Clonal Dissemination and mupA Gene Polymorphism of Mupirocin-Resistant Staphylococcus aureus Isolates from Long-Term-Care Facilities in South Korea

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Received 21 June 2005/Returned for modification 1 August 2005/Accepted 12 October 2005

We identified 25 high-level mupirocin-resistant (MuH) and 21 low-level mupirocin-resistant (MuL) Staphylococcus aureus isolates from eight long-term-care facilities (LTCFs). The pulsed-field gel electrophoresis patterns of 19 MuH and 19 MuL isolates from two facilities were identical for 18 and 15 isolates, respectively. The most predominant mupA restriction fragment length polymorphism type was found in 21 MuH isolates. We conclude that clonal transmission of MuH and MuL S. aureus strains occurred in these LTCFs. This is the first report of clonal transfer of mupirocin resistance in LTCFs.

Colonization and infection with Staphylococcus aureus are common in older people in long-term-care facilities (LTCFs) (1, 2, 4). The prevalence of S. aureus colonization and infection, which result primarily from methicillin-resistant strains, has recently been reported by chronic care facilities worldwide (1, 3, 6, 7). Mupirocin calcium ointment is a topical antibiotic indicated for the eradication of nasal carriage of staphylococci, including methicillin-resistant strains. Mupirocin alone or in combination with other antimicrobial agents decreases S. aureus colonization among residents of LTCFs (3, 7, 8, 20). Several outbreaks of methicillin-resistant S. aureus colonization and infection in LTCFs have been reported, and until now, it was thought that the application of mupirocin ointment might help break the chain of transmission. However, the extensive use of this agent has led to the rapid emergence of mupirocin-resistant strains in different parts of the world (9, 16, 11, 13, 18, 19). To our knowledge, there are no reports on the prevalence and outbreak of mupirocin-resistant S. aureus in LTCFs. In South Korea, mupirocin ointment has been used since 1994 to eradicate staphylococcal infection in hospitals, and the prevalence and mechanisms of mupirocin-resistant staphylococci were first reported in 2003 (23).

We investigated the clonal transmission of high-level mupirocin-resistant (MuH) and low-level mupirocin-resistant (MuL) S. aureus and the mupA gene polymorphisms of MuH S. aureus strains in LTCFs. Seven hundred forty-nine swab specimens were obtained from patients of eight LTCFs from July to August 2002. Nasal swab specimens were obtained from 632 patients (one isolate per patient), and 117 infection swab samples were obtained from infected sites (e.g., sore, wound, or trachea) present in these patients. Swab specimens were cultured on staphylococcal broth (Trypticase soy broth [TSB]) medium for 24 h at 35°C. Mannitol salt agar and mannitol salt oxacillin agar supplemented with 6 μg/ml oxacillin were used to isolate S. aureus and methicillin-resistant S. aureus, respectively. Initial identification was based on colony morphology, Gram staining, the coagulase test using the Staphaurex latex agglutination kit (Murex Biotech Ltd., Dartford, United Kingdom), and thermonuclease production with DNase medium (Becton Dickinson, Franklin Lakes, NJ). When necessary, further confirmatory tests were performed using a Vitek system (bioMerieux, Marcy l’Etoile, France). Of the 749 swab samples, 407 S. aureus isolates (54.3%) were recovered and 259 isolates were resistant to oxacillin. These values ranged from 36.6% to 80.0% in the various LTCFs.

All isolates were screened for resistance to mupirocin on Muller-Hinton agar (Difco Co., Detroit, MI) with a mupirocin disk (5 μg; Oxoid Co., Hampshire, United Kingdom). The Etest (AB Biodisk, Solna, Sweden) was used to determine the MICs for mupirocin-resistant isolates. Susceptibility testing was conducted by disk diffusion according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) (15). The following 12 antibiotics were also tested: oxacillin, penicillin, cefazolin, ampicillin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, gentamicin, rifampin, ofloxacin, tetracycline, and vancomycin. Among the 407 S. aureus isolates, 46 were mupirocin resistant, 25 were classified as MuH (EN62 and ES62 were isolated from the nasal and infection sites of one patient), and 21 were classified as MuL. MuH S. aureus strains were isolated from four LTCFs and MuL S. aureus strains were isolated from five LTCFs. In South Korea, mupirocin resistance was detected in 5% of S. aureus and 27% of coagulase-negative staphylococcus isolates in a tertiary care hospital (23). We identified mupirocin-resistant S. aureus isolates in five of eight LTCFs; the mupirocin resistance rate was 11.3%. Most MuH and MuL strains were collected from one LTCF, and 42 of 46 strains were isolated from nasal samples. All mupirocin-resistant isolates showed resistance to oxacillin, penicillin, and erythromycin (except FW02, which showed intermediate resistance) and were susceptible to ampicillin and vancomycin. All MuL isolates showed resistance to gentamicin.

Molecular typing of the mupirocin-resistant isolates was performed by pulsed-field gel electrophoresis (PFGE) analysis of Smal-restricted chromosomal DNA (10). PFGE analysis...
showed that MuH strains fell into four distinct PFGE clone groups (A, B, C, and D). However, 20 of 25 isolates belonged to PFGE group A, and 18 isolates of PFGE group A were isolated from one LTCF (Fig. 1A). MuL S. aureus strains were grouped into two different clone groups (A and B); clone group A was the most dominant, comprising 20 of 21 strains (Fig. 1B). Eighteen of the 21 MuL isolates showed the same PFGE band patterns, and 16 of these isolates were also isolated from one LTCF (a different LTCF from that which produced the MuH group A isolates). These results indicate that clonal transmission of mupirocin-resistant S. aureus occurred in two LTCFs. A few groups have reported mupirocin resistance in LTCFs (2, 7, 21), but to our knowledge, our data are the first to show MuH and MuL S. aureus clonal transmission in LTCFs.

Southern analysis of EcoRI-, HindIII-, and ClaI-digested plasmid DNA confirmed the plasmid of the ileS2 gene (mupA gene) in all 25 MuH isolates. We found three different mupA gene polymorphs: (i) EcoRI-digested fragment of 4.2 kb, HindIII-digested fragment of 8 kb, and ClaI-digested fragments of 23-kb and 2.1-kb fragments (polymorph I; 21 isolates); (ii) EcoRI-digested fragment of 6 kb, HindIII-digested fragment of 5 kb, and ClaI-digested fragment of 23-kb fragment (polymorph II; isolates HN41 and HN42); and (iii) EcoRI-digested fragment of 4.6 kb, HindIII-digested fragment of 6 kb, and ClaI-digested fragment of 23 kb, and a 2.1-kb fragment (polymorph III; isolates HN41 and HN42).
isolates identified from different LTCFs showed different polymorph III; isolates GN28 and GN40) (Table 1). The MuH high-level mupirocin resistance isolates should be established in LTCFs. mupA gene was conserved but that variation occurs near the mupA locus. Low-level mupirocin resistance isolates of older residents in LTCFs and easy dissemination of antibiotic resistance among these strains. J. Clin. Microbiol. 37:2781–2788.

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TABLE 1. PFGE DNA patterns of mupirocin-resistant S. aureus and mupA restriction fragment length polymorphism patterns of high-level mupirocin-resistant S. aureus isolates

| Isolate | PFGE pattern | mupA polymorph |
|---------|--------------|----------------|
| EN44, EN45, EN59, EN72, EN81, ES63, FN31, EN48 | A1 | I |
| EN57 | A2 | I |
| EN68 | A3 | I |
| FW02 | A4 | I |
| EN02, EN05, ES62 | A5 | I |
| EN62, EN70 | A6 | I |
| EN34, EN74, EN79, ET30 | A7 | I |
| FN41, HN42 | B | II |
| EN09 | C | I |
| GN28, GN40 | D | III |

| Isolate | PFGE pattern | mupA polymorph |
|---------|--------------|----------------|
| FN1, FN5, FN29, FN41, FN43, FN47, FN55, FN29, FN37, FN39, FN28, FN42, FN53, FN30, FN09, EN57, AN45, CW04 | A1 | |
| FN13 | A2 | |
| HN20 | A3 | |
| FN16 | B | |

morph III; isolates GN28 and GN40) (Table 1). The MuH isolates identified from different LTCFs showed different polymorph types. These data suggest that the mupA gene may be conserved but that variation occurs near the mupA loci. The polymorphs characterized by the EcoRI-hybridizing band of about 4 kb have been detected in several countries (5, 12, 14, 17, 22), but it is not known whether they are identical or different HindIII and ClaI fragments. The increasing number of older residents in LTCFs and easy dissemination of antibiotic resistance to prevent the dissemination of resistant organisms. Our data showing clonal dissemination of mupirocin-resistant S. aureus isolates indicate that adequate infection control strategies against mupirocin-resistant S. aureus isolates should be established in LTCFs.

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Volume 50, no. 1, p. 365–367, 2006. Page 367: The Acknowledgments section was not included. It should read, “This study was supported by an intramural research grant of the Korea Centers for Disease Control and Prevention (2910-213).”