Nosocomial Spread of an Unusual Methicillin-Resistant
Staphylococcus aureus Clone That Is Sensitive to All
Non-β-Lactam Antibiotics, Including Tobramycin

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Between January and December 1999, in Hippokration General Hospital, Thessaloniki, Greece, a large proportion of the methicillin-resistant Staphylococcus aureus isolates (34.4%) exhibited susceptibility to virtually all alternative non-β-lactam antibiotics, including tobramycin. Twenty-five of them were selected randomly for further testing; all belonged to a unique genotype and were characterized as heterogeneously resistant to oxacillin. The aadD gene, encoding tobramycin resistance, failed to be amplified in all cases, indicating absence of the gene or the entire plasmid pUB110 from the mec DNA. The increased incidence of methicillin-resistant Staphylococcus aureus (MRSA) in many countries during the last decades has been accompanied by the appearance of multidrug-resistant clones, replacing other MRSA lineages (1). Tobramycin in particular is unlikely to be effective against MRSA because the gene (aadD) encoding tobramycin and kanamycin resistance is present within plasmid pUB110, which is considered a stable part of the mec DNA (3, 6). Recently, MRSA strains sensitive to gentamicin but resistant to tobramycin have emerged in French hospitals (5, 7, 8), while several MRSA strains sensitive to kanamycin have been occasionally isolated in the United Kingdom; they generally retained resistance to several alternative antibiotics (16). We report a hospital outbreak of clinical infections due to MRSA that exhibits susceptibility to virtually all alternative non-β-lactam antibiotics, including tobramycin.

Between January and December 1999, 247 S. aureus isolates were recovered from clinical infection of separate patients in Hippokration General Hospital, Thessaloniki, northern Greece. Identification to species level was performed by using the Vitek automated system (bioMerieux, Hazelwood, Mo.). MICs of oxacillin and tobramycin were tested using an agar dilution method (10) for susceptibility to ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, nitrofurantoin, rifampin, tetracycline, trimethoprim, and vancomycin. The isolates were also tested for high-level resistance to spectinomycin (500 μg/ml), which has been associated with carriage of transposon Tn554 (12). The mec DNA was confirmed by population analysis profiles using four dilutions of the bacterial cultures (4, 12). The MIC was defined as the lowest concentration of the antibiotic that inhibited 99.9% of the bacterial cells.

PCR was performed for the tobramycin-sensitive MRSA isolates to amplify a 449-bp product within the mecA gene (2). The method was also used for the detection of genes _ermA_ (139-bp product), which is part of transposon Tn554, and _aadD_ (165-bp product), which is part of plasmid pUB110, using published primers (9, 14).

The protocol used for the determination of _Smal_ macrorestriction patterns was described previously (15). The digested chromosomal DNAs were separated with a contour-clamped homogeneous electric field DRIII apparatus (Bio-Rad, Birmingham, United Kingdom). Concatameric bacteriophage lambda DNA molecules (48.5 kb; Bio-Rad) were used as size standards. Banding patterns of the strains were compared visually according to the criteria proposed by Tenover et al. (13).

The 25 isolates that were recovered from Hippokration Hospital remained susceptible to ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, nitrofurantoin, rifampin, tetracycline, trimethoprim, and vancomycin. Among the four isolates that were recovered from two different hos-
pitals in Thessaloniki (Table 1), two were also susceptible to all non-β-lactam antibiotics; the remaining two exhibited additional resistance to ciprofloxacin and tetracycline. The PCR test for the mecA gene was positive in the 29 isolates, while aadD and ermA genes failed to be amplified in all cases. The isolates were susceptible to spectinomycin at 500 μg/ml, required a MIC of oxacillin equal to or higher than 16 mg/liter, and were characterized by disk diffusion and population analysis as heterogeneously resistant, belonging to class II or II/III (12).

Smal macrorestriction showed that the 25 isolates from Hippokration Hospital and 2 from AHEPA hospital (Table 1) belonged to a unique genotype (pattern I; Table 1). The remaining two isolates, which were recovered from different hospitals (Agios Dimitrios and AHEPA), exhibited distinct pulsortypes (patterns II and III, respectively; Fig. 1; Table 1). Patterns II and III differed from each other by only five bands. The isolation of tobramycin-sensitive MRSA in two other tertiary hospitals in our region, two isolates from which exhibited a clonal identity with those recovered in our hospital, may indicate the geographical spread of this clone. The common pulsed-field gel electrophoresis (PFGE) pattern of the Greek isolates seems to differ substantially from those of other multidrug-susceptible MRSA clones that have been described previously (5, 7, 8, 12, 16), although these strains were not run under the same conditions. However, most of the latter MRSA strains exhibited resistance to some non-β-lactam antibiotics, such as ciprofloxacin and tobramycin.

Tobramycin seldom retains activity against MRSA because the gene conferring resistance (aadD) is present within plasmid pUB110, which is usually part of the mec DNA (3), although recently mec elements lacking this plasmid have been described (11). pUB110 was integrated during the period when mec DNA was being formed and prior to the emergence of the first outbreaks of MRSA infections in European hospitals in the early 1960s (3). This was the era of pre-MRSA, in which transcription of the mecA gene was strongly repressed by the mcl gene encoding a repressor function. A mutation in the rep gene of pUB110 possibly served for the stabilization of the plasmid after its integration into the chromosome of S. aureus (6). The appearance of MRSA was followed by various patterns of resistance to antibiotics, but tobramycin resistance was consistently recorded (1, 5, 6). In our isolates, aadD failed to be amplified, indicating the absence of the gene or the entire plasmid from the mec DNA. Also, the susceptibility of the isolates to spectinomycin at 500 μg/ml suggested the absence of transposon Tn554, which has been related to resistance to several alternative antibiotics (3).

The MRSA strain of this report has spread into different departments of a general hospital where antibiotic usage is heavy. This observation might indicate the virulence of this strain, which survived and became widespread in the hospital.
Despite its susceptibility to a wide range of antimicrobials. It should be noted that the antibiotics mainly in use in this as well as other Greek hospitals are β-lactam–β-lactamase inhibitor combinations, expanded-spectrum cephalosporins, monobactams, carbapenems, and glycopeptides. The overuse of most of these drugs might have facilitated the spread of this MRSA strain.

Multidrug-resistant MRSA has become a major nosocomial pathogen, and vancomycin is at present the antibiotic of choice for systemic infection. However, it is believed that the widespread use of vancomycin in regions with high proportions of MRSA will hasten the emergence of resistance in staphylococci and may also select for vancomycin-resistant enterococci. In view of the intrahospital spread of “new” multidrug-susceptible MRSA clones, we feel that additional therapeutic options are presented and vancomycin usage could be considerably reduced. Also, several of the antibiotics used on less of an intrahospital basis, such as macrolides, tetracyclines, lincosamides, and cotrimoxazole, could be used more extensively. This is of more value for regions such as Greece where the prevalence of MRSA is one of the highest in Europe. Nevertheless, vancomycin might still be the most appropriate agent for life-threatening infections. It has yet to be determined whether dissemination of this particular clone resulted from strains with enhanced virulence or genetic adaptability or from selection caused by changing consumption of antibiotics.

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