International Journal of Medical Microbiology 311 (2021) 151471

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

A longitudinal study shows intermittent colonization by *Staphylococcus aureus* with a high genetic diversity in hemodialysis patients

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ARTICLE INFO

Keywords:
Hemodialysis
Colonization
Staphylococcus aureus
Longitudinal analysis
Molecular epidemiology

ABSTRACT

Staphylococcus aureus colonization increases the risk of invasive infections in different groups of patients. We analyzed the dynamics and factors associated with *S. aureus* colonization in hemodialysis patients. A longitudinal study was conducted at a dialysis center associated with a tertiary health care institution. *S. aureus* colonization was assessed three times in nostrils and on the skin and was classified as absent, intermittent or persistent. The molecular analysis included pulsed-field gel electrophoresis (PFGE) and *spa*-typing. Clonal complex was inferred from *spa*-typing. A model of generalized estimating equations was performed to determine the factors associated with colonization. A total of 210 patients were included. Colonization by methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) isolates was 29.1 % vs. 4.8 %, 29.2 % vs. 6.7 % and 24.1 % vs. 7.1 % in the first, second and third screenings respectively. Most of the colonized patients were intermittent carriers (77.8 %, n = 63). PFGE and *spa*-typing revealed a high genetic diversity. One third (33.3 %) of the carriers classified as persistent had different clones during follow-up. Clonal complex 8 was frequent among MSSA (28 %) and MRSA (59 %) isolates. Current smoking (OR:7.22, 95 %CI 2.24–23.27), Charlson index (OR:1.22, 95 %CI 1.03–1.43) and previous infection by *S. aureus* (OR:2.41; 95 %CI:1.09−5.30) were associated with colonization by this microorganism. Colonization increased the risk of bacteremia (HR = 4.9; 95 % CI: 1.9–12.9). In conclusion, the colonization by *S. aureus* in hemodialysis patients changes over time and acquisition of new clones is a frequent event. These results evidence that patients are repeatedly recolonizing from hospitals, dialysis units and their homes. On the other hand, factors not associated with healthcare, as smoking, can increase the risk of colonization.

1. Introduction

Colonization by *Staphylococcus aureus* has been described as an important risk factor for the development of invasive infections in different groups of patients (Maamoun et al., 2011). Patients undergoing hemodialysis are a particularly affected group, greatly exceeding colonization rates reported in other types of healthcare exposure (Maamoun et al., 2011; Rao et al., 2010). This is due to the prolonged use of catheters, the frequent administration of antibiotics, multiple hospitalizations, immune response alterations, the presence of different comorbidities, and close contact with healthcare settings (Centers for Disease Control and Prevention, 2005). *Staphylococcus aureus* causes up to 70 % of vascular access infections in hemodialysis patients, and the problem worsens by the dissemination of methicillin-resistant *S. aureus* (MRSA) isolates (Maamoun et al., 2011). About 90 % of patients with MRSA infections require hospitalization, and 17 % die during hospital stay (Rao et al., 2010; Centers for Disease Control and Prevention, 2005).

Colonization is even more frequent than infection, and patients can remain colonized for a long time, increasing not only the risk of

Abbreviations: CC, clonal complex; MIC, minimum inhibitory concentration; UPGMA, unweighted pair group method using arithmetic averages; MRSA, methicllin-resistant *Staphylococcus aureus*; MSSA, methicllin-susceptibe *Staphylococcus aureus*; CLSI, clinical and laboratory standards Institute; ICU, intensive care unit; CDC, Centers for Disease Control and Prevention; PFGE, pulsed-field gel electrophoresis; GEE, generalized estimating equation; MLST, multilocus sequence typing; ERIC, enterobacterial repetitive intergenic consensus.

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https://doi.org/10.1016/j.ijmm.2020.151471
Received 21 August 2020; Received in revised form 29 October 2020; Accepted 21 December 2020
Available online 26 December 2020

infections but also the possibility of transmission of *S. aureus* isolates between the dialysis center, hospitals and the community since hemodialysis patients circulate frequently in these settings (Patel et al., 2011; Price et al., 2015).

Colonization is an event that could change over time, so a cross-sectional approach is not sufficient to estimate the frequency and associated factors with this outcome (Bogut et al., 2007). Follow-up studies with at least three screenings are necessary to establish the carrier status that does not underestimate the overall results (Bogut et al., 2007). Furthermore, it is necessary to apply molecular typing methods with high discriminatory power that allow the differentiation of strains from several samples in the same patient, the identification of persistent carriers, and the evaluation of the existence of horizontal transmission of colonizing isolates between hemodialysis patients to suggest sources of transmission (Bogut et al., 2007).

Taking into account the above, the aim of this study was to analyze the dynamics and the factors associated with colonization by *S. aureus* in a group of hemodialysis patients in a renal center in Medellín (Colombia). This information will allow the establishment of measures to prevent the spread of this bacteria in hemodialysis patients and to decrease the risk of invasive infections such as bacteremia.

2. Materials and methods

2.1. Study design

A longitudinal study that assessed the change over time of colonization by *S. aureus* was conducted. Colonization screenings were performed at three times: at the beginning of the study, two months after, and then six months after. Carrier status was defined according to CDC (Centers for Disease Control and Prevention) as the presence of bacteria in a body site without signs or symptoms of a disease (Horan et al., 2008). Colonization status was classified according to phenotypic methods as absent, intermittent, and persistent in patients with three completed screenings: absent if all the screenings were negative, intermittent if one or two screenings were positive and persistent if three screenings were positive. The clearance of colonization was defined as the presence of two consecutive negative cultures.

2.2. Setting

The study was conducted at an ambulatory dialysis center associated with a hospital in Medellín (Colombia), from October 2017 to October 2019. This center has 72 stations and provides outpatient hemodialysis to approximately 350 adults. Standard precautions are followed by all healthcare workers, according to CDC recommendations (Centers for Disease Control and Prevention, 2001). Active surveillance for the detection of colonization by *S. aureus* was not implemented either before or during this study. In this regard, routinely swab for MSSA and MRSA is not performed and decolonization protocols are not used in the dialysis center.

2.3. Participants

All patients of 18 years old or older, with chronic kidney disease and central venous catheter in hemodialysis were included. The exclusion criteria were an imminent change of dialysis center, rejection of the informed consent, an imminent change to peritoneal dialysis, mental illness, and transplantation.

2.4. Variables

The primary end point was the change over time of colonization by *S. aureus,* measured as a categorical variable (1 = colonized, 0 = non-colonized) in each one of the three screenings (baseline, and at two and six months later). The exposures evaluated included time on dialysis (less than 1 year), use of beta-lactam antibiotics in the last six months, current smoking, comorbidities (measured as Charlson index) (Crooks et al., 2015), Karnosfky index (Perez Valdivieso et al., 2007), previous hospitalization, history of *S. aureus* infection, and catheter type.

2.5. Epidemiological data

The epidemiological information was obtained from medical records and interviews with each patient. The information included socio-demographic, clinical and dialysis characteristics like age, sex, previous hospitalization, antimicrobial use, intensive care unit (ICU) stay, site of colonization, comorbidities, catheter type, ward, and dialysis time. Additionally, the time-to-first-bacteremia was analyzed using the baseline status and time-dependent nature of colonization at 12-month follow-up. The interviews were performed and the medical records reviewed by two trained investigators using a questionnaire previously standardized in a pilot study, which was conducted immediately before starting the recruitment. The consistency of the information obtained from the questionnaires and that registered on a database was evaluated for each patient.

2.6. Colonization screenings

All patients were screened for *S. aureus* colonization in the nostrils and on the skin surrounding the catheter using sterile cotton swabs with a sterile 0.9 % saline solution. Each swab was inserted about 1 cm and rotated three or four times along the nasal septum of both anterior nares. Regarding the catheter, the same procedure was repeated, applying slight pressure to the skin. The swabs were subsequently placed in an Amies transport medium with charcoal, enriched in trypticase soy broth (TSB) overnight at 37 °C, and then cultured on mannitol salt agar. The preliminary identification of *S. aureus* was conducted by standard laboratory methods based on colony morphology in sheep blood agar, and positive catalase and coagulase tests. The antibiotic susceptibility was determined using the automated Vitek 2 system (bioMérieux, Marcy l'Etoile, France) according to 2018 CLSI cutoff points (CLSI, 2018). Three colonies were picked for phenotypic and molecular characterization of the isolates. Additionally, when two or more morphotypes in the culture, each one of them was identified and evaluated to confirm if they belong to the same clone.

2.7. Molecular confirmation of methicillin-resistant S. aureus (MRSA)

DNA was extracted from the isolates using the Wizard Genomic DNA purification kit (Promega) according to the manufacturer's instructions. The presence of the *nuc* (species-specific thermonuclease) and *mec*A (determinant of methicillin resistance) genes was verified by a polymerase chain reaction (PCR) as previously described (Brakstad et al., 1992; Kondo et al., 2007). Similarly, SCC*mec* types and subtypes were determined using two sets of multiplex PCR reactions according to protocols previously reported (Kondo et al., 2007; Milheiriço et al., 2007).

2.8. Molecular typing

Genetic relatedness of isolates was assessed using pulsed-field gel electrophoresis (PFGE), which was performed using the *Sma*I restriction enzyme (Thermo Scientific, United States) (Mulvey et al., 2001). DNA fragment patterns were normalized using the bacteriophage lambda ladder PFGE marker (New England BioLabs, United Kingdom). Electrophoresis was performed on a CHEF DR III (Bio-Rad Laboratories, Hercules, CA) at 11 °C. Cluster analysis was performed using the Dice coefficient with BioNumerics software version 6.0 (Applied Maths, SintMartens-Latem, Belgium). Dendrograms were generated by the unweighted pair group method using average linkages (UPGMA), with 1% tolerance and 0.5 % optimization settings. A similarity cutoff of 80 %

was used to define genetically related strains. Strains with a cutoff of 100 % were considered indistinguishable genetically. Additionally, the polymorphic X region of the protein A gene (*spa*) was amplified and sequenced, and corresponding *spa*-types were assigned using the *spa*-typing website (http://www.spaserver.ridom.de/) (Shopsin et al., 1999). Clonal complexes (CC) were inferred by *spa* repeat pattern analysis in a sample of 138 (61.9 %) *S. aureus* isolates representing the most frequent patterns obtained by PFGE (Mathema et al., 2008).

2.9. Statistical analyses

Categorical variables were described as absolute and relative frequencies. Continuous variables were expressed as mean and standard deviation or median and interquartile range, according to the assumption or not of the normality.

To determine associated factors with colonization, a model of generalized estimating equations (GEE) was performed, as colonization was measured on three occasions and can change over time. As a result, the observations are not independent, and a correlation structure should be assumed. Therefore, the odds of colonization by *S. aureus* were estimated using a GEE model for a binomial distribution, assuming an exchangeable correlation and a robust estimator of variance. Each exposure was analyzed in a different model and confounding variables were included, as previously suggested in literature, associated with each exposure and with the colonization by *S. aureus* (Kluytmans and Wertheim, 2005; Karanika et al., 2015; Wang et al., 2012; Robicsek et al., 2011). Measures of association (odds ratio – OR) were expressed with their corresponding 95 % confidence intervals (95 % CI) and p-value. The time-to-first-bacteremia was analyzed using the baseline status and time-dependent nature of colonization with methods of survival analysis for staggered entries. Statistical analyses were carried out using STATA software, version 14.0.

3. Results

Of 393 eligible patients, 259 patients met the inclusion criteria and 210 were enrolled in the study of which 178 completed the second screening and 141 the third one (Fig. 1). Regarding to the population characteristics, 50.5 % (n = 106) were female and the median age was 62 years (IQR: 51.9−71.1). The medical histories of patients revealed frequent hospitalization history (69 %, n = 145) and the use of antibiotics within the previous six months (59 %, n = 124). The most frequent comorbidities were arterial hypertension (90.5 %, n = 190), diabetes mellitus (46.7 %, n = 98), followed by heart failure (23.3 %, n = 49) and coronary arterial disease (19.5 %, n = 41). The Charlson index mean was 5.6 (SD: 2.3). Thirty-four percent of patients (n = 72) did not want to change their catheter for a fistula (Table 1).

3.1. Staphylococcus aureus colonization

Longitudinally, 50.9 % (n = 107) of the patients were colonized by *S. aureus,* with at least one positive result. The percentages of colonization by methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA) were 29.1 % vs. 4.8 % in the first screening, 29.2 % vs. 6.7 % in the second one and 24.1 % vs. 7.1 % in the third one respectively. All MRSA isolates were positive for the *mec*A gene. The SCC*mec* type IVa was the most common (40.5 %, n = 15), followed by IVc (24.3 %, n = 9), V (18.9 %, n = 7) and IVb (8.1 %), n = 3. The SCC*mec* type was non-typeable in three isolates. Among carriers, colonization only in the nostrils was observed in 56.3 % of the samples (n = 94), followed by colonization in the nostrils and on the skin (29.3 %, n = 49), and only on the skin (14.4 %, n = 24) in the three screenings.

The susceptibility profile was evaluated among 37 MRSA isolates; 51.3 % (n = 19) were resistant to tetracycline followed by resistance to erythromycin (18.9 %; n = 7) (Table 2). The most frequent resistance profile among MRSA isolates was only oxacillin 37.8 % (n = 14), oxacillin + tetracycline in 48.6 % (n = 18), followed by oxacillin + erythromycin (18.9 %; n = 7) and oxacillin + erythromycin + tetracycline (13.5 %; n = 5). One hundred and eighty-seven MSSA isolates were evaluated, which showed resistance mainly to tetracycline (14 %; n = 23) (Table 2).

3.2. Dynamics of colonization

The cumulative incidence of colonization was 14 carriers per 100 patients after the first screening and 6 carriers per 100 patients after the second one. Of the 141 patients who completed the three screenings, 57.4 % (n = 81) were positive in at least one of the three screenings, and intermittent carriers (77.8 %, n = 63) were more common than persistent carriers (22.2 %, n = 18) (Fig. 2). Two patients had persistent colonization by MRSA. Clearance of colonization after the first screening was not frequent and was observed in 8.5 % (n = 12) of patients colonized by MSSA and in 0.7 % (n = 1) of patients colonized by MRSA.

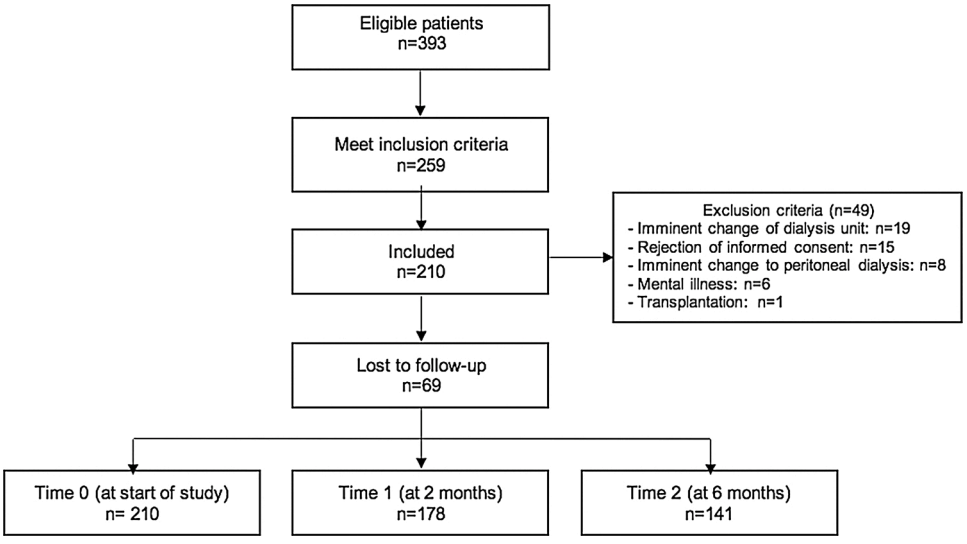

Fig. 1. Flowchart illustrating inclusion of patients and follow-up.

Table 1
Baseline characteristics of hemodialysis patients according to colonization status by *Staphylococcus aureus*.

Characteristic	Total n = 210	*S. aureus* colonization n = 71 (%)	*S. aureus* non-colonization n = 139 (%)
Sex			
Male	104 (49.5)	31 (43.7)	73 (52.5)
Female	106 (50.5)	40 (56.3)	66 (47.5)
Age Median (IQR)	62.0 (51.9–71.1)	61.12 (46.2–71.4)	62.25 (53.4–70.9)
Socioeconomic status[a]			
Low	181 (86.2)	63 (88.7)	118 (84.9)
Medium	20 (9.5)	4 (5.6)	16 (11.5)
High	9 (4.3)	4 (5.6)	5 (3.6)
Currently smoking	8 (3.8)	6 (8.4)	2 (1.4)
Time on dialysis (months) Median (IQR)	16.1 (2.3–50.4)	12.5 (2.3–50.2)	16.10 (2.2–5.0)
Comorbidities			
Arterial hypertension	190 (90.5)	61 (85.9)	129 (92.8)
Coronary arterial disease	41 (19.5)	13 (18.3)	28 (20.1)
Tumor or hematologic malignancy	20 (9.5)	9 (12.7)	11 (7.9)
Chronic obstructive pulmonary disease	19 (9.0)	10 (14.9)	9 (6.5)
Diabetes mellitus	98 (46.7)	34 (47.9)	64 (46.0)
Heart failure	49 (23.3)	20 (28.2)	29 (20.9)
Connective tissue disease	13 (6.2)	3 (4.2)	10 (7.2)
Charlson index Mean (SD)	5.62 (2.3)	5.66 (2.4)	5.60 (2.2)
Karnofsky index (≤70)	65 (30.9)	20 (28.2)	45 (32.37)
Presence of tunneled catheter	190 (90-5)	65 (91.5)	125 (89.9)
Jugular catheter	195 (92.9)	65 (91.5)	130 (93.5)
Catheter time (months) Median (IQR)	3 (0.9–13.8)	3.2 (1.0–13.0)	2.8 (0.9–14.2)
Refusal of fistula use	72 (34.3)	21 (29.6)	51 (36.7)
Surgery in the last year	49 (23.3)	18 (25.3)	31 (22.3)
History in the last 6 months of:			
S. aureus infection	13 (6.2)	3 (4.2)	10 (7.2)
Stay in another renal center	83 (39.5)	32 (45.1)	51 (36.7)
Peritoneal dialysis	15 (7.1)	7 (9.9)	8 (5.7)
Hospitalization	145 (69.0)	45 (63.4)	100 (71.9)
Intensive care unit	55 (26.2)	20 (28.2)	35 (25.2)
Domiciliary medicine	72 (34.3)	27 (38.0)	45 (32.4)
Immunosuppressive therapies	33 (15.7)	12 (16.9)	21 (15.1)
Invasive medical devices	37 (17.0)	11 (15.5)	26 (18.7)
Stay in nursing home, temporary shelter or convent	13 (6.2)	7 (9.9)	6 (4.3)
Antibiotic use (in the last 6 months)	124 (59.0)	40 (56.3)	84 (60.4)
Penicillin	33 (15.7)	16 (22.5)	17 (12.2)
First generation cephalosporins	36 (17.1)	12 (16.9)	24 (17.3)
Third generation cephalosporins	12 (5.7)	2 (2.8)	10 (7.2)
Glycopeptides	51 (24.3)	14 (19.7)	37 (26.6)
Aminoglycosides	39 (18.6)	12 (16.9)	27 (19.4)
Albumin Median (IQR)	3.99 (3.5–4.2)	3.97 (3.7–4.3)	4 (3.5–4.2)

[a] Socioeconomic status was defined according to the Colombian government classification system as low or middle/high.

3.3. Molecular typing

The colonizing population of *S. aureus* in hemodialysis patients was very heterogeneous. Hence, PFGE (Figs. A1–A3) and *spa*-typing (Fig. 3) revealed a high genetic diversity in the isolates obtained in each screening. The MSSA isolates were more diverse and presented 30 *spa*-types, while the MRSA strains presented 12 types. The *spa*-types predominating among MSSA isolates included t304, t189, t008, and t922. In contrast, t1610 and t148 were common among the MRSA strains (Fig. 3). On the other hand, of 38 patients with isolates from the skin and nostrils, 26.3 % (n = 10) had strains with different *spa*-types in both samples. In relation to carriers classified as persistent, the spa-typing showed that one third (33.3 %) of patients had different clones in at least one of the screenings; no particular clone was more likely in the persistent carriers (Fig. 2). Similarly, PFGE revealed the presence of non-genetically related isolates in almost 50 % of the patients (Fig. 4). Regarding MLST, inferred-CC8 (up to 28 %) and inferred-CC188 clones (up to 15 %) predominated among MSSA isolates (Fig. 5). The frequency of other clones, such as CC45, CC1, CC30, and CC15, was variable in each screening. The inferred clonal complexes found among MRSA isolates were mainly CC8 (up to 59 %) and CC5 (up to 30 %) (Figs. 4 and 5). The two persistent MRSA carriers had different clones including CC88, CC121 and CC8.

To combine *spa*-types and inferred clonal complexes, CC8-t304 and CC188-t189 were the most frequent among MSSA isolates, while CC8-t1610 was the most common among MRSA isolates (Fig. 3). Inferred-CC5 with new *spa*-type was also observed with percentages up to 30 % in MRSA isolates (Fig. 3).

Table 2
Resistance percentages among MRSA and MSSA isolates colonizing hemodialysis patients.

Resistance percentage	MRSA isolates n = 37 (%)	MSSA isolates n = 187 (%)
Tetracycline	19 (51.3)	23 (14)
Erythromycin	7 (18.9)	19 (11.6)
Clindamycin	0	0
Gentamicin	2 (5.4)	0
Mupirocin	1 (2.7)	0
Ciprofloxacin	1 (2.7)	3 (1.6)
Linezolid	1 (2.7)	0
Trimethoprim/ sulfamethoxazole	1 (2.7)	1 (0.5)

3.4. Factors associated with colonization

In the bivariate analysis, current smoking was the only variable associated with colonization by *S. aureus* (OR: 5.87, 95 % IC 1.81–19.00, p value = 0.003) (Table 3). This association was maintained in the multivariate analysis even after the adjustment for sex, age, and the Charlson index, presenting an increase in the effect size (OR: 7.22, 95 % IC: 2.24–23.27). Thus, the risk of colonization by *S. aureus* is up to six times higher in current smokers in comparison with non-smokers. Charlson index and previous infection by *S. aureus* were associated with colonization by this bacterium in the multivariate analysis after adjusting by confounding variables (OR: 1.22, 95 %IC: 1.03–1.43 and OR: 2.41, 95 IC: 1.09–5.30; respectively) (Table 3).

3.5. Staphylococcus aureus bacteremia

During the 12 months of follow-up, seventy-one cases of bacteremia were observed, most of them caused by *S. aureus* (n = 28; 39.4 %). Colonization status was associated with an increased risk of bacteremia (HR = 4.9; 95 % CI: 1.9–12.9) and PFGE revealed that 77.8 % of the patients were infected with the same strain that had been previously identified colonizing them. The frequency of bacteremia among persistent and intermittent carriers was similar. All patients with *S. aureus* colonization and bacteremia needed hospitalization with a median length of hospital stay of 15 days (IQR: 13–19). Four patients with MRSA colonization developed bacteremia and recurrent infections by this microorganism. Three patients died during hospital stay (all of them with MSSA infection).

Phenotypic methods							Molecular typing (*spa*)					
Isolate	**T0**	**T1**	**T2**	**n=141**	**%**	**Colonization type**	**T0**	**T1**	**T2**	**n=141**	**%**	**Clone**
MSSA	■	■	■	16	11.3	Persistent	▒	▒	▒	10	7.1	Same
							▒	■	■	6	4.2	New
							▒	▒	■			
	■			12	8.5	Intermittent	■			12	8.5	NA
	■	■		11	7.8		▒	▒		6	4.2	Same
							▒	■		5	3.6	New
		■		8	5.7			■		8	5.7	NA
			■	7	5.0				■	7	5.0	NA
		■	■	6	4.2			▒	▒	3	2.1	Same
								▒	■	3	2.1	New
	■		■	5	3.6		▒		▒	4	2.9	Same
							▒		■	1	0.7	New
MRSA	■	■	■	2	1.4	Persistent	▒	▒	▒	2	1.4	Same
			■	5	3.6	Intermittent			■	5	3.6	NA
		■	■	3	2.1			▒	▒	1	0.7	Same
								▒	■	2	1.4	New
	■	■		3	2.1		▒	▒		1	0.7	Same
							▒	■		2	1.4	New
		■		2	1.4			■		2	1.4	NA
	■			1	0.7		■			1	0.7	NA
NONE				60	42.6	Absent				60	42.6	NA

Fig. 2. Classification of colonization by *S. aureus* in patients with three screenings performed. Dark gray: *Staphylococcus aureus* detected. White: without colonization. Light gray: colonization by the same clone of *S. aureus*. Black: colonization by a new clone of *S. aureus*. NA: not applicable, patients with only a positive screening.

4. Discussion

In this study, the colonization by *S. aureus* was an event changing over time, and diverse clones were found even among carriers classified as persistent. *Staphylococcus aureus* continues to be a microorganism that colonizes and causes infection with high frequency in hemodialysis patients (Calfee, 2013). The percentage of colonization found in this study is similar to other reports with frequencies around 27 % with a single observation (Bogut et al., 2007; Kluytmans and Wertheim, 2005). However, to include the results of the three screenings, 57.4 % of the patients had at least one positive result. This agrees with other longitudinal studies that have reported that between 49 % and 62 % of hemodialysis patients have at least one positive culture for *S. aureus* when a follow-up is performed (Maamoun et al., 2011; Price et al., 2015). Furthermore, in this study, *S. aureus* colonization increased the risk of bacteremia and molecular typing confirmed that patients were infected with the same strain that had been previously identified colonizing them. Thus, these results show that it is necessary a permanent monitoring of the colonization status to detect all cases, because most patients are intermittent carriers and, as was evidenced in this study, the clearance of colonization is not frequent (Patel et al., 2011; Bogut et al., 2007).

These findings have several implications because a higher number of screenings allow the detection of more carriers and therefore identifies all patients with a greater risk of developing infections such as bacteremia; even more in countries like ours where more than 34.3 % patients refuse the fistula use as vascular access (Table 1) (Maamoun et al., 2011). A previous study showed that the carriers who failed to eradicate *S. aureus* were more likely to have bacteremia compared with those who were successfully decolonized with mupirocin and chlorhexidine (Price et al., 2015). This suggests the consideration of prophylactic therapies in colonized patients with a greater risk of invasive infections to decrease adverse outcomes such as hospitalizations, complications, and death (Price et al., 2015).

In relation to MRSA colonization, a meta-analysis revealed that the frequency of MRSA carriers in hemodialysis patients is 7.2 %, which is a higher percentage than patients on peritoneal dialysis 1.3 % (Karanika et al., 2015). The percentage of colonization by MRSA found that this study is similar to that previously reported but notably increased over time, being 4.8 % in the first screening, 6.7 % in the second one, and 7.1 % in the third one. The above can be explained by antibiotic pressure that can be generated during the course of the hemodialysis therapy or the acquisition of new strains, but it contrasts with other studies that suggest that MRSA frequency remains stable over time in dialysis patients (Bogut et al., 2007; Karanika et al., 2015). Although infections by MRSA have been associated with a worse prognosis, the risk of infection in patients colonized by *S. aureus* refers to both susceptible and resistant strains (Wertheim et al., 2005).

On the other hand, PFGE revealed the circulation of different strains among colonized patients in each screening. This finding suggests that horizontal transmission (patient-patient) of colonizing isolates is not frequent in hemodialysis patients; instead there are different colonization sources out of the renal center, as has been found in other reports (Bogut et al., 2007). Additionally, in the case of resistant isolates, the high genetic diversity could also suggest excessive antibiotic pressure resulting in the selection of these strains (Bogut et al., 2007).

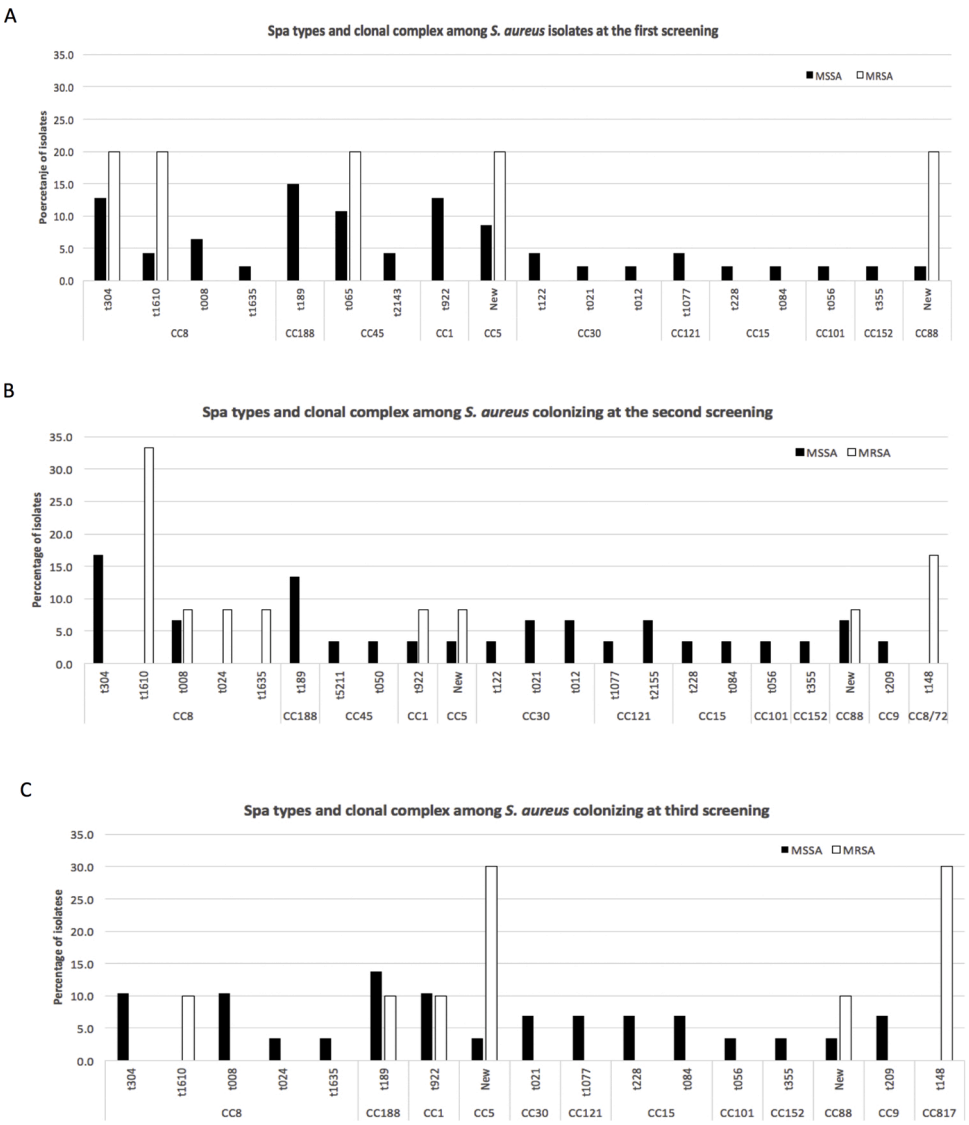

Fig. 3. *Spa*-types and inferred clonal complex among MSSA and MRSA isolates colonizing hemodialysis patients. A, B and C are the first, second and third screenings respectively.

Further, results obtained from PFGE and *spa*-typing showed the presence of different clones in samples from the skin and nostrils in the same patient, as well as the acquisition of new strains even in carriers classified as persistent by phenotypic methods. This evidences that the acquisition or recolonization by new clones of *S. aureus* occurs frequently in hemodialysis patients, as has been reported not only in this group of patients but also in healthy people (Bogut et al., 2007; Wertheim et al., 2005; Muthukrishnan et al., 2013). It is important to consider that hemodialysis patients circulate permanently not only in hospitals and renal centers but also in the community, and in these places they can acquire strains through contact with other patients, health workers, households or fomites (Maamoun et al., 2011; Kang et al., 2012). Moreover, the utilization of phenotypic methods to classify colonization status is not sufficient; it is necessary to apply molecular methods to detect true persistent carriers.

Regarding inferred MLST, four clones were commonly found among MSSA and MRSA isolates (CC8, CC5, CC30 and CC45), which correspond to the major globally disseminated clones of *S. aureus* and had been found in hemodialysis patients (Lakhundi and Zhang, 2018; Monaco et al., 2017; Scheuch et al., 2019). Predominant clones in the United States include CC30, CC8, and CC45, while in Europe CC5, CC8 and CC22 are common, and CC5, CC30, and CC8 clones are common in Latin America (Lakhundi and Zhang, 2018; Monaco et al., 2017). Both CC7 and CC8 harbor the fibrinogen binding protein (efb/fib), which contributes to the initiation of foreign body infections (Herman-Bausier et al., 2015). In contrast, the collagen binding adhesion mediating bacterial adherence to collagen substrates and collagenous is not expressed in CC8 and CC7 but in CC30 (Switalski et al., 1993). These genetic differences illustrate the strong influence of the medical environment on the *S. aureus* population and in consequence on the distribution of lineage-associated virulence factors.

MSSA population is more heterogeneous than MRSA, as the introduction of SCCmec occurs in MSSA lineages that are permissive and can maintain it (Monaco et al., 2017). For this reason, molecular epidemiology data is mainly focused on MRSA and is limited for MSSA (Monaco et al., 2017). In this study particularly, the CC8 clone harboring SCC*mec* IV was the most frequent among MRSA isolates. This clone, which frequently includes community-associated strains, is becoming predominant in Colombian hospitals, displacing previously reported healthcare-associated CC5 clones. Community-associated strains have overlapped healthcare-associated clones and cause endemic hospital infections all over the world (Jimenez et al., 2012; Jiménez et al., 2013). Additionally, community-associated strains cause about 25 % of the infections in hemodialysis patients and are the main cause of infection in

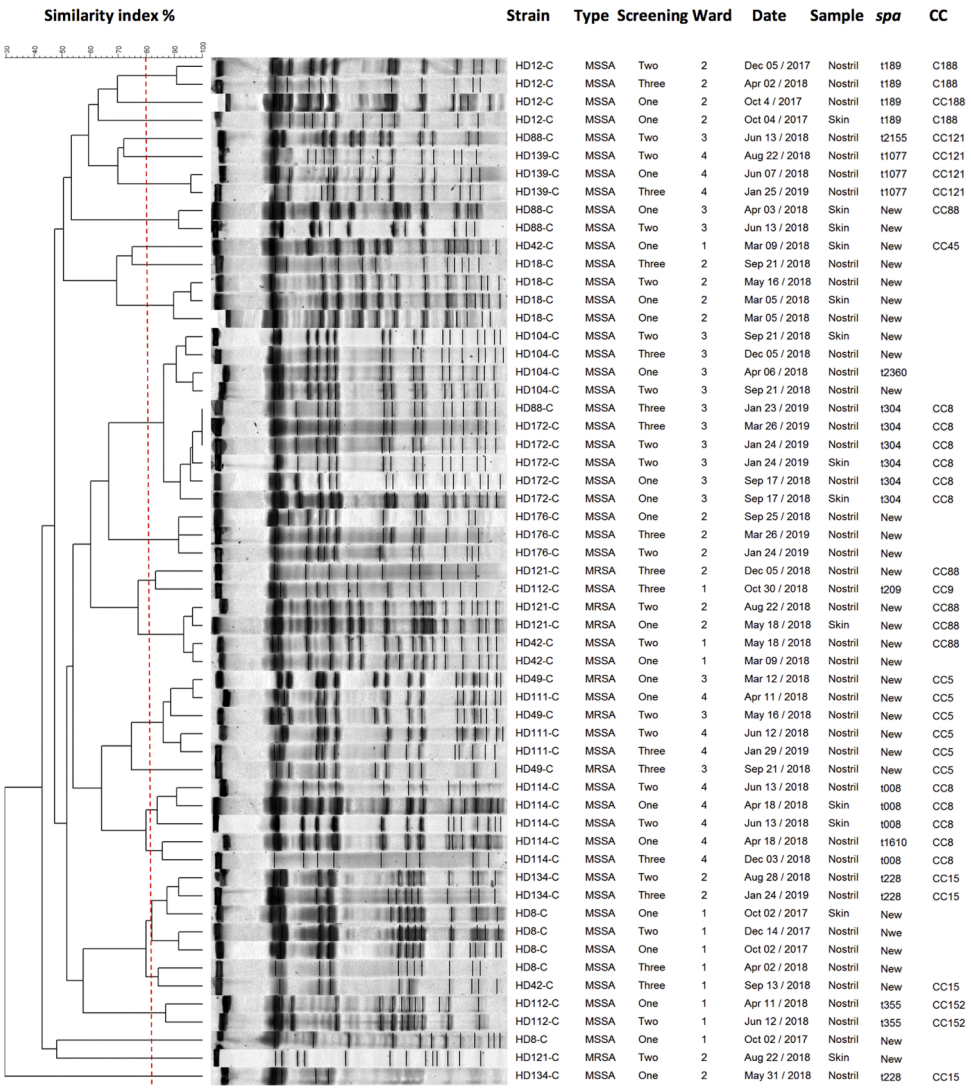

Fig. 4. Genetic relatedness in *S. aureus* isolates colonizing persistent carriers. The broken line corresponds to the cutoff level (80 %) used to define PFGE clones as related.

the skin and soft tissues, a frequent infection in this group of patients (Chua et al., 2011; Snyder and D'Agata, 2012).

In relation to the risk factors associated with colonization, in this study Charlson index and previous infection by *S. aureus* were associated with colonization by this bacteria as has been suggested by studies conducted in this and other types of patients (Patel et al., 2011; Karanika et al., 2015; Wang et al., 2012). Interestingly, current smoking had a higher effect on this outcome. This association is not new and has been observed in some studies (Choi et al., 2019; Neupane et al., 2018; Neidhart et al., 2018; Sakr et al., 2018). Similarly, a recent study showed that smoking increases the average nasal load of *S. aureus* (2.6 × 104 CFU/swab) in smoking patients compared to the load observed in healthy non-smoking individuals (1.7 × 103 CFU / swab) (Cole et al., 2018). This situation leads to the need to include additional characteristics to those associated with healthcare to allow the identification of colonized patients. This is due to other characteristics, such as the habits and behavior of the patient. The conditions of the residential environment should also be evaluated, and this is why some studies have evidenced the absence of healthcare factors associated with colonization by *S. aureus* in hemodialysis patients (Maamoun et al., 2011; Kang et al., 2012). In this way, a study conducted in a renal center found that 6.8 % of the household cohabitants were colonized with MRSA isolates genetically related to those found in hemodialysis patients, suggesting the transmission of this bacteria in the residential environment (Lu et al., 2008).

Regarding limitations, this study was performed in a single facility; therefore, it may not be representative of epidemiology in other dialysis centers. However, the facility where the research was carried out is one of the biggest in the city and attends a heterogenous population from different places. Secondly, health-care workers, dialysis machines, and the environment were not evaluated, which may suggest unrecognized sources for *S. aureus* transmission within the renal center. Thirdly, other methods as whole genome sequencing needs to be performed, to capture in a more precise way, the diversity of *S. aureus* in studies about colonization, infection and transmission (Paterson et al., 2015). This method provides information on pathogen identification, epidemiological typing and drug susceptibility, allows to establish transmission directions, details virulence patterns and monitors clones in the same patient over time (Paterson et al., 2015).

Finally, in this study we picked three colonies for phenotypic and molecular characterization and therefore the bacterial population diversity within single patient could have been not fully evaluated. However, this study has several strengths, such as the longitudinal evaluation of colonization status, the screening of the skin to increase in the sensitivity of detection, the application of molecular typing methods with high discriminatory power as PFGE and the identification of risk

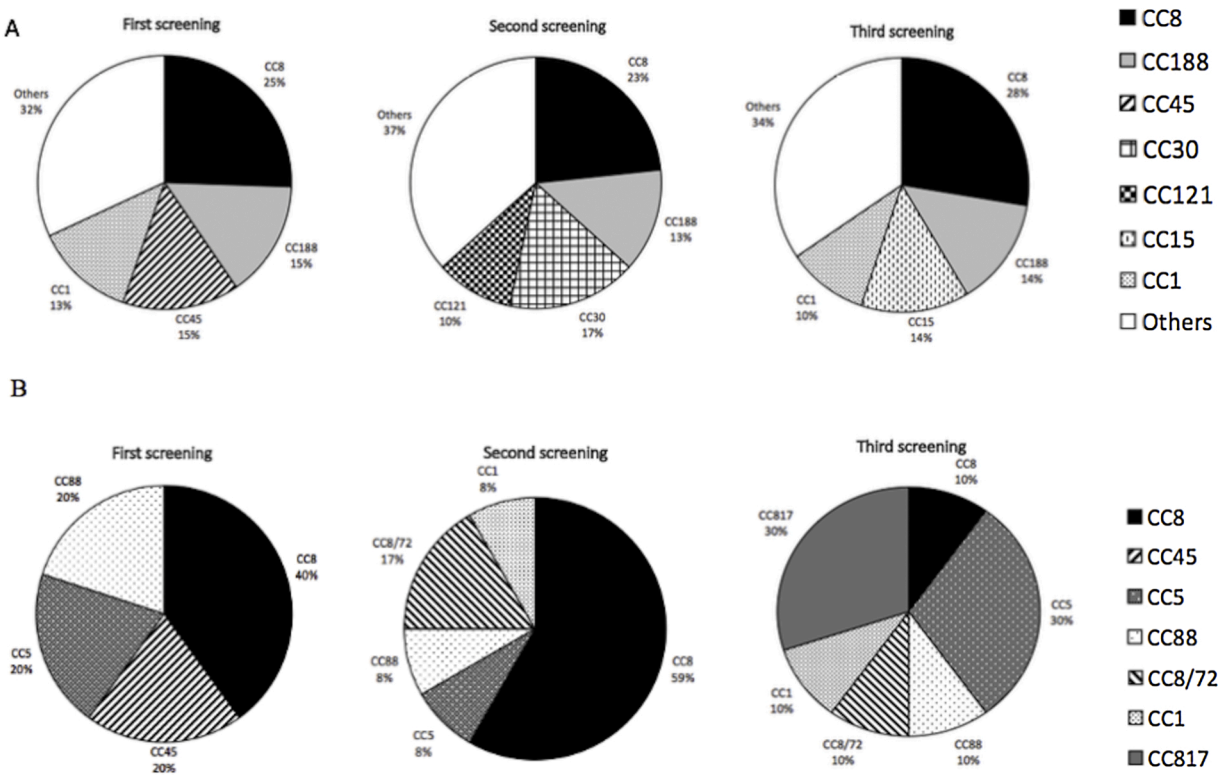

Fig. 5. Inferred Clonal complex (CC) among MSSA and MRSA isolates colonizing hemodialysis patients. A: MSSA; B: MRSA.

Table 3
Bivariate and multivariate analysis for identification of factors associated with colonization by *S. aureus*.

Variable	Bivariate analysis				Multivariate analysis			
	OR	95 % CI		p-value	OR	95 % IC		p-value
Current smoking[a]	5.87	1.81	19.00	0.003	7.22	2.24	23.27	0.001
Charlson index[b]	0.97	0.88	1.07	0.514	1.22	1.03	1.43	0.019
Karfnosky index[c]	1.01	0.99	1.03	0.247	0.70	0.41	1.21	0.207
Jugular catheter[d]	0.55	0.21	1.48	0.240	0.64	0.23	1.80	0.402
History of hospitalization[e]	0.99	0.70	1.40	0.956	0.98	0.61	1.56	0.919
Use of beta-lactam antibiotics[f]	0.82	0.57	1.18	0.288	0.81	0.55	1.17	0.264
Time on dialysis[g]	1.06	0.69	1.65	0.774	1.09	0.70	1.68	0.706
Previous infection by *S. aureus*[h]	1.89	0.87	4.12	0.109	2.41	1.09	5.30	0.029

[a] Adjusted by age, sex, and Charlson index.
[b] Adjusted by age, albumin, and history of hospitalization.
[c] Adjusted by age, sex, Charlson index, and time on dialysis.
[d] Adjusted by time on dialysis, and previous infection by *S.* aureus.
[e] Adjusted by age, time on dialysis, Charlson index, and albumin.
[f] Adjusted by stay in nursing homes, Charlson index, and previous infection by *S. aureus*.
[g] Adjusted by Charlson index, and age.
[h] Adjusted by time on dialysis, history of hospitalization, and previous use of antibiotics.

factors associated with colonization.

5. Conclusion

In conclusion, colonization by *S. aureus* in hemodialysis patients is changing over time, and the acquisition of new strains is a frequent event. Molecular methods evidenced high genetic diversity among carriers meaning that patients are repeatedly recolonizing from hospitals, dialysis units and their homes. Colonization increased the risk of infection and it justifies the need to surveillance the colonization status in patients with recurrent infections. In addition, these results show the importance to improve strategies about catheter handling by the healthcare worker and to educate the patients about the care of this vascular access to prevent infections by MSSA and MRSA isolates. On the other hand, the presence of habits, such as smoking, increased the risk of colonization in this study, leading to the need to consider other factors not associated with healthcare for the identification of colonized patients.

Funding

This research was supported by Comité para el Desarrollo de la Investigación –CODI- Universidad de Antioquia, Colombia Project: 2017-15526 and Departamento Administrativo de Ciencia, Tecnología e innovación – Colciencias- (MINCIENCIAS COLOMBIA) Project: 111577756947.

Ethics approval

All procedures performed in this study were in accordance with the ethical standards of Bioethics Committee for Human Research at the University of Antioquia (CBEIH-SIU) (approval no. 17-65-689) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Authors' contributions

Conceived and designed the study: JMV LS JNJ. Performed the experiments: LS MG. Analyzed the data: JMV LS MG JNN. Contributed reagents/materials/analysis tools: JMV LS MG JNN. Wrote the paper: JMV LS MG JNN.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

To Ana Lucía Arbelaez and Luz Adriana Patiño for the collection and storage of strains. To Fresenius Medical Care for the approval of this study. To MINCIENCIAS COLOMBIA for supporting a Research Young Scholarship Marlon Gallego contract -752-2018.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijmm.2020.151471.

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