Prevalence and Characterization of Community Acquired Methicillin Resistant *Staphylococcus aureus* Colonization in High-Risk Individuals in Toronto, Canada.

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Abstract:

Introduction: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has emerged as a common pathogen causing skin and soft tissue infections (SSTI's). In many parts of North America, CA-MRSA has now replaced methicillin-susceptible *S. aureus* (MSSA) as the primary cause of SSTI's. There are several commonly cited risks for CA-MRSA infection, yet little is known about colonization rates in high-risk individuals.

Methods: Between July 10 and August 18, 2007, 295 consenting male residents of a Toronto community shelter provided information regarding risk factors for MRSA colonization, and swabs from their nares, axilla, and any visibly open sores. Swabs were enriched and selectively cultured for for MRSA and MSSA, which were identified using standard methods. MRSA were typed by *SmaI* PFGE, and *SCCmec* type and presence of PVL was determined by PCR.

Results: Overall, 110 (37%) and 12 (4.1%) or residents screened positive for MSSA and MRSA, respectively. MRSA were of 5 distinct types, the largest cluster included 6 residents positive from 11 sites, including a post-frostbite foot infection. This unusually resistant (R; R to clindamycin and fusidin) cluster was closely related to the PVL-positive community strain, CMRSA-10 (USA-300). Two other residents carried the typical CMRSA-10/USA-300 strain responsible for the surge of CA-MRSA in North America. The remaining 4 MRSA (all PVL-negative) included 2 CMRSA-2 (USA-800) with community-acquired *SCCmec*-IVa (R to ß-lactams only), 1 CMRSA-2 (USA-100) with *SCCmec*-II (the common nosocomial strain), and 1 CMRSA-1 (USA-600) also carrying the community *SCCmec*-IVa cassette.

Conclusion: The most common isolate of CA-MRSA found in this cohort of high-risk individuals was an unusually drug resistant variant of the most common strain of CA-MRSA. The implications of colonization with this strain are yet to be determined.

Introduction:

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged in Canada in the early 1980's; however it was primarily a nosocomial infection and has only recently emerged as a community-associated infection (CA-MRSA). *S. aureus* is frequently associated with skin and soft tissue infections (SSTI's), and in many parts of North America, CA-MRSA is replacing methicillin-sensitive *S. aureus* (MSSA) as the most common cause of SSTI's. CA-MRSA has predominately been associated with a single strain, which is characteristically PVL-positive, *msrA*-positive, and belonging to the CMRSA-10/USA300 pulsed field type.

Several risk factors, including homelessness, have been associated with CA-MRSA infection. In 2003, surveillance screening in ~300 homeless men in a Toronto shelter identified no individuals colonized with CA-MRSA. A notable increase in CA-MRSA symptomatic infections in Toronto emergency departments has prompted us to re-examine the colonization rates in this population.

The purpose of this study was to determine the CA-MRSA colonization rate in a downtown Toronto shelter for homeless men.

Table 1

Demographic Information	Number (%)	
	MRSA - (N=284)	MRSA + (N=12)
Age (median, range)	48 yrs (25-83)	48yrs (33-69)
First Nations/Aboriginal ethnicity	10 (3%)	3 (25%)**
Admitted to hospital in last 12 months	80 (28%)	6 (50%)*
ED visit in last 12 months	131 (46%)	6 (50%)
Skin infection in last 12 months	30 (11%)	3 (25%)*
History of MRSA colonization	3 (1.0%)	0
Antibiotics within the last 3 months	45 (15%)	1 (9%)
Lived in another shelter in the last 12 months	83 (29%)	3 (25%)
Lived on the street in the last 12 months	71 (25%)	4 (33%)
Years homeless (median, range)	2.0 yrs (0-40)	1.5 yrs (0-17)
Share personal items with other men at shelter	28 (10%)	1 (8%)
Gay/Lesbian/Bisexual	13 (5%)	0
IV Drug user	60 (21%)	1 (8%)
HIV positive	7 (2%)	0
Chronic skin conditions (e.g. psoriasis)	30 (10%)	1 (8%)
Prison in the last year	67 (24%)	4 (33%)

Methods:

Approval for this prospective observational study was obtained from the Research Ethics Board's of both Mt Sinai and St Michaels Hospital's. Between July 10, and August 18, 2007, adult male residents of a large (500 bed) shelter for homeless men were asked to participate.

Consenting individuals had swabs taken from their anterior nares and axilla, as well as from any open wound, if reported by the resident. Swabs were placed in Starswab II Charcoal transport medium (Starplex Scientific Inc, Toronto, Ontario), and held at room temperature until processing (done the evening following swab collection). Swabs were processed by placing them in 2 ml of BHI broth (Oxoid, Nepean ON) for overnight incubation at 37^{0}C, and then plating in Mannitol Salt agar (MSA; Difco) for selective isolation of MSSA, and Denim Blue agar (DBA) for MRSA detection the following morning. DBA plates were incubated (in darkness) for at least 24 hours, and MSA were incubated for at least 48 hours, before being discarded as negative.

Characterization of S. aureus isolates (antibiotic susceptibility testing, molecular characterization by PCR for *nuc*, *pvl* and *SCCmec* type, chromosomal typing by pulsed field gel electrophoresis), was according to standard laboratory methods.

Table 2

Swab location and type	Total No.	MSSA No (%)	MRSA No (%)
Residents Screened	295	110 (37.3)	12 (4.1)
Swabs Collected	599	139 (23.2)	18 (3.0)
Nasal Swabs	295	93 (31.5)	11 (3.7)
Axillary Swabs	295	45 (15.3)	6 (2.0)
Wound Swabs	9	1 (11.1)	1 (11.1)

Results:

Between July 10 and August 18, 2007, 295 male homeless shelter residents consented to participate. A total of 599 swabs were collected, including 295 nasal swabs, 295 axilla swabs, and 9 wound swabs.

Overall, 122 (41%) of residents grew S. aureus from one or more specimens. Of these, 110 (37%) grew MSSA from 139 specimens, and 12 (4.1%) grew MRSA from 18 specimens. Of the 12 MRSA positive residents, 8 were colonized with CMRSA-10/USA300. Of these CMRSA-10/USA300 positive individuals, 4 grew MRSA from both axilla and nasal swabs, 4 were only positive on nasal swabs, and only 1 resident had an isolated axilla positive swab. One of 9 (11%) suppurative wound swabs in this population were positive for MRSA, and this individuals nasal and axillary swabs were also MRSA positive.

The only characteristic associated with MRSA colonization was aboriginal ethnicity (25% vs 3%, P=0.01; Table 1). Residents who were MRSA positive were somewhat more likely to have been hospitalized in the last year (50% vs 26%, P=.10) and to report having a skin infection in the prior 12 months (25% vs 11%, P=.13)

MRSA isolates in this population belonged to 5 distinct clusters. Specific details regarding lineage and resistance patterns are shown in Table 3. Of note, the largest cluster comprised 6 individuals, and was closely related to the epidemic community (CMRSA-10/USA300) strain, but was unusually drug resistant with constitutive *ermA/C*-mediated resistance to clindamycin rather than the more typically associated *msrA* gene and clindamycin susceptibility. This strain also displayed a high level of fusidic acid resistance (MIC >8mg/L) resulting from point mutations in the chromosomal EF-G region of the *fusA* gene, suggesting exposure to fusidic acid ointment available over the counter in Canada.

Table 3

MRSA Cluster No. of Residents (No. of Isolates)	*Sma*IPFGE Canadian-Type CDC-Type	SCC*mec* Type Association PVL gene	Resistance Profile* *(gene)* Associated with Strain Type
1. 6 (11)	CMRSA-10 USA300	IVa (community) PVL-positive	-ß-lactams -ciprofloxacin -erythromycin -clindamycin (*ermA/C*) -fusidic acid (*fusA*)
2. 2 (2)	CMRSA-10 USA300	IVa (community) PVL-positive	- ß-lactams -ciprofloxacin -erythromycin *(msrA)*
3. 2 (3)	CMRSA-2 USA800	IVc (community) PVL-negative	-ß-lactams only
4. 1 (1)	CMRSA-2 USA100	II (nosocomial) PVL-negative	-ß-lactams -ciprofloxacin -erythromycin -clindamycin (*ermA/C*)
5. 1(1)	CMRSA-1 USA600	IVa (community) PVL-negative	-ß-lactams -ciprofloxacin -erythromycin *(msrA)*

*No MRSA isolates were resistant to tetracycline or trimethoprim/sulfamethoxazole

Conclusions:

Five separate clusters of MRSA derived from 3 distinct genetic lineages were identified during this 2007 survey. This was in contrast to **no** MRSA being identified in a similar survey performed at this same Toronto homeless shelter in 2003.
All but one of these MRSA clusters carried *SCCmec*-IV type cassettes, indicating that their acquisition was most likely in the community.

Of concern is that the predominant strain was a variant of the epidemic CMRSA-10/USA300, that had not only acquired an *ermA/C*-type gene that enabled its resistance to clindamycin, a drug commonly used in the treatment of community infections, but it had also developed resistance to the over-the-counter antibiotic fusidic acid. While this cluster was centered around an individual with a suppurative foot infection, the implications of colonization with this strain remains to be determined.

Future Directions

The association between colonization with MRSA strains, and the development of clinical infection is not well understood. Tracking health outcomes in the study population is presently underway.

A repeat analysis of this population will be conducted in the upcoming months to determine if patients remain colonized, and to determine if antibiotic resistance patterns have changed.

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