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Reduced pro-inflammatory responses to *Staphylococcus aureus* bloodstream infection and low prevalence of enterotoxin genes in isolates from patients on haemodialysis

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Abstract

Patients with end stage renal failure undergo regular haemodialysis (HD) and often develop episodes of *Staphylococcus aureus* bloodstream infection (BSI) which can reoccur. However, clinically, patients on HD, with *S. aureus* BSI, respond well to treatment, rarely developing overt signs of sepsis. We investigated the contributions of bacterial virulence and cytokine responses to the clinical course of *S. aureus* BSI in HD and non-HD patients. Seventy patients were recruited including 27 (38 %) patients on HD. Isolates were *spa*-typed and virulence and antimicrobial resistance gene carriage was investigated using DNA microarray analysis. Four inflammatory cytokines, IL-6, RANTES, GROγ and leptin were measured in patient plasma on the day of diagnosis and after seven days. There was no significant difference in the prevalence of genotypes or antimicrobial resistance genes in *S. aureus* isolates from HD *Vs* non-HD patients. The enterotoxin gene cluster (containing staphylococcal enterotoxins *seg, sei, sem, sen, seo, seu*) was significantly less prevalent among BSI isolates from HD patients compared to non-HD. Comparing inflammatory cytokine response to *S. aureus* BSI in HD patients to non-HD, IL-6 and GROγ were significantly lower ($p=0.021$ and $p=0.001$) in HD patients compared to other patients on the day of diagnosis and RANTES levels were significantly lower ($p=0.025$) in HD patients on day seven following diagnosis. Lowered cytokine responses in HD patients and a reduced potential for super-antigen production by infecting isolates may partly explain the favourable clinical responses to episodes of *S. aureus* BSI in HD patients that we noted clinically.

Key words: Haemodialysis, *Staphylococcus aureus*, bloodstream infection, cytokines, virulence.

Introduction

Patients on haemodialysis (HD) due to end stage renal failure are at increased risk of developing bloodstream infection (BSI). Among the factors that contribute to this increased risk are; the frequent necessity for intravascular devices, regular large bore needle puncture, regular contact with healthcare facilities and altered immune responses to renal dysfunction. *Staphylococcus aureus* is the predominant etiologic agent identified in HD patients with BSI, being responsible for 29-43% of reported cases [1-3]. In one study, the incidence of catheter-associated BSI among patients on HD was reported as 7.6-14.4 episodes/100 patient-years, with *S aureus* accounting for 56% of episodes [4]. Furthermore, recurrence rates of *S. aureus* BSI among patients on HD of 14% are reported [5]. Regular exposure to healthcare facilities and colonisation with *S. aureus* are among the factors contributing to recurrent episodes among HD patients. Recent studies on nasal *S. aureus* carriage rates in HD patients reveal a carriage rate of 43-57% [6-9].

The scientific literature report rates of infective endocarditis (IE) in HD patients as high as 20 %, related to the higher burden of co-morbidities in this setting [10, 11]. In comparison, we reported rates of 7.6 % IE among HD in Beaumont Hospital. Two studies report overall complication rates in HD to be 33 % and 31 % [12, 13] but do not present comparative rates in the absence of HD. Clinical observations of patients in our hospital on HD who experienced episodes of *S. aureus* BSI suggest that they generally respond well to treatment displaying no severe signs of sepsis. A favourable prognosis among HD patients was also reported by Franciolli *et al.*, [14] who noted that among 37 patients investigated, systemic complications were rarely life-threatening.

In this prospective study we investigated the genotype and virulence gene repertoire of *S. aureus* isolates causing BSI in HD patients compared with other patients with BSIs and

identified differential circulating cytokine responses over the course of infection in HD patients compared to non-HD patients.

Methods

Setting, patients and definitions

A prospective cohort study was performed over a two year period in 70 patients (27 on HD HD and 43 non-HD) with *S. aureus* BSI in Beaumont Hospital (BH) Dublin, Ireland, (64 patients) and the Mater Misericordiae University Hospital (MMUH), Dublin (6 patients). BH is an 800-bed tertiary referral centre and a national centre for renal and pancreatic transplantation, neurosurgery and cochlear implantation. The BH dialysis centre provides acute and out-patient maintenance HD services, both within the region and Ireland, delivering 30,000 HD treatments annually. The MMUH, Dublin is a 570-bed tertiary referral centre and national centre for cardiothoracic (including heart and lung transplantation) and spinal injuries. MMUH also provides services under several medical and surgical specialties including renal, general and vascular surgery and urology. Consenting patients with positive *S. aureus* BSI diagnosis (as outlined below) were recruited to the study.

The definitions of nosocomial, healthcare-acquired (HCA) or community-acquired (CA) BSI described by Friedman *et al* were used [15]. In this classification, CA BSI (BSI obtained as outpatients or identified within 48 h of hospital admission) is sub-classified into two; CA BSI and HCA BSI. The HCA BSI's were identified from patients with recent hospital admission or exposure to significant medical care in community or outpatient settings, while CA BSI's described other community onset BSI that did not have significant prior healthcare exposure. Nosocomial infections were defined by positive blood culture obtained from patients hospitalized for 48 h or longer or if a patient was transferred from another hospital, the duration of inpatient stay was calculated from the date of first hospital

admission [15]. A complicated infection was defined here as persistent *S. aureus* BSI despite at least three days of appropriate antibiotics (e.g. flucloxacillin for methicillin-susceptible *S. aureus* (MSSA), or vancomycin for methicillin-resistant *S. aureus* (MRSA), and disseminated infection such as osteomyelitis, or IE [16].

Patient sample collection

S. aureus BSI was confirmed as 'proven BSI' by the diagnostic laboratories of BH and MMUH based on the recovery of *S. aureus* from the initial blood culture taken from patients prior to commencing antibiotics with evidence of clinical infection, e.g. fever, other signs of sepsis. Patients with isolates representing possible contamination were excluded (defined as the presence of *S. aureus* in one of two blood cultures but infection considered clinically insignificant). A further blood culture was taken at least three days after the initial *S. aureus* BSI diagnosis. In addition blood samples (10 ml) were taken from patients for cytokine analysis on the day of diagnosis of *S. aureus* BSI and seven days later. Blood was collected in Li-Heparin blood bottles (S-Monovette, Germany), centrifuged at 4000 x *g* and the plasma was decanted and stored in aliquots at -80^{o}C.

Identification of *S. aureus* from blood culture

Blood was cultured from patients with suspected BSI based on clinical signs (e.g. tachycardia, fever, hypotension) by inoculation of a least 10 ml blood into BACTEC Plus aerobic/F^{TM} culture bottles and using the BACTEC^{TM} 9240 continuous blood monitoring system (Becton Dickinson, CA USA). *S. aureus* was initially identified from blood culture by colony morphology on Columbia Blood Agar (Oxoid Ltd, UK), Gram stain pattern, positive catalase test and positive slide or tube coagulase test (Staphaurex Plus, Remel, Oxoid Ltd, UK) and definitively by DNA microarray analysis (see below). MRSA was identified based

on the production of pink colonies on MRSA select chromogenic agar (Biorad, Fannin Healthcare, Ireland) and by automated antibiotic susceptibility testing (PM67 panel) using the BD Phoenix^{TM} Automated Microbiology System (BD, Pharmingen, CA, USA). Isolates were stored on cryoprotect beads (Cruinn Diagnostics Ltd, Ireland) at -80^{o}C until required.

Patient details

Patient demographic and clinical information was collected from patient charts, nursing notes and clinical microbiological team laboratory records. Data collected included age, sex, co-morbid conditions (HDs, diabetes mellitus (DM), chronic obstructive pulmonary disorder, cardiac disease), sources of *S. aureus* BSI, fever defervescence, clinical outcomes, and *S. aureus* acquisition (HCA, CA, nosocomial as defined above) Complications such as the development of IE were also recorded.

Patient cytokine assays

The levels of IL-6, GRO-γ, RANTES and leptin were determined in patient plasma samples using immunometric sandwich enzyme-linked immunosorbant assay (ELISA) (R and D systems, Abington, UK (IL-6, RANTES, leptin) or Acris Antibodies Ltd., Germany (GRO-γ). These cytokines were selected following analysis of plasma from sub-groups of patients with *S. aureus* BSI (complicated versus uncomplicated infection as previously defined [16]) using a cytokine antibody array in a related study described by McNicholas *et al*[16]. For ELISA's the manufacturer's instructions were followed using 50-100 μl of plasma. Plasma protein concentrations were measured using the Bradford assay [17] and cytokine levels were normalised to plasma protein concentration to account for variability in blood processing and biological variations in plasma protein concentrations between patients, which may occur in HD patients.

148

149 **Typing and characterisation of *S. aureus* isolates**

150 Genomic DNA from *S. aureus* isolates was extracted using a DNeasy® Blood and Tissue kit
151 (Qiagen, Crawley, UK). *Spa* typing was carried out on genomic DNA according to the
152 protocol and primers described on the SeqNet website (http://www.seqnet.org). Sequencing
153 was performed by Beckman Coulter Genomics (Takeley, UK) and Source BioScience
154 (Tramore, Waterford, Ireland). Genetic characterisation of isolates was undertaken by DNA
155 microarray profiling, including detection of known virulence-associated and antimicrobial
156 resistance genes, and assignment of isolates to multilocus sequence type (ST) or clonal
157 complexes (CCs) and, for MRSA only, to STs and staphylococcal cassette chromosome *mec*
158 (SCC*mec*) types, using the StaphyType Kit 2 (Alere Technologies Germany) as described
159 previously [18, 19].

160

161 **Statistical analyses**

162 Fisher's exact test was used to analyze categorical variables. The significance of differences
163 between the groups was expressed as a p-value. Mann-Whitney tests were used to compare
164 cytokine data between groups and were calculated using GraphPad Prism. p values of ≤ 0.05
165 were considered significant.

166

167 **Results**

168 **Epidemiological characteristics of patients with *S. aureus* BSI**

169 Of the 70 patients recruited, 27 (37%) were on HD. Relevant patient and clinical details for
170 all patients are summarized in Table 1. Similar proportions of male gender and age >65 y
171 were recorded in each group. Cardiac disease was the predominant co-morbidity recorded in
172 both groups followed by DM. A central venous catheter (CVC) was the most common source

of *S. aureus* BSI in HD patients (17/27, 62.9%) and was significantly associated with HD (p=0.0001). Sources of BSI among non-HD patients were variable and included peripheral venous catheters (PVC) (14/43, 32.5%), but no source was identified in a third of patients (14/43, 32.5%). More HD patients had HCA onset of BSI compared to non-HD patients (66.6% Vs 23.2%, p=0.0004). However, fewer HD patients had a nosocomial onset compared to other patients (29.6 % Vs 60.5 %, p=0.015). There was no significant difference in the rate of complicated BSI infections (e.g. persistent BSI, MRSA BSI or disseminated BSI, such as osteomyelitis, IE etc) among HD patients compared to other patients. Furthermore using duration of fever as a pseudo-measure of time to recovery, it appears that patients on HD had more timely resolution of infection (2/8 patients with persistent fever at 72 h were on HD *Vs* 6/8 non-HD).

Genotype of *S. aureus* isolates

The isolates belonged to a variety of CCs including CC22, CC30 and CC45, each containing a variety of *spa* types as detailed in Table 2 with *agr* and capsule types. CC45 isolates were significantly less prevalent (1/27, 3.7%) among HD patients compared to non-HD patients (10/43, 23.2%); p=0.041. All other CCs were distributed similarly between the two groups (Figure 1a). Immune evasion cluster (IEC) types A to G were represented among *S. aureus* isolates independent of whether they were from patients receiving HD or not (Table 2). The largest IEC group identified was IEC type B (encoding *sak, chp, scn*) and HD isolates accounted for 28% of these (11/39) (Figure 1b). Two HD isolates were IEC-negative, harbouring an untruncated *hlb* (CC30 and CC7), indicating possible animal origin. No significant difference in the distribution of capsule types was found between isolates from either group. In total, 10/27 (37%) of isolates from HD patients were capsule type 5 and the remainder were capsule type 8, whereas 23/43 (53%) of isolates from non-HD patients were

capsule type 5 and the remainder were capsule type 8 (p=0.222). No statistical differences in the distribution of *agr* types were found among HD *vs* non-HD isolates, although a greater number of *agr III* isolates were found among isolates from HD patients (7/27, 25.9%) compared to isolates from non-HD patients (4/43, 9.3%), p=0.092) (Table 3).

Virulence and antibiotic resistance gene carriage

The virulence and antibiotic resistance genes detected by DNA microarray analysis of the *S. aureus* isolates are listed by CC in Table 2 and their prevalence in HD and non-HD groups is summarised in Table 3. The prevalence was similar in the HD and non-HD groups for most genes investigated. However, the enterotoxin gene cluster (*egc*) which contains the staphylococcal enterotoxin genes, *seg, sei, sem, sen, seo, seu* was significantly more prevalent in non-HD isolates compared to HD isolates (35/43, 82% *vs* 15/27, 55% (p=0.029). The predominant antimicrobial agent resistance mechanisms based on gene carriage, were resistance to β-lactams (*bla*-mediated, 86%), multidrug resistance mediated through *sdrM* (72%), fosfomycin resistance mediated through *fos*B (49%), erythromycin resistance mediated through *erm*(C) (21%) and β-lactam resistance mediated through *mecA* (21%). However, there was no significant difference in the proportional prevalence of these genes between the two isolate groups (Table 3). Five MRSA isolates were recovered from HD patients and 11 from non-HD patients.

Patient cytokine responses

Both IL-6 and GROγ levels were significantly lower in HD patients compared to non-HD patients on the day of diagnosis of *S. aureus* BSI (p=0.021, p=0.001) (Figure 2.A). RANTES levels increased significantly on day seven following diagnosis compared to the day of diagnosis in both patient groups (p=0.020, p<0.0001) (Figure 3). However, the levels reached

on day seven in the HD group were significantly lower than those of non-HD patients (p=0.025) (Figure 3). There were no significant differences in leptin levels between the two patient groups.

Discussion

The setting for this study (and the center from which the majority of patients were recruited) included a large tertiary hospital, the national referral centre for renal transplantation and has a large patient cohort on renal replacement therapy. The most common source of *S. aureus* BSI identified among patients on HD was CVCs (65 %). A twelve year review conducted in our hospital reported CVCs as the source of *S. aureus* BSI in 83 % (328/394) of HD patients [20]. A recent case-control study from a Brazilian HD unit reported that patients with a CVC had an 11.2-fold increased risk of developing BSI compared to those with an arteriovenous fistulae (AVF) for vascular access [2]. A UK study reported a six-fold increased risk associated with CVCs compared to AVF [21]. An AVF was identified as the source of infection in only three HD patients in the present study. The definitions of CA, HCA and nosocomial BSI are of particular significance in this study as they take account of BSI acquired in patients with regular out-patient contact which would previously have been classed as CA. This distinction revealed that more HD patients had HCA onset BSI and this likely reflects regular contact with out-patients services of healthcare. Contrary to this, the majority of non-HD patients had nosocomial onset, likely reflecting their recent admission to hospital.

Despite patients on HD having an increased risk of developing *S. aureus* BSI, the complication rate was lower in HD patients, although not significantly (4/27, 14.8 % *Vs* 11/43, 25.6%), (p=0.37). The twelve year review mentioned above, conducted in the same hospital found a rate of 11% complications among 394 patients, the most common of which was IE at 7.6% [20]. The rate of IE found in HD patients in this study was 11.1%, which is within the range of 1-17% reported in five cohort reviews of HD patients [20]. The 30 day mortality rate for HD patients in this study was lower (2/70, 2.8%) than that reported

elsewhere, e.g., 15.2 % [3, 22] and previously in our own institution, i.e. 9.8 % [20]. However, rates of MRSA causing *S. aureus* BSI had decreased nationally at the time of the present study by 18% (41.9 % in 2006, compared to 24.0 % in 2011[23]). As MRSA is reported to be associated with a higher mortality rate in patients with BSI than MSSA, the lower mortality rate overall found here may reflect a lower proportion of MRSA among *S. aureus* than found in previous studies.

The genotypic features of *S. aureus* isolates causing BSI in our study were generally similar for HD patients and non-HD patients with similar distributions of CCs found reflecting the common CCs reported in clinical *S. aureus* collections [24] (i.e. CC5, 8, 45, 30, 15, 22). The one exception was a single CC45 isolate causing *S. aureus* BSI in the HD group compared to 10 in non-HD patients. The CC45 isolates were recovered from patients from both hospitals and from different locations within each and therefore the possibility that this was a clinical cluster was unlikely.

Clinical isolates of *S. aureus* can encode a vast array of virulence factors including adhesins, toxins and immune evasion genes, the profiles of which are usually lineage specific and this was also noted here. Associations between pathogenic features of isolates, specific patient cohorts and the clinical course of infection are still unclear [25, 26]. However recently, multivariate statistical analysis (discriminant analysis of principal components) of *S. aureus* isolates from IE compared to BSI revealed that isolate groups can be distinguished based on subtle combinations of genetic markers suggesting that bacterial features contribute to the clinical course of *S. aureus* BSI [27, 28]. Interestingly, in the present analysis the enterotoxin gene cluster, *egc* was less frequently detected in HD isolates compared to non-HD isolates and while this may reflect the relative proportion of lineages causing BSI in non-HD isolates, the lack of the *egc* locus may contribute to a favourable clinical course for HD patients. The role of *egc*-encoded superantigens in *S. aureus* pathogenesis is unclear but it

may facilitate *S. aureus* survival on mucosal surfaces. Studies comparing the effects of isogenic *S. aureus egc* mutants in *in-vivo* invasive murine infection models suggest some aggravating effects of these superantigens in the BSI setting [29]. However, a negative correlation between *egc* detection and severity of infection was previously reported when comparing patients with *S. aureus* sepsis to those with *S. aureus* septic shock suggesting a protective role [30]. Among patients on HD, we did not observe statistically significant differences in the levels of any of the studied cytokines in patients infected with egc^+ *S. aureus* compared to egc^- *S. aureus*. It is possible that other cytokines, particularly TH1 and TH2-types may be affected. However, given that questions remain regarding the *in-vivo* expression, regulation and role of super-antigens encoded by the *egc*, alternative approaches may be more appropriate to investigate these speculations.

An attenuated pro-inflammatory response involving IL-6, GROγ and RANTES was identified in HD patients in the present study. These cytokines were selected from a panel of pro-inflammatory cytokines because they showed the greatest differential levels between complicated and uncomplicated *S. aureus* BSI in pooled representative plasma samples in an earlier study [16]. Previous studies of cytokine alterations in HD have given contradictory findings. For example, increased levels of IL-1, IL-6, Il-8 and TNF-α during a single HD session were reported [31] while other studies reported no change [32]. The pattern of reduced levels of pro-inflammatory cytokines, at least for those cytokines selected for investigation here, supports our clinical experience that patients on HD rarely develop overt clinical signs of sepsis when bacteraemic. We propose that this attenuated response contributes to a lesser sepsis phenotype. We previously reported significantly increased circulating RANTES levels in patients with *S. aureus* BSI [16]. Although RANTES levels increased in HD patients in this study between the day of diagnosis of *S. aureus* BSI and day seven following diagnosis, the levels remained significantly lower than in non-HD patients

with *S. aureus* BSI. RANTES' main function is as a chemotactic agent for leucocytes facilitating their recruitment to infection sites [33] and elevated circulating levels are a feature of ongoing infection. In the context of HD, the finding of significantly lower RANTES levels in HD compared to non-HD patients supports the more favourable response to *S. aureus* infection that we have observed clinically. Patients on HD are at risk of recurrent episodes of *S. aureus* BSI with rates of 1.06 episodes per 100 patients reported in a recent UK review [21]. While none of the HD patients in this study had a confirmed recurrent episode of *S. aureus* BSI during the study period, the possibility of previous sub-clinical infection in this group cannot be excluded. Regular exposure to *S. aureus* in HD patients may invoke different innate immune responses to that of acute exposure typical of non-HD patients, characterised by high fever/rigors and hypotension.

There were limitations to this study. Only 70 patients were recruited over 28 months due to logistical issues related to obtaining samples from very ill patients or the movement or discharge of patients. Therefore these results need to be confirmed in larger multi-centre studies. Furthermore, due to variations in plasma quantities available, cytokine analysis was carried out on 57 patients (day of diagnosis sample) and 61 patients (7 days following diagnosis sample) only. The first blood sample was taken on the day of laboratory diagnosis of *S. aureus* BSI and not when it was first suspected on clinical grounds. This time difference was variable and was a time period during which cytokine levels may have changed. Complicated *S. aureus* BSI was under-represented possibly because of the difficulty in obtaining patient consent or assent from relatives of very ill or rapidly deteriorating patients. Although similar prevalence rates of co-morbidities were found in HD and non-HD patients in this study, the contribution(s) of co-morbid conditions associated with *S. aureus* BSI (e.g. concurrent infection, autoimmune conditions, recent surgery etc.) to the cytokine response cannot be excluded. In addition, only HD and non-HD patients with *S. aureus* infection were

included in the study and therefore we cannot exclude that factors independent of *S. aureus* infection may contribute to altered cytokine levels in these patient groups.

The innate and adaptive immune responses in HD are complex even in the absence of BSI due to the retention of uremic toxins, therapeutic interventions and co-morbidities [34]. However, in this patient group we have shown host-mediated differences compared to other patients with *S. aureus* BSI, i.e. lower IL-6 and GROγ at diagnosis and RANTES at seven days. These findings or differences in other immune signatures may partly explain the favourable response to episodes of *S. aureus* BSI in HD patients that we have noted clinically and that are reflected in the more timely resolution of infection in these patients. In addition, interrogation of the virulence factor gene profile of BSI isolates revealed a lower prevalence of *egc* enterotoxin genes amongst HD isolates which warrants further investigation. The identification of modifiable host or bacterial-mediated factors (eg production of damaging toxins) in this way, could improve the clinical outcomes for HD or other patient groups who develop *S. aureus* BSI. Furthermore, vaccine development for *S. aureus* BSI has to date been hampered by a lack of efficacy in humans despite promising pre-clinical findings and this can be improved by the identification of more appropriate epitopes that represent protective responses. Increasingly, there is recognition that future strategies will require identification of immune signatures that contribute to protective infection outcomes.

Compliance with Ethical Standards

HH is in receipt of research funding from Pfizer (Ireland) and Astellas and has received lecture fees from Cepheid. All other authors declare no conflict of interest. This work was supported by an Educational Award to SMN from Pfizer Ireland, Grant number WS 376235. Approval for the study was obtained from Beaumont Hospital and Mater Misericordiae Hospital Ethics (Research)

Committees. Only consenting patients (informed consent from patient/relative) were recruited to the study.

Table 1. Clinical details for haemodialysis (HD) and non-haemodialysis patients

Patient characteristic	**HD n=27 n (%)**	**Non-HD n=43 n (%)**	***p value***
Male sex	18 (66.6)	26(60.5)	0.623
Age >65yrs	11 (40.7)	18 (41.8)	1.00
Comorbidities			
Diabetes mellitus	7 (25.9)	10 (23.2)	1.000
COPD	2 (7.4)	8 (18.6)	0.297
Cardiac disease	12 (44.4)	13 (43)	0.306
Autoimmune disease	2 (7.4)	4(9.3)	1.000
Malignancy	0 (0)	5 (11.6)	0.149
Source of BSI			
CVC	17 (62.9)	5 (11.6)	0.0001*
PVC	1 (3.7)	10 (23.2)	0.04*
Unknown	4 (14.8)	14 (32.5)	0.159
Other[a]	5 (18.5)	14 (32.5)	0.272
Onset of BSI[b]			
Healthcare-associated	18 (66.6)	10 (23.2)	0.0004*
Community-acquired BSI	1 (3.7)	7 (16.3)	0.141
Nosocomial	8 (29.6)	26 (60.5)	0.015*
Clinical course of infection			
Uncomplicated	23 (85.2)	32(74.4)	0.376
Complicated	4 (14.8)	11 (25.6)	0.371
Infectious endocarditis	3 (11.1)	8 (18.6)	0.511
Other complications[c]	1 (3.7)	3 (7.0)	1.000
30 day mortality	0 (0)	2 (4.6)	0.524

[a] Other sources of BSI included skin and soft tissue infection, arteriovenous fistula, implanted cardiac device. [b]Nosocomial, healthcare-associated (HA) and community-acquired (CA) infection were defined as described by Friedman *et al.* [15]. [c]Other complications include discitis, septic arthritis and persistent positive blood culture. * indicates a statistical significance with $p<0.05$.

361 **Table 2 Characterisation of 70 *S. aureus* bloodstream isolates by DNA microarray profiling and *spa* typing**

Patient type	CC (n)	*spa* types[a]	*agr*/ capsule type	Antimicrobial[b] resistance genes	Toxin genes [b, c]	MSCRAMM [b, d] adhesion & biofilm genes	Immune evasion genes[e]
HD	**CC1(1)**	t127	III/8	*fusC, sdrM,*	*sea, seh, sek/q*	*bbp, cna, ebh, fnBPB, sdrD, sasG, vwb*	IEC type D, *mprf*
HD	**CC5 (2)**	t179 (1) t3720 (1)	II/5	*blaZ, erm*(C) (1), *sdrM, fosB.*	*tst* (1), *sea* (1), *sed/j/r* (1), *egc, hlgA*	*bbp, ebh, fnBPB, sdrD, sasG, vwb*	IEC type F (1), IEC type D (1), *mprf*
Non-HD	**CC5 (5)**	t002(3) t548(2)	II/5	*blaZ, fusB* (1), *fosB, sdrM, msrA*(1)	*sep, egc, hlgA*	*bbp, ebh, fnBPB sdrD, sasG, vwb*	IEC type F (5), *mprf*
HD	**CC7 (2)**	t091	I/8	*blaZ, aadD* (1), *sdrM, linA* (1), *tet*(K) (1).	*hlgA*	*bbp, ebh, fnBPB, sdrD* (1), *fib, vwb*	IEC type G(1), UN*hlb* (1) *mprf* (1)
Non-HD	**CC7 (1)**	t091	I/8	*sdrM*	*hlgA*	*bbp, ebh, fnBPB, vwb*	IEC type G
HD	**CC8 (1)**	t304	I/5	*sdrM, fosB,*	*hlgA*	*bbp, ebh, fib fnBPB, sdrD, sasG, vwb*	IEC type B, *mprf*
Non-HD	**CC8 (2)**	t008	I/5	*blaZ, sdrM, fosB*	*sed/j/r* (1), *sek/q* (1), *hlgA*	*bbp, ebh, fib, fnBPB, sdrD, sasG, vwb*	IEC type E (1) IEC type B (1), *mprf*
Non-HD	**CC9 (1)**	t209	II/5	*blaZ, erm*(A), *sdrM, fosB*	*etA, egc, hlgA*	*ebh, fnBPB, sdrD, vwb*	IEC type B, *mprf*
HD	**CC12 (1)**	t160	II/8	*sdrM, fosB*	*seb, sep, hlgA*	*cna, ebh, fib, fnBPB, vwb*	IEC type G, *mprf,*
Non-HD	**CC12 (1)**	t2133	II/8	*blaZ, sdrM, fosB*	*etB, seb, sep, hlgA*	*cna, ebh, fib, fnBPB, vwb*	IEC type G, *mprf*
HD	**CC15 (4)**	t084 (3) t9569 (1)	II/8	*blaZ , erm*(C) (1), *sdrM, fosB*	*etA* (1), *hlgA , lukF-PV and lukS-PV* (1),	*bbp* (3), *ebh, fib, fnBPB, sdrD, sasG, vwb*	IEC type C, *mprf*
Non-HD	**CC15 (4)**	t084(1) t228 (1) t1885 (1) t4579 (1)	II/8	*blaZ, erm*(C) (1), *aad D(1), sdrM, fosB, qacA(1)*	*hlgA* (3)	*bbp* (3), *ebh, fib, fnBPB, sasG, sdrD* (3)	IEC type C, *mprf*
HD	**CC22 (6)[f]**	t032(3) t557(2) t1214(1)	I/5	*blaZ, erm*(C) (3), *mecA* (4),	*sec/l* (5), *egc, hlgA*(4)	*bbp (5), cna, sasG, sdrD* (5), *vwb*	IEC type B, *mprf*

Non-HD	**CC22 (14)[§]**	t032 (5) t515(2) t557(1) t1214 (2) t891 (1) t5276 (1) t9570 (1) t9854 (1)	I/5	*blaZ* (13), *erm*(C) (9), *mecA* (11)	*seb* (1), *sec/l* (9), *egc*, *hlgA*(5)	*bbp (13), cna*, *sasG*, *sdrD, vwb*.	IEC type B, *mprf*
Non-HD	**CC25 (1)**	t078	I/5	*sdrM, fosB*	*etD*, *egc*, *hlgA*	*bbp*, *ebh*, *fnBPB, sdrD, vwb*	IEC type B, *mprf*,
HD	**CC30 (6)**	t012(2) t021(1) t019 (1) t1872 (1) t2643 (1)	III/8	*blaZ*, *erm*(A)(2), *sdrM*, *fosB*	*tst* (5), *sea* (2), *egc* (5), *hlgA* (5)	*bbp*, *cna*, *ebh*, *fnBPB* (1), *sdrD* (4), *vwb*	IEC type A (2), IEC type B (2), IEC type C (1), UN*hlb*(1), *mprf*
Non-HD	**CC30 (4)**	t012(2) t021 (1) t9566 (1)	III/8	*blaZ*, *sdrM*, *fosB*	*tst* (1), *sea* (3), *egc*, *hlgA*	*bbp*, *cna*, *ebh*, *sdrD*, *vwb*	IEC type A (3), IEC type B (1), *mprf*
HD	**CC45 (1)**	t2784	I/8	*blaZ*, *sdrM*, *fosB*	*seb*, *sec/l egc hlgA*	*bbp*, *cna*, *ebh*, *fnBPB*, *sdrD*, *vwb*	IEC type B, *mprf*
Non-HD	**CC45 (10)**	t026 (1) t015 (1) t065 (1) t067 (1) t230 (1) t1510 (1) t1574 (1) t3344 (1) t9899 (1) non-typeable (1)	I/8	*blaZ (8)*, *ermA*(2), *sdrM*, *fusB* (1)	*seb*(1),*sec/l* (6), *egc*, *hlgA*, *etA*(1), *tst* (2)	*bbp* (7), *cna*, *ebh* *fnBPB*(9), *sdrD*(8), *vwb*	IEC type B, *mprf* (7)
HD	**CC121 (1)**	t159	IV/8	*sdrM*, *fosB*, *mupA*	*etB*, *egc*, *hlgA*	*bbp*, *cna*, *ebh*, *fib*, *fnBPB*, *vwb*	IEC type E, *mprf*,
HD	**CC188 (1)**	t189	I/8	*blaZ*, *sdrM*	*hlgA*	*cna*, *ebh*, *fib*, *fnBPB*, *sdrD*, *vwb*	IEC type E, *mprf*,
HD	**CC398 (1)**	t1149	I/5	*sdrM*	*hlgA*, *etD*	*bbp*, *ebh*, *fnBPB*, *sdrD*, *vwb*	IEC type B,

[a]Where more than one *spa* type was identified among isolates of a given CC, the number of isolates belonging to each *spa* type is shown in parenthesis

[b]The number of positive isolates are indicated in parenthesis if not all isolates within a CC were positive for the gene indicated.

[c]Toxins detected include enterotoxins, exfoliative toxins and haemolysins. Where egc is indicated this refers to the enterotoxin gene cluster containing the enterotoxin genes *seg, sei, sem, sen, seo, seu*

[d]MSCRAMM, Microbial surface components recognizing adhesive matrix molecules,

[e]IEC, immune evasion cluster. IEC typeA - *sea, sak, chp, scn*, IEC type B - *sak, chp, scn*, IEC type C - *chp, scn*, IEC type D - *sea, sak, scn*, IEC type E - *sak, scn*, IEC type F - *sep, sak, chp, scn*, IEC type G - *sep, sak, scn, UNhlb* - untruncated *hlb* (IEC-negative).

[f]The 15 MRSA isolates in the study that belonged to CC22, were further characterised by the array as belonging to ST22-MRSA-IV.

All isolates harbored the following genes; *hla* (except one CC22 isolate), *hld*, *clfA, clfB, eno, ebps*, *fnBPA, map* (except one CC22 isolate), *sdrC, icaA, icaC* (except one CC22 isolate), *icaD, isaB, isdA*.

385

386 **Table 3. Gene carriage in *S. aureus* isolates from HD and non-HD patients with BSI**

Gene/gene cluster[a]	Encoded protein	HD (n=27) n (%)	Non-HD (n=43) n (%)	*p* value[b]
Virulence				
tst	Toxic shock toxin	6 (22)	3 (7)	0.079
sea	Staphylococcal enterotoxin A	4 (15)	3(7)	0.417
seb	Staphylococcal enterotoxin B	2(7)	2 (5)	0.637
sec/l[c]	Staphylococcal enterotoxin C/L	6(22)	14 (32)	0.296
egc[c]	Enterotoxin gene cluster	15 (55)	35(82)	0.029*
sed/j/r[c]	Staphylococcal enterotoxin D/J/R	1 (4)	1(2)	1.000
cna	Collagen adhesin	17(63)	29(67)	0.788
ebh	Extracellular matrix binding homologue	21 (78)	29 (67)	0.420
Accessory gene regulator				
agr I	Accessory gene regulator I	12 (44)	28(65)	0.130
agrII	Accessory gene regulator II	7 (26)	11 (26)	1.000
agr III	Accessory gene regulator III	7 (26)	4(9)	0.092
agr IV	Accessory gene regulator IV	1(4)	0 (0)	-
Antibiotic resistance				
mecA	Penicillin binding protein 2A	4 (15)	11 (26)	0.376
blaZ	β-lactamase	22(81)	38 (88)	0.493
ermC	Methyltransferase	5 (18)	10 (23)	0.769
sdrM	Tetracycline efflux pump	22(81)	29 (67)	0.272
fosB	Fosfomycin inactivating enzyme	16(59)	18 (42)	0.219

387

388 [a] Only a selection of genes are shown including toxin and adhesin genes, the accessory
389 gene regulator *(agr*) and antibiotic resistance genes. [b] *p* values determined by Fisher's exact
390 test [c] These genes are clustered together on pathogenicity islands or plasmids and therefore
391 the *p* value calculated applies to each gene within the cluster, *egc*, enterotoxin gene cluster,
392 containing the enterotoxin genes *seg, sei, sem, sen, seo, seu.*

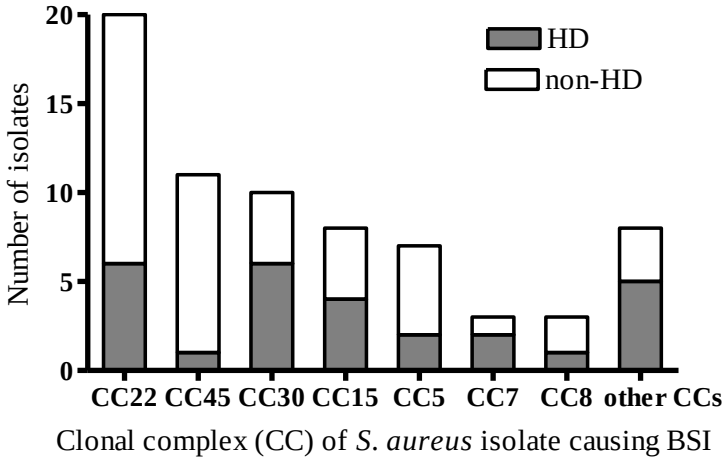
393

394

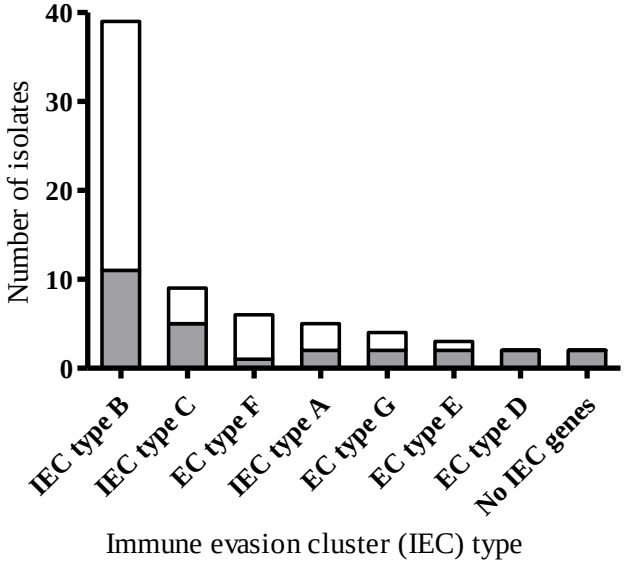
395

Figure 1.

A


20
15
10
5
0
Number of isolates
HD
non-HD
CC22
CC45
CC30
CC15
CC5
CC7
CC8
other CCs
Clonal complex (CC) of S. aureus isolate causing BSI

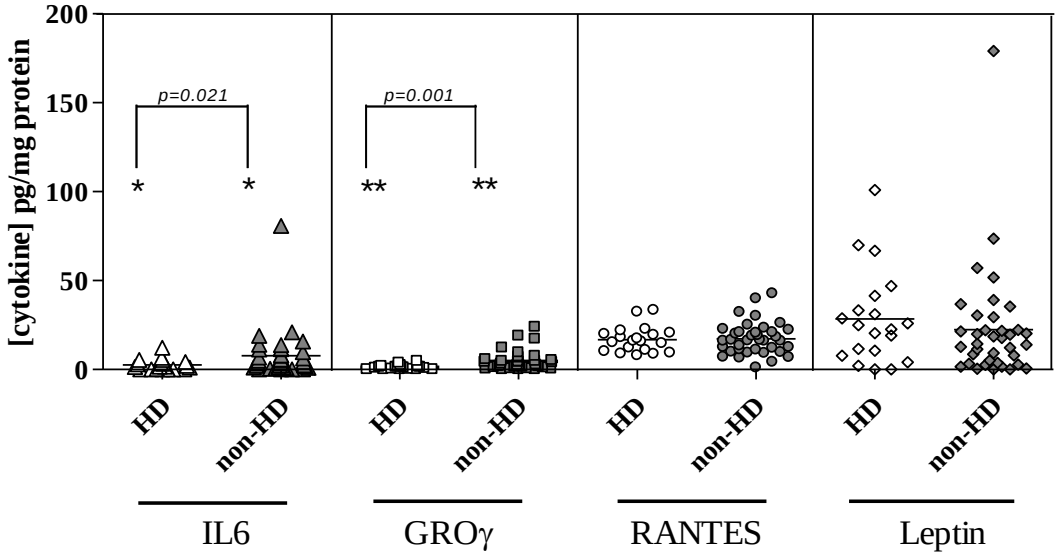
B


40
30
20
10
0
Number of isolates
IEC type B
IEC type C
EC type F
IEC type A
EC type G
EC type E
EC type D
No IEC genes
Immune evasion cluster (IEC) type

406 **Figure 2**

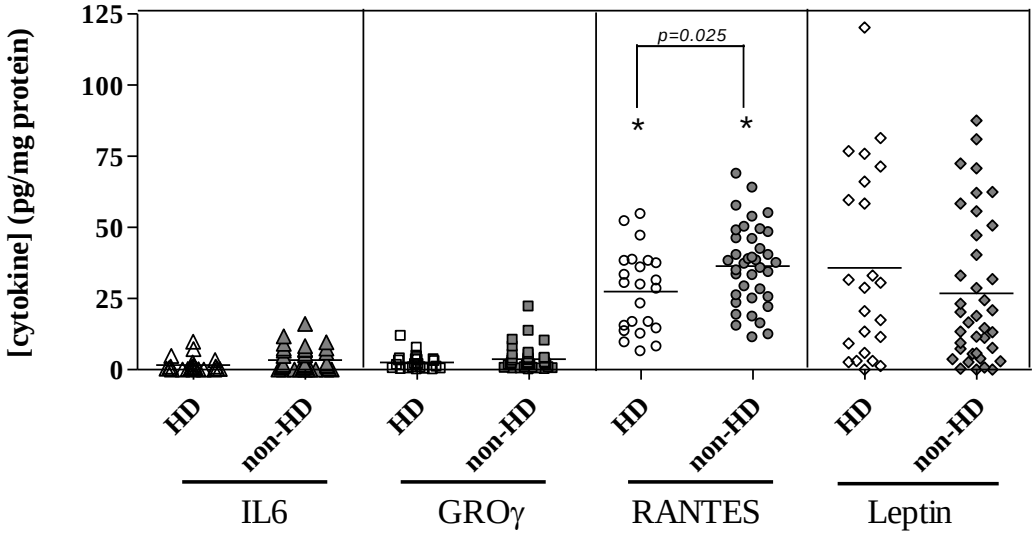
407

408 A



409

410 B



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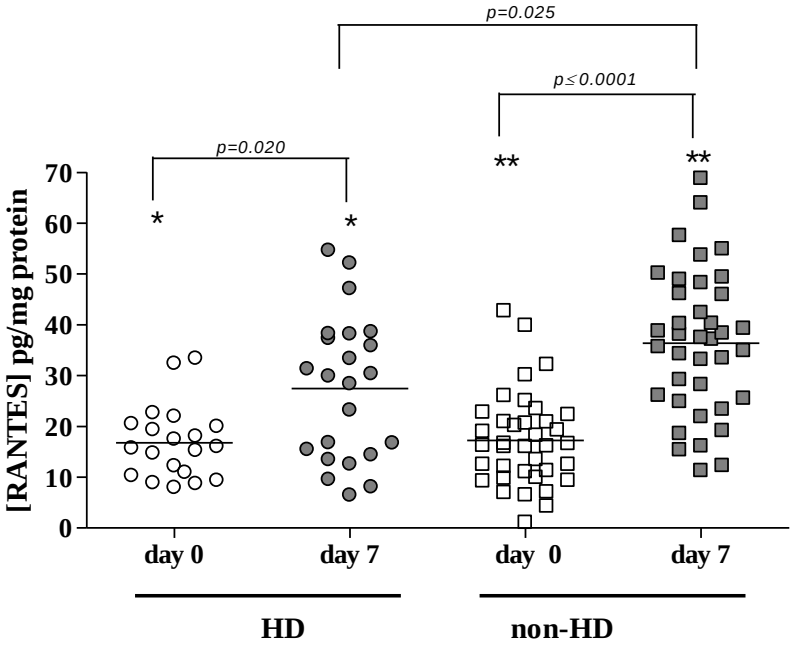

414

415 **Figure 3**

416

Legends

Figure 1. Distribution of clonal complexes (CC) (A) and immune evasion complex (IEC) types (B) amongst *S. aureus* isolates recovered from patients with bloodstream infection on haemodialysis (HD) compared to those not undergoing HD (non-HD). Other CCs included single isolates belonging to CC1, CC9, CC25, CC121, CC188, CC398 and two CC12 isolates.

Figure 2. Comparison of cytokine levels in haemodialysis (HD) and non-HD patients with *S. aureus* bloodstream infection. Scatter plots of cytokine levels in HD patients compared to non-HD patients on day 0 (A) and day 7 (B) following diagnosis of *S. aureus* BSI. Horizontal bars denote mean values.

Figure 3. Comparison of RANTES levels over the course of *S. aureus* BSI on haemodialysis (HD) and non-HD patients**.** Horizontal bars denote mean values.

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