Acapsular clinical *Staphylococcus aureus* isolates lack *agr* function

J. Fischer[1,2], J. C. Lee[2], G. Peters[1] and B. C. Kahl[1]

1) *Institute of Medical Microbiology, University Hospital Münster, Münster, Germany and* 2) *The Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA,*

Abstract

Staphylococcus aureus is a major human pathogen causing community- and hospital-acquired infections. Capsule production of *S. aureus* confers protection against host defence. There is a lack of information concerning the association of capsular polysaccharide (CP) expression and activity of the accessory gene regulator (*agr*) in clinical *S. aureus* isolates. Production of CP and *agr* expression were assessed in 195 *S. aureus* isolates from infected patients at a German University Hospital. Northern blot analysis revealed that *S. aureus* strains with a non-functional *agr* locus were more likely to be CP-negative than strains with a functional *agr* locus.

Keywords: *agr*, capsule, clinical isolates, *Staphylococcus aureus*, virulence

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Corresponding author: B. C. Kahl, Institute of Medical Microbiology, University Clinics of Muenster, Domagkstr.10, 48149 Münster, Germany

E-mail: kahl@uni-muenster.de

Staphylococcus aureus is a major human pathogen that causes a wide spectrum of community- and hospital-acquired diseases, ranging from local infections such as skin and soft tissue infections, e.g. impetigo, carbuncle, furuncle and cellulitis, to more life-threatening infections such as sepsis, pneumonia and endocarditis [1].

To cause such a variety of infections, *S. aureus* is equipped with numerous virulence factors, many of which are differentially regulated by RNAIII encoded by the *agr* operon [2].

Agr functions as a quorum sensing system by secreting an auto-inducing peptide [3]. Sequence variations within the peptide and its transmembrane receptor *agrC* distinguish four *agr*-specificity groups [4]. The *agr* locus positively regulates capsule expression, which confers resistance to phagocytosis [5].

The majority of *S. aureus* clinical isolates (c.70%) produce capsular polysaccharides (CP) with serotype 8 (CP8) being most prevalent followed by serotype 5 (CP5) with some geographical variations [6].

The results of experimental infection studies have revealed that *agr*-mutants are significantly attenuated in animals [7,8]. Moreover, two separate studies indicated that *S. aureus* strains with a defect in *agr* function were associated with persistent bacteraemia and poor clinical outcome [9,10]. The authors speculated that this might be because of increased production of cell surface proteins that facilitate biofilm formation, immune evasion and persistent infection.

There is a lack of information concerning the association of CP expression and *agr* activity in clinical *S. aureus* isolates. We hypothesized that lack of CP production by clinical isolates is the result of a non-functional *agr* locus in these strains. During a 12-month period we collected 195 *S. aureus* isolates (Table 1) from infected or colonized patients treated at the tertiary University Hospital of Münster (1400 beds). In our study out of 195 clinical strains, 17 strains were penicillin-susceptible *S. aureus* (9%), 168 strains were methicillin-susceptible *S. aureus* (86%) and ten strains were methicillin-resistant *S. aureus* (5%). Production of CP was evaluated by colony immunoblot (Fig. 1a) [11] and RNAIII transcription was assessed by Northern blot analysis (Fig. 1b) [12]. Multiplex PCR was performed to determine *agr* and capsule genotypes [13]. Chi-squared test was used to analyse the statistical significance of the results.

All 195 isolates were genotypically and phenotypically characterized for capsule types and expression. Multiplex PCR (Fig. 1a) revealed that all were either *cap5* ($n = 78$, 40%) or *cap8* ($n = 117$, 60%). Forty-five isolates (23%) expressed CP5, 80 (40%) expressed CP8 and 70 strains (36%) were phenotypically non-typeable (NT) as indicated by immunoblot analysis, suggesting that they expressed no capsule (Fig. 1b).

As *agr* function is known to vary in clinical isolates, and was shown in laboratory strains to regulate capsule expression [11], its function among these clinical isolates was tested. In Northern blot analysis we identified 49% of our clinical isolates as having an inactive *agr* locus, as shown by the lack of an RNAIII transcript (Table 1). This finding is in contrast to that of Traber *et al.* [14], who reported that <23% of their clinical isolates from a single hospital had a dysfunctional *agr* locus.

TABLE 1. **Analysis of *agr* expression and capsule phenotype in clinical *Staphylococcus aureus* strains from different sources**

	agr activity (%)		Capsule phenotype (%)		
Source of isolate	**Positive (*n* = 100)**	**Negative (*n* = 95)**	**CP5 (*n* = 45)**	**CP8 (*n* = 80)**	**NT (*n* = 70)**
Abscess (*n* = 23)	16 (70)	7 (30)	4 (17.4)	11 (47.8)	8 (34,8)
Arthritis (*n* = 6)	5 (82)	1 (18)	3 (50)	0 (0)	3 (50)
Bone (*n* = 15)	6 (40)	9 (60)	2 (13.3)	5 (33.3)	8 (53,3)
Catheter (*n* = 19)	11 (57.9)	8 (42.1)	3 (15.8)	11 (57.9)	5 (26,3)
CNS (*n* = 19)	14 (73.7)	5 (26.3)	4 (21.1)	7 (36.8)	8 (33,3)
Eye (*n* = 3)	3 (100)	0 (0)	0 (0)	2 (66.7)	1 (33,3)
Nasal colonization (*n* = 46)	20 (43.5)	26 (56.5)	5 (10.9)	25 (54.4)	16 (34,7)
Otitis (*n* = 9)	3 (33.3)	6 (66.7)	4 (44.4)	3 (33.3)	2 (22,2)
Pneumonia (*n* = 11)	6 (46.2)	7 (53.8)	1 (9.1)	8 (72.7)	2 (18,2)
Prosthesis (*n* = 11)	6 (54.5)	5 (45.5)	2 (18.2)	5 (45.5)	4 (36,4)
Sepsis (*n* = 5)	3 (60)	2 (40)	2 (40)	2 (40)	1 (20)
Skin (*n* = 11)	3 (27.3)	8 (72.7)	6 (54.5)	3 (27.3)	2 (18,2)
Soft tissue (*n* = 25)	10 (40)	15 (60)	8 (32)	8 (32)	9 (36)
Urinary tract (*n* = 2)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)
Wound (*n* = 25)	11 (44)	14 (56)	4 (16)	9 (36)	12 (48)

CNS, central nervous system, CP, capsular polysaccharide; NT, non-typeable.

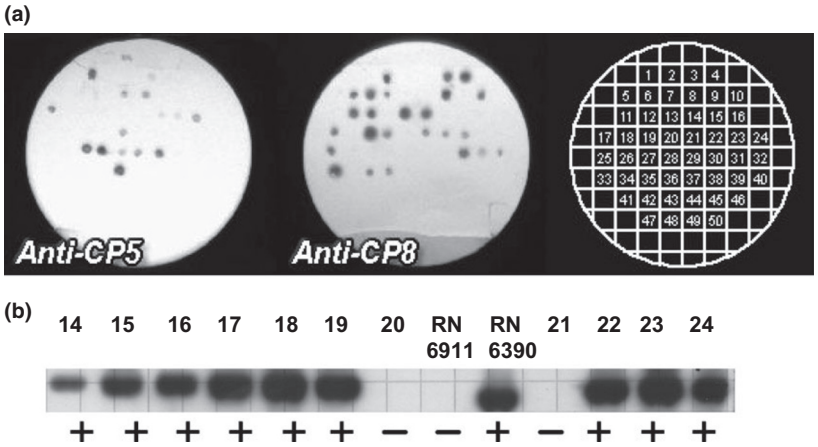

FIG. 1. (a) After overnight culture of the clinical isolates, total cellular RNA was isolated using the FastPrep system (Fast prep FP120 Instrument; Qbiogene, Heidelberg, Germany). Ten micrograms of RNA was electrophoresed through a 1.5% 0.66 M formaldehyde gel in morpholinepropane sulfonic acid (MOPS) running buffer. Every gel was controlled for equal loading of RNA. RNA was transferred using standard procedures. The membranes were hybridized with the digoxigenin-labelled probe and detected by chemiluminescence (Roche, Mannheim, Germany). As a positive control RN6390 [21] and as a negative control RN6311 [22] were used. The results were defined as positive in the case of visible bands and negative if no band could be detected. (b) Capsule typing of all 195 isolates was performed according to ref. [11] by colony immunoblot method with capsular polysaccharide 5 (CP5) or CP8-specific antibodies. The reactivities of the clinical isolates were evaluated in comparison to those of control *Staphylococcus aureus* strains included on each filter membrane (JL278 CP5; PS80 CP8; JL801 non-typeable). Isolates with no reaction to CP5 and CP8 antibodies were defined as non-typeable.

TABLE 2. **Loss of *agr* activity is associated with a non-encapsulated phenotype in clinical *Staphylococcus aureus* strains. *Agr* activity was assessed by Northern blot analysis, capsule expression by colony immunoblot and *agr* groups by *agr* multiplex PCR in 195 clinical *S. aureus* strains**

	Capsule phenotype (%)			Capsule genotype (%)		*agr* groups (%)			
***agr* activity**	**CP5 (*n* = 45)**	**CP8 (*n* = 80)**	**NT (*n* = 70)**	***cap5* (*n* = 78)**	***cap8* (*n* = 117)**	**I (*n* = 90)**	**II (*n* = 59)**	**III (*n* = 32)**	**IV (*n* = 14)**
Positive (*n* = 100)	31 (68.9)	42 (52.5)	27 (38.6)	45 (45)	55 (55)	54 (54)	28 (28)	16 (16)	2 (2)
Negative (*n* = 95)	14 (31.1)	38 (47.5)	43 (61.4)	33 (34.7)	62 (65.3)	36 (37.9)	31 (32.6)	16 (16.8)	12 (12.6)
			p 0.0079		p 0.188				p 0.013

CP, capsular polysaccharide; NT, non-typeable.