 (53.6)	20 (58.8)	17 (48.6)	8 (32.0)
icaA	41 (59.4)	15 (44.1)	26 (74.3)*,**, §, §§	5 (20.0)
sea	38 (55.1)	17 (50.0)	21 (60.0)	9 (36.0)
sem	14 (20.3)	5 (14.7)	9 (25.7)	17 (68.0)#
seo	15 (21.7)	8 (23.5)	7 (20.0)	16 (64.0)#,##

Statistical significance was determined by both univariate (odds ratio and Fisher's exact test) and multivariate (logistic regression) analysis, with 95 % confidence intervals

n, sample size; SC, clinical isolates from superficial infections; IC, clinical isolates from invasive infections; * and **, significant association with invasive isolates compared to nasal colonizers on univariate and multivariate analysis respectively; [*icaA*, $P<0.001$ and *cna* and *sej*, $P<0.01$ (univariate analysis); *icaA*, $P<0.001$ and *sej*, $P<0.01$ (multivariate analysis)]; # and ##, significant association with nasal colonizers compared to invasive and/or superficial isolates on univariate and multivariate analysis respectively; [*seo* and *sem* $P<0.001$ and $P<0.01$ respectively (univariate analysis); seo, $P<0.01$ (multivariate analysis)]; § and §§, significant association with invasive isolates than superficial isolates on univariate and multivariate analysis respectively; ¤, significantly more ($P<0.05$) in nasal colonizers compared to other groups; ¶significantly more ($P<0.05$) in superficial isolates compared to other groups.

cartilage, and is an important virulence factor in osteomyelitis, arthritis, sepsis, endocarditis and keratitis[6]. Most of the invasive isolates in this study (62.8%) were also from similar infections, with 62.9 per cent of these being positive for *cna*. *sej* is a plasmid-borne genetic determinant encoding for a pyrogenic, super-antigenic enterotoxin[7]. While nearly 57 per cent of the invasive isolates in this study were positive for *sej*, only 20 per cent of the nasal colonizers harboured this gene. Likewise, *ica*A, encoding for the polysaccharide intercellular adhesion (PIA) required during biofilm formation[8,9], was present at a considerably higher proportion in invasive isolates (74%) than in nasal colonizers (20%). Previous studies have also reported a significant association of *cna*, *ica* and *sej*, as well as *fnb*A, *sdr*E, *hlg* and *eta*, in invasive compared with carriage isolates[6-9]. However, O'Donnell *et al*[10] reported no significant difference in the distribution of *ica* and *cna* amongst invasive and colonizing MRSA.

None of the virulence determinants tested showed a significant association with superficial isolates compared to nasal colonizers (Tables I and II). *sem* and *seo* were significantly more prevalent in colonizing isolates than invasive and/or superficial isolates [*seo* and *sem*, $P<0.001$ and $P<0.01$, respectively (univariate analysis); *seo*, $P<0.01$ (multivariate analysis)] (Table I). These genes are encoded by *egc*, a locus encoded by a mobile genetic element which may augment the carriage potential of *S. aureus* by inactivation of a crucial sequence or loss of another mobile genetic element carrying virulence gene during its insertion[11]. Several previous studies have also reported the predominance of *egc* locus in carriage isolates[5,11-16].

Comparison of *S. aureus* isolates from invasive and superficial infections (Tables I and II) demonstrated a significantly greater presence of *cna* and *ica*A in invasive isolates on univariate analysis ($P<0.05$), and of *ica*A on multivariate analysis ($P<0.05$). *sej* was

Table II. Odds ratios and 95% confidence intervals for the presence of eleven virulence determinants in *S. aureus* isolates from nasal colonization, superficial and invasive infections

Gene	Odds ratio (95 % confidence intervals)			
	Total clinical isolates vs. nasal colonizers	SC vs. nasal colonizers	IC vs. nasal colonizers	SC vs. IC
*agr*I	0.87 (0.32-2.33)	1.49 (0.50-4.39)	0.44 (0.13-1.48)	3.38 (1.11-10.30)
*agr*II	0.99 (0.37-2.65)	0.65 (0.20-2.08)	1.42 (0.48-4.17)	0.46 (0.16-1.30)
*agr*III	0.29 (0.09-0.94)	0.16 (0.03-0.86)	0.43 (0.12-1.55)	0.38 (0.07-2.08)
*agr*IV	4.37 (0.94-20.36)	4.14 (0.81-21.21)	4.60 (0.91-23.26)	0.90 (0.31-2.59)
*fnb*A	1.86 (0.60-5.81)	2.37 (0.59-9.51)	1.53 (0.43-5.44)	1.55 (0.40-6.07)
cna	3.89 (1.31-11.53)	2.18 (0.65-7.29)	6.77 (2.05-22.39)	0.32 (0.12-0.86)
*sdr*E	1.86 (0.69-5.05)	1.40 (0.46-4.31)	2.43 (0.81-7.27)	0.58 (0.22-1.51)
sej	3.67 (1.24-10.89)	2.48 (0.75-8.22)	5.33 (1.63-17.48)	0.46 (0.18-1.22)
eta	1.75 (0.58-5.29)	1.91 (0.57-6.45)	1.60 (0.47-5.44)	1.20 (0.43-3.34)
hlg	2.46 (0.94-6.45)	3.04 (1.03-8.97)	2.01 (0.69-5.85)	1.51 (0.58-3.92)
ica	5.86 (1.97-17.45)	3.16 (0.96-10.39)	11.56 (3.35-39.90)	0.27 (0.09-0.75)
sea	2.18 (0.85-5.61)	1.78 (0.61-5.12)	2.67 (0.92-7.70)	0.67 (0.26-1.73)
sem	0.12 (0.04-0.33)	0.08 (0.02-0.29)	0.16 (0.05-0.51)	0.50 (0.15-1.68)
seo	0.16 (0.06-0.42)	0.17 (0.05-0.54)	0.14 (0.04-0.45)	1.23 (0.39-3.87)

SC, clinical isolates from superficial infections; IC, clinical isolates from invasive infections

also more frequently present in invasive isolates but the association was not significant. In the superficial isolates, the presence of *eta* and *hlg* was higher compared to invasive ones, though not significant.

Amongst the *agr* subgroups (Tables I and II), the presence of *agr* III and *agr* I type was significantly greater in isolates from nasal colonization and superficial infections, respectively ($P<0.05$ on univariate and multivariate analysis).

The isolates from each group were also evaluated for the total number of virulence genes present (Figure). While most of the superficial isolates (74.28%) harboured 2-4 genes, majority of the invasive isolates (88.57%) harboured 3-6 genes. None of the nasal colonizers contained more than four virulence genes. Nearly two-thirds (68.58%) of the invasive isolates contained all the three or at least two of the genes out of *ica*A, *cna* and *sej*. In contrast, two-third (68.57%) of the superficial isolates harboured either one or none of the three genes. Also, 56 per cent of the nasal colonizers did not harbour *ica*A, *cna* or *sej*; 28 and 16 per cent of these isolates carried one and two of the three genes, respectively, and none of these isolates were positive for all the three genes.

In conclusion, the present study suggests a considerable difference in the virulence potential of colonizing vs. superficial vs. invasive *S. aureus* isolates. Majority of the invasive isolates were found to harbour a greater number of virulence genes compared to the superficial or carriage isolates. Of the 11 major virulence markers tested, *ica*A, *cna* and *sej* were significantly associated with the invasive isolates, and detection of these genes may be a likely indicator of *S. aureus* invasiveness in a clinical setting. Isolates from superficial infections predominantly exhibited an *agr* I background, without a specific association with any of the virulence factors tested. In contrast, nasal colonizers showed *agr* III background and carried *sem* and *seo* more frequently. The presence of *sem* and *seo* may thus be suggestive of an isolate's carriage potential.

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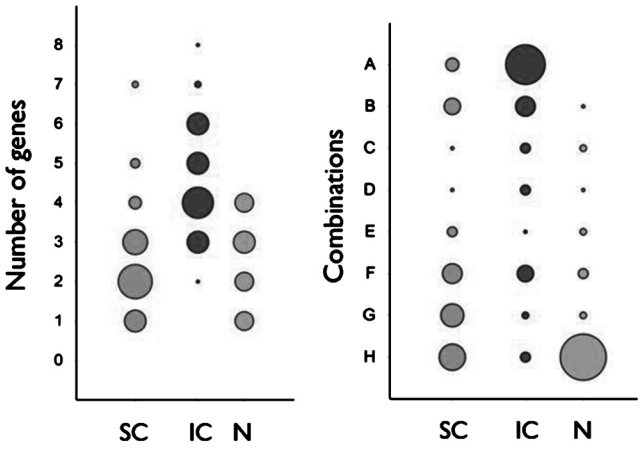

Fig. Distribution of *S. aureus* isolates from nasal colonization (n = 25), superficial infections (n = 34) and invasive infections (n = 35) according to the number of virulence genes present, and different combinations of *ica*A, *cna* and *sej*. The diameter of the circles represents the number of isolates. A, *cna* +, *ica* +, *sej* +; B, *cna* +, *ica* +, *sej* -; C, *cna* +, *ica* -, *sej* +; D, *cna* -, *ica* +, *sej* +; E, *cna* +, *ica* -, *sej* -; F, *cna* -, *ica* +, *sej* -; G, *cna* -, *ica* -, *sej* +; H, *cna* -, *ica* -, *sej* -. SC, clinical isolates from superficial infections; IC, clinical isolates from invasive infections; N, nasal colonizers.

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