JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2008, p. 3868–3869 Vol. 46, No. 11
0095-1137/08/$08.00+0 doi:10.1128/JCM.01035-08

Occurrence of *mecA* in Nonstaphylococcal Pathogens in Surface Waters[▽]

The presence of antibiotics and other pharmaceuticals in natural waters and overland runoff has selected for highly resistant bacteria in nonclinical settings (13, 19). Of particular interest are methicillin-resistant staphylococci shed by humans and animals, as well as the gene conferring their resistance to ß-lactam antibiotics, *mecA*. Although *mecA* is most commonly associated with staphylococci (9), the detection of a *mecA* homologue in *Enterococcus hirae* (5) has led to the speculation that *mecA* also occurs in nonstaphylococcal genera (7). Despite continual increases in the community prevalence of methicillin-resistant staphylococci (16), there is little understanding of the role of the natural environment as a reservoir of staphylococci and other potentially pathogenic bacteria that harbor *mecA*. Based on these concerns, we investigated the occurrence of methicillin-resistant staphylococci and *mecA* in surface waters impacted by suburban runoff and fecal pollution.

Water samples (1 liter) were collected from 13 locations in Bay Village, OH, including a recreational beach (5 sites) and a tributary (8 sites) that empties into Lake Erie adjacent to the beach. These waters are known to contain elevated densities of fecal pollution indicator bacteria (*Escherichia coli* and enterococci) and have been implicated in outbreaks of bacterial disease, including gastroenteritis, dermatitis, and acute respiratory illness (3). One hundred milliliters of each sample was filtered through a nitrocellulose membrane (0.45-μm pore size), following which the membranes were incubated in Mueller-Hinton broth supplemented with oxacillin (6 mg liter^{-1}) and polymyxin B (25,000 IU) for 18 h at 35°C. An inoculum (50 μl) from each sample (all 13 samples exhibited growth) was transferred to Baird-Parker agar base supplemented with egg yolk-tellurite emulsion (1, 12) along with cultures of preidentified control bacteria, including methicillin-resistant *Staphylococcus aureus* (SB-01) (20), *S. aureus* (ATCC 6538), and *Staphylococcus epidermidis* (ATCC 146). The DNA from 65 isolates that exhibited a phenotype consistent with that of *Staphylococcus* spp. (black, convex, shiny colonies) and the control strains were subjected to multiplex PCR analysis (12) targeting a *Staphylococcus* species-specific segment of the 16S rRNA gene (17), an *S. aureus*-specific segment of *femB* (11), and *mecA*, which encodes penicillin-binding protein 2a (11). While none of the environmental isolates were identified as staphylococci, *mecA* was detected in 44 isolates. The putative identities of 25 *mecA*-containing isolates representing all of the study sites were assessed by sequencing approximately 1,000 bp of the 16S rRNA gene (10), followed by BLAST analysis. Further confirmation of identity was achieved using the API 20E and 20 Strep systems (Biomerieux) in addition to a series of genetic and other biochemical assays (Table 1). The *mecA*-containing isolates were identified as *Proteus vulgaris* ($n = 18$), *Morganella morganii* ($n = 4$), and *Enterococcus faecalis* ($n = 3$). Although Baird-Parker agar is most commonly used to isolate staphylococci, the growth of these bacteria was not surprising, since each is known to tolerate the supplements used to select against non-lecithinase-producing bacteria (tellurite) (18, 21) and gram-negative species (polymyxin B) (6, 8). The validity of this result was further confirmed when preidentified strains of *P. vulgaris* (ATCC 6896), *M. morganii* (NRRL B-1663), and *E. faecalis* (ATCC 29212) exhibited a phenotype on Baird-Parker agar similar to that of control strains of *S. aureus* (ATCC 6538) and *S. epidermidis* (ATCC 146).

It has been suggested that *mecA* originated in *Staphylococcus sciuri* or *Enterococcus hirae* and then became established in species of clinically significant staphylococci, including *S. aureus* and *S. epidermidis* (5, 7, 9). To determine the relationship of *mecA* detected in the current study to previously determined *mecA* sequences, approximately 250 bp of *mecA* from a representative isolate of *P. vulgaris*, *M. morganii*, and *E. faecalis* was commercially sequenced (GenBank accession no. EU710763, EU710764, and EU736140). BLAST analysis and sequence alignment (ClustalW) revealed 100% similarity only to sequences of *mecA* found in *S. aureus* and other staphylococci that harbored the staphylococcal cassette chromosome *mec* (SCC*mec*). The similarity of the *mecA* sequences among species of *Proteus*, *Morganella*, *Enterococcus*, and *Staphylococcus* indicates that *mecA* is more widely distributed in the environment than previously understood and also hints at the potential for SCC*mec* transfer among differing bacteria genera. Although the transfer of SCC*mec* among genera is thought to be relatively rare, segments of penicillin-binding-protein-encoding genes can be acquired by marginally resistant bacteria, including *Haemophilus*, *Neisseria*, and *Streptococcus* (4), effectively increasing their resistance to β-lactam antibiotics.

P. vulgaris, *M. morganii*, and *E. faecalis* are causal agents of urinary tract, wound, and respiratory infections and are known to exhibit resistance to multiple antibiotics. Antibiotic sensitivity assays were performed with the *mecA*-containing isolates according to CLSI guidelines (2), which revealed that all of the isolates were resistant to three β-lactam antibiotics: oxacillin (MIC $>$ 128 mg liter^{-1}), ampicillin (MIC $>$ 128 mg liter^{-1}), and amoxicillin (MIC $>$ 128 mg liter^{-1}). The isolates were also resistant to antibiotics representing other classes, including glycopeptides (vancomycin [MIC $>$ 128 mg liter^{-1}]), macrolides (erythromycin [MIC $>$ 128 mg liter^{-1}]), and tetracyclines (tetracycline [MIC $>$ 32 mg liter^{-1}]). Although tolerance to glycopeptides and macrolides is expected for members of the *Enterobacteriaceae*, the resistance patterns exhibited by all of the isolates suggest that multiple resistance mechanisms characterize these environmental pathogens. All of the isolates were susceptible to trimethoprim (MIC $<$ 8 mg liter^{-1}) and ciprofloxacin (MIC $<$ 1 mg liter^{-1}). The antibiotic susceptibility of control strains was also assayed to determine if the environmental isolates exhibited unique susceptibility patterns. In all cases, the environmental strains exhibited resistance to a greater number of antibiotics than the control strains. Specifically, *P. vulgaris* (ATCC 6896) was susceptible to tetracycline, *M. morganii* (NRRL B-1663) was susceptible to erythromycin and tetracycline, and *E. faecalis* (ATCC 29212) was susceptible (or displayed intermediate resistance) to all of the antibiotics tested. These results are of particular interest, since they not only illustrate a difference in resistance between control and environmental strains of the same bacteria species but also show that *mecA* can be maintained in natural reservoirs.

Although methicillin-resistant staphylococci were not detected in natural surface waters associated with a recreational beach, other resistant pathogens were isolated, including *mecA*-containing enterococci and members of the *Enterobacteriaceae*. Our results underscore the role of the natural envi-

TABLE 1. Results of biochemical, growth, and genetic assays with *mecA*-harboring isolates[a]

Identity of *mecA*-containing isolates (n[b])	Test result						
	Biochemical test		Growth/appearance on diagnostic medium[c]			Species-specific PCR analysis[d]	
	Gram stain	Catalase	Swarming	mEI	Slanetz and Bartley	*P. vulgaris*	*E. faecalis*
P. vulgaris (18)	−	+	+	−	−	+	−
M. morganii (4)	−	+	−	−	−	+/−[e]	−
E. faecalis (3)	+	−	−	+	+	−	+

[a] Preidentified strains of *Proteus vulgaris* (ATCC 6896), *Morganella morganii* (NRRL B-1663), and *Enterococcus faecalis* (ATCC 29212) were used as controls in each assay. +, positive result; −, negative result, +/−, intermediate result.
[b] *n*, no. of isolates.
[c] mEI (BD Diagnostics) and Slanetz and Bartley (Oxoid) media are differential and selective for *Enterococcus* spp.
[d] For *P. vulgaris*, see reference 14; for *E. faecalis*, see reference 15.
[e] Isolates of *M. morganella* exhibited a weak PCR signal.

ronment as a reservoir of clinically significant pathogens that harbor *mecA*. Combined with the historic implication of these waters in disease outbreaks, these results further emphasize the need for environmental pathogen surveillance to support ongoing epidemiology programs.

Nucleotide sequence accession numbers. 16S rRNA gene sequences determined in this work have been deposited in GenBank under accession numbers EU710760 to EU710762 and EU736118 to EU736139.

This study was funded by grants from the United States Department of Agriculture (CSREES grant number 2007-31100-06039), the Cuyahoga County Board of Health, and the Sigma Xi Grants-in-Aid of Research program.

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▽ Published ahead of print on 24 September 2008.