Molecular Fingerprinting of Mupirocin-Resistant Methicillin-Resistant *Staphylococcus aureus* from a Burn Unit

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ABSTRACT

Objectives: To characterize mupirocin-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients in a burn unit by pulsed-field gel electrophoresis and plasmid contents.

Methods: A total of 53 methicillin-resistant *S. aureus*, consisting of 48 mupirocin-resistant and 5 mupirocin-susceptible MRSA were compared by plasmid content and pulsed-field gel electrophoresis of *Sma* I digested genomic DNA.

Results: Of the 48 mupirocin-resistant isolates, 39 expressed high-level, and 9 expressed low-level mupirocin resistance. Plasmids were detected in all of the 53 isolates; however, only the high-level mupirocin-resistant isolates contained a 38 kb-conjugative plasmid that encoded high-level mupirocin resistance. Pulsed-field gel electrophoresis divided the isolates into four patterns designated types I to IV. Forty-three isolates consisting of 34 high-level, 5 low-level mupirocin-resistant and 4 mupirocin-susceptible isolates defined the type-I pattern. Eight isolates, five high-level and three low-level mupirocin-resistant isolates had the type-II pulsed-field pattern. The type-III and type-IV pulsed-field patterns consisted of a single isolate each. The type-I and type-II pulsed-field patterns were related and only differed by four *Sma* I bands.

Conclusions: Results of typing the mupirocin-resistant MRSA from the burn unit with pulsed-field gel electrophoresis indicated that closely related MRSA clones previously circulating in the unit had acquired a high-level mupirocin-resistant plasmid, and spread aided by mupirocin use.

Key Words: *burn unit, methicillin-resistant* S. aureus, *mupirocin-resistance, pulsed-field gel electrophoresis*

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Since its introduction for clinical use in the United Kingdom in 1985, mupirocin (Bactroban, SmithKline Beecham, Rixensart, Belgium) has become available in more than 90 other countries worldwide.[1] It has been used for the treatment of different types of skin infections and as prophylaxis before skin surgery.[2–9] Mupirocin has been particularly useful in the elimination of nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in patients and health care workers,[10–13] thereby serving as an important agent in the control of MRSA outbreaks. However, as its use has increased worldwide, mupirocin resistance has also emerged and is increasing.[14–21] Mupirocin resistance has been attributed to prolonged use of the antibiotic in some instances,[16,22] although resistance has also been found in coagulase-negative staphylococci isolated long before mupirocin was available for clinical use.[23] Mupirocin resistance can be low level (minimum inhibitory concentration [MIC] 8–256 mg/L) or high-level (MIC > 512 mg/L). The high-level resistance (mupH) is clinically significant, often resulting in treatment failures.[1] High-level mupirocin resistance is encoded by plasmid-borne *mupA* genes, whereas genes with low-level mupirocin resistance (mupL) are located on the bacterial chromosome.[1] Isolates expressing mupL carry mutations in the host's isoleucyl-tRNA synthetase (IRS), whereas isolates expressing mupH contain two biochemically distinct IRS: a native mupirocin-sensitive IRS plus an additional enzyme that is not sensitive to inhibition by mupirocin.[24]

The burn unit at Ibn Sina Hospital, Kuwait, is a 70-bed special facility for treating burn patients of all ages and sexes. It is the only such facility for the state of Kuwait. It has an intensive care unit with 12 beds. The bed occupancy in the burn unit is 70 to 100%. Mupirocin was introduced for clinical use in this facility in 1992 and has been used consistently since then for the treatment of MRSA infections or for the elimination of nasal MRSA carriage. Both the nasal and skin preparations are available and are administered to the patients according to the guidelines provided by the manufacturer.[25] A study of 395 staphylococci isolated between April 1994 and May 1995 after more than 2 years of mupirocin use in the unit failed to detect mupH in any of the *S. aureus* iso-

lates.[26] However, 5% of them expressed mupL, and one isolate of *Staphylococcus haemolyticus* expressed mupH.[26] It was at that time recommended that, although the incidence of mupirocin resistance was low, MRSA isolated from the burn unit should be tested routinely for mupirocin resistance, so that any resistant isolates could be detected early and to facilitate the early institution of infection control measures. No further high-level mupirocin staphylococci were isolated in the burn unit in the following year after this study. However, high-level mupirocin resistance was detected among MRSA isolates submitted for typing to the Staphylococcal Research Laboratory, Department of Microbiology, Faculty of Medicine, Kuwait University, from the burn unit, in November 1996. Because MRSA has been a persistent pathogen in the burn unit, the observed mupirocin-resistant MRSA could have evolved by the acquisition by MRSA already circulating in the burn unit of a plasmid-borne high level mupirocin resistance, which later proliferated, aided by mupirocin use. They could also have represented a new mupirocin-resistant MRSA clone introduced into the unit from outside, probably by a patient, which later spread among other patients. To address this question, mupirocin-resistant MRSA isolated from patients in the burn unit between November 1996 and May 1997 were compared with representatives of mupirocin-susceptible MRSA from the same unit, using a combination of antibiogram, plasmid analysis, and pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

Methicillin-Resistant *Staphylococcus aureus*

A total of 53 MRSA isolates were studied. They were among MRSA isolated from patients in the burn unit, Ibn Sina Hospital, Kuwait, and submitted for typing to the Staphylococcal Research Laboratory, Department of Microbiology, Faculty of Medicine, Kuwait University. The isolates were identified as *S. aureus* at the Clinical Microbiology Laboratory of Ibn Sina Hospital, Kuwait, by cultural characteristics, colony morphology, positive results for catalase, and tube coagulase test using rabbit plasma (Difco Laboratories, Detroit, MI).

Susceptibility to Antimicrobial Agents

Susceptibility to antimicrobial agents was tested by the disk diffusion method on Mueller-Hinton agar as described previously,[27] using commercial antibiotic disks (Oxoid, Basingstoke, England). Susceptibility to mupirocin was determined using disks containing 200 μg and 5 μg of mupirocin. Growth to the edge of the 200-μg mupirocin disk indicated high-level resistance, whereas growth within a 14-mm zone of inhibition with the 5-μg mupirocin disk detected low-level resistance. Minimum inhibitory concentration of mupirocin was determined using E-test strips (AB Biodisk, Solna, Sweden), according to the manufacturer's instructions. For testing their susceptibility to heavy metals and nucleic acid-binding compounds, 6-mm disks impregnated with cadmium acetate (130 μg), propamidine isethionate (50 μg), and ethidium bromide (60 μg) were used. *Staphylococcus aureus* strain ATCC25923 was used as the control strain.

Plasmid Isolation and Transfer

Plasmids were isolated by the cetyltrimethylammonium bromide method, separated by agarose gel electrophoresis and sized as previously described.[28]

Plasmids were transferred in phage-mediated conjugation and conjugation experiments. For phage-mediated conjugation experiments, 0.1 mL each of an overnight culture of a donor strain and a recipient strain, WBG1876, lysogenized with staphylococcal phage J,[29] was added to 5.0 mL of Tris-buffered saline (TSB) containing 0.01 M calcium chloride and incubated at 35°C overnight with gentle shaking. The mixture was then pelleted by centrifugation, the supernatant was discarded, and the deposit was spread onto selective media containing appropriate antibacterial agents. Controls, consisting only of the donor and recipient cells were set with the tests. Transfer was considered to have occurred when growth was obtained from donor-recipient mixtures and not from controls. Transcipients were obtained on brain heart infusion agar (BHIA) plates containing fusidic acid 5 mg/L, rifampicin 2.5 mg/L, and one of mupirocin 5 mg/L, chloramphenicol 10 mg/L, cadmium 60 mg/L, and ethidium bromide 60 mg/L. Conjugation experiments were performed as previously described,[29] and transconjugants were obtained on selection plates as for the phage-mediated conjugation experiment.

Pulsed-Field Gel Electrophoresis

Cells were grown overnight in tryptic soy broth and used to prepare agarose blocks by a modification of a method previously described.[29] Then, 1.5 mL of overnight culture was centrifuged in a microcentrifuge at 2000 times gravity for 5 minutes, and washed twice in 1.0 mL of 50 mM EDTA, pH 8.0. The cells were resuspended in 0.5 to 1.0 mL EC buffer (6 mM Tris; 1 M NaCl; 100 mM EDTA; Brij 58 [Sigma Chemical, St. Louis, MO, USA] 0.5%). Sodium deoxycholate, 0.2%, sodium lauryl sarcosine, 0.5%, pH 7.5, were added to give a cell density of approximately 3×10^8 colony-forming units (cfu)/mL. In a fresh microfuge tube, 50 μL of lysostaphin, 200 mg/mL, was mixed with 100 μL of cell suspension to which 150 μL of agarose (chromosomal grade, Bio-Rad Laboratories, Richmond, CA, USA) was added, mixed, and transferred by pipetting into block-forming wells (Bio-Rad) and allowed to solidify. The formed blocks were incubated in 1.0 mL EC buffer for 3 hours, followed by an overnight incubation at 50°C in

proteinase K buffer (0.5 M EDTA 5 mM Tris; sodium laurylsarcosine, 1%, pH 7.5) containing 1 mg/mL proteinase K. The blocks were then washed in 50 mM EDTA for 2 hours, with the buffer changed after every 30 minutes, and stored at 4°C in 50 mM EDTA. The blocks were digested with *Sma* I (Gibco BRL), according to the manufacturer's instructions. Electrophoresis was performed with 1% agarose gel in 0.5×TBE buffer, using a CHEF DR III apparatus (Bio-Rad Laboratories). The gel was run at 14°C, 6V/cm and 120°C switch angle for 20 hours. Linear ramp of switch times were 0.5 to 40.0 seconds. The gels were stained in 0.5 mg/L ethidium bromide and photographed under ultraviolet illumination. The chromosomal patterns were examined and compared by eye and assigned to PFGE types. The relatedness of the strains was determined according to the recommendation of Tenover et al.[30]

RESULTS

Fifty-three MRSA isolates from 34 patients were studied. These consisted of 48 mupirocin-resistant and 5 mupirocin-susceptible isolates. The MRSA were cultured from different clinical samples obtained from the throat, burn wounds, blood, nose, groin, and axillary (Table 1).

Resistance to Antimicrobial Agents

Results of disk susceptibility tests with the mupirocin disks revealed that 39 isolates from 26 patients expressed mupH and 9 isolates, from 9 patients, expressed mupL. Results of MIC determination with E-test strips demonstrated that all of the isolates that expressed high-level resistance by growing to the edge of 200-μg mupirocin disks, had MICs higher than 1024 μg/mL. The nine isolates with low-level mupirocin resistance had MICs between 32 and 128 μg/mL. Six of them had MICs of 32 mg/L, two isolates had MICs of 64 mg/L, and one isolate had a MIC of 128 mg/L (see Table 1). With regard to their susceptibility to other antimicrobial agents tested with the disk diffusion method, it was demonstrated that, irrespective of their level of mupirocin resistance, they were all resistant to methicillin, penicillin G, gentamicin, kanamycin, trimethoprim, cadmium, and mercuric chloride, but they were varied in their resistance to streptomycin, erythromycin, tetracycline, chloramphenicol, ciprofloxacin, ethidium bromide, and propamidine isethionate. Thirty-three isolates were resistant to streptomycin, 21 were resistant to erythromycin, 47 were resistant to tetracycline, 44 were resistant to chloramphenicol, and 47 were resistant to ciprofloxacin. Fifty isolates were resistant to propamidine and ethidium bromide. They were all susceptible to vancomycin and teicoplanin. Because of the similarity of their resistance patterns, antibiogram was not an effective typing tool with these isolates.

Table 1. Characteristics of Methicillin-Resistant *Staphylococcus aureus* Isolates

Patient	*MRSA Isolates*	*Source of Specimens*	*Mupirocin-Resistant MIC (mg/L)*	*PFGE Types**
1	1–7	Canulla sites (2), right hand, axilla, right & left legs, thigh	HL	I (7)
2	8, 10, 11	Back, nose, burn wound	HL	I (3)
	9	Right leg	HL	II
3	12	Nose	HL	I
4	13	Burn wound	HL	I
5	14	Left leg	HL	I
6	15	Throat	LL (32)	II
7	16	Blood	HL	I
8	17	Left leg	HL	II
	18, 19	Throat, burn wound	HL	I
9	20	Nose	HL	II
	21–23	Burn wound, groin, left leg	HL	I (3)
10	24	NS	LL (64)	I
11	25	Groin	HL	I
	26	Right hand	LL (32)	II
12	27	NS	S	IV
	28	Right leg	HL	I
13	29	Throat	LL (32)	II
14	30	Face	HL	I
15	31, 32	Burn wound, nose	HL	I
15	33	Throat	LL (64)	I
16	34	Right leg	HL	I
17	35	Blood	HL	I
18	36	NS	LL (128)	I
19	37	Burn wound	LL (32)	III
20	38	Right thigh	HL	I
21	39	Burn wound	LL (32)	I
22	40	Nose	S	I
23	41	Axilla	S	I
24	42	NS	S	I
25	43	NS	HL	I
26	44	Throat	HL	I
27	45	NS	S	I
28	46	Nose	LL (32)	I
29	47, 48	NS, burn wound	HL	I
30	49	Blood	HL	I
31	50	Throat	HL	I
32	51	Nose	HL	I
33	52	Burn wound	HL	I
34	53	Nose	HL	II

*Parentheses: number of isolates with PFGE patterns. MIC = minimum inhibitory concentration; HL = high-level resistance; LL = low-level resistance; S = mupirocin-susceptible; NS = not specified. Only the MIC of mupL isolates are given since the mupH isolates were all over 1024 mg/L.

Examination of the patients' medical files revealed that 28 of the 34 patients from whom mupirocin-resistant isolates were obtained had been treated with mupirocin on admission.

Plasmid Analysis

The plasmid content of all 53 MRSA isolates is summarized in Table 2. They harbored three to five plasmids, which varied in size between 2.91 kilobases (kb) and 38 kb. A 38-kb plasmid was present in all mupH isolates and absent in the mupL and mupirocin-susceptible isolates (Figure 1). The other plasmid types were present in all of the isolates, irrespective of their level of mupirocin resistance, and were not useful in discriminating among the isolates.

Table 2. Plasmid Contents of Methicillin-Resistant *Staphylococcus aureus* Isolates

Plasmid Type	*Plasmid Contents (kb)*	*HL (n = 39)*	*LL (n = 9)*	*Mup-S (n = 5)*
Type 1	38, 26, 4.4, 3.3, 2.9	32	—	—
Type 2	38, 26, 4.4, 3.3	7	—	—
Type 3	–, 26, 4.4, 3.3, 2.9	—	4	1
Type 4	–, 26, 4.4, 3.3	—	5	4

HL = high-level mupirocin-resistant isolates; LL = low-level mupirocin-resistant isolates; Mup-S = mupirocin-susceptible.

Six representative isolates consisting of three with mupH and three with mupL were selected and used as donors in conjugation and phage-mediated conjugation experiments in attempts to isolate the different plasmids and determine their resistance phenotypes. High-level mupirocin resistance was transferred from all three resistant isolates (isolates 16, 28, and 30) in conjugation experiments. Transfer of the mupH was accompanied by the transfer of the 38 kb plasmid alone or with the cotransfer of a 4.4 kb plasmid encoding chloramphenicol resistance when isolates 16 and 30 were used as donors (Table 3). None of the three mupL isolates transferred any of their resistance in conjugation experiments. However all six isolates transferred resistance to cadmium, mercuric chloride, propamidine isethionate, and ethidium bromide in phage-mediated conjugation experiments, and their transfer in all instances was accompanied by the transfer of a 26 kb plasmid. The plasmids transferred and their resistance phenotypes are presented in Table 3.

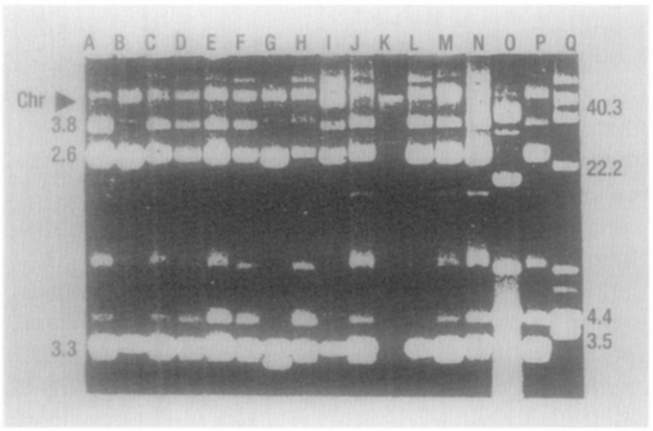

Figure 1. Plasmid contents of representative MRSA isolates. Lanes A to P contain plasmids from representative MRSA studied. Lane Q contains plasmids of strain WBG4483 used as size markers. The sizes are given in kilobases. Only covalently close circular (CCC) DNA are labelled. Chr = chromosomal DNA band. Lanes A, C, D, E, F, I, J, L, M, N, and P are representative of the mupH isolates and contain the 38-kb plasmid; lanes B and G are representative of mupL isolates and do not contain the 38-kb plasmid. Lanes K and O contain MRSA corresponding to pulsed-field types III and IV, respectively. These also lack the 38-kb plasmid.

Table 3. Plasmids Transferred from Mupirocin-Resistant MRSA

MRSA Isolate	*Mupirocin Resistance*	*Mode of Transfer**	*Resistance Transferred†*	*Plasmids Transferred (kb)*
15	Low	P	Cd, Hg, Pi, Eb	26
16	High	C	Mupirocin	38
		C	Cm	4.4
		P	Cd, Hg, Pi, Eb	26
28	Low	C	Mupirocin	38
		P	Cd, Hg, Pi, Eb	26
30	High	C	Mupirocin	38
			Cm	4.4
		P	Cd, Hg, Pi, Eb	26
35	Low	P	Cd, Hg, Pi, Eb	26
39	Low	P	Cd, Hg, Pi, Eb	26

*P = phage-mediated conjugation; C = conjugation; †Cd = cadmium; Hg = mercuric chloride; Pi = propamidine isethionate; Eb = ethidium bromide; Cm = chloramphenicol.

Pulsed-Field Gel Electrophoresis

Four pulsed-field patterns, labelled types I to IV, were obtained. The PFGE patterns of individual isolates are presented in Table 1. Forty-three of the 53 isolates had

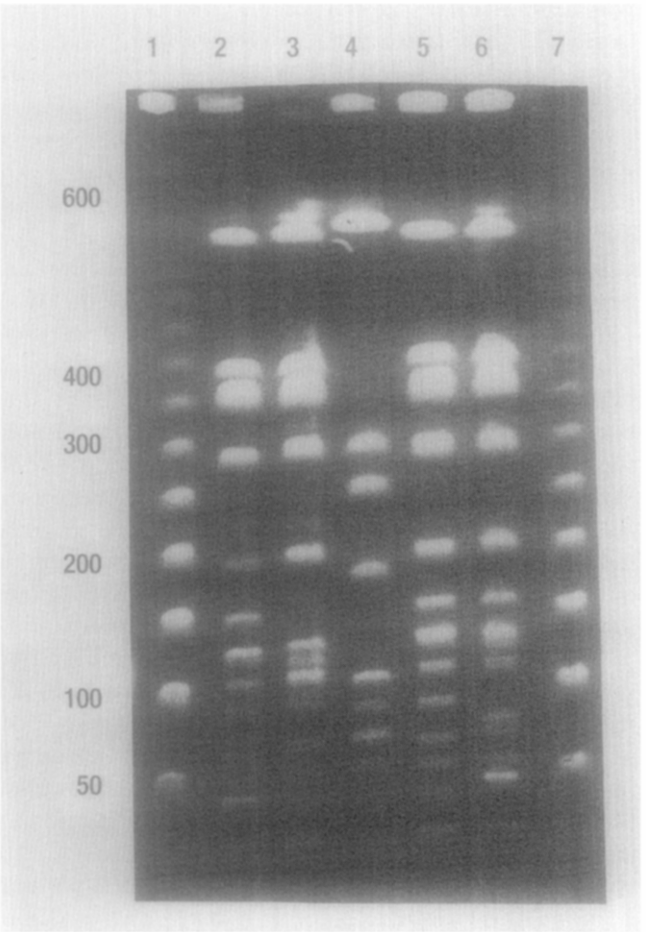

Figure 2. PFGE patterns of representative MRSA isolates. Lane 1 contains phage lambda concatemer used as size markers. Sizes are in kilobase pairs. Lanes 2 and 5 are representative of PFGE type-I; lane 3, PFGE type-III; lane 4, PFGE type-IV; and lane 6, PFGE type-II. Lane 7 provides size markers.

type-I pulsed-field pattern. This consisted of 34 of the 39 high-level resistant, 5 of 9 low-level resistant, and 4 of 5 mupirocin-susceptible isolates. Eight isolates had type II pulsed-field pattern consisting of five high-level and three low-level mupirocin resistant isolates. The types I and II pulsed-field patterns were related but not similar, and differed from each other by a total of four DNA bands in the 50- to 100-kb fragment region. One type had two DNA bands that were absent in the other type (Figure 2). The type-III and type-IV pulsed-field patterns consisted of a single isolate each.

DISCUSSION

This report presents the first known major incidence of high-level mupirocin resistance among *S. aureus* in a Kuwait hospital. Until now only three mupirocin-resistant staphylococci were seen in two hospitals in Kuwait (unpublished observation). These consisted of one MRSA and two coagulase-negative staphylococci. One of the coagulase-negative staphylococci, *S. haemolyticus*, was isolated from a patient in the same burn unit.[26] This finding revealing the presence of a major problem with mupirocin-resistant MRSA in the burn unit is of concern because of its implication for the continued use of mupirocin on patients in the burn unit. The findings warrant a review of the protocol for the use of mupirocin in the unit. Mupirocin therapy should be given after susceptibility testing of MRSA isolates, not only from the burn unit but also from other wards in the hospital. Although high-level mupirocin resistance in MRSA is still low worldwide,[1] the experience reported here in which high-level mupirocin resistance existed in the burn unit but was not detected because it was not being tested suggests that a similar situation may exist elsewhere. To maintain the usefulness of mupirocin therapy, mupirocin resistance should be tested for routinely even in facilities where mupirocin is not being used, because mupirocin-resistant MRSA strains can be introduced into such facilities, where it can spread among patients.

Results of transfer experiments demonstrated that the 38-kb plasmid in the high-level resistant isolates is a self-transmissible plasmid that encodes high-level mupirocin resistance. High-level mupirocin resistance has been found in self-transmissible and non-self-transmissible plasmids in different countries.[14,17,23,26] The 4.4-kb plasmid encoded chloramphenicol resistance and the 26-kb plasmid encoding linked resistance to cadmium, mercuric chloride, propamidine isethionate, and ethidium bromide. As none of the plasmids was associated with low-level mupirocin resistance, its determinants were chromosomal in these isolates, which is consistent with other reports that low-level mupirocin resistance genes are chromosomal.[1]

Typing of the isolates revealed that they were of different clones. However, the majority of them were related and belonged to PFGE type I and type II. They also had similar resistance and plasmid profiles. As shown in Table 2 and Figure 1, plasmid analysis could identify isolates expressing high-level mupirocin resistance because of the presence of the 38-kb plasmid in them, but could not distinguish those expressing low-level mupirocin resistance from mupirocin-susceptible isolates. In contrast to resistance and plasmid analysis, PFGE typing was more discriminatory and demonstrated that the mupH MRSA were related to the mupL MRSA and to some mupirocin-susceptible MRSA isolated from the same unit. The type-I pulsed-field pattern was found among isolates expressing high-level and in those expressing low-level mupirocin resistance as well as in mupirocin-susceptible isolates. These results suggest that previously mupirocin-susceptible MRSA circulating in the burn unit had acquired the 38-kb conjugative plasmid encoding high-level mupirocin resistance genes and had spread among different patients, its successful maintenance and spread being enhanced by mupirocin use. The recovery of mupirocin-resistant isolates from 28 patients treated with mupirocin supports the suggestion that mupirocin use may have enhanced their spread. The emergence of mupirocin resistance in staphylococci following the prolonged use of the antibiotic has been documented elsewhere.[15,16] However, mupirocin-resistant isolates also were recovered from patients who had not been treated with mupirocin. These patients probably acquired them through cross-contamination. The similarities in pulsed-field patterns observed between mupH and mupL MRSA suggests that the MRSA clones acquired both resistance determinants separately. They first acquired mupL and later mupH since, in a previous study, 5% of the isolates already expressed mupL.[26] The presence of the 26-kb plasmid encoding resistance to cadmium, mercuric chloride, propamidine isethionate, and ethidium bromide in both mupH and mupL isolates further supports the relatedness of the isolates and the notion that they evolved from MRSA previously circulating in the burn unit.

The mupH MRSA appeared to have a higher capacity to spread more readily than the mupL isolates since they were isolated more frequently than the mupL isolates from different body sites in different patients (see Table 1), reflecting both colonization and infection. This could be because the mupH determinant was plasmid-borne, and plasmid carriage of resistance may facilitate resistance transmission much more easily than chromosomal carriage. Also the presence of different MRSA in the same patients would facilitate transfer of the self-transmissible mupirocin-resistance plasmid between the MRSA isolates.

Finally this study has demonstrated the value of a central typing laboratory in helping to detect an ongoing mupirocin-resistance problem in a burn unit that was not

known to exist because it was not being tested for in the local hospital laboratory. Routine testing of MRSA for mupirocin resistance will facilitate early detection of resistance and can help control the spread of mupirocin-resistant MRSA.

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