Staphylococcal Resistance to Aminoglycosides before and after Introduction of Amikacin in Two Teaching Hospitals

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A prospective study was conducted to determine the prevalence of aminoglycoside-resistant Staphylococcus aureus and coagulase-negative staphylococci before and after the introduction of amikacin as the sole aminoglycoside used in our burn unit, adult intensive care unit, and neonatal intensive care unit. Pharyngeal or endotracheal cultures, as well as superficial surveillance cultures, were collected weekly during the following four study periods: all units for 4 months before amikacin introduction, all units 4 to 8 months after, all units 12 to 13 months after, and the neonatal intensive care unit 30 months after. A total of 2,613 strains of coagulase-negative staphylococci and 316 strains of S. aureus were obtained from 916 patients. During the course of the study, amikacin-resistant coagulase-negative staphylococci increased from 0 to 22%, colonizing 43% of patients, whereas no amikacin-resistant S. aureus was detected. During the preamikacin survey, 68% of the coagulase-negative staphylococci and 12% of the S. aureus strains were resistant to tobramycin and gentamicin. This resistance did not decrease after amikacin was introduced. Initially, 83% of the aminoglycoside-resistant coagulase-negative staphylococci were resistant to both tobramycin and gentamicin. During the last surveillance this value dropped to 40%, and 48% of the strains had become resistant to all three aminoglycosides. Resistance to aminoglycosides, including amikacin, develops quickly in coagulase-negative staphylococci from clinical areas where these antimicrobial agents are widely used. However, aminoglycoside resistance in S. aureus is much less frequent.

Although aminoglycosides are predominantly used for the treatment of gram-negative infections, they are also known to have antistaphylococcal activity (15). This becomes an important consideration when patients are treated empirically for suspected sepsis with an aminoglycoside and ureidopenicillin, other penicillins, or cephalosporins susceptible to staphylococcal beta-lactamases. This type of empiric treatment is commonly administered to premature neonates, to patients with neutropenia, and occasionally to patients with suspected nosocomial infections (17, 21) due to enhanced clinical activity of such antimicrobial agent combinations in gram-negative sepsis (2). In addition, the emergence of methicillin-resistant Staphylococcus aureus (22) and the widespread use of central lines with bacteremia due to Staphylococcus epidermidis (7) are increasing the clinical importance of the antistaphylococcal activity of aminoglycosides. Little is known about the emergence of amikacin resistance in staphylococci; nearly all epidemiological surveys have focused on the emergence of resistance in gram-negative bacteria (6, 10, 16, 18, 24).

Resistance to tobramycin and gentamicin was observed both in coagulase-negative staphylococci and S. aureus in the intensive care units (ICUs) and burn unit at two McMaster University teaching hospitals during a period of exclusive tobramycin and gentamicin use. At that time, no resistance to amikacin was apparent. In view of the lack of clinically significant resistance developing in gram-negative bacteria when amikacin is introduced as the aminoglycoside of choice (6, 10, 16, 18, 24), this prospective study was undertaken to determine the prevalence of resistance of coagulase-negative staphylococci and S. aureus to gentamicin, tobramycin, and amikacin before and after the introduction of amikacin as the only prescribed aminoglycoside in those units.

MATERIALS AND METHODS

Prevalence studies. Three different geographical areas were surveyed during four periods: the neonatal ICU, adult ICU, and burn unit. The ICUs were located at one hospital and the burn unit at another. The neonatal ICU is a 35-bed unit caring almost exclusively for premature babies. Gentamicin was used extensively in that unit. The adult ICU has a 15-bed capacity, and the burn unit has 6 beds. In both units, tobramycin was used. Surveillance studies were conducted in all three units for 4 months before switching to amikacin, from 4 to 8 months after, and from 12 to 13 months after. A fourth surveillance period was conducted only in the neonatal ICU from 30 to 31 months after switching to amikacin. During the course of the study, amikacin was the only aminoglycoside used in the study areas. The percentages of patients colonized or infected with aminoglycoside-resistant strains and the percentages of resistant strains of staphylococci were calculated for each surveillance period.

Microbiology. Nasal and superficial swabs were collected from all patients on admission and at weekly intervals thereafter. Endotracheal tube suction samples were obtained from entubated patients. Superficial sites consisted of open wounds in children and adults and umbilical stumps in neonates. Axillary swabs were obtained in the absence of open wounds. Swabs were transported in charcoal medium (NCS Diagnostics, Inc., Mississauga, Ontario, Canada) to the microbiology laboratory and inoculated onto MacConkey agar and blood agar plates (Oxoid Canada, Inc., Nepean, Ontario, Canada). After overnight incubation at 35°C in 5% CO2, colonies characteristic of staphylococci were Gram stained and tested for catalase production. Large, well-isolated colonies consisting of catalase-positive, gram-positive cocci were tested for coagulase production by a commercial tube coagulase test (BBL Microbiology Systems, Cockeysville, Md.). Coagulase-positive isolates were

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considered to be \(S.\) \textit{aureus}. Coagulase-negative isolates were not further identified and are therefore referred to as coagulase-negative staphylococci. Antimicrobial agent susceptibilities were determined by agar dilution. The method is similar to that described in the National Committee for Clinical Laboratory Standards performance standard M7-A but used Sensitest agar (Oxoid) in place of modified Mueller-Hinton agar. The antimicrobial-agent-containing medium was quality controlled daily with \textit{Escherichia coli} ATCC 25922, \(S.\) \textit{aureus} ATCC 29213, and \textit{Pseudomonas aeruginosa} ATCC 27853. At least once per week, each batch of antimicrobial-agent-containing media was tested with an expanded range of 15 strains of control organisms having known and discriminatory MICs. The breakpoint for gentamicin and tobramycin was 4 \(\mu\)g/ml, for amikacin the breakpoint was 16 \(\mu\)g/ml, and it was 2 \(\mu\)g/ml for oxacillin. Detection of gentamicin-resistant subpopulations was determined by subculturing isolated colonies onto brain heart infusion agar (Oxoid) containing 5 \(\mu\)g of gentamicin per ml (23).

### RESULTS

A total of 2,613 strains of coagulase-negative staphylococci and 316 strains of \(S.\) \textit{aureus} were obtained from 916 patients during the four surveillance periods. The numbers of patients colonized and the numbers of isolates obtained during each surveillance period are summarized in Table 1.

During the course of the study, the percentage of patients colonized with amikacin-resistant coagulase-negative staphylococci increased from 0\% before to 43\% 30 to 31 months after amikacin was introduced (Table 2). The percentage of patients colonized with tobramycin- and gentamicin-resistant strains did not appear to decrease after amikacin was introduced and remained at approximately 80\%. The percentage of amikacin-resistant coagulase-negative staphylococci increased from 0 to 22\% during the course of the study. \(S.\) \textit{aureus} resistance to amikacin was not observed. However, only a small number of strains were obtained during the last two surveillance periods due to the low prevalence of colonization. Only two isolates with gentamicin-resistant subpopulations were encountered during the study. These subcultures were retested and found to be resistant to tobramycin and gentamicin. Combined tobramycin and gentamicin resistance occurred in 83\% of aminoglycoside-resistant coagulase-negative staphylococci during the first survey. Gentamicin resistance, as it developed, was linked to tobramycin and gentamicin resistance (Table 3). During the last surveillance, 48\% of resistant strains were resistant to all three aminoglycosides.

### DISCUSSION

This is a survey of aminoglycoside resistance in \(S.\) \textit{aureus} and coagulase-negative staphylococci before and after the introduction of amikacin. The vast majority of isolates merely colonized patients. The prevalence of infecting strains was too low that comparative analysis of colonizing versus invasive strains was not deemed worthwhile. Invasive strains of coagulase-negative staphylococci are usually multiply resistant (7, 14). This most likely reflects the previous exposure of the patient to antimicrobial agents or admission to units where antimicrobial agents are widely used. Therefore, the practical concern is not that multiply resistant coagulase-negative staphylococci become more virulent but that they may be more difficult to treat and that they harbor resistance plasmids which may be transferred to other bacteria on skin and mucosal surfaces (13).

Widespread aminoglycoside resistance developing in coagulase-negative staphylococci was previously reported. Weinstein et al. reported that 80\% of infants in a special care nursery with a previous outbreak of gentamicin-resistant \(S.\) \textit{aureus} became colonized with gentamicin-resistant coagulase-negative staphylococci (23). Richardson and Marples reviewed the antimicrobial agent susceptibilities of clinically significant strains of \(S.\) \textit{epidermidis} between 1976 and 1980 and found that concomitant gentamicin and tobramycin resistance increased from 7 to 33\% (19). In the National Nosocomial Infections Study, the incidence of hospital-acquired gentamicin-resistant coagulase-negative staphylococci rose from 2 to 24\% and that of gentamicin-resistant \(S.\) \textit{aureus} increased from 1 to 13\% between 1975 and 1979 (1). Archer et al. found that the prevalence of gentamicin-resistant staphylococci have increased.
resistant coagulase-negative staphylococci in a cardiac surgery unit increased from 20 to 68% over a period of 4 years (4).

Based on our findings, it appears that amikacin resistance also develops quickly in coagulase-negative staphylococci after this aminoglycoside is introduced, but without a concomitant decrease in gentamicin and tobramycin resistance. In contrast, amikacin resistance does not appear to increase appreciably in gram-negative bacteria when this aminoglycoside is widely used (6, 10, 16, 24). Furthermore, hospitals which switched from gentamicin or tobramycin to amikacin experienced a decrease over time in the prevalence of gram-negative bacilli to gentamicin and tobramycin (6, 10, 16, 24).

The amikacin resistance which we observed in coagulase-negative staphylococci was clearly associated with resistance to the other two aminoglycosides. A total of 99% of amikacin-resistant strains were also resistant to tobramycin and gentamicin. Whereas 83% of aminoglycoside-resistant strains were both gentamicin and tobramycin resistant before amikacin was introduced, this decreased to 40% in the last survey, in which 48% were resistant to all three aminoglycosides. This probably represents the emergence of genetically linked multiaminoglycoside resistance. Oxacillin resistance was also associated with aminoglycoside resistance, as was previously reported for methicillin (3, 19).

Linkage of multiple resistance on a single plasmid may account for the continuing high prevalence of tobramycin and gentamicin resistance even after the use of these antimicrobial agents was discontinued. This is currently being investigated by aminoglycoside-modifying enzyme analysis, plasmid isolation, and restriction endonuclease typing. In this study, isolates have not been tested for susceptibility to other aminoglycosides.

It is of interest that amikacin resistance did not develop in S. aureus. A much lower prevalence of gentamicin-resistant S. aureus than of coagulase-negative staphylococci was observed previously (1, 23). There are a number of possibilities which may account for this. The number of patients colonized and the total number of bacteria are much higher for coagulase-negative staphylococci than for S. aureus. Hence, by probability, it is more likely to resist to be observed in coagulase-negative staphylococci if resistance plasmids are equally likely to be acquired by different species of staphylococci. However, resistance plasmids may also be more easily acquired and maintained by coagulase-negative staphylococci than by S. aureus. This concept is supported by two observations. First, gentamicin resistance plasmids are more easily lost in vitro from S. aureus than from S. epidermidis (H. Bialkowski-Hobotzanka, personal communication), and second, a larger number of resistance plasmids are present in clinical isolates of S. epidermidis than in isolates of S. aureus (20).

The appearance of similar resistance plasmids have been observed in both coagulase-negative staphylococci and S. aureus. Cohen et al. demonstrated that a gentamicin resistance plasmid appeared in S. epidermidis during an outbreak with S. aureus carrying the same plasmid. A year earlier, S. epidermidis with a similar resistance pattern was observed (8). Interspecies transfer of resistance plasmids, including those conferring amikacin resistance, was demonstrated in staphylococci in vitro and is believed to occur by conjugative transfer (5, 9). It is possible that such transfer of amikacin resistance from coagulase-negative staphylococci to S. aureus may yet occur in our neonatal ICU, where amikacin is still used. Since differential phenotypic expression of resistance has been reported (11, 12), it is tempting to speculate that resistance may already have been transferred to some strains of S. aureus but is not being phenotypically expressed. This is yet another possibility to account for the absence of resistance in S. aureus. We believe this deserves further study.

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LITERATURE CITED


