A multistrain cluster of methicillin-resistant *Staphylococcus aureus* based in a native community

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ABSTRACT: Since 1986 the authors' hospital has experienced increased numbers of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates linked to residents of a native Indian community infected or colonized on admission. A survey of 422 consecutive persons from that community admitted to hospital over a three year period identified 21 (4.9%) carrying MRSA. In a case control study of 34 carriers compared to noncarriers from the community, only prior hospitalization within the past 12 months was identified as being significantly

associated with the carrier state, but a specific hospital associated with this risk was not identified. A study of subsets of MRSA isolates in these patients revealed multiple strains present, identified by antibiograms, phage typing profiles and plasmid analysis. Community-based clusters of MRSA have only rarely been previously identified. **Can J Infect Dis 1990;1(4):121-126**

Key Words: Methicillin-resistant *Staphylococcus aureus*, Phage type, Plasmid

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) is an increasing problem in hospitals across North America (1,2) and elsewhere (3). Community foci of MRSA have only occasionally been identified, and when studied, have been shown to be due to a single strain (4,5). Since 1986, at the University of Alberta Hospitals (UAH) the authors have experienced increased numbers

of patients with community-acquired MRSA, which in a previous preliminary communication appeared to be associated with an Indian community (6). They now report a more detailed description of these patients, including evidence that multiple strains are involved.

PATIENTS AND METHODS

Patients with *Staph aureus* isolates with high level resistance to methicillin detected in the microbiology laboratory are referred to the hospital's infection control unit for follow-up. In 1986, when the increasing numbers of patients admitted with MRSA were noticed, a case control study was carried out which suggested com-

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Figure 1) Frequency of new methicillin-resistant *Staphylococcus aureus* patients at the University of Alberta Hospitals, 1977-89

munity A, a native community near Edmonton, as the source (6).

Community A: Community A is a native community located 80 km from Edmonton. The current population is about 5800 native Indians, plus a small number of non-natives and transient individuals. A major portion of the community's revenue is derived from oil reserves. Conditions on the reservation range from very poor and crowded to very high standards of living. Drug and alcohol abuse have been identified as major problems in the community. There are no hospital facilities on the reservation. Most elective or emergency medical care is provided by a community hospital which also provides care to other non-native communities. Cases requiring medical care not available in the community hospital are usually transferred to the city of Edmonton.

Beginning in 1987, following identification of community A as a likely source, all admissions to the authors' hospital from that community underwent screening for MRSA. Swabs were taken of anterior nares and evident skin lesions and plated on mannitol salt agar for isolation of *Staph aureus*.

In an attempt to determine whether subpopulations or clinical factors were present within com-

munity A responsible for the increased MRSA carrier rate, a case control study based on a retrospective chart review was performed on the 34 MRSA carriers seen at the hospital between 1986 and 1989 and 68 noncarriers from community A admitted to hospital, thus matching each carrier to two randomly selected noncarriers admitted during the same calendar year. Factors examined included: age, sex, hospitalization in the past year, previous admission (if any) to the community hospital serving community A (hospital B), antibiotic use within the previous two months, history of intravenous drug use or alcohol abuse, trauma as an admitting diagnosis, or chronic medical illness (eg, chronic renal failure, diabetes, cancer).

Strain identification and sensitivity testing:

Strains of *Staph aureus* were identified by standard laboratory methods. Every strain underwent full sensitivity testing using the Kirby Bauer method (NCCLS standard Ms-A3) (7) and the agar dilution method (NCCLS standard M7-A) (8). MRSA was defined as an isolate with a zone of less than 10 mm around a 1 µg oxacillin disc and an oxacillin minimal inhibitory concentration (MIC) greater than 4.0 mg/mL.

Phage typing: Phage typing was performed on the

TABLE 1

Case control study of methicillin-resistant *Staphylococcus aureus* carriers and noncarriers from community A

	Carriers (n=34)	Noncarriers (n=68)
Mean age (years)	19	17
Sex (male/female)	18/16	40/28
Prior hospitalization	31 (91%)	39 (57%)*
Admission to hospital B	28 (82%)	43 (63%)**
Antibiotic use	13 (38%)	14 (24%***)
Intravenous drug use	2 (6%)	0 (0%)
Alcohol abuse	12 (35%)	17 (25%)
Trauma	12 (35%)	21 (31%)
Chronic disease	8 (23%)	6 (9%)

* $P < 0.01$ by χ^2 test; odds ratio 7.7; 95% confidence level 1.9 to 26.3.** $P = 0.08$. *** $P = 0.10$

TABLE 2

Phage patterns in 19 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in 18 patients from community A

MRSA strain	Phage pattern	Antibiogram pattern
1A	29-42E/53/75/77/83A; 81/82	2
1B	29-77/83A; 95	1
2	53+ (84+ 85+ 90++)	3
3	53/84+ (75+ 90+)	4
4	53	2
5	75, 95	1
6	Nontypeable	1
7	29; 53; 81	1
8	29/52A; 42E/47/54/75/77/83A	1
9	81/82	1
10	81/82	1
11	42E/47/54/75/77; 81/82/95	1
12	29-42E/47/53/54/75/77; 81/82/95	1
13	27-42E/47/75/77/83A; 81/82/95+ (54+ 77+)	1
14	29-42E/47/75/77/83A; 81/82+ (54+ 95+)	1
15	42E/47/75/77/83A; 81/82+ (29+ 95+)	1
16	42E/47/54/75/77; 81/82/95+ (20+)	1
17	29-42E/47/53/54/75/77; 81/82/95	2
18	Nontypeable	1

first 19 MRSA isolates from 18 community A patients (two isolates from one patient) by the Canadian *Staphylococcus* Phage Typing Reference Centre of the Laboratory Centre for Disease Control, at routine test dilution and 100 x routine test dilution according to the standard methodology of the World Health Organization Reference Centre for *Staph aureus* Phage Typing, PHLS Colindale, London, England (9).

Plasmid profiles: Plasmid studies were also carried out on the first 19 isolates. Plasmid DNA was extracted using an alkaline sodium dodecylsul-

TABLE 3

Antibiotic resistance patterns in 34 methicillin-resistant *Staphylococcus aureus* isolates

	Resistant to	Sensitive to	No.	%
1	p/o/e/ceph/clinda	g/cotrim/v	24	71
2	p/o/e/ceph	g/cotrim/clinda/v	7	21
3	p/o/ceph/g	e/cotrim/clinda/v	1	3
4	p/o/ceph	e/g/cotrim/clinda/v	1	3
5	p/o/e/ceph/clinda/g	v	1	3

p Penicillin; o Oxacillin; e Erythromycin; ceph Cephalothin; clinda Clindamycin; g Gentamicin; cotrim Cotrimoxazole; v Vancomycin

phate procedure. Briefly, cells were suspended in 100 μ L of 15% sucrose, 40 mM Tris, 2 mM EDTA at pH 7.9 containing 200 μ g/mL lysostaphin and incubated at 37°C for 5 mins. They were placed in ice; two volumes of alkaline solution (0.18 N sodium hydroxide; 1.2% sodium dodecylsulphate) were added and the cells were incubated for 5 mins. Ice cold 3 M potassium acetate at pH 5.0 (150 μ L) was added and the mixture kept on ice for a further 10 mins. After centrifugation in an Eppendorf centrifuge for 5 mins at 5°C, two volumes of ice cold ethanol were added to supernatant which was kept frozen at -20°C for 30 mins or longer. DNA was obtained by centrifugation and visualized after electrophoresis on 0.7% agarose gels in Tris-acetate buffer.

RESULTS

The increased incidence of MRSA cases at the authors' hospital first detected in 1986 has stabilized through 1989 (Figure 1). Prospective culturing of 422 patients admitted to hospital from community A in the period 1987-89 identified 21 different individuals (4.9%) infected with or carrying MRSA on admission. There has been no change in the incidence of MRSA carriage in screened patients in the past two years (eight of 174 in 1988, and nine of 174 in 1989) compared to the earlier period. Most would not have been detected had routine surveillance cultures not been performed, though many later developed clinical infection. No instances of UAH nosocomial transmission from community A patients was documented after the screening program was implemented. In total, MRSA was isolated during 37 admissions from 34 patients from community A in the period 1986-89, representing 61% of all MRSA seen at hospital during that period. Non-community A MRSA isolates during that period included patient transfers from American hospitals and other sporadic community-based isolates. No sustained UAH nosocomial acquisition occurred. The 34 patients from community A constitute the population which was studied in more detail.

The case control study of 34 MRSA patients and

Figure 2) Electrophoresis in 0.7% agarose of plasmid DNA of 19 methicillin-resistant *Staphylococcus aureus* strains. Lane A shows strain 1a; lane B strain 1b; lane C strain 2; lanes D-K strains 4-11, respectively; and lanes M-T strains 12-19, respectively. Lanes L and T contain marker plasmids of *Bacillus thuringiensis*, with molecular weights shown on the right (17). kb Kilobases; chrom. chromosome

68 randomly selected noncarriers admitted to UAH from community A identified hospitalization within the past 12 months as a risk factor (Table 1); hospital B, the community hospital associated with community A, just failed to reach statistically significant association.

Table 2 shows phage typing results performed at the Laboratory Centre for Disease Control (Ottawa) on the first 19 MRSA isolates from community A. Only two strains, 6 and 18 were nontypeable. By this method, small clusters of strains were noted: 12 and 17, 9 and 10, and 2 and 4 were probably related within the clusters but not between them; strains 11 and 16, and 14 and 15 were possibly related; and strain 3 showed possible relation to strains 2 and 4.

Five different antibiotic resistance patterns were seen in 34 isolates (Table 3). The most common pattern, seen in 71%, demonstrated considerable resistance including, in addition to oxacillin, resistance to erythromycin and clindamycin; these retained aminoglycoside and cotrimoxazole sensitivity. All isolates were sensitive to vancomycin.

Plasmid pattern analysis is shown in Figure 2. In each of five strains – 1a, 1b, 2, 4 and 5 – two plasmids were present. One plasmid (approximately 125 kb) was common to all strains. The other plasmid varied between strains; strains

1b and 4 contained an approximately 85 kb plasmid, while strains 1a, 2 and 5 contained plasmids of varying sizes (45 to 100 kb). Strains 12 to 19 each contained a single plasmid, approximately 100 kb in size.

DISCUSSION

The authors' previous report identified a potential community focus of MRSA in Alberta (6). This report confirms that ongoing endemic transmission is occurring in community A. Since 1987 when screening began, a stable incidence of about 5% MRSA carriage was found in admissions from community A. Since this is a hospital-based survey, the data do not necessarily reflect the situation in the community as a whole.

The case control study of MRSA carriers and noncarriers from community A suggests that hospitalization was an important risk factor for MRSA patients. While there was a trend towards association with previous admission to hospital B, this trend did not achieve statistical significance. It remains possible that patient-to-patient transmission within hospital B is a source of some of the cases. Hospital B serves a number of communities besides community A; only rarely have members of these other communities been identified as being MRSA carriers. Furthermore, since some MRSA carriers from community A have not

been previously hospitalized, some person-to-person transmission of MRSA must be occurring within community A. Community foci of MRSA have previously been reported in chronic care facilities such as nursing homes, or in intravenous drug users (4,10-12). The case control study also showed a statistically significant trend in favour of carriers for a number of other factors, including antibiotic use (38% versus 24%), chronic medical disease (23% versus 9%) and even intravenous drug use (6% versus 0%).

The identification of other epidemiological factors important in the development and perpetuation of this focus of MRSA was very likely prevented by the small number of cases available, the fact that controls were matched at only a 2:1 ratio, and the fact that the study was based on a retrospective chart review.

Furthermore, MRSA cases in this study were identified by two different methods: standard clinical testing (principally before 1987) and routine admission screening (1987-89). This may also interfere with interpretation of the case control study results. The authors are currently planning a prospective epidemiological survey to accompany their microbiological survey of admissions from community A in an attempt to explore these matters further.

The mechanism by which MRSA was introduced into this community and by which ongoing transmission occurs was not determined by this study. It is possible that the organisms have arisen *de novo* in this community. Alternatively, it is possible that the source was a hospital – either the local community hospital or another. However, as there has been no reported endemic focus of MRSA in western Canadian hospitals, it seems likely that the organism was introduced from elsewhere. Members of this community are highly mobile and travel extensively between other native communities in western Canada and the north-western United States. It cannot be excluded that such travel has resulted in the organism being brought into this community.

Unfortunately, the retrospective methodology of the case control study did not allow this factor nor the familial interrelationships between carriers to be examined (there were some instances in which several individuals from the same family were identified as MRSA carriers).

A disturbing feature of this cluster is the evidence that multiple strains may be involved. Antibiotic sensitivity testing, phage typing and plasmid profiles all demonstrated multiple strains. It would, however, seem highly unlikely if not impossible to have multiple strains introduced into the community from a single source such as

a hospital or other institution. This makes understanding of the pathogenesis and spread of MRSA in this community even more difficult.

The present study underlines the poor reliability of current methodologies in identifying particular staphylococcal strains associated with an outbreak situation. Use of antibiograms as a means of evaluating strain 'relatedness' can be questioned for several reasons, including difficulties with susceptibility methodology (especially for MRSA), presence of a multiply resistant strain before an epidemic situation supervenes, or changes in resistance pattern caused by changes in microbial genetic make-up. Phage typing is itself subject to variation in performance, interpretation and reproducibility of results (13,14). The value of these tests depends on the stability of the phage patterns and several other factors. Inability to type MRSA properly has frequently been reported in other outbreaks (13). None of the available epidemiological markers appears to be reliable by itself in the investigation of outbreaks of MRSA infection.

Plasmid pattern analysis is recognized by many as a useful epidemiological tool because of the stability of plasmid patterns over time and temperatures (13,14). They should serve as useful markers even for the differentiation of isolates with similar antibiograms or phage patterns. Technical problems with the procedure itself as well as interpretation of patterns – especially for Gram-positive organisms – make these techniques unreliable as epidemiological tools (16). The loss or gain of antibiotic resistance, the existence of a single plasmid in different molecular forms, and the presence of one plasmid only or none at all contribute to problems with this method.

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