JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2008, p. 477–479 Vol. 46, No. 2
0095-1137/08/$08.00+0 doi:10.1128/JCM.01596-07

Methicillin-Resistant *Staphylococcus aureus* Nasal Carriage among Injection Drug Users: Six Years Later▽

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Received 10 August 2007/Returned for modification 2 October 2007/Accepted 13 November 2007

A survey in 2000 to detect methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in Vancouver downtown east side injection drug users (IDUs) revealed an MRSA nasal colonization incidence of 7.4%. This is a follow-up study to determine the current prevalence of MRSA colonization and to further characterize the isolates and risk factors for colonization. In this point prevalence study of MRSA nasal carriage among IDUs, nasal swabs were cultured to detect *S. aureus*. Isolates were studied for their antimicrobial susceptibility patterns and the presence of *mecA* and Panton-Valentine leukocidin (PVL) genes and by pulsed-field gel electrophoresis (PFGE). *S. aureus* was isolated from 119 of 301 (39.5%) samples; three (2.5%) participants had both methicillin-sensitive *S. aureus* (MSSA) and MRSA, resulting in 122 isolates. Of these, 54.1% were MSSA and 45.9% were MRSA, with an overall MRSA rate of 18.6%. USA-300 (CMRSA-10) accounted for 75% of all MRSA isolates; 25% were USA-500 (CMRSA-5). None of the USA-500 isolates were positive for PVL; 41 (97.6%) USA-300 isolates contained PVL. One MSSA isolate, from an individual also carrying USA-300, was positive for PVL. The PFGE pattern of this MSSA isolate was related to that of the MRSA strain. The antibiograms of USA-300 compared to USA-500 isolates showed 100% versus 7.1% susceptibility to trimethoprim-sulfamethoxazole (TMP-SMX) and 54.8% versus 7.1% susceptibility to clindamycin. MRSA nasal colonization in this population has increased significantly within the last 6 years, with USA-300 replacing the previous strain. Most of these strains are PVL positive, and all are susceptible to TMP-SMX.

Illicit drug use is a universal health problem, with an estimated 13 million injection drug users (IDUs) worldwide (20). Skin and soft tissue infections are the leading cause for emergency department visits and subsequent hospitalizations of IDUs; *Staphylococcus aureus* and *Streptococcus pyogenes* are important and common pathogens in this setting (18). *S. aureus* nasal carriage, present in about 20% of the general population, has been identified as a risk factor for the subsequent development of community-acquired and nosocomial staphylococcal infections (14, 22).

People who misuse drugs have a higher rate of nasal or skin colonization with *S. aureus* than the general population (2, 14). Furthermore, *S. aureus* nasal carriage has been found to be associated with an increased risk of subsequent infections in IDUs (2).

A recent upsurge in skin infections, abscesses, and more invasive infections among IDUs in North America has been reported in the literature (4, 12). The causative agent of these outbreaks, referred to as community-associated methicillin-resistant *S. aureus* (MRSA), was distinguished by the mobile genetic element staphylococcal chromosomal cassette (SCC) *mec* type IV and the presence of the Panton-Valentine leukocidin (PVL) toxin. (16).

This study was a follow-up to a previous point prevalence survey of IDUs performed 6 years ago (7). Among 229 IDUs surveyed in 2000, 27% had *S. aureus* nasal colonization, with an overall MRSA colonization rate of 7.4%. Molecular typing showed that all isolates had one clonal pattern (USA-500, or CMRSA-5). The objectives of the current study were to determine the prevalence of methicillin-susceptible *S. aureus* (MSSA) and MRSA nasal colonization in IDUs, to compare it to the available data from 2000, to identify risk factors for colonization, and to characterize the isolates by using molecular methods.

MATERIALS AND METHODS

An opportunistic point prevalence study of MRSA nasal carriage among IDUs was conducted in the downtown east side (DTES) of Vancouver, British Columbia, Canada, from 1 April to 1 May 2006. Subjects were recruited voluntarily from social and health service centers including three medical clinics, two drop-in centers, and a safe injection site.

Participants must have injected drugs within the preceding year to be eligible for the study. Each individual signed a written consent, and a single swab of both nares was obtained (Venturi Transystem; Copan Diagnostics, Corona, CA). The swab was inserted approximately 2 cm into the nares and rolled a few times in each nostril. In addition, a brief anonymous questionnaire was administered to determine other risk factors including (i) hospitalization within the last 3 months, (ii) travel outside Vancouver within the last 12 months, and (iii) usage of antibiotics within the last 12 months. Exclusion criteria included cessation of injection drug use more than a year prior to the study period and an inability or unwillingness to give written informed consent. The study protocol and consent forms were approved by the Vancouver Coastal Health Research Institute and the University of British Columbia Clinical Research Ethics Board.

Population demographics. DTES is an inner-city neighborhood comprising 10 square blocks with an estimated population of 16,000. Approximately 9,000 (56.3%) of the residents are IDUs. Males comprise 54% of the population, compared with 50% in Vancouver overall. Aboriginals represent 8.4% of DTES residents, compared to 4.4% of the population of British Columbia. Poor or

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▽ Published ahead of print on 26 November 2007.

TABLE 1. Nasal colonization and microbiological characteristics of isolates from 2000 versus 2006

Characteristic	Value for:		*P*
	2000 (total participants, 299)	2006 (total participants, 301)	
No. (%) of participants with *S. aureus*	81 (27.1)	119 (39.5%)[a]	0.001
No. (%) of *S. aureus* isolates			0.008
MSSA	59 (72.8)	66 (54.1)	
MRSA	22 (27.2)	56 (45.9)	
Overall MRSA colonization rate (%)	7.4	18.6	0.0001
PFGE pattern of MRSA (no. [%])			0.0001
USA-500	22 (100)	14 (25)	
USA-300	0	42 (75)	
PVL (%)			
MSSA	NA[b]	1 (1.5%)	
USA-500	0	0	0.0001
USA-300		41 (97.6%)	

[a] Three participants had mixed colonization with both MSSA and MRSA; thus, the total number of isolates was 122.
[b] NA, not available for analysis.

TABLE 2. Antibiotic susceptibility profile and risk factors for colonization with USA-300 versus USA-500

Characteristic	No. (%) of isolates in indicated cluster		*P*
	USA-300	USA-500	
Total	42 (75)	14 (25)	
Susceptibility to:			
TMP-SMX	42 (100)	1 (7.1)	0.0001
Ciprofloxacin	2 (4.8)	0	1.0
Tetracycline	40 (95.2)	0	0.0001
Clindamycin	23 (54.8)	1 (7.1)	0.002
Erythromycin	1 (2.4)	1 (7.1)	0.44
Recent hospitalization	12 (28.6)	3 (21.4)	0.74
Recent antibiotic use	36 (85.7)[a]	12 (85.7)	1.0
Recent travel	17 (40.5)[a]	4 (28.6)	0.53

[a] One participant was unsure.

substandard living conditions in the DTES, including poverty, crowded housing, homelessness, poor nutrition and hygiene, substance abuse, and chronic illness, place inhabitants at high risk for communicable diseases including human immunodeficiency virus and hepatitis B and C (3).

Laboratory methods. Specimens were transported to the Vancouver General Hospital laboratory and cultured within 24 h of collection. Nasal specimens were inoculated on Columbia agar plates with 5% sheep blood and incubated under 5% CO_2 at 35°C for 24 h. *S. aureus* was identified by growth characteristics and by the subsequent detection of catalase and coagulase activities. Screening for MRSA was done using cefoxitin disks (30 μg) as recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (5). The presence of the *mecA* gene and the *S. aureus*-specific *nuc* gene was confirmed by PCR. Molecular typing of MRSA isolates was performed by pulsed-field gel electrophoresis (PFGE) following SmaI restriction digestion (1), and each isolate was compared to each of the other isolates and to the 10 Canadian and U.S. epidemic MRSA strains (17, 19).

All *S. aureus* isolates were assessed for the presence of the PVL genes *lukF-PV* and *lukS-PV* (16). Antibiotic susceptibility testing was performed using the disk diffusion method according to CLSI performance standards (5). The antibiogram included trimethoprim-sulfamethoxazole (TMP-SMX), tetracycline, ciprofloxacin, clindamycin, erythromycin, and the D-test when indicated (10).

Statistics. Association tests on contingency tables (e.g., PVL positivity versus USA-300/USA-500, tetracycline sensitivity versus USA-300/USA-500, etc.) were calculated using Fisher's exact test to test whether there were significant differences between observed and expected frequencies. Results were determined to be statistically significant if the *P* value was <0.05. All tests were performed using GraphPad software (http://graphpad.com/quickcalcs/index.cfm).

RESULTS

During the study period, a total of 429 participants were interviewed, 301 of whom met the inclusion criteria and agreed to have a nasal swab performed. *S. aureus* grew in 122 specimens from 119 participants (39.5% overall carriage) (Table 1); 3 individuals had mixed colonization with both MSSA and MRSA. Of these 122 isolates, 66 (54.1%) were MSSA and 56 (45.9%) were MRSA.

PFGE typing of the MRSA isolates revealed two clusters: 42 (75%) were identified as USA-300 (CMRSA-10) and 14 (25%) as USA-500 (CMRSA-5). The USA-300 cluster contained six subtypes, and the USA-500 cluster contained four subtypes. Of the 42 USA-300 isolates, 41 (97.6%) were positive for PVL, whereas none of the USA-500 were PVL positive (Table 1).

MSSA isolates were negative for PVL with the exception of one strain that was isolated from an individual also carrying a USA-300 strain. The PFGE pattern of this MSSA strain differed from that of the MRSA strain by only two bands, consistent with a deletion of less than 50 kb in the MSSA strain.

MRSA isolates demonstrated lower rates of susceptibility than MSSA isolates to TMP-SMX, ciprofloxacin, tetracycline, clindamycin, and erythromycin. USA-300 strains were more likely than USA-500 strains to retain susceptibility to TMP-SMX (100%), tetracycline (95.2%), and clindamycin (54.8%) (Table 2).

Risk factors for MRSA colonization were not significantly different from those for MSSA colonization: 85.7% versus 69.7% for antibiotic use ($P = 0.05$) and 26.8% versus 15.2% for hospitalization ($P = 0.12$). Furthermore, no differences for carriage of different MRSA strains were noticed with regard to hospitalization, antibiotic use, or travel between the participants (Table 2).

DISCUSSION

Nasal carriage of MRSA plays a key role in the epidemiology and pathogenesis of staphylococcal infections. This study is unique in that it documents the changing pattern of MRSA genotypes within a defined high-risk population. The 2000 study population of IDUs in the DTES was approached by a team of two nurses for needle exchange. In the current study, the same DTES population was recruited through the community clinics and the recently established safe injection site.

The increase in *S. aureus* nasal colonization was a direct result of a significant increase in MRSA carriage, while the absolute numbers of persons with MSSA remained relatively stable. A new clonal type, USA-300, accounted for the majority of MRSA isolates, supplanting the previous 100% prevalence of USA-500 in this population of IDUs. The antimicrobial susceptibility profile reflected this shift, with an overall increase in susceptibility to TMP-SXT, tetracycline, and clindamycin. This novel strain has previously been recognized as a major cause of infections in a high-risk population such as IDUs (11, 12).

Recent hospitalization and antibiotic usage rates were higher, though not statistically significantly so, for participants with MRSA colonization than for those with MSSA. However, no differences in risk factors for colonization with either USA-500 or USA-300 were noted; in particular, no differences in recent hospital stay were observed. This may be due to the less stringent definition of prior hospitalization within a 3-month period used in this study. Regardless, the relative contribution of cross-contamination in the community versus transmission of the "community" strain (USA-300) in local health care facilities will remain unknown until more definitive epidemiological surveys can be performed. The additional burden that USA-300 will place on health care facilities and community resources has yet to be fully determined (15, 23). Vancouver General Hospital (which is the health care facility for approximately 20% of the DTES walk-in emergency visits and approximately 40% of the ambulance intake for this area) alone has noted an increase in the number of admitted patients with community-associated MRSA as a percentage of all MRSA cases from 4% (2003) to 16% (2004) and 27% (2005) (21). An obvious concern is that intermingling of the two MRSA "populations" will eventually result in "hospital" strains that acquire the virulence factors predisposing to aggressive soft tissue infection, while "community" strains become more resistant to antimicrobials.

The ability of MRSA strains to change their molecular profile was neatly demonstrated in this study by the case of a patient who was dually colonized with MSSA and USA-300. The MSSA strain was, surprisingly, PVL positive and had a PFGE pattern similar to that of the USA-300 isolate. This molecular aberration was likely due to the loss of the SCC*mec* cassette or a portion thereof and is consistent with the relatively high rate of excision and integration of SCC*mec* IV (6, 8, 9, 13).

This study highlights the need for interventional measures in high-risk groups, not only to minimize further acquisition in these populations but also to prevent the spread of community strains within health care facilities and the general population.

ACKNOWLEDGMENTS

We are grateful to Reg Daggitt and the staff of the Downtown Community Health Centre, Jane Porter and the staff of the Pender Clinic, Chris Buchner and the staff of INSITE, the DTES safe injection site, and Steve Adilman and Tina Braun of Vancouver Native Health Clinic for help with participant enrollment and specimen collection. We thank Leslie Forrester for help with the statistical analysis and Steve Reynolds for his contribution in the initial design of the study. We also thank Leane Kishi and Linda Wishart, who performed the laboratory/molecular testing.

All authors declare no conflicts of interest.

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