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Fluoroquinolone Impact on Nasal Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus* Colonization Durations in Neurologic Long-Term-Care Facilities

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***Staphylococcus aureus* nasal carriage is a risk factor for subsequent infection. Estimates of colonization duration vary widely among studies, and factors influencing the time to loss of colonization, especially the impact of antibiotics, remain unclear. We conducted a prospective study on patients naive for *S. aureus* colonization in 4 French long-term-care facilities. Data on nasal colonization status and potential factors for loss of colonization were collected weekly. We estimated methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) colonization durations using the Kaplan-Meier method and investigated factors for loss of colonization using shared-frailty Cox proportional hazards models. A total of 285 *S. aureus* colonization episodes were identified in 149 patients. The median time to loss of MRSA or MSSA colonization was 3 weeks (95% confidence interval, 2 to 8 weeks) or 2 weeks (95% confidence interval, 2 to 3 weeks), respectively. In multivariable analyses, the methicillin resistance phenotype was not associated with *S. aureus* colonization duration ($P = 0.21$); the use of fluoroquinolones (hazard ratio, 3.37; 95% confidence interval, 1.31 to 8.71) and having a wound positive for a nonnasal strain (hazard ratio, 2.17; 95% confidence interval, 1.15 to 4.07) were associated with earlier loss of MSSA colonization, while no factor was associated with loss of MRSA colonization. These results suggest that the methicillin resistance phenotype does not influence the *S. aureus* colonization duration and that fluoroquinolones are associated with loss of MSSA colonization but not with loss of MRSA colonization.**

Staphylococcus aureus is a human commensal bacterium and a common cause of hospital-acquired infections, ranging from mild skin infections to bacteremia. The anterior nares are the most common *S. aureus* niche, although the presence of extranasal sites of colonization is increasingly recognized (1, 2). Approximately 30 to 50% of humans are asymptomatically colonized with *S. aureus*, and nasal carriage is a major risk factor for subsequent infection (3, 4). However, the role of long-term nasal *S. aureus* persistence remains unknown. Notably, persistent but not intermittent nasal *S. aureus* carriage is a risk factor for continuous peritoneal dialysis-related infections (5). Moreover, long-term methicillin-resistant *S. aureus* (MRSA) carriers remain at risk for subsequent infection and death (6).

Estimates of MRSA colonization duration vary widely among studies (7–15) and range from 12 days (8) to 40 months (13). A meta-analysis estimated that the median time to clearance was 88 weeks after documented, untreated MRSA colonization (16). Some factors associated with a longer MRSA colonization duration have already been identified, e.g., ≥2 MRSA-positive body sites or a skin break at readmission (9, 14, 17), prior fluoroquinolone use (17), human immunodeficiency virus positivity (18), and household contacts with MRSA, young age, or *spa* type t002 (9). Conversely, self-sufficiency in daily activities (10) or having an antibiotic-treated documented infection (9) was associated with a shorter MRSA carriage time.

This study was undertaken in long-term-care facilities (LTCFs) to evaluate prospectively nasal *S. aureus* (MRSA and/or methicillin-sensitive *S. aureus* [MSSA]) colonization duration and identify factors influencing the time to loss of colonization.

MATERIALS AND METHODS

Settings and study design. The Antibiotic Use and *Staphylococcus aureus* Resistant to Antibiotics (ASAR) study is a prospective cohort of patients naive for *S. aureus* colonization established in France from January 2008 to October 2010 in 4 neurologic LTCFs. Patients were included if they

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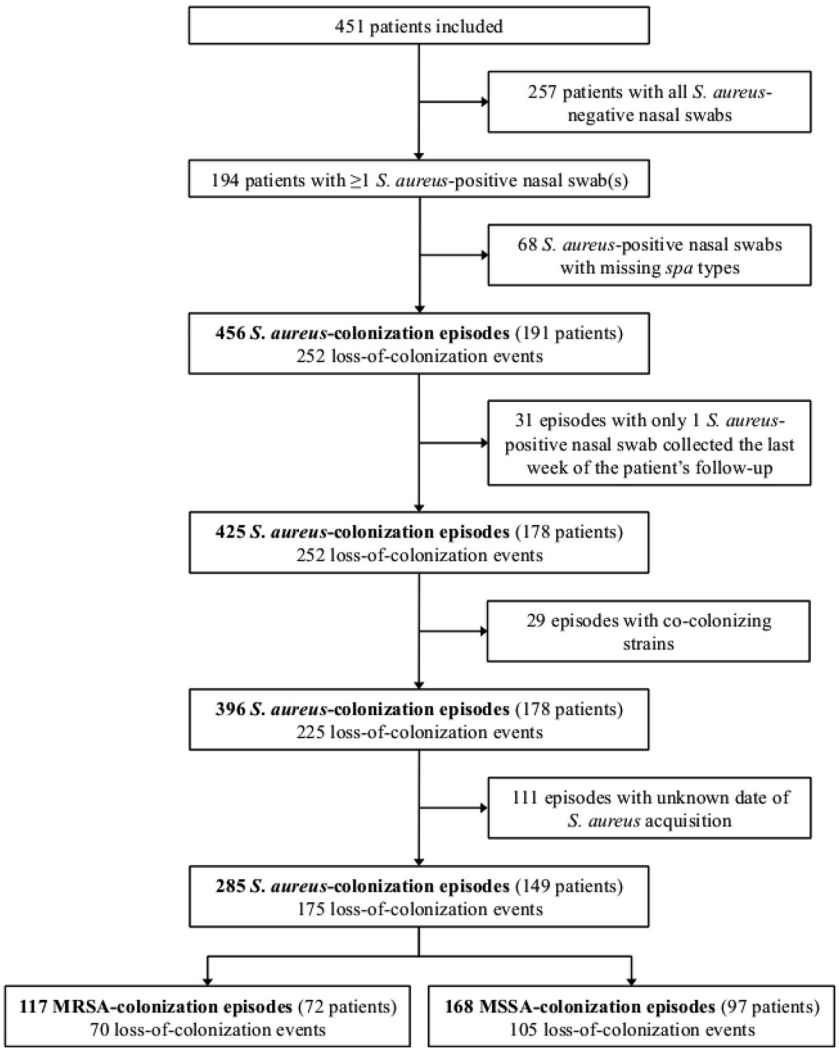

FIG 1 Flowchart of the study population.

were not colonized with *S. aureus* at admission (negative nasal and perineal swab specimens), ≥18 years old, and hospitalized for a neurologic disorder with an expected stay of ≥2 months, as detailed previously (19). An institutional review board (Comité de Protection des Personnes Île-de-France XI) approved the study protocol (reference 07032); no written informed consent was obtained from the patients because the study interventions were standard care. Authorization was also obtained from the Commission Nationale de l'Informatique et des Libertés (French Data Protection Agency; reference 907302).

Each patient was tested weekly for his or her status of nasal colonization with *S. aureus*. At inclusion, sociodemographic data (sex and age) and diabetes mellitus status were recorded. Every week, a standardized questionnaire was used to collect prospective data on strict isolation (single room or cohorting), contact isolation, use of an invasive medical device (catheter, tracheostomy, enteral nutrition, and/or urination device), body-washing assistance, and wound swabbing for *S. aureus* identification. Daily antimicrobial use was recorded either in computerized pharmacy records or on specific standardized questionnaires. Antibiotics were classified as follows: fluoroquinolones (Anatomical Therapeutic Chemical Group J01MA), aminoglycosides (J01G), and other anti-MSSA agents (including β-lactamase-resistant penicillins [J01CF], combined penicillins and β-lactamase inhibitors [J01CR], third- and fourth-generation cephalosporins [J01DD/DE], and carbapenems [J01DH]). Macrolides, lincosamides, streptogramins, and other anti-*S. aureus* agents (including vancomycin, fusidic acid, fosfomycin, and rifampin) were rarely prescribed and were not considered in the analyses.

Microbiologic techniques. To assess *S. aureus* colonization, an alginate swab was rotated inside each nostril and then placed in Stuart's transport medium (Transwab; Medical Wire and Equipment) and kept at room temperature until arrival at the Microbiology Laboratory of Raymond-Poincaré University Hospital. Screening for MRSA and antimicrobial susceptibility testing were performed as previously described (20). To determine *S. aureus* clonal diversity, all strains isolated from positive nasal swabs were subjected to *spa* typing.

Definition of colonization episodes. A colonization episode was defined as the period between the acquisition and loss of colonization with a given *S. aureus* strain. *S. aureus* strains were defined by their methicillin resistance phenotype and *spa* type. *S. aureus*-positive swabs for which the *spa* type was not identified were considered missing samples for the analyses. *S. aureus* strain acquisition was defined as a positive nasal swab following either negative nasal and perineal swabs at admission or following two consecutive nasal swab specimens negative for that strain during follow-up. This definition and missing samples meant that the date of colonization acquisition was unknown for many episodes and data for those episodes were excluded from the analyses (Fig. 1).

The event of interest was the loss of *S. aureus* colonization, defined as two consecutive nasal swab specimens negative for the episode strain. The time to loss of colonization was the number of weeks from the date of acquisition of a given strain. Episodes were right censored during follow-up at the last positive swab when consecutive missing samples prevented a precise loss-of-colonization determination or at the end of observation.

Statistical analyses. Median MRSA, MSSA, and *S. aureus* colonization durations were estimated using the Kaplan-Meier method to account for right-censored observations. Associations between factors and the loss of MRSA, MSSA, or *S. aureus* colonization were investigated using Cox proportional hazards models (21). Because a patient could have several colonization episodes, a shared-frailty term was used to model a lack of independence between a given patient's events (22). Episodes with a single *S. aureus*-positive nasal swab collected during the last week of the patient's follow-up were not analyzed, because survival analysis requires that each episode be observed over some nonnull time interval.

Isolation precautions, the use of an invasive medical device, body-washing assistance, antibiotic use, and having one or several *S. aureus*-positive wounds were included as time-dependent covariates in the models. We distinguished wounds colonized with the nasal strain (i.e., the strains had the same methicillin resistance phenotype and the same *spa* type) from wounds colonized with a nonnasal strain (i.e., the strains had different methicillin resistance phenotypes and/or *spa* types). For nasal swabs positive for two cocolonizing strains, we created a time-dependent covariate (having another nasal *S. aureus* strain) for exposure to the strain different from that of the ongoing episode. Time-dependent covariates were analyzed as binary variables (exposed/nonexposed), and exposure was defined for the time interval between the collection of two nasal swab specimens. The dates of antibiotic prescriptions were compared with the estimated dates of *S. aureus* acquisition and loss of colonization, which were considered the midpoint between the times for the last negative and the first positive nasal swabs and the midpoint between the times for the last positive and the first negative nasal swabs, respectively.

Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated for all variables. Those associated with time to loss of colonization in univariate analyses (2-sided $P < 0.20$) were included in the multivariable models and maintained in the final models when their HR estimates remained statistically significantly associated (2-sided $P < 0.05$) with loss of colonization using a manual backward stepwise procedure, yielding the final adjusted models. All univariate and multivariable models were adjusted for center and fitted with the Efron method for handling ties (23). Interactions between the strain's methicillin resistance phenotype and all the covariates remaining in the final model of loss of *S. aureus* colonization were systematically evaluated.

Sensitivity analyses (i) included only the first colonization episode per patient, (ii) used survival time recorded as calendar dates (i.e., nasal swabbing dates) rather than the time to loss of colonization, where time zero corresponded to the time of *S. aureus* acquisition (for this approach, episodes with an unknown date of acquisition could be included because the event of interest was the loss of *S. aureus* colonization on a given calendar date rather than the time from acquisition), and (iii) defined the loss of *S. aureus* colonization as three consecutive nasal swab specimens negative for the episode strain.

Analyses were performed using SAS (version 9.3) software (SAS Institute, Cary, NC). The PHREG procedure and the RANDOM statement were used to fit a shared-frailty model to clustered data with normally distributed random effects.

RESULTS

Study population. Among the 451 patients naive for *S. aureus* colonization at admission and included in the ASAR study, 194 (43.0%) had ≥1 *S. aureus*-positive nasal swab specimens during follow-up (Fig. 1), and the 149 patients included in the analyses were from centers 1 to 4: 17 (11.4%), 53 (35.6%), 50 (33.6%), and 29 (19.5%) patients, respectively (Table 1). Their median age was 52.6 years (range, 18.7 to 86.5 years), and 101 (67.8%) were male. The median follow-up duration was 20 weeks (range, 4 to 65 weeks), and the median number of colonization episodes per patient was 1 (range, 1 to 12). Figure S1 in the supplemental material reports the results for the nasal swabs obtained from each patient during follow-up. At admission, the characteristics of the patients included in the analyses were comparable to those of the 45 patients that acquired *S. aureus* and were excluded from the analyses (data not shown).

TABLE 1 Characteristics of the 149 patients colonized with *Staphylococcus aureus*

Variable	Value
No. (%) of patients at center[a]:	
1	17 (11.4)
2	53 (35.6)
3	50 (33.6)
4	29 (19.5)
No. (%) of male patients	101 (67.8)
Median (IQR[b]) age (yr)	52.6 (39.2–61.8)
Median (IQR) no. of colonization episodes	1 (1–2)
Median (IQR) follow-up duration (wk)	20 (13–35)

[a] French centers 1 to 4 (city in France) were, respectively, Maritime Hospital (Berck), Raymond-Poincaré University Hospital (Garches), Jacques-Calvé Center (Berck), and Sainte-Barbe Center (Fouquières-lès-Lens).
[b] IQR, interquartile range.

Considering the 194 patients who had ≥1 *S. aureus*-positive nasal swab specimens during follow-up, the protocol called for a total of 4,728 swab specimens to be obtained (1 per week), but 450 (9.5%) were missing. The rate of missing data (8.5%) was similar for the 149 patients analyzed.

Colonization episodes. During follow-up, 285 *S. aureus* colonization episodes (117 episodes with MRSA colonization and 168 episodes with MSSA colonization) were identified in 149 patients; among those episodes, loss of colonization occurred in 175 (61.4%) (70 [59.8%] and 105 [62.5%] for MRSA and MSSA colonization episodes, respectively).

The median Kaplan-Meier-estimated time to loss of *S. aureus* colonization was 3 weeks (95% CI, 2 to 4 weeks). The respective median times to loss of MRSA and MSSA colonization were 3 weeks (95% CI, 2 to 8 weeks) and 2 weeks (95% CI, 2 to 3 weeks), respectively. Figure 2 shows the estimated Kaplan-Meier curves of the MRSA or MSSA colonization duration. A loss of colonization occurred after 1 week for 156 (54.7%) episodes: 63 (53.8%) MRSA colonization episodes and 93 (55.4%) MSSA colonization episodes.

Seventy-two *spa* types were identified, with the most predominant *spa* types being t008, t777, and t002 (Table 2). Among the 285 *S. aureus* strains, 152/168 (90.5%) MRSA strains and 4/117 (3.4%) MSSA strains were fluoroquinolone resistant.

Among the 149 patients, 44 (29.5%) were colonized with ≥2 different *S. aureus* strains, including 1 patient who was colonized with 5 different ones. Forty-three (28.9%) patients had ≥2 colonization episodes with the same *S. aureus* strain during their follow-up.

Factors influencing time to loss of colonization. Among 315 *S. aureus*-positive wound swab specimens collected from 96 colonization episodes, 215 (68.3%) were colonized with the nasal

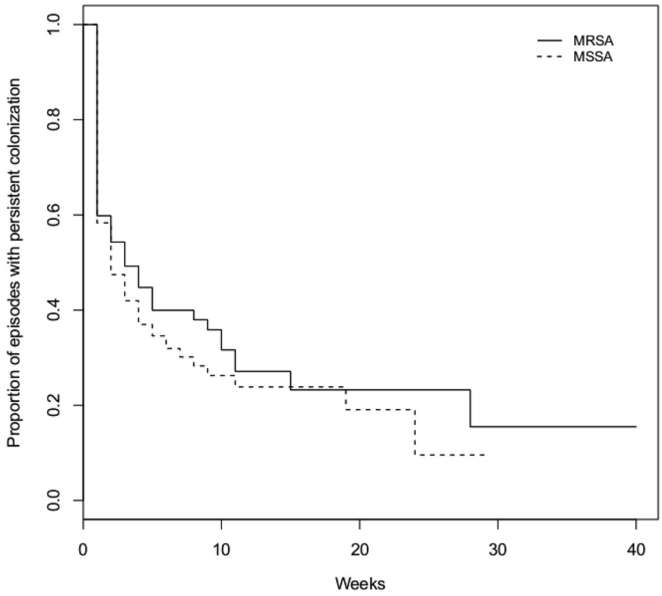

FIG 2 Estimated Kaplan-Meier curves of MRSA or MSSA colonization duration.

strain. Among the 100 wounds colonized with a nonnasal strain (45 wounds colonized with MRSA, 55 wounds colonized with MSSA), the methicillin resistance phenotype of the nonnasal strain differed from that of the nasal strain for 78. Only 12 patients (8 MRSA and 9 MSSA colonization episodes) received fluoroquinolones during their colonization episodes, 6 patients (4 MRSA and 3 MSSA colonization episodes) took aminoglycosides, and other anti-MSSA agents were prescribed to 13 patients (9 MRSA and 9 MSSA colonization episodes).

Univariate Cox analyses identified only the adjusting center variable ($P = 0.025$) to be significantly associated with loss of MRSA colonization (Table 3). Fluoroquinolone use and a nonnasal strain-positive wound were significantly associated with earlier loss of MSSA and *S. aureus* colonization. The methicillin resistance phenotype did not impact the *S. aureus* colonization duration ($P = 0.21$).

Multivariable analyses retained fluoroquinolone use and a nonnasal strain-positive wound as being significantly and independently associated with earlier loss of MSSA colonization and retained fluoroquinolone use, a nonnasal strain-positive wound, and *spa* type t571 as being significantly and independently associated with earlier loss of *S. aureus* colonization (Table 4). No interaction between fluoroquinolone use and the methicillin resistance phenotype was found in the final model of loss of *S. aureus* colonization ($P = 0.31$).

Considering the first colonization episode per patient, only *spa* type t2903 or t571 was an independent factor for loss of MRSA or *S. aureus* colonization, respectively (see Table S1 in the supplemental material). Using survival time recorded as calendar dates, fluoroquinolone use and a nonnasal strain-positive wound were significantly and independently associated with earlier loss of *S. aureus* colonization (see Table S2 in the supplemental material). Those risk factors were not significant for MSSA episodes, while the use of other anti-MSSA agents was independently associated with earlier loss of colonization. Finally, when the loss of *S. aureus* colonization was defined as three consecutive nasal swab specimens negative for the episode strain, fluoroquinolones were independently associated with loss of MSSA and *S. aureus* colonization, a wound positive for a nasal strain and *spa* type t2903 were independently associated with loss of MRSA and *S. aureus* colonization, *spa* type t571 was independently associated with loss of *S. aureus* colonization, and a wound positive for a nonnasal strain was independently associated with loss of MRSA, MSSA, and *S. aureus* colonization (see Table S3 in the supplemental material).

DISCUSSION

Our study showed that estimates of nasal MRSA and MSSA colonization durations were similar and that fluoroquinolone use and a wound positive for a nonnasal strain were independently and significantly associated with shorter MSSA colonization durations. Notably, the methicillin resistance phenotype was not associated with longer duration of *S. aureus* colonization, suggesting that in the human population, *S. aureus* fitness, which depends on bacterial growth and clearance rates, is not influenced by methicillin susceptibility and, thus, that the biologic cost associated with methicillin resistance might be limited. Although methicillin resistance was inversely associated with the growth rate *in vitro* (24), the compensatory mutations that can counterbalance the cost conferred by resistance may be differently selected *in vivo* (25) and, by extension, in the human population. Studies examining whether MRSA and MSSA strains compete for nasal colonization have yielded conflicting results (26–29): some found no evidence of competition, while others showed that MSSA colonization protected against MRSA acquisition in the nares.

The median time to loss of MRSA colonization of 3 weeks observed in the present study was shorter than that described previously (9–15). Several explanations could account for the variability of MRSA colonization duration: different health care set-

TABLE 2 *spa* type distribution according to methicillin resistance phenotype[a]

	No. (%) of episodes or patients					
	MRSA		MSSA		*S. aureus*	
spa type	Episodes ($n = 117$)	Patients ($n = 72$)	Episodes ($n = 168$)	Patients ($n = 97$)	Episodes ($n = 285$)	Patients ($n = 149$)
t008	38 (32.5)	27 (37.5)	26 (15.5)	17 (17.5)	64 (22.5)	42 (28.2)
t777	27 (23.1)	19 (26.4)	9 (5.4)	9 (9.3)	36 (12.6)	26 (17.4)
t002	7 (6.0)	6 (8.3)	11 (6.5)	8 (8.2)	18 (6.3)	14 (9.4)
t571	0	0	12 (7.1)	9 (9.3)	12 (4.2)	9 (6.0)
t2903	12 (10.3)	6 (8.3)	0	0	12 (4.2)	6 (4.0)
Others	33 (28.2)	26 (36.1)	110 (65.5)	70 (72.2)	143 (50.2)	95 (63.8)

[a] Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S. aureus*.

TABLE 3 Univariate shared-frailty Cox proportional hazards regression models for loss of MRSA, MSSA, or *S. aureus* colonization[f]

Variable	MRSA		MSSA		*S. aureus*	
	HR[a] (95% CI)	*P* value[b]	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Patient characteristics						
Center[c]						
1	Reference	**0.025**	Reference	0.42	Reference	**0.006**
2	0.55 (0.27–1.10)		0.76 (0.42–1.38)		0.63 (0.41–0.97)	
3	0.36 (0.18–0.72)		0.62 (0.34–1.15)		0.47 (0.31–0.73)	
4	0.34 (0.12–0.98)		0.62 (0.31–1.24)		0.49 (0.29–0.84)	
Male sex	1.84 (0.87–3.89)	0.11	1.20 (0.76–1.90)	0.43	1.40 (0.96–2.03)	0.08
Age ≥ 60 yr	0.84 (0.46–1.52)	0.56	1.41 (0.89–2.24)	0.14	1.07 (0.75–1.51)	0.73
Diabetes mellitus	0.86 (0.34–2.18)	0.74	0.82 (0.45–1.50)	0.52	0.83 (0.50–1.36)	0.45
Bacterial characteristics						
Methicillin resistance	NA		NA		0.82 (0.60–1.12)	0.21
spa type						
Other *spa* types	Reference	0.19	Reference	0.29	Reference	0.11
t002	1.70 (0.45–6.39)		1.53 (0.65–3.57)		1.39 (0.71–2.71)	
t008	0.66 (0.31–1.42)		1.33 (0.71–2.50)		0.80 (0.53–1.20)	
t777	1.44 (0.69–3.00)		0.88 (0.33–2.33)		1.15 (0.72–1.83)	
t571			2.16 (1.02–4.58)		2.17 (1.08–4.36)	
t2903	1.65 (0.61–4.48)				1.37 (0.64–2.93)	
Antimicrobials used						
Fluoroquinolones	1.65 (0.49–5.56)	0.42	3.23 (1.26–8.33)	**0.015**	2.48 (1.19–5.17)	**0.016**
Aminoglycosides	1.06 (0.14–8.07)	0.95	2.03 (0.26–15.76)	0.50	1.40 (0.34–5.77)	0.64
Other anti-MSSA agents[d]	0.94 (0.28–3.11)	0.91	1.66 (0.63–4.38)	0.31	1.32 (0.63–2.76)	0.46
Other follow-up variables						
Strict isolation	0.78 (0.32–1.90)	0.58	1.00 (0.53–1.90)	1.00	0.93 (0.55–1.55)	0.77
Contact isolation	0.50 (0.22–1.12)	0.09	1.28 (0.74–2.22)	0.37	0.89 (0.57–1.40)	0.61
Invasive medical device use[e]	0.79 (0.45–1.40)	0.42	1.18 (0.78–1.79)	0.44	1.04 (0.74–1.44)	0.84
Body-washing assistance	0.88 (0.48–1.60)	0.67	1.18 (0.76–1.83)	0.45	1.04 (0.74–1.48)	0.81
Having a wound positive for a nasal strain	0.65 (0.35–1.22)	0.18	0.85 (0.49–1.47)	0.56	0.79 (0.53–1.18)	0.25
Having a wound positive for a nonnasal strain	1.88 (0.92–3.86)	0.09	2.12 (1.13–3.96)	**0.019**	2.04 (1.28–3.25)	**0.003**
Having another nasal *S. aureus* strain			2.75 (0.64–11.89)	0.18	1.01 (0.24–4.16)	0.99

[a] All analyses were adjusted for center.
[b] Boldface *P* values represent statistically significant differences.
[c] French centers 1 to 4 (city in France) were, respectively, Maritime Hospital (Berck), Raymond-Poincaré University Hospital (Garches), Jacques-Calvé Center (Berck), and Sainte-Barbe Center (Fouquières-lès-Lens).
[d] Including β-lactamase-resistant penicillins (Anatomical Therapeutic Chemical Group, J01CF), combinations of penicillins and β-lactamase inhibitors (J01CR), third- and fourth-generation cephalosporins (J01DD/DE), and carbapenems (J01DH).
[e] Including catheter, tracheostomy, enteral nutrition, and/or urination device.
[f] Abbreviations: CI, confidence interval; HR, hazard ratio; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S. aureus*; NA, not applicable.

tings (acute care facilities versus LTCFs), definitions of loss of colonization, follow-up times, frequencies of *S. aureus* sampling, and numbers of anatomical sites screened; the use of *spa* typing to identify strains; and the treatment used to decolonize the patients. Some authors investigated the MRSA colonization duration in rehospitalized patients (8, 12–14). The designs of those studies might have overestimated loss-of-colonization times, if the lag time between discharge and readmission was long. In addition, some exclusion criteria may prevent the accurate estimation of loss-of-colonization times, including shorter colonization episodes, in the analyses. For example, one study excluded patients readmitted ≤3 months postdischarge, resulting in an estimated median time to MRSA clearance of 8.5 months (14), whereas another excluded transient carriers, defined as patients having only 1 MRSA-positive culture and a negative culture within a week (9). Others investigated the MRSA colonization duration at different times after hospital discharge, e.g., 6 months after the initial detection of MRSA, yielding longer colonization duration estimates (10, 15, 30). However, one study conducted in a rehabilitation center estimated the median nasal carriage time to be 44 days, which is not very different from our estimate (7). Moreover, a recent study that collected specimens from patients with MRSA skin and soft tissue infections and their household members for MRSA surveillance cultures every 2 weeks for 6 months reported median MRSA colonization duration of 21 days, a finding which is also close to our findings (31).

Pertinently, fluoroquinolone use in the present study was associated with earlier loss of MSSA colonization but not with loss of MRSA colonization. Fluoroquinolones act mainly against MSSA and not against MRSA (32), which could explain that observation. Consistently, 96.6% of the colonizing MSSA strains were fluoroquinolone sensitive, while 90.5% of the colonizing MRSA strains were fluoroquinolone resistant. Although not statistically significant, the fluoroquinolone effect also favored shorter MRSA

TABLE 4 Multivariable shared-frailty Cox proportional hazards regression models for loss of MRSA, MSSA, or *S. aureus* colonization[d]

Variable	MRSA		MSSA		*S. aureus*	
	HR[a] (95% CI)	*P* value[b]	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Center[c]						
1	Reference	**0.025**	Reference	0.82	Reference	0.18
2	0.55 (0.27–1.10)		1.17 (0.57–2.41)		1.00 (0.58–1.72)	
3	0.36 (0.18–0.72)		0.95 (0.45–2.00)		0.71 (0.41–1.23)	
4	0.34 (0.12–0.98)		0.95 (0.42–2.15)		0.65 (0.33–1.27)	
spa type						
Other *spa* types					Reference	**0.025**
t002					1.48 (0.76–2.86)	
t008					0.75 (0.50–1.12)	
t777					1.12 (0.71–1.78)	
t571					2.31 (1.16–4.57)	
t2903					1.72 (0.81–3.63)	
Fluoroquinolones			3.37 (1.31–8.71)	**0.012**	2.75 (1.32–5.76)	**0.007**
Having a wound positive for nonnasal strain			2.17 (1.15–4.07)	**0.016**	2.32 (1.44–3.74)	**<0.001**

[a] All analyses were adjusted for center.
[b] Boldface *P* values represent statistically significant differences.
[c] French centers 1 to 4 (city in France) were, respectively, Maritime Hospital (Berck), Raymond-Poincaré University Hospital (Garches), Jacques-Calvé Center (Berck), and Sainte-Barbe Center (Fouquières-lès-Lens).
[d] Abbreviations: CI, confidence interval; HR, hazard ratio; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S. aureus*.

colonization durations. Moreover, no interaction between fluoroquinolone use and the methicillin resistance phenotype was found in the final model of loss of *S. aureus* colonization, suggesting that the association between fluoroquinolone use and colonization duration did not differ according to the strain's methicillin resistance phenotype. In contrast, Harbarth et al. reported that prior fluoroquinolone therapy was independently associated with MRSA persistence (17). However, our definition of persistence and fluoroquinolone exposure as a time-dependent covariate differed, enabling assessment of short-term effects (1 week) on the loss of colonization.

Having a wound positive for a nonnasal strain was independently and significantly associated with shorter MSSA or *S. aureus* colonization durations. Univariate analysis indicated the same trend for MRSA colonization durations ($P = 0.09$). *S. aureus* is frequently isolated from hospitalized patients' wounds and may be a source of cross-contamination with the anterior nares (33, 34). A nonnasal strain isolated from a wound may compete with the nasal strain and thereby shorten the nasal *S. aureus* colonization duration. Indeed, some reported observations suggested microbial competition during nasal colonization (26, 27, 35). However, among the 50 *S. aureus* colonization episodes immediately followed by a colonization episode with a different strain, only 5 (10%) were concomitant with colonization of a wound with a nonnasal strain corresponding to that of the subsequent episode.

In earlier investigations, skin lesions or >1 MRSA-positive body sites were predictors of prolonged MRSA colonization (11, 12, 14, 17, 36, 37). We did not include the same variables, but a wound positive for a nasal strain favored longer MRSA, MSSA, and *S. aureus* colonization durations in univariate analyses, albeit not significantly. Our results suggesting opposite effects of having a wound colonized with a nasal or nonnasal strain on the colonization duration highlight the importance of distinguishing between them.

The major strength of our prospective study was the use of a protocol that allowed precise estimation of the *S. aureus* colonization duration, i.e., weekly sampling of patients with a relatively long duration of monitoring (median follow-up, 5 months), made possible by the study setting in LTCFs, where the average stay is 3 months. Moreover, similar results were found when the loss of *S. aureus* colonization was defined as 3 consecutive nasal swab specimens negative for the episode strain, indicating the good sensitivity of the *S. aureus* detection method. Furthermore, *spa* typing enabled the better characterization of the *S. aureus* strains and thereby provided more precise estimates of the colonization duration for each episode. Lastly, the use of shared-frailty models ensured maximum exploitation of the data. Standard survival analysis requires independent data, and the simplest way to study recurrent events is to examine only the time to the first event. That first sensitivity analysis failed to find any association between fluoroquinolone use or a nonnasal strain-positive wound and earlier loss of *S. aureus* or MSSA colonization, thereby illustrating the advantage of this methodology. Conversely, when the sensitivity analyses used survival time recorded as calendar dates, fluoroquinolone use and a nonnasal strain-positive wound were independent risk factors for loss of *S. aureus* colonization, supporting our main analysis outcome.

This study has several limitations. First, missing data made us exclude 111 colonization episodes from the analyses, to avoid underestimating their durations. Moreover, if a patient had been repeatedly colonized with the same strain, we could have considered the episode to be several colonization episodes because of missing data, while it might have been a single episode. In that context, only the colonization episode for which the acquisition interval was known was included, perhaps underestimating colonization durations, even if right censoring was considered in the survival analysis. In contrast, our definition of loss of colonization, intended to avoid possible false-negative swabs, might have overestimated colonization durations.

Second, although colonization pressure was not evaluated, all analyses were adjusted for center to limit potential cofounding by colonization pressure. Moreover, because of interval censoring, we could not discern whether a patient was colonized for 1 week or decolonized and then recolonized very rapidly because of a given strain's high colonization pressure. Nevertheless, 43 (28.9%) patients had ≥2 episodes of colonization with the same *S. aureus* strain during follow-up, suggesting that the persistent carriage frequently described in the literature could be the result of recolonization (4, 38). Furthermore, our second sensitivity analysis using calendar time to better control colonization pressure yielded results consistent with those from our main analysis.

Third, only nasal swab samples were used to determine *S. aureus* colonization status, as the study protocol did not include swabbing of extranasal body sites. Identification of the strains was based only on *spa* typing and methicillin susceptibility, whereas whole-genome sequencing would have increased the resolution of *S. aureus* characterization. Moreover, no information regarding the treatment used for *S. aureus* decolonization was collected during the study, which prevented us from considering this variable in the analyses.

In conclusion, for the LTCF patient cohort described here, the estimated median times to loss of MRSA or MSSA colonization were similar, while fluoroquinolone use and a nonnasal strain-positive wound were independent predictors of the loss of MSSA colonization. These results suggest that fluoroquinolone use shortens the MSSA colonization duration, but fluoroquinolone use is also a risk factor for MRSA acquisition (19); therefore, it is essential that they be prescribed with caution in health care facilities. Finally, because a longer duration of colonization has important implications for infection control policies and patient care and may be associated with an increased risk of infection, further research on the natural history of nasal and extranasal *S. aureus* colonization and on this bacterium's fitness is needed.

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REFERENCES

1. **Williams REO.** 1963. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. Bacteriol Rev **27:**56–71.
2. **McKinnell JA, Huang SS, Eells SJ, Cui E, Miller LG.** 2013. Quantifying the impact of extranasal testing of body sites for methicillin-resistant *Staphylococcus aureus* colonization at the time of hospital or intensive care unit admission. Infect Control Hosp Epidemiol **34:**161–170. http://dx.doi.org/10.1086/669095.
3. **von Eiff C, Becker K, Machka K, Stammer H, Peters G.** 2001. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N Engl J Med **344:**11–16. http://dx.doi.org/10.1056/NEJM200101043440102.
4. **Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL.** 2005. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis **5:**751–762. http://dx.doi.org/10.1016/S1473-3099(05)70295-4.
5. **Nouwen JL, Fieren MW, Snijders S, Verbrugh HA, van Belkum A.** 2005. Persistent (not intermittent) nasal carriage of *Staphylococcus aureus* is the determinant of CPD-related infections. Kidney Int **67:**1084–1092. http://dx.doi.org/10.1111/j.1523-1755.2005.00174.x.
6. **Datta R, Huang SS.** 2008. Risk of infection and death due to methicillin-resistant *Staphylococcus aureus* in long-term carriers. Clin Infect Dis **47:**176–181. http://dx.doi.org/10.1086/589241.
7. **Aeilts GD, Sapico FL, Canawati HN, Malik GM, Montgomerie JZ.** 1982. Methicillin-resistant *Staphylococcus aureus* colonization and infection in a rehabilitation facility. J Clin Microbiol **16:**218–223.
8. **Haverkate MR, Derde LP, Brun-Buisson C, Bonten MJ, Bootsma MC.** 2014. Duration of colonization with antimicrobial-resistant bacteria after ICU discharge. Intensive Care Med **40:**564–571. http://dx.doi.org/10.1007/s00134-014-3225-8.
9. **Larsson AK, Gustafsson E, Nilsson AC, Odenholt I, Ringberg H, Melander E.** 2011. Duration of methicillin-resistant *Staphylococcus aureus* colonization after diagnosis: a four-year experience from southern Sweden. Scand J Infect Dis **43:**456–462. http://dx.doi.org/10.3109/00365548.2011.562530.
10. **Lucet JC, Paoletti X, Demontpion C, Degrave M, Vanjak D, Vincent C, Andremont A, Jarlier V, Mentre F, Nicolas-Chanoine MH, Staphylococcus aureus Resistant a la Meticilline en Hospitalisation a Domicile (SARM HAD) Study Group.** 2009. Carriage of methicillin-resistant *Staphylococcus aureus* in home care settings: prevalence, duration, and transmission to household members. Arch Intern Med **169:**1372–1378. http://dx.doi.org/10.1001/archinternmed.2009.217.
11. **Marschall J, Muhlemann K.** 2006. Duration of methicillin-resistant *Staphylococcus aureus* carriage, according to risk factors for acquisition. Infect Control Hosp Epidemiol **27:**1206–1212. http://dx.doi.org/10.1086/507917.
12. **Mattner F, Biertz F, Ziesing S, Gastmeier P, Chaberny IF.** 2010. Long-term persistence of MRSA in re-admitted patients. Infection **38:**363–371. http://dx.doi.org/10.1007/s15010-010-0038-8.
13. **Sanford MD, Widmer AF, Bale MJ, Jones RN, Wenzel RP.** 1994. Efficient detection and long-term persistence of the carriage of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis **19:**1123–1128. http://dx.doi.org/10.1093/clinids/19.6.1123.
14. **Scanvic A, Denic L, Gaillon S, Giry P, Andremont A, Lucet JC.** 2001. Duration of colonization by methicillin-resistant *Staphylococcus aureus* after hospital discharge and risk factors for prolonged carriage. Clin Infect Dis **32:**1393–1398. http://dx.doi.org/10.1086/320151.
15. **Vriens MR, Blok HE, Gigengack-Baars AC, Mascini EM, van der Werken C, Verhoef J, Troelstra A.** 2005. Methicillin-resistant *Staphylococcus aureus* carriage among patients after hospital discharge. Infect Control Hosp Epidemiol **26:**629–633. http://dx.doi.org/10.1086/502592.
16. **Shenoy ES, Paras ML, Noubary F, Walensky RP, Hooper DC.** 2014. Natural history of colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE): a systematic review. BMC Infect Dis **14:**177. http://dx.doi.org/10.1186/1471-2334-14-177.
17. **Harbarth S, Liassine N, Dharan S, Herrault P, Auckenthaler R, Pittet D.** 2000. Risk factors for persistent carriage of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis **31:**1380–1385. http://dx.doi.org/10.1086/317484.
18. **Alexander EL, Morgan DJ, Kesh S, Weisenberg SA, Zaleskas JM, Kaltsas A, Chevalier JM, Silberzweig J, Barron Y, Mediavilla JR, Kreiswirth BN, Rhee KY.** 2011. Prevalence, persistence, and microbiology of *Staphylococcus aureus* nasal carriage among hemodialysis outpatients at a major New York hospital. Diagn Microbiol Infect Dis **70:**37–44. http://dx.doi.org/10.1016/j.diagmicrobio.2010.12.005.
19. **Couderc C, Jolivet S, Thiébaut ACM, Ligier C, Remy L, Alvarez AS, Lawrence C, Salomon J, Herrmann JL, Guillemot D, Antibiotic Use and *Staphylococcus aureus* Resistant to Antibiotics (ASAR) Study Group.** 2014. Fluoroquinolone use is a risk factor for methicillin-resistant *Staphylococcus aureus* acquisition in long-term care facilities: a nested case-case-control study. Clin Infect Dis **59:**206–215. http://dx.doi.org/10.1093/cid/ciu236.

20. **Alvarez AS, Remy L, Allix-Beguec C, Ligier C, Dupont C, Le Minor O, Lawrence C, Supply P, Guillemot D, Gaillard JL, Salomon J, Herrmann JL.** 2014. Patient nostril microbial flora: individual-dependency and diversity precluding prediction of *Staphylococcus aureus* acquisition. Clin Microbiol Infect **20:**70–78. http://dx.doi.org/10.1111/1469-0691.12208.
21. **Cox DR.** 1972. Regression models and life-tables. J R Stat Soc Series B Stat Methodol **34:**187–220.
22. **Amorim LD, Cai J.** 2015. Modelling recurrent events: a tutorial for analysis in epidemiology. Int J Epidemiol **44:**324–333. http://dx.doi.org/10.1093/ije/dyu222.
23. **Hertz-Picciotto I, Rockhill B.** 1997. Validity and efficiency of approximation methods for tied survival times in Cox regression. Biometrics **53:**1151–1156. http://dx.doi.org/10.2307/2533573.
24. **Ender M, McCallum N, Adhikari R, Berger-Bachi B.** 2004. Fitness cost of SCCmec and methicillin resistance levels in *Staphylococcus aureus*. Antimicrob Agents Chemother **48:**2295–2297. http://dx.doi.org/10.1128/AAC.48.6.2295-2297.2004.
25. **Bjorkman J, Nagaev I, Berg OG, Hughes D, Andersson DI.** 2000. Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. Science **287:**1479–1482. http://dx.doi.org/10.1126/science.287.5457.1479.
26. **Dall'Antonia M, Coen PG, Wilks M, Whiley A, Millar M.** 2005. Competition between methicillin-sensitive and -resistant *Staphylococcus aureus* in the anterior nares. J Hosp Infect **61:**62–67. http://dx.doi.org/10.1016/j.jhin.2005.01.008.
27. **Huang SS, Datta R, Rifas-Shiman S, Kleinman K, Placzek H, Lankiewicz JD, Platt R.** 2011. Colonization with antibiotic-susceptible strains protects against methicillin-resistant *Staphylococcus aureus* but not vancomycin-resistant enterococci acquisition: a nested case-control study. Crit Care **15:**R210. http://dx.doi.org/10.1186/cc10445.
28. **Krebes J, Al-Ghusein H, Feasey N, Breathnach A, Lindsay JA.** 2011. Are nasal carriers of *Staphylococcus aureus* more likely to become colonized or infected with methicillin-resistant *Staphylococcus aureus* on admission to a hospital? J Clin Microbiol **49:**430–432. http://dx.doi.org/10.1128/JCM.02039-10.
29. **Landelle C, Iten A, Uckay I, Sax H, Camus V, Cohen G, Renzi G, Schrenzel J, Pittet D, Perrier A, Harbarth S.** 2014. Does colonization with methicillin-susceptible *Staphylococcus aureus* protect against nosocomial acquisition of methicillin-resistant *S. aureus*? Infect Control Hosp Epidemiol **35:**527–533. http://dx.doi.org/10.1086/675825.
30. **Rogers C, Sharma A, Rimland D, Stafford C, Jernigan J, Satola S, Crispell E, Gaynes R.** 2014. Duration of colonization with methicillin-resistant *Staphylococcus aureus* in an acute care facility: a study to assess epidemiologic features. Am J Infect Control **42:**249–253. http://dx.doi.org/10.1016/j.ajic.2013.09.008.
31. **Cluzet VC, Gerber JS, Nachamkin I, Metlay JP, Zaoutis TE, Davis MF, Julian KG, Royer D, Linkin DR, Coffin SE, Margolis DJ, Hollander JE, Mistry RD, Gavin LJ, Tolomeo P, Wise JA, Wheeler MK, Bilker WB, Han X, Hu B, Fishman NO, Lautenbach E.** 2015. Duration of colonization and determinants of earlier clearance of colonization with methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis **60:**1489–1496. http://dx.doi.org/10.1093/cid/civ075.
32. **Hooper DC.** 2002. Fluoroquinolone resistance among Gram-positive cocci. Lancet Infect Dis **2:**530–538. http://dx.doi.org/10.1016/S1473-3099(02)00369-9.
33. **Almeida GC, dos Santos MM, Lima NG, Cidral TA, Melo MC, Lima KC.** 2014. Prevalence and factors associated with wound colonization by *Staphylococcus* spp. and *Staphylococcus aureus* in hospitalized patients in inland northeastern Brazil: a cross-sectional study. BMC Infect Dis **14:**328. http://dx.doi.org/10.1186/1471-2334-14-328.
34. **Bessa LJ, Fazii P, Di Giulio M, Cellini L.** 2015. Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. Int Wound J **12:**47–52. http://dx.doi.org/10.1111/iwj.12049.
35. **Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR.** 2010. The human nasal microbiota and *Staphylococcus aureus* carriage. PLoS One **5:**e10598. http://dx.doi.org/10.1371/journal.pone.0010598.
36. **MacKinnon MM, Allen KD.** 2000. Long-term MRSA carriage in hospital patients. J Hosp Infect **46:**216–221. http://dx.doi.org/10.1053/jhin.2000.0807.
37. **Robicsek A, Beaumont JL, Peterson LR.** 2009. Duration of colonization with methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis **48:**910–913. http://dx.doi.org/10.1086/597296.
38. **van Belkum A, Verkaik NJ, de Vogel CP, Boelens HA, Verveer J, Nouwen JL, Verbrugh HA, Wertheim HF.** 2009. Reclassification of *Staphylococcus aureus* nasal carriage types. J Infect Dis **199:**1820–1826. http://dx.doi.org/10.1086/599119.