Emergence of Sequence Type 398 as a Community- and Healthcare-Associated Methicillin-Susceptible *Staphylococcus aureus* in Northern Manhattan

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The methicillin-susceptible *Staphylococcus aureus* (MSSA) clone sequence type (ST) 398 has increasingly been identified as a pathogen in diverse geographic settings, yet its epidemiology remains incompletely understood. In this case-control study of MSSA infections, we identified ST398 MSSA as both a major community- and hospital-associated MSSA pathogen in the Dominican neighborhood of northern Manhattan.

Keywords. *S. aureus*; MSSA; CC398; human ST398; case-control study.

Over the past decade, infections with *Staphylococcus aureus* sequence type 398 (ST398) have emerged worldwide [1–5]. There is mounting epidemiological and genetic evidence that methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) ST398 constitute at least 2 clinically and biologically distinct lineages, with MSSA ST398 the likely precursor of MRSA ST398 [2, 6]. Whereas MRSA ST398 has been associated with exposure to livestock and is rarely encountered beyond immediate animal contacts, MSSA ST398 appears readily transmissible within community households [2].

Received 26 February 2013; accepted 19 May 2013; electronically published 31 May 2013.

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Clinical Infectious Diseases 2013;57(5):700–3

DOI: 10.1093/cid/cit375

However, the epidemiology of human MSSA ST398 infections remains poorly described. Most notably, MSSA ST398 accounted for a high proportion of *S. aureus* infections in China and has been described in invasive infections in the Netherlands, France, and the United States [2–5]. Although it is present as one of the most common infectious MSSA clones in the New York City area [2], ST398 has been absent from other MSSA sample collections across the United States [7, 8].

Here, we systematically surveyed MSSA infections for the presence of ST398 at a tertiary care hospital in northern Manhattan over a 2-year period. We then carried out a case-control study to assess demographic, clinical, and microbiological risk factors for ST398 MSSA infections compared to non-ST398 MSSA infections.

METHODS

This study was approved by the Columbia University Medical Center (CUMC) institutional review board. Between January 2010 and January 2012, all available MSSA specimens (which excluded bloodstream isolates) cultured at the clinical microbiology laboratory at CUMC were prospectively collected and tested with a ST398-lineage specific polymerase chain reaction (PCR) [9].

Using a random-number generator, each ST398 case was matched with 3 MSSA controls from the same sample collection. All isolates were characterized by *S. aureus* protein A (*spa*) typing using Ridom StaphType software and clustered into *spa* clonal complexes (spa-CCs) and screened for the presence of Panton-Valentine leukocidin (PVL) as described elsewhere [10]. *Spa* types consistent with the ST398 lineage were further verified by multilocus sequence typing [10].

Demographic and clinical information was extracted from the patient's electronic medical records by retrospective chart review. Minimum inhibitory concentrations for standard gram-positive antimicrobials were determined using broth microdilution panels purchased from MicroScan (Dade Behring, Sacramento, California). Expression of inducible clindamycin resistance due to the *erm* gene was determined by the Kirby-Bauer D-test [10].

Univariate analysis was performed using Fisher exact tests. All variables with a P value of <.2 in univariate models were entered into the initial multivariate logistic regression model. The stepwise selection method with entry and stay level of $P = .1$ was used to build the final multivariate logistic regression

Table 1. Study Population Characteristics, by Case-Control Status

Characteristic	Cases, No. (%) (n = 64)	Controls, No. (%) (n = 192)	*P* Value[a]
Demographics			
Female	30 (47)	93 (48)	.89
Age category, y			**<.01**
<5	7 (11)	57 (30)	
5–18	8 (13)	29 (15)	
19–65	38 (59)	73 (38)	
>65	11 (17)	33 (17)	
Hispanic ethnicity	42 (66)	57 (30)	**<.0001**
Race			.66
Asian	0 (0)	5 (2.6)	
Black	8 (13)	21 (11)	
White	19 (30)	65 (35)	
Unknown	37 (58)	101 (53)	
Northern Manhattan/ Bronx zip code	53 (83)	109 (57)	**<.001**
Comorbidities			
Diabetes mellitus	9 (14)	25 (13)	.83
Alcohol dependence	6 (9.4)	2 (1.0)	**<.01**
Cancer	6 (9.4)	16 (8.3)	.80
Immunosuppressive therapy	3 (4.7)	14 (7.3)	.57
Cystic fibrosis	1 (1.6)	14 (7.3)	.13
Anatomic malformation	0 (0)	27 (14)	**.001**
Underlying skin disease	4 (6.3)	19 (10)	.46
Cardiovascular disease	15 (23)	56 (29)	.42
Pulmonary disease	4 (6.3)	29 (15)	.08
End-stage renal disease (hemodialysis)	1 (1.6)	4 (2.1)	1.0
Liver disease	5 (7.8)	4 (2.1)	**<.05**
Type of infection			
Skin and soft tissue infection	40 (63)	117 (61)	.88
Pneumonia			.56
Community-acquired	2 (3.1)	4 (2.1)	
Hospital-acquired	2 (3.1)	5 (2.6)	
Ventilator-associated	5 (7.8)	8 (4.2)	
Urinary tract infections	2 (3.1)	3 (1.6)	.60
Eye/ear infections	3 (4.7)	10 (5.2)	1.0
Osteomyelitis	3 (4.7)	7 (3.6)	.71
Secondary bacteremia	4 (6.3)	3 (1.6)	.07
Colonizer	6 (9.4)	33 (17)	.16
Community vs hospital infection			
Admitted to hospital at onset of infection	27 (42)	79 (41)	1.0
Admitted for infection	16 (25)	35 (18)	.28
Hospital onset, >48 h admission	8 (13)	34 (18)	.48
Hospital admission past 6 mo	26 (41)	42 (22)	**<.01**
Nursing home resident	0 (0)	10 (5.2)	.07
Community-associated[b]	34 (53)	127 (66)	.07
Prior surgery	10 (16)	44 (23)	.29
Postsurgical infection	2 (3.1)	6 (3.1)	1.0
ICU stay	8 (13)	34 (18)	.55
Death	4 (6.3)	8 (4.2)	.50
Drug resistance			
Penicillin	16 (25)	90 (47)	**<.001**
Levofloxacin	2 (3.1)	13 (6.8)	.18
Erythromycin	62 (97)	77 (40)	**<.0001**
Clindamycin (Kirby-Bauer)	62 (97)	48 (25)	**<.0001**
Tetracycline	0 (0)	8 (4.2)	.26
PVL presence	8 (13)	46 (24)	.12

Table 1 continued.

Abbreviations: ICU, intensive unit; PVL, Panton-Valentine leukocidin.

[a] Fisher exact test.

[b] Community associated defined as culture positive <48 hours after admission and lack of hospitalization or long-term care facility residence over past 6 months.

model. The adjusted odds ratio (OR) and 95% confidence interval (CI) for the variables selected in the final model were reported. All statistical analyses were carried out using SAS software, version 9.1 (SAS Institute, Cary, North Carolina).

RESULTS

We screened 1607 successive nonbloodstream MSSA cultures from individual patients at CUMC with ST398 lineage-specific PCR and identified 64 (4.0%) positive samples [9]. The majority of these samples were *spa* type t571 (n = 51 [80%]), whereas other *spa* types included t1451 (n = 7) and 1 each of t5635, t6440, t6605, t6608, and t7331. These *spa* types were verified as ST398 by MLST typing.

Most patients with ST398 infections lived in the hospital's catchment area in northern Manhattan (n = 33), in the Bronx (n = 20), and only rarely in other boroughs of the city (n = 5), New York State (n = 5), or New Jersey (n = 1). Remarkably, nearly half of the patients (n = 26 [41%]) had been admitted to the hospital in the 6 months prior to their ST398 infection. In addition, 8 patients (13%) who acquired the infection during their hospital stay.

In a case-control study, we compared each ST398 case with 3 randomly matched non-ST398 MSSA controls (Table 1). Among the 192 control MSSA isolates, spa-CC t008-ST8 (n = 34 [18%]), spa-CC t665-ST30 (n = 31 [16%]), spa-CC t002-ST5 (n = 24 [13%]), and spa-CC t084 (n = 18 [9.4%]) were most frequently encountered.

The frequency of PVL positivity was similar in both groups (Table 1). ST398 and control MSSA isolates were highly

susceptible to most antimicrobials tested. However, almost all ST398 isolates were resistant to erythromycin and clindamycin (97%) compared to only 38% ($P < .0001$) of controls.

In both groups, more men than women were infected. Cases and controls differed in their age distribution, ethnicity, and area of residence. Compared to controls, ST398 patients were more likely aged 19–65 years (59% vs 38%, $P = .007$), whereas more controls were <5 years of age. Compared to controls, ST398 patients identified themselves more frequently as Hispanic (66% vs 30%; $P < .0001$) and resided in the area surrounding the hospital (82% vs 38%; $P = .0004$).

Both cases and controls exhibited a predominance of skin and soft tissue infections (SSTIs), and shared similar measures of hospital admission and mortality (Table 1). ST398 cases were more frequently associated with secondary bacteremias, but this did not reach statistical significance (6.3% vs 1.6%, $P = .07$). ST398 cases had been more frequently admitted to the hospital over the 6 months preceding their positive culture (41% vs 22%, $P = .005$), whereas overall both ST398 and controls were frequently consistent with community-associated *S. aureus* infections (53% vs 66%, $P = .07$).

Although many potentially predisposing comorbidities for MSSA infections were shared between cases and controls (such as diabetes mellitus, cancer, or underlying skin disease), more patients with ST398 had liver cirrhosis and alcohol dependence than did controls. In contrast, controls more frequently had congenital malformations (Table 1).

In multivariate analysis, living in the northern Manhattan/Bronx zip codes (OR, 3.1 [95% CI, 1.3–7.5]), Hispanic ethnicity (OR, 3.4 [95% CI, 1.7–7.1]), admission to the hospital in the 6 months prior to infection (OR, 2.7 [95% CI, 1.3–5.7]), and a diagnosis of alcohol abuse (OR, 6.0 [95% CI, 1.0–35.4]) were significantly associated with ST398 MSSA infection.

DISCUSSION

Infections with ST398 MSSA have been encountered worldwide, but in the United States most cases appear to be restricted to New York City [7, 8, 11, 12]. The current study examined the epidemiology of nonbacteremic ST398 infections at a tertiary care hospital in northern Manhattan, a neighborhood with high ST398 colonization in community households [2]. Hispanic ethnicity and residence in the hospital catchment area were independently associated with ST398 infections, suggesting that the colonized community serves as the source of infections. Unexpectedly, patients with ST398 infections were also more frequently hospitalized over the 6 months prior to infection. Our findings suggest that ST398 has emerged as both a community- and hospital-associated pathogen.

We noted a trend to more frequent episodes of secondary invasive disease with ST398, which is consistent with a recent survey of French bloodstream infections [4]. An increase in invasive disease may indicate yet unidentified virulence features of ST398 MSSA. Alternatively, it may also reflect underlying comorbidities or an immunosuppressed state of the affected host. Poorer health of a subset of ST398 patients in our population is indirectly suggested by the higher proportion of alcohol abuse, cirrhosis, and recent hospitalization. In addition, this could suggest nosocomial acquisition of ST398 MSSA, where patients acquired the clone in the hospital and subsequently developed an infection in the community.

Our study demonstrated that ST398 infections were common among Hispanics living in the Dominican community of Washington Heights. As such, we hypothesize that this strain was introduced to New York by Dominicans traveling between the 2 countries and that it continues to spread within this group [10]. Although some of the differences between cases and controls may be intrinsic to ethnicity, several ST398-infected patients were non-Hispanic and resided in other parts of the city. This suggests local transmission or alternative reservoirs of ST398.

Several limitations to our study need to be considered. First, data collection was based on a retrospective review of medical records, which may have introduced a potential information bias. Second, matching cases and controls by variables such as age or site of infection could have influenced outcomes. Third, the control group consisted of genetically diverse MSSA with 4 predominant spa-CCs, which might have biased the comparisons. Last, this study represents the experience at a single tertiary care hospital and may not be generalized to other settings.

Although ST398 MSSA has been suggested as the precursor of livestock-associated–ST398, this strain has been notably absent from large European and American MSSA sample collections from the 1990s and early 2000s [7, 8, 13, 14]. These studies likely only provide an incomplete picture of the global molecular epidemiology of MSSA infections. Nevertheless, the combined results argue for a more recent clonal dissemination of ST398. We speculate that in part this may have been driven by the almost universal presence of erythromycin and clindamycin resistance in human ST398 MSSA isolates. Clindamycin is part of the empirical coverage for community-associated MRSA in outpatients, especially children, with SSTI.

Taken together, we observed that ST398 MSSA is a frequent cause of both community and hospital-associated MSSA infections in northern Manhattan. Clinical predictors of ST398 infections were mainly associated with people living in the largely Dominican neighborhood surrounding the hospital. The unexpectedly high frequency of recent healthcare contact among ST398 patients highlights the importance of community colonizers as potential reservoirs for nosocomial pathogens. This study also emphasizes the utility of MSSA surveillance programs, rather than efforts solely to monitor MRSA, as MSSA continues to account for the majority of *S. aureus* infections worldwide.

Notes

Acknowledgments. We thank the clinical microbiology laboratory and Malcolm Rothman for collection of methicillin-susceptible *Staphylococcus aureus* specimens.

Financial support. This research was supported by the National Institutes of Health (K08 AI090013 to A.-C. U.) and (R01 AI077690 and R01 AI077690-S1 to F. D. L.) and by the Paul A. Marks Scholarship (to A.-C. U.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. van Cleef BA, Monnet DL, Voss A, et al. Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. Emerg Infect Dis **2011**; 17:502–5.
2. Uhlemann AC, Porcella SF, Trivedi S, et al. Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. MBio **2012**; 3:e00027–12.
3. Fan J, Shu M, Zhang G, et al. Biogeography and virulence of *Staphylococcus aureus*. PLoS One **2009**; 4:e6216.
4. Valentin-Domelier AS, Girard M, Bertrand X, et al. Methicillin-susceptible ST398 *Staphylococcus aureus* responsible for bloodstream infections: an emerging human-adapted subclone? PLoS One **2011**; 6: e28369.
5. Verkade E, Bergmans AM, Budding AE, et al. Recent emergence of *Staphylococcus aureus* clonal complex 398 in human blood cultures. PLoS One **2012**; 7:e41855.
6. Price LB, Stegger M, Hasman H, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. MBio **2012**; 3:e00305–11.
7. Campbell SJ, Deshmukh HS, Nelson CL, et al. Genotypic characteristics of *Staphylococcus aureus* isolates from a multinational trial of complicated skin and skin structure infections. J Clin Microbiol **2008**; 46:678–84.
8. Goering RV, Shawar RM, Scangarella NE, et al. Molecular epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from global clinical trials. J Clin Microbiol **2008**; 46: 2842–7.
9. Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L. Rapid PCR detection of *Staphylococcus aureus* clonal complex 398 by targeting the restriction-modification system carrying sau1-hsdS1. J Clin Microbiol **2011**; 49:732–4.
10. Uhlemann AC, Dumortier C, Hafer C, et al. Molecular characterization of *Staphylococcus aureus* from outpatients in the Caribbean reveals the presence of pandemic clones. Eur J Clin Microbiol Infect Dis **2012**; 31: 505–11.
11. Orscheln RC, Hunstad DA, Fritz SA, et al. Contribution of genetically restricted, methicillin-susceptible strains to the ongoing epidemic of community-acquired *Staphylococcus aureus* infections. Clin Infect Dis **2009**; 49:536–42.
12. Mediavilla JR, Chen L, Uhlemann AC, et al. Methicillin-susceptible *Staphylococcus aureus* ST398, New York and New Jersey, USA. Emerg Infect Dis **2012**; 18:700–2.
13. Rijnders MI, Deurenberg RH, Boumans ML, Hoogkamp-Korstanje JA, Beisser PS, Stobberingh EE. Population structure of *Staphylococcus* aureus strains isolated from intensive care unit patients in the Netherlands over an 11-year period (1996 to 2006). J Clin Microbiol **2009**; 47:4090–5.
14. Crisostomo MI, Westh H, Tomasz A, Chung M, Oliveira DC, de Lencastre H. The evolution of methicillin resistance in *Staphylococcus aureus*: similarity of genetic backgrounds in historically early methicillin-susceptible and -resistant isolates and contemporary epidemic clones. Proc Natl Acad Sci U S A **2001**; 98:9865–70.