

Virulence Markers in *Staphylococcus aureus* Strains Isolated from Hemodialysis Catheters of Mexican Patients

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* is an increasingly important cause of nosocomial bacterial infections worldwide. *S. aureus* is responsible for several pathologies, including skin infections, endocarditis, meningitis, deep-tissue ulcers, and sepsis. *S. aureus* biofilm formation on catheters and other medical devices is a major post-operative concern, because biofilms are often the source of persistent and difficult to treat bacterial infections. While catheter-related *S. aureus* infections have been reported, the strains responsible for these infections have not been genetically characterized. We genetically characterized *S. aureus* strains isolated from hemodialysis catheters in Mexican patients. The frequency of 35 genes coding for adhesins, toxins, and other virulence-associated products in the 55 isolated *S. aureus* strains was determined using PCR, while real-time PCR was used to examine the level of gene expression. Of the 55 *S. aureus* strains isolated from 109 patients, 45 (81.8%) were determined to be methicillin-resistant. The *icaA*, *rbf*, *sarA*, and *agr* genes are involved in biofilm formation and bacterial dispersion and were detected in 96.3%, 40.0%, 74.5%, and 100% of *S. aureus* strains, respectively, and 70.9% of the strains formed a detectable biofilm. Interestingly, 67.2% of the strains contained the *icaA*, *agr*, *spa*, *clfA*, *sdrC*, *sdrD*, *sdrE*, *seg*, *seh*, and *sei* genes, suggesting that this gene combination is important for successful catheter colonization. The results of this study provide significant insight into the virulence gene make-up of catheter-colonizing *S. aureus* strains, and will assist in developing a more targeted treatment approach for persistent *S. aureus* biofilm contamination of medical devices.

Keywords: *S. aureus*; Hemodialysis; Catheter; Genotyping

1. Introduction

Staphylococcus aureus is a frequent cause of catheter-related infections in patients [1]. The worldwide emergence of methicillin-resistant *S. aureus* (MRSA) is of major concern, as its emergence has dramatically reduced the number of antibiotics available for the prevention and treatment of infections in both hospitals and communities [2]. In 2005 in the United States, invasive MRSA infections occurred in 42.5/1000 dialysis population, a rate exceeding that in the general population by 100-fold [3]. MRSA exhibits a great capacity for biofilm formation on surfaces, making endovascular catheters a favorable nidus for infection. *S. aureus* formation of biofilms requires the synthesis of PNAG (polymeric N-acetylglucosamine), and the enzymes responsible for its synthesis are encoded by the *icaADBC* operon [4].

Additionally, the *rbf* gene promotes biofilm formation by *S. aureus* via repression of *icaR*, a negative regulator of the *icaADBC* operon [5]. The SarA protein, which is encoded by the *sarA* locus, is a positive regulator of PNAG-dependent *S. aureus* biofilm formation [6,7].

The microbial adherence to cells and extracellular matrix is considered as an essential first step in the process of colonization and infection [8]. A well characterized family of staphylococcal surface adhesins, called MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) are known to mediate adherence to host extracellular matrix components, such as fibrinogen, fibronectin and collagen [9].

Among the *S. aureus* adhesin-coding genes, *fnbA*, *fnbB*, *spa*, *clfA*, *clfB*, *cna*, *bbp*, *ebps*, *map/eap*, *sdrC*, *sdrD* and *sdrE* are found (see **Table 1**). *S. aureus* strains harboring *fnbA* and *fnbB* have been found associated with invasive infections such as endocarditis, septic ar-

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Table 1. Virulence-related genes studied in the *S. aureus* strains [25].

Gene	Protein name	Function
Adhesins		
<i>clfA</i>	Clumping factor A (ClfA)	Adhesin specific for fibrinogen
<i>clfB</i>	Clumping factor B (ClfB)	Adhesin specific for fibrinogen
<i>fnbpA</i>	Fibronectin-binding protein A (FnBPA)	Adhesin specific for fibronectin
<i>fnbpB</i>	Fibronectin-binding protein B (FnBPB)	Adhesin specific for fibronectin
<i>cna</i>	Collagen adhesin (Cna)	Adhesin specific for collagen
<i>spa</i>	<i>Staphylococcus</i> protein A (Spa)	Binds the Fc domain of immunoglobulins and von Willibrand factor
<i>bbp</i>	Sialoprotein-binding protein (Bbp)	Adhesin specific for bone sialoprotein
<i>ebps</i>	Elastin-binding protein of <i>Staphylococcus aureus</i> (EbpS)	Adhesin specific for elastin
<i>map/eap</i>	MHC class II analog protein or extracellular adhesion protein (Map/Eap)	Major histocompatibility complex class II analogue
<i>sdrC, sdrD</i> and <i>sdrE</i>	Serine aspartate repeat protein (SdrC, SdrD and SdrE)	Unknown; putative adhesins
Toxins		
<i>sea, seb, sec, sed, see, seg, seh, sei</i> and <i>sej</i>	Enterotoxins A, B, C, D, E, G, H, I and J.	Exotoxins with superantigen activity
<i>tst</i>	Toxic shock syndrome toxin 1 (TSST-1)	Exotoxin with superantigen activity
<i>eta</i> and <i>etb</i>	Exfoliative toxins A and B	Exotoxin with superantigen activity
<i>pvl</i>	Panton-Valentine leukocidin (PVL)	Bicomponentleukocidin
<i>hlg</i>	Alpha-toxin (Hlg)	Bicomponentleukocidin
Other genes		
<i>efb</i>	Extracellular fibrinogen-binding protein (Efb)	Extracellular fibrinogen-binding protein
<i>v8</i>	Serine protease V8	Serine protease
<i>chp</i>	Chemotaxis inhibitory protein of <i>Staphylococcus aureus</i> (CHIP)	Innate immune modulators
<i>arcA</i>	Arginine deiminase pathway	Important role in growth and survival

thritis and osteomyelitis [10]. It has also been shown that protein A (Spa) is an important virulence factor in septic arthritis models and subcutaneous infections in mice [11]. *S. aureus* strains deficient in CNA showed impaired capacity to produce endocarditis [12], osteomyelitis [13] and keratitis [14]. It has been shown that clumping factor A (ClfA) is an important *S. aureus* virulence factor in several experimental infection models, including rat endocarditis [15], mice arthritis [16] and rabbit infective endocarditis [17]. ClfA also inhibits phagocytosis by human polymorphonuclear leucocytes [18].

S. aureus is able to produce a great number of extracellular proteins, including enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI and SEJ), Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin 1 (TSST-1), exfoliative toxin (ETA and ETB) and alpha toxin (Hlg), some of which can act as superantigens (**Table 1**).

Other virulence factors are encoded by the genes *chp*, *v8*, *arcA* and *coa* [19]. The expression of most of the virulence factors in *S. aureus* is controlled by the *agr*

locus [20].

Despite the fact that severe catheter-related *S. aureus* infections have been reported [21–23], and that these infections have become important causes of morbidity, mortality, and a source of concern in the primary and emergency care context over the past decade [24], the *S. aureus* strains responsible for these infections have not been genetically characterized. Therefore, in order to gain some insight into the combinations of genes present in catheter-colonizing *S. aureus* strains, the presence of 35 virulence genes coding for adhesins, toxins, or other products was determined in 55 *S. aureus* strains isolated from tunneled hemodialysis catheters of Mexican patients with chronic renal failure being treated with hemodialysis. Hence we screened by PCR for the presence of 35 common *S. aureus* virulence-associated genes, including adhesin, toxin, superantigen, and biofilm formation genes. We then used real-time PCR to measure the expression of *icaA*, *rbf*, and *sarA* biofilm formation genes, as well as that of the global virulence gene regulator, *agr*. This study is the first report on the prevalence

of the 35 most common virulence-associated genes in *S. aureus* strains isolated from hemodialysis catheters. These findings will be of significant interest to health professionals and researchers alike, as the discovery of gene sets required for catheter colonization will allow for a more targeted approach to the development of treatment strategies, which would be of great interest to vaccine researchers.

2. Materials and Methods

2.1. Patients

A total of 109 patients presenting with symptoms of infection (e.g., erythema, inflammation, pain, and yellow suppuration) at the insertion site of a Mahurkar-type catheter were selected during September 2009 to May 2010. All of the patients had visited hemodialysis services at four public hospitals located in Estado de Mexico, Mexico [40.4% ($n = 44$) of the samples were from Hospital General Regional 72 del IMSS; 26.6% ($n = 29$) from Hospital General Regional N° 196 Aragón; 21.1% ($n = 23$) from ISSEMyM Satélite; and 11.9% ($n = 13$) of the samples were from Unidad Médica de Atención Ambulatoria (UMAA) N° 199]. The patients ranged in age from 17 - 77 years old, with 56% ($n = 61$) of the patients less than 50 years old. The causes of the chronic renal insufficiency were diabetes mellitus (33% $n = 36$), glomerulonephritis (17.4%, $n = 19$), hypertension (10.1%, $n = 11$), polycystic kidney disease (1.84%, $n = 2$), renal failure secondary to neurogenic bladder, and congenital malformation, lupus nephritis, or preeclampsia (each with 0.92%, $n = 1$); the cause was not determined in 34% of the patients ($n = 37$). The local ethics committee of each hospital approved the study. Inclusion criteria for choosing patients were: persons suffering chronic renal failure, with symptoms of infection and accepting to participate in the study by sign in the informed consent letter. Patients who were under treatment with antibiotics at the moment of sampling or during the last 30 days were excluded. Patients not accepting participate in the study were also excluded.

2.2. Sampling, Bacteria Identification, and PCR Conditions

After obtaining informed consent from each patient, samples were taken from the exterior terminal end of the catheter using sterile cotton swabs. The samples were placed in Stuart transport medium (BD Bioxon, Cuautitlán Izcalli, Edo. de Mexico, Mexico) and taken to the laboratory for further cultivation. For the primary phenotypical species identification of the *S. aureus* strains, tube-coagulase test (Bactident-coagulase, Merck, Darmstadt, Germany) and Api 32 Staph system (BioMerieux, Durham, NC, USA) were applied. This was confirmed by

molecular detection of the *S. aureus* specific genes encoding 23S rRNA, thermostable nuclease (*nuc*), clumping factor (*clfA*), coagulase (*coa*) and protein A region X (*spa*), and the *femA* and *femB* genes. **Table 1** shows the virulence-related genes studied in the *S. aureus* strains. Bacterial DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI). One ml of an overnight culture was centrifuged at 13,000 rpm for 2 minutes and the pelleted cells were resuspended in 480 μ L of 50 mM EDTA. A mixture of 60 μ L of 10 mg/mL lysozyme (Sigma Cat. # L7651) and 60 μ L of 10 mg/mL lysostaphin (Sigma Cat. # L7386) were added and the sample was incubated at 37°C for 60 minutes. Afterwards sample was centrifuged 2 minutes at 13,000 rpm and the supernatant was removed. Six hundred microliters of Nuclei Lysis solution were added in order to resuspend the cells. Sample was incubated at 80°C for 5 minutes to lyse the cells and then cooled to room temperature. Three microliters of RNase solution were added and the tube was inverted 5 times to mix. Sample was incubated at 37°C for 60 minutes and then cooled to room temperature; 200 μ L of Protein Precipitation Solution were added to the RNase-treated cell lysate and the mixture was vortexed vigorously at high speed for 20 seconds. Sample was incubated on ice for 5 minutes, centrifuged at 13,000 rpm for 3 minutes, and the supernatant containing the DNA was transferred to a clean 1.5 mL microcentrifuge tube containing 600 μ L of room temperature isopropanol. The tube was mixed by inversion and centrifuged at 13,000 rpm for 2 minutes. Supernatant was poured off and the tube was drained on absorbent paper. Afterwards, 600 μ L of room temperature 70% ethanol were added and the tube was gently inverted several times to wash the DNA pellet. The tube was centrifuged at 13,000 rpm for 2 minutes and the ethanol was aspirated. The tube was drained on absorbent paper and the pellet was allowed to air-dry for 15 minutes. One hundred microliters of DNA rehydration solution were added to the tube and the sample was incubated at 65°C for 1 hour. DNA was stored at -20°C until it was used.

PCR amplification of genetic markers was performed in a Corbette Research Thermocycler using PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Piscataway, New Jersey, USA). The amplified products were stained with ethidium bromide after electrophoresis in a 1% or 2% agarose gel. PCR primers, primers and probes for real time PCR and conditions used in PCR and real time PCR assays are listed in **Table 2**. The ATCC 33592 *S. aureus* strain was used as a positive control for PCR amplification of *fnbA*, *fnbB*, *spa*, *clfA*, *clfB*, *cna*, *ebps*, *map/eap*, *sdrC*, *sdrD*, *sdrE*, *icaA*, *rbf*, *sarA* and group I *agr* loci. For *bbp*, *eta*, *etb*, *hlg*, *pvl*, *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *tst*, *chp*, *efb*, *V8*, *arcA* and *coa*, the following strains from our collection were used, respec-

Table 2. PCR primers, primers and probes for real time PCR and conditions used in PCR and real time PCR assays.

Gene	Primers and probes descriptions	Sequence (5'-3')	Size of amplified product (bp)	PCR and Real Time PCR conditions cycling
23S rRNA	Forward	ACGGAGTTACAAAGGACGAC	1251	94°C 5 min; 37 × (94°C 40 s, 64°C 60 s, 72°C 75 s); 72°C 10 min.
	Reverse	AGC TCAGCCTTAACGAGTAC		
<i>femA</i>	Forward	AGACAAATAGGAGTAATGAT	509	94°C 5 min; 25 × (94°C 60 s, 57°C 60 s, 72°C 60 s); 72°C 10 min.
	Reverse	AAATCTAACTGAGTGATA		
<i>femB</i>	Forward	TTACAGAGTTAACTGTTACC	651	
	Reverse	ATACAAATCCAGCACGCTCT		
<i>nuc</i>	Forward	GCGATTGATGGTGATACGGTT	279	94°C 5 min; 37 × (94°C 60 s, 55°C 30 s, 72°C 30 s); 72°C 7 min.
	Reverse	ACGCAAGCCTTGACGAACTAAAGC		
<i>mecA</i>	Forward	GTAGAAATGACTGAACGTCCGATAA	310	94°C 4 min; 30 × (94°C 45 s, 50°C 45 s, 72°C 60 s); 72°C 2 min.
	Reverse	CCAATTCCACATTGTTTCGGTCTAA		
<i>fnbA</i>	Forward	GCGGAGATCAAAGACAA	1279	94°C 5 min; 30 × (94°C 30 s, 50°C 30 s, 72°C 60 s); 72°C 7 min.
	Reverse	CCATCTATAGCTGTGTGG		
<i>fnbB</i>	Forward	GGAGAAGGA ATTAAGGCG	812	
	Reverse	GCCGTCGCCTTGAGCGT		
<i>spa</i> (X-region)	Forward	CAAGCACAAAAGAGGAA	Size polymorphisms	94°C 5 min; 30 × (94°C 60 s, 60°C 60 s, 72°C 60 s); 72°C 7 min.
	Reverse	CACCAGGTTTAACGACAT		
<i>clfA</i>	Forward	GGCTTCAGTGCTTGTAGG	Size polymorphisms	94°C 5 min; 35 × (94°C 60 s, 57°C 60 s, 72°C 60 s); 72°C 7 min.
	Reverse	TTTTTCAGGGTCA ATATAAGC		
<i>clfB</i>	Forward	TGGCGGCAAATTTTACAGTGACAGA	404	
	Reverse	AGAAATGTTTCGCGCCATTTGGTTT		
<i>cna</i>	Forward	TTCACAAGCTTGGTATCAAGAGCATGG	452	
	Reverse	GAGTGCCTTCCCAAACCTTTTGAGC		
<i>bbp</i>	Forward	TCAAAAGAAAAGCCAATGGCAAACG	500	
	Reverse	ACCGTTGGCGTGTAACCTGCTG		
<i>ebpS</i>	Forward	GCAAGTAATAGTGCTTCTGCCGCTTCA	550	
	Reverse	CATTTTCCGGTGAACCTGAACCGTAGT		
<i>map/eap</i>	Forward	GCATGATAGAGGTATCGGGGAACGTG	665	95°C 15 min; 32 × (95°C 60 s, 60°C 90 s, 72°C 60 s); 72°C 10 min.
	Reverse	TCCCTTGATCATTGCCATTGCTG		
<i>sdrC</i>	Forward	CGCATGGCAGTGAATACTGTTGCAGC	731	
	Reverse	GAAGTATCAGGGGTGAACTATCCACAAATTG		
<i>sdrD</i>	Forward	CCACTGGAAATAAAGTTGAAGTTCAACTGCC	467	
	Reverse	CCTGATTTAACTTTGTCATCAACTGTAATTTGTG		
<i>sdrE</i>	Forward	GCAGCAGCGCATGACGGTAAAG	894	
	Reverse	GTCGCCACCGCCAGTGTCTATA		
<i>eta</i>	Forward	CGCTGCGGACATTCTACATGG	676	
	Reverse	TACATGCCCGCCACTTGCTTGT		
<i>etb</i>	Forward	GAAGCAGCCAAAACCCATCGAA	419	
	Reverse	TGTTGTCCGCCTTACCCTGTGAA		

Continued

<i>hlg</i>	Forward	TTGGCTGGGGAGTTGAAGCACA	306	
	Reverse	CGCCTGCCCAGTAGAAGCCATT		
<i>pvl</i>	Forward	TGCCAGACAATGAATTACCCCAT	894	
	Reverse	TCTGCCATATGGTCCCAACCA		
<i>sea</i>	Forward	TTGCAGGGAACAGCTTTAGGCAATC	252	
	Reverse	TGGTGTACCACCCGCACATTGA		
<i>seb</i>	Forward	GACATGATGCCTGCACCAGGAGA	355	
	Reverse	AACAAATCGTTAAAAACGGCGACACAG		
<i>sec</i>	Forward	CCCTACGCCAGATGAGTTGCACA	602	
	Reverse	CGCCTGGTGCAGGCATCATATC		
<i>sed</i>	Forward	GAAAGTGAGCAAGTTGGATAGATTGCGGCTAG	830	
	Reverse	CCGCGCTGTATTTTCTCCGAGAG		
<i>see</i>	Forward	TGCCCTAACGTTGACAACAAGTCCA	532	
	Reverse	TCCGTGTAAATAATGCCTGCCTGAA		
<i>seg</i>	Forward	TGCTCAACCCGATCCTAAATTAGACGA	117	
	Reverse	CCTCTTCCTCAACAGGTGGAGACG		
<i>seh</i>	Forward	CATTCACATCATATGCGAAAGCAGAAG	358	
	Reverse	GCACCAATCACCCTTTCTGTGC		
<i>sei</i>	Forward	TGGAGGGGCCACTTTATCAGGA	220	
	Reverse	TCCATATTCTTTGCCTTACCAGTG		
<i>sej</i>	Forward	CTCCCTGACGTTAACACTACTAATAACCC	432	
	Reverse	TATGGTGGAGTAACACTGCATCAAAA		
<i>tst</i>	Forward	AGCCCTGCTTTTACAAAAGGGGAAAA	306	
	Reverse	CCAATAACCACCCGTTTTATCGCTTG		
<i>chp</i>	Forward	AACGGCAGGAATCAGTACACACCATC	479	
	Reverse	GGCAAGTTATGAAATGTCTGCCAAACC		
<i>efb</i>	Forward	CGGTCCAAGAGAAAAGAAACCAGTGAG	303	
	Reverse	TGTGCTTTTCTGTGTGCACTGACAGTATG		
<i>V8</i>	Forward	CAACGAATGGTCATTATGCACCCGTA	529	
	Reverse	TTTGGTACACCGCCCAATGAA		
<i>arcA</i>	Forward	CACGTAACCTGCTAGAACGAG	724	
	Reverse	GAGCCAGAAGTACGCGAG		
<i>icaA</i>	Forward	TCAGACACTTGCTGGCGCAGTC	936	
	Reverse	TCACGATTCTCTCCCTCTCTGCCATT		
<i>rbf</i>	Forward	GAATTCTAGAAAAGAGGTAAAGTTATGGC	1200	
	Reverse	CACTCATAAAAGCTTCTTC		
<i>sarA</i>	Forward	CAATCACTGTGTCTAATGAA	700	95°C 5 min; 40 × (95°C 30 s, 60°C 60 s, 72°C 30 s); 72°C 10 min.
	Reverse	GTGCCATTAGTGCAAACCTC		
<i>coa</i>	Forward	ATAGAGATGCTGGTACAGG	Size polymorphisms	95°C 5 min; 30 × (94°C 60 s, 58°C 60 s, 72°C 60 s); 72°C 10 min.
	Reverse	GCTTCCGATTGTTTCGATGC		

Continued

Agr group-specific multiplex PCR				
	PAN	ATGCACATGGTGCACATGC		
	agr1	GTCACA AGTACTATA AGCTGCGAT	441	
<i>agr</i>	agr2	TATTACTAATTGAAA AGTGCCATAGC	575	94°C 5 min; 26 × (94°C 30 s, 55°C 30 s, 72°C 60 s); 72°C 10 min.
	agr3	GTA ATGTAATAGCTTGATAATAATACCCAG	323	
	agr4	CGATAATGCCGTAATACCCG	659	
Real Time PCR				
	Probe	TGGATGTTGGTTCCAGAAACATTGGGAG		
<i>icaA</i>	Forward	TGAACCGCTTGCCATGTG		
	Reverse	CACGCGTTGCTTCCAAAGA		
	Probe	CCGCCACCGCCGAATTTACCACCA		
<i>gyrB</i>	Forward	AGTAACGGATAACGGACGTGGTA		95°C 5 min (Hot Start activation); 40 × (95°C 5 s, annealing/extension 60°C 10 s).
	Reverse	CCAACACCATGTAAACCACCAGAT		
<i>rbf</i>	Forward	TTAGAAGGAATCTTTAA AACCTTATTGAATAA		
	Reverse	TTGTGAATTTTCTTCTTCGGACA		
<i>sarA</i>	Forward	TTTTTTTACGTTGTTGTGCATTAACA		
	Reverse	CATTTAAACTACAAACAACCACAAGTTG		
<i>agr</i>	Forward	TGAAATTCGTAAGCATGACCCA		
	Reverse	CCATCGCTGCAACTTTGTAGAC		

tively: sa93, sa19, sa55, sa75, sa59, sa9, sa32, sa79, sa66, sa73, sa31, sa33, sa62, sa15, sa63, sa110, sa111, sa43, sa105 and sa35.

The expression of *gyrB*, *icaA*, *rbf*, *sarA* and *agrA* was measured using RT-PCR. To 500 µL of bacterial culture, grown in TSB at 37°C for 24 h, 1000 µL of RNA Protect Bacteria Reagent (Qiagen, Hilden, Germany) were added. Sample was vortexed for 30 seconds and incubated at room temperature for 5 minutes. Sample was centrifuged at 9400 rpm for 10 minutes and the bacterial cells were resuspended in 200 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) containing 10 mg/mL lysozyme and 40 mg/mL lysostaphin. Sample was vortexed by 10 seconds and incubated at room temperature for 5 minutes. Afterwards, extraction and purification of total RNA was done using the RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, including on-column DNase treatment. The concentration and purity of total RNA were analyzed using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Inc.).

To obtain cDNA, a QuantiTec Reverse transcription kit (Qiagen), which includes genomic DNA elimination, was used according to the manufacturer's instructions. The *icaA* probe contained the fluorescent reporter dye FAM covalently linked to the 5' end and BHQ-1 covalently linked to the 3' end as a quencher. The *gyrB* probe

contained the fluorescent reporter dye JOE covalently linked to the 5' end and BHQ-1 covalently linked to the 3' end as a quencher. A Rotor Gene Probe PCR Kit (Qiagen, Hilden, Germany) and Rotor Gene SYBR Green PCR Kit (Qiagen) kit were used for the real-time PCR assays.

The sa22, sa103 *S. aureus* clinical strains from our collection, *S. epidermidis* ATCC 35984 and *E. coli* ATCC 11775 were used as negative controls for the real-time PCR assays. *S. aureus* ATCC 33592 was used as the positive control.

2.3. Determination of Biofilm Formation

S. aureus biofilm formation was determined using the microtiter plate assay, as described in [15]. *S. epidermidis* ATCC 35984 and *S. epidermidis* ATCC 12228 were used as positive and negative controls, respectively. In this assay, polystyrene-adhered bacteria were stained with safranin, and the absorbance at 492 nm was recorded. Strains with an A_{492} less than 0.2, between 0.2 and 1.0, or greater than 1.0 were considered biofilm negative, weak biofilm formers, or strong biofilm formers, respectively [26].

2.4. Statistical Analysis

The frequencies of the virulence-related gene combina-

tions among the MRSA (*mecA*⁺) and MSSA (*mecA*⁻) *S. aureus* strains were analyzed using Chi-squared tests. A *p* value less than 0.005 was considered significant.

3. Results

Half of the catheter-isolated bacteria (55/109) were identified as *S. aureus* based on PCR amplification of the 23S rRNA, *nuc*, *clfA*, *coa*, *spa*, *femA* and *femB* genes. The remaining isolates (*n* = 54) were identified as *S. epidermidis*.

The most frequently detected virulence genes in the analyzed strains were i) adhesin-coding genes, including *spa* (100%), *clfA* (100%), *sdrD* (94.5%), *sdrE* (92.7%), *sdrC* (89%) and *ebps* (85.4%); ii) toxin-coding genes, including *hlg* (96.3%), *seg* (94.5%), *sei* (94.5%) and *seh* (78.1%); and iii) genes implicated in biofilm formation, including *icaA* (96.3%), *sarA* (74.5%) and *rbf* (40%) (Table 3). The *agr* operon was detected in all *S. aureus* strains; 16 strains harbored the group I *agr* locus, 29 the group II *agr* locus and 10 the group III *agr* locus (Table 3). Almost 82% of the strains were *mecA*⁺. None of the strains harbored the *pvl* or *sej* genes (Table 3).

The strains contained combinations of 15 to 25 of the 35 examined virulence genes (Table 4). Six strains (11%) carried a combination of 25 genes, with 12 genes coding for adhesins with affinity for fibronectin, fibrinogen and collagen and 5 genes coding for toxins, some of which can act as superantigens (Tables 1 and 4). Sixty percent of the strains carried a combination of the *seg*, *seh* and *sei* toxin genes (Table 4).

Fifteen strains carried six (*fnbA*, *cna*, *sdrE*, *eta*, *hlg*, *ica*) of the seven virulence genes that have been reported to be significantly more common in invasive *S. aureus* strains [19]. Six of these fifteen strains carried the 25-gene combination (Table 4), and the remaining nine strains each harbored a unique combination of virulence genes (data not shown).

3.1. Detection of *IcaA*, *Rbf*, *SarA* and *AgrA* Expression Using Real Time PCR

All of the *S. aureus* strains carrying *icaA* (*n* = 53), *rfb* (*n* = 22), *sarA* (*n* = 41) or *agr* (*n* = 55) expressed the genes (Figures 1(a)-(d)). Constitutive *gyrB* expression was used as a control (data not shown). The *T_m* was determined for each real time PCR run, when Rotor Gene SYBR Green PCR Kit was used, to differentiate specific amplification from nonspecific amplification (data not shown).

3.2. Determination of Biofilm Formation

Among the *S. aureus* strains studied, 70.9% (*n* = 39) were able to form a biofilm on polystyrene; 51% (*n* = 28) were weak biofilm formers, and 20% (*n* = 11) were strong biofilm formers.

Table 3. Frequency of *mecA* and virulence-related genes in *S. aureus* strains.

Gene	N° of strains positive for the gene (% of total)
Adhesins	
<i>spa</i>	55 (100)
<i>clfA</i>	55 (100)
<i>sdrD</i>	52 (94.5)
<i>sdrE</i>	51 (92.7)
<i>sdrC</i>	49 (89.0)
<i>ebps</i>	47 (85.4)
<i>clfB</i>	45 (81.8)
<i>bbp</i>	43 (78.2)
<i>cna</i>	43 (78.1)
<i>map/eap</i>	35 (63.6)
<i>fnbB</i>	31 (56.3)
<i>fnbA</i>	19 (34.5)
Toxins	
<i>hlg</i>	53 (96.3)
<i>seg</i>	52 (94.5)
<i>sei</i>	52 (94.5)
<i>seh</i>	43 (78.1)
<i>sea</i>	26 (47.2)
<i>eta</i>	23 (42.0)
<i>see</i>	15 (27.2)
<i>tst</i>	14 (25.4)
<i>etb</i>	13 (23.6)
<i>seb</i>	13 (23.6)
<i>sed</i>	6 (10.9)
<i>sec</i>	3 (5.4)
<i>pvl</i>	0 (0.0)
<i>sej</i>	0 (0.0)
Other genes	
<i>icaA</i>	53 (96.3)
<i>ebf</i>	51 (92.7)
<i>v8</i>	51 (92.7)
<i>arcA</i>	50 (90.9)
<i>chp</i>	49 (89.0)
<i>mecA</i>	45 (81.8)
<i>sarA</i>	41 (74.5)
<i>rbf</i>	22 (40.0)
Agr groups	
<i>agrII</i>	29 (52.7)
<i>agrI</i>	16 (29.1)
<i>agrIII</i>	10 (18.2)

Table 4. Combinations of virulence-related genes in *S. aureus* strains¹.

Virulence gene combinations in the <i>S. aureus</i> strains (n = 55)	N° of strains (%)	N° of genes per combination (n = 35) No. %
Adhesins: <i>spa, clfA, clfB, bbp, ebps, sdrC, sdrD, sdrE.</i>		
Toxins: <i>seg, seh, sei, hlg.</i>	10 (18.2)	20 (57.1)
Other genes: <i>icaA, sarA, chp, efb, v8, arcA, agr, mecA.</i>		
Adhesins: <i>spa, clfA, fnbA, fnbB, clfB, cna, bbp, ebps, map/eap, sdrC, sdrD, sdrE.</i>		
Toxins: <i>eta, hlg, seg, seh, sei.</i>	6 (11.0)	25 (71.4)
Other genes: <i>icaA, sarA, chp, efb, v8, arcA, agr, mecA.</i>		
Adhesins: <i>spa, clfA, bbp, ebps, map/eap, sdrC, sdrD, sdrE.</i>		
Toxins: <i>seg, seh, sei.</i>	5 (9.0)	17 (48.5)
Other genes: <i>icaA, chp, efb, v8, arcA, agr.</i>		
Adhesins: <i>spa, clfA, bbp, map/eap, sdrC, sdrD, sdrE.</i>		
Toxins: <i>hlg, seg, seh, sei.</i>	2 (3.6)	17 (48.5)
Other genes: <i>icaA, chp, efb, v8, arcA, agr.</i>		
Adhesins: <i>spa, clfA, clfB, cna, bbp, ebps, map/eap, sdrC, sdrE.</i>		
Toxins: <i>eta, tst, sea, hlg, seg, seh, sei.</i>	2 (3.6)	22 (62.8)
Other genes: <i>icaA, chp, efb, v8, agr, mecA.</i>		
Adhesins: <i>spa, clfA, cna, bbp, ebps, map/eap, sdrE.</i>		
Toxins: <i>hlg, seg, seh, sei.</i>	2 (3.6)	17 (48.5)
Other genes: <i>icaA, sarA, v8, arcA, agr, mecA.</i>		
Adhesins: <i>spa, clfA, clfB, ebps, sdrE.</i>		
Toxins: <i>hlg, seg, seh, sei.</i>	2 (3.6)	15 (42.8)
Other genes: <i>icaA, chp, efb, v8, agr, mecA.</i>		
Adhesins: <i>spa, clfA, fnbA, fnbB, clfB, cna, sdrD.</i>		
Toxins: <i>hlg, seg, sei.</i>	2 (3.6)	15 (42.8)
Other genes: <i>icaA, chp, arcA, agr, mecA.</i>		
Adhesins: <i>spa, clfA, sdrC, sdrD, sdrE.</i>		
Toxins: <i>hlg, seg, sei.</i>	2 (3.6)	15 (42.8)
Other genes: <i>icaA, sarA, chp, efb, arcA, agr, mecA.</i>		
Adhesins: <i>spa, clfA, cna, sdrC, sdrD, sdrE.</i>		
Toxins: <i>hlg, seg, seh, sei.</i>	2 (3.6)	18 (51.4)
Other genes: <i>icaA, rbf, sarA, efb, v8, arcA, agr, mecA.</i>		
Adhesins: <i>spa, clfA, fnbB, clfB, cna, ebps, sdrC, sdrD, sdrE.</i>		
Toxins: <i>hlg, seg, seh, sei.</i>	2 (3.6)	21 (60.0)
Other genes: <i>icaA, sarA, chp, efb, v8, arcA, agr, mecA.</i>		
Adhesins: <i>spa, clfA, fnbB, clfB, cna, bbp, ebps, sdrC, sdrD, sdrE.</i>		
Toxins: <i>hlg.</i>	2 (3.6)	18 (51.4)
Other genes: <i>icaA, chp, efb, v8, arcA, agr, mecA.</i>		
Other combinations	16 (29.0)	
The most common combination of genes in the strains		
<i>icaA, agr, spa, clfA, sdrC, sdrD, sdrE, seg, seh, sei.</i>	37 (67.2)	10 (28.5)

¹There were no statistically significant differences in the frequencies of the virulence-related gene combinations among the MRSA (*mecA*+) and MSSA (*mecA*-) strains ($p < 0.005$).

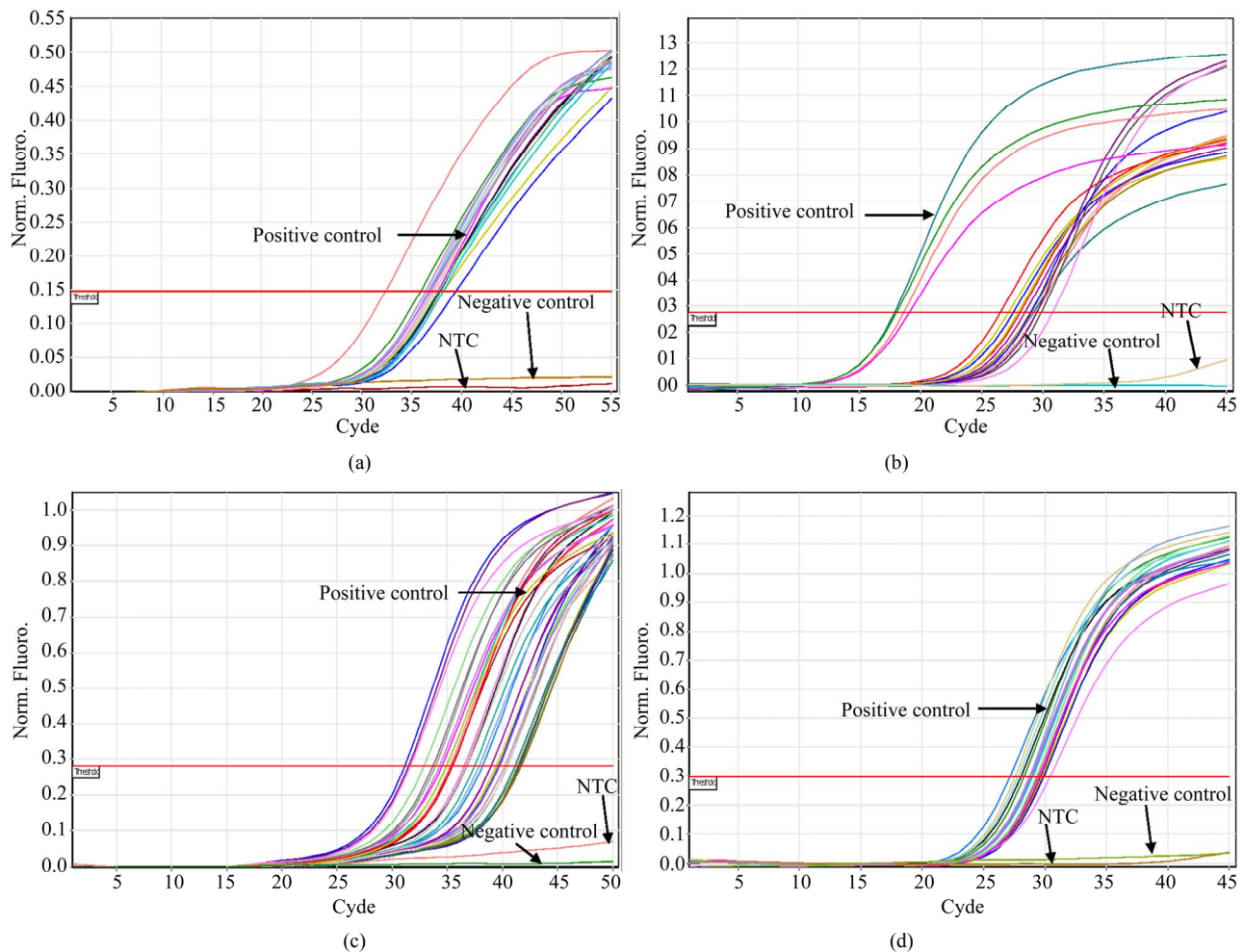


Figure 1. Detection of *icaA* (a), *rbf* (b), *sarA* (c), and *agrA* (d) expression in *S. aureus* strains isolated from catheters using real time PCR. *S. aureus* ATCC 33592 was used as a positive control; the negative controls were *S. epidermidis* ATCC 35984 (a), sa22 (b), sa103 (c) and *E. coli* ATCC 11775 (d). NTC indicates the no DNA control.

4. Discussion

In this study, we found that 55 of 109 catheters in hemodialysis patients harbored *S. aureus*. This high incidence of *S. aureus*-positive catheters may be due to a deficiency in the management of patients within the different hospital settings and/or the high rate of autoinfection by the patients' own indigenous *S. aureus*, which is present in their anterior nares [27].

Despite reports of severe catheter-related *S. aureus* infections [21-23,28], the *S. aureus* strains responsible for these infections have not been genetically characterized. While the genotype of *S. aureus* has been suggested to contribute to the severity of the infection [29], the *S. aureus* virulence factors that are involved in catheter colonization and the subsequent bacteremia are not known. Bacterial adhesins, toxins, and genes involved in bacterial dispersion may be involved in the development of catheter-related infections. Therefore, to gain some insight into the combinations of genes present in catheter-

colonizing *S. aureus* strains, the presence of 35 virulence genes coding for adhesins, toxins, or other products was determined in *S. aureus* strains isolated from tunneled hemodialysis catheters. As expected, a large number of strains carried genes for at least five adhesins (maximum of 12, average of 7; **Table 4**), with the combination of *spa*, *clfA*, *sdrC*, *sdrD* and *sdrE* being the most frequently detected combination. The Sdr proteins in *S. aureus* are members of the MSCRAMMs family (Microbial Surface Components-Recognizing Adhesive Matrix Molecules) that are encoded by the tandemly arrayed *sdrC*, *sdrD* and *sdrE* genes [30]. Although the precise role of Sdr adhesins in staphylococcal infection is not known, a strong correlation between the *sdr* genes of *S. aureus* and certain diseases has been reported. There is a significantly increased prevalence of the *sdrE* gene in invasive *S. aureus* strains [31], in *S. aureus* strains responsible for osteomyelitis [32], and in *S. aureus* isolates responsible for bone infections [33].

Among the toxin genes, *hlg* (96.3%), *seg* (92.7%), *sei* (85.4%) and *seh* (78.1%) were the most frequently detected; no *sej*-positive or *pvl*-positive strains were found, and the most frequently observed combination was *seg*, *seh*, and *sei* (74.5%; **Table 4**). The frequency of toxin-encoding genes in the catheter-colonizing strains was higher than the previously reported frequency in a series of 100 MRSA isolates from hospital infections; with *seg* (77%) and *sei* (77%) being the most frequently detected toxin-encoding genes [34]. However, it has been reported that invasive *S. aureus* strains were not more likely than noninvasive strains to carry the *seg*, *seh* and *sei* genes [31].

The virulence markers *ebf*, *v8*, *arcA* and *chp* were identified in most *S. aureus* strains (**Table 2**), with frequencies similar to those reported for *S. aureus* strains isolated from complicated skin and skin-structure infections [35].

All strains carrying genes involved in biofilm formation and bacterial dispersion expressed the genes (**Figure 1**), and 70.9% of the examined strains formed a biofilm. This is an important bacterial virulence property as it has been reported that staphylococci account for more than 60% of all identified pathogens in central venous catheter-related infections [36]. Moreover, in a Center for Disease Control report of 2005, 15% of all reported MRSA infections occurred in dialysis patients [3], and a rate of 0.88 MRSA bacteremias/100 hemodialysis patients/year was reported in England for 2008-2009 [37].

Thirty-seven *S. aureus* isolates (67.2%) had the virulence gene combination of *icaA*, *agr*, *spa*, *clfA*, *sdrC*, *sdrD*, *sdrE*, *seg*, *seh* and *sei* (**Table 4**), a combination that was detected in 16 different gene patterns (**Table 4**); 30 of these strains were *mecA*⁺. This result suggests that this gene combination may be important for successful catheter colonization. We conclude that these *S. aureus* strains containing such array of virulence factors are an element of risk for catheterized Mexican patients, which may acquire bacteremia or other pathologies.

These findings could be used to develop more targeted treatment strategies for *S. aureus* infections, which would be of great interest to vaccine researchers. The discovered gene combination could also be used to quickly identify *S. aureus* strains that are a greater threat to catheterized patients, which would warrant more aggressive treatment in a hospital setting.

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REFERENCES

- [1] P. Viale and S. Stefani, "Vascular Catheter-Associated Infections: A Microbiological and Therapeutic Update," *Journal of Chemotherapy*, Vol. 18, No. 3, 2006, pp. 235-249.
- [2] I. M. Gould, "The Clinical Significance of Methicillin-Resistant *Staphylococcus aureus*," *The Journal of Hospital Infection*, Vol. 61, No. 4, 2005, pp. 277-282. doi:10.1016/j.jhin.2005.06.014
- [3] Centers for Diseases Control, "Invasive Methicillin-Resistant *Staphylococcus aureus* Infections among Dialysis Patients—United States, 2005," *Morbidity and Mortality Weekly Report*, Vol. 56, No. 9, 2007, pp. 197-199.
- [4] F. Götz, "Staphylococcus and Biofilms," *Molecular Microbiology*, Vol. 43, No. 6, 2002, pp. 1367-1378. doi:10.1046/j.1365-2958.2002.02827.x
- [5] D. Cue, M. G. Lei, T. T. Luong, L. Kuechenmeister, P. M. Dunman, S. O'Donnell, S. Rowe, J. P. O'Gara and C. Y. Lee, "Rbf Promotes Biofilm Formation by *Staphylococcus aureus* via Repression of *icaR*, a Negative Regulator of *icaADBC*," *Journal of Bacteriology*, Vol. 191, No. 20, 2009, pp. 6363-6373. doi:10.1128/JB.00913-09
- [6] K. E. Beenken, J. S. Blevins and M. S. Smeltzer, "Mutation of *SarA* in *Staphylococcus aureus* Limits Biofilm Formation," *Infection and Immunity*, Vol. 71, No. 7, 2003, pp. 4206-4211. doi:10.1128/IAI.71.7.4206-4211.2003
- [7] J. Valle, A. Toledo-Arana, C. Berasain, J. M. Ghigo, B. Amorena, J. R. Penadés and I. Lasa, "SarA and Not Sigma-B Is Essential for Biofilm Development by *Staphylococcus aureus*," *Molecular Microbiology*, Vol. 48, No. 4, 2003, pp. 1075-1087. doi:10.1046/j.1365-2958.2003.03493.x
- [8] E. Mongodin, O. Bajolet, J. Cutrona, N. Bonnet, F. Dupuit, E. Puchelle and S. de Bentzmann, "Fibronectin-Binding Proteins of *Staphylococcus aureus* Are Involved in Adherence to Human Airway Epithelium," *Infection and Immunity*, Vol. 70, No. 2, 2002, pp. 620-630.
- [9] W. Navarre and O. Schneewind, "Surface Proteins of Gram-Positive Bacteria and Mechanisms of Their Targeting to the Cell Wall Envelope," *Microbiology and Molecular Biology Reviews*, Vol. 63, No. 1, 1999, pp. 174-229.
- [10] S. J. Peacock, N. P. J. Day, M. G. Thomas, A. R. Berendt and T. J. Foster, "Clinical Isolates of *Staphylococcus aureus* Exhibit Diversity in *fnb* Genes and Adhesion to Human Fibronectin," *The Journal of Infection*, Vol. 41, No. 1, 2000, pp. 23-31. doi:10.1053/jinf.2000.0657
- [11] N. Palmqvist, T. Foster, A. Tarkowski and E. Josefsson, "Protein A Is a Virulence Factor in *Staphylococcus aureus* Arthritis and Septic Death," *Microbial Pathogenesis*, Vol. 33, No. 5, 2003, pp. 239-249. doi:10.1006/mpat.2002.0533
- [12] S. A. Hienz, T. Schennings, A. Heimdahl and J. L. Flock, "Collagen Binding of *Staphylococcus aureus* Is a Virulence Factor in Experimental Endocarditis," *The Journal of Infectious Diseases*, Vol. 174, No. 1, 1996, pp. 83-88. doi:10.1093/infdis/174.1.83
- [13] M. O. Elasmri, J. R. Thomas and R. A. Skinner, "*Staphylococcus aureus* Collagen Adhesin CONTRIBUTES to the

- Pathogenesis of Osteomyelitis," *Bone*, Vol. 30, No. 1, 2002, pp. 275-280. doi:10.1016/S8756-3282(01)00632-9
- [14] M. N. Rhem, E. M. Lech, J. M. Patti, D. McDevitt, M. Höök, D. B. Jones and K. R. Wilhelmus, "The Collagen-Binding Adhesin Is a Virulence Factor in *Staphylococcus aureus* Keratitis," *Infection and Immunity*, Vol. 68, No. 6, 2000, pp. 3776-3779. doi:10.1128/IAI.68.6.3776-3779.2000
- [15] P. Moreillon, J. M. Entenza, P. Francioli, D. McDevitt, T. J. Foster, P. Francois and P. Vaudaux, "Role of *Staphylococcus aureus* Coagulase and Clumping Factor in Pathogenesis of Experimental Endocarditis," *Infection and Immunity*, Vol. 63, No. 12, 1995, pp. 4738-4743.
- [16] E. Josefsson, O. Hartford, L. O'Brien, J. M. Patti and T. Foster, "Protection against Experimental *Staphylococcus aureus* Arthritis by Vaccination with Clumping Factor A, a Novel Virulence Determinant," *The Journal of Infectious Diseases*, Vol. 184, No. 12, 2001, pp. 1572-1580. doi:10.1086/324430
- [17] J. Vernachio, A. S. Bayer, T. Le, Y. L. Chai, B. Prater, A. Schneider, B. Ames, P. Syribeys, J. Robbins and J. M. Patti, "Anti-Clumping Factor A Immunoglobulin Reduces the Duration of Methicillin-resistant *Staphylococcus aureus* Bacteremia in an Experimental Model of Infective Endocarditis," *Antimicrobial Agents and Chemotherapy*, Vol. 47, No. 11, 2003, pp. 3400-3406. doi:10.1128/AAC.47.11.3400-3406.2003
- [18] J. Higgings, A. Louhman, K. P. van Kessel, J. A. van Strijp and T. J. Foster, "Clumping Factor A of *Staphylococcus aureus* Inhibits Phagocytosis by Human Polymorphonuclear Leucocytes," *FEMS Microbiology Letters*, Vol. 258, No. 2, 2006, pp. 290-296.
- [19] S. Watanabe, T. Ito, T. Sasaki, S. Li, I. Uchiyama, K. Kishii, K. Kikuchi, R. L. Skov and K. Hiramatsu, "Genetic Diversity of Staphylocoagulase Genes (*coa*): Insight into the Evolution of Variable Chromosomal Virulence Factors in *Staphylococcus aureus*," *PLoS One*, Vol. 4, No. 5, 2009, Article ID: e5714. doi:10.1371/journal.pone.0005714
- [20] G. Ji, R. C. Beavis and R. P. Novick, "Cell Density Control of Staphylococcal Virulence Mediated by an Octapeptide Pheromone," *Proceedings of the National Academy of Science USA*, Vol. 92, No. 26, 1995, pp. 12055-12059. doi:10.1073/pnas.92.26.12055
- [21] J. J. Ayala-Gaytán, M. C. Alemán-Bocanegra, C. E. Guajardo-Lara and S. B. Valdovinos-Chávez, "Catheter-Associated Bloodstream Infections. Review of Five-Year Surveillance among Hospitalized Patients," *Revista Médica del Instituto Mexicano del Seguro Social*, Vol. 48, No. 2, 2010, pp. 145-150.
- [22] G. Jean, "Incidence and Risk Factors for Infections from Hemodialysis Catheters," *Néphrologie*, Vol. 22, No. 8, 2001, pp. 443-448.
- [23] G. Jean, B. Charra, C. Chazot, T. Vanel, J. C. Terrat, J. M. Hurot and G. Laurent, "Risk Factors Analysis for Long-Term Tunneled Dialysis-Catheter Related Bacteremia," *Nephron*, Vol. 91, No. 3, 2002, pp. 399-405. doi:10.1159/000064279
- [24] M. G. Parker and B. N. Doebbeling, "The Challenge of Methicillin-Resistant *Staphylococcus aureus* Prevention in Hemodialysis Therapy," *Seminars in Dialysis*, Vol. 25, No. 1, 2012, pp. 42-49. doi:10.1111/j.1525-139X.2011.00999.x
- [25] K. Plata, A. E. Rosato and G. Wegrzyn, "*Staphylococcus aureus* as an Infectious Agent: Overview of Biochemistry and Molecular Genetics of Its Pathogenicity," *Acta Biochimica Polonica*, Vol. 56, No. 4, 2009, pp. 597-612.
- [26] T. Møretrø, L. Hermansen, A. L. Holck, M. S. Sidhu, K. Rudi and S. Langsrud, "Biofilm Formation and the Presence of the Intercellular Adhesion Locus *ica* among Staphylococci from Food and Food Processing Environments," *Applied and Environmental Microbiology*, Vol. 69, No. 9, 2003, pp. 5648-5655. doi:10.1128/AEM.69.9.5648-5655.2003
- [27] K. Toshkova, C. Annemüller, O. Akineden and C. Lämmler, "The Significance of Nasal Carriage of *Staphylococcus aureus* as Risk Factor for Human Skin Infections," *FEMS Microbiology Letters*, Vol. 202, No. 1, 2001, pp. 17-24. doi:10.1111/j.1574-6968.2001.tb10774.x
- [28] R. Stolić, G. Trajković, V. Perić, A. Jovanović, D. Stolić, S. Sovtić, T. Lazarević, Z. Zivić and G. Subarić-Gorgieva, "Central Venous Catheters in Hemodialysis: To Accept Recommendations or to Stick to Own Experience," *Vojnosanitetski Pregled*, Vol. 65, No. 1, 2008, pp. 21-26. doi:10.2298/VSP0801021S
- [29] V. G. Fowler Jr., C. L. Nelson, L. M. McIntyre, B. N. Kreiswirth, A. Monk, G. L. Archer, J. Federspiel, S. Naidich, B. Remortel, T. Rude, P. Brown, L. B. Reller, G. R. Corey and S. R. Gill, "Potential Associations between Hematogenous Complications and Bacterial Genotype in *Staphylococcus aureus* Infection," *The Journal of Infectious Diseases*, Vol. 196, No. 5, 2007, pp. 738-747. doi:10.1086/520088
- [30] E. Josefsson, K. W. McCrea, D. Ni Eidhin, D. O'Connell, J. Cox, M. Höök and T. J. Foster, "Three New Members of the Serine-Aspartate Repeat Protein Multigene Family of *Staphylococcus aureus*," *Microbiology*, Vol. 144, No. 12, 1998, pp. 3387-3395. doi:10.1099/00221287-144-12-3387
- [31] S. J. Peacock, C. E. Moore, A. Justice, M. Kantzanou, L. Story, K. Mackie, G. O'Neill and N. P. Day, "Virulent Combinations of Adhesin and Toxin Genes in Natural Populations of *Staphylococcus aureus*," *Infection and Immunity*, Vol. 70, No. 9, 2002, pp. 4987-4996. doi:10.1128/IAI.70.9.4987-4996.2002
- [32] A. Sabath, D. C. Melles, G. Martirosian, H. Grundmann, A. van Belkum and W. Hryniewicz, "Distribution of the Serine-Aspartate Repeat Protein-Encoding *sdr* Genes among Nasal and Invasive *Staphylococcus aureus* Strains," *Journal of Clinical Microbiology*, Vol. 44, No. 3, 2006, pp. 1135-1138. doi:10.1128/JCM.44.3.1135-1138.2006
- [33] S. Trad, J. Allignet, L. Frangeul, M. Davi, M. Vergassola, E. Couve, A. Morvan, A. Kechrid, C. Buchrieser, P. Glaser and N. El-Sohl, "DNA Microarray for Identification and Typing of *Staphylococcus aureus* Isolates," *Journal of Clinical Microbiology*, Vol. 42, No. 5, 2004, pp. 2054-2064. doi:10.1128/JCM.42.5.2054-2064.2004
- [34] P. Sauer, J. Síla, T. Stosová, R. Vecerová, P. Hejnar, I.

- Vágnerová, M. Kolár, V. Raclavsky, J. Petzelová, Y. Lovecková and D. Koukalová, "Prevalence of Genes Encoding Extracellular Virulence Factors among Methicillin-Resistant *Staphylococcus aureus* Isolates from the University Hospital, Olomouc, Czech Republic," *Journal of Medical Microbiology*, Vol. 57, No. 4, 2008, pp. 403-410. [doi:10.1099/jmm.0.47413-0](https://doi.org/10.1099/jmm.0.47413-0)
- [35] S. J. Campbell, H. S. Deshmukh, C. L. Nelson, I. G. Bae, M. E. Stryjewski, J. J. Federspiel, G. T. Tonthat, T. H. Rude, S. L. Barriere, R. Corey and V. G. Fowler Jr., "Genotypic Characteristics of *Staphylococcus aureus* Isolates from a Multinational Trial of Complicated Skin and Skin Structure Infections," *Journal of Clinical Microbiology*, Vol. 46, No. 2, 2008, pp. 678-684. [doi:10.1128/JCM.01822-07](https://doi.org/10.1128/JCM.01822-07)
- [36] J. S. Groeger, A. B. Lucas, H. T. Thaler, H. Friedlander-Klar, A. E. Brown, T. E. Kiehn and D. Armstrong, "Infectious Morbidity Associated with Long-Term Use of Venous Access Devices in Patients with Cancer," *Annals of Internal Medicine*, Vol. 119, No. 12, 1993, pp. 1168-1174.
- [37] R. Fluck, J. Wilson and C. R. Tomson, "UK Renal Registry 12th Annual Report (December 2009): Chapter 12: Epidemiology of Methicillin Resistant *Staphylococcus aureus* Bacteraemia amongst Patients Receiving Dialysis for Established Renal Failure in England in 2008: A Joint Report from the UK Renal Registry and the Health Protection Agency," *Nephron Clinical Practice*, Vol. 115, No. 1, 2010, pp. 261-270. [doi:10.1159/000301235](https://doi.org/10.1159/000301235)