Anaerobic Resistance of Clinical Isolates of *Staphylococcus aureus* to Aminoglycosides†

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The anaerobic minimum inhibitory concentration of six aminoglycosides (amikacin, gentamicin, kanamycin, netilmicin, sisomicin, and tobramycin) averaged over 10-fold greater than the aerobic minimum inhibitory concentration for 50 clinical isolates of *Staphylococcus aureus*. Cultures from osteomyelitis and blood generally showed a somewhat greater increase in minimum inhibitory concentration due to anaerobiosis than did cultures from abscesses and wounds, and amikacin activity was most affected by anaerobiosis.

Infections caused by staphylococci may be superficial and aerobic in nature (e.g., ecthyma and impetigo) or deep-seated and essentially anaerobic (e.g., osteomyelitis and some abscesses). Several investigators have reported that anaerobiosis decreases the effectiveness of aminoglycosides against facultatively anaerobic bacteria including *Staphylococcus* (5, 7, 8, 11–13, 15). It has been suggested that the redox potential in some inflamed or necrotizing lesions may be low enough to decrease the clinical effectiveness of aminoglycosides (1, 6). At present, essentially all antibiotic susceptibility determinations on facultatively anaerobic bacteria are conducted under aerobic conditions. This study was designed to investigate quantitatively the effect of anaerobiosis on the minimum inhibitory concentration (MIC) of aminoglycosides for clinical isolates of *Staphylococcus aureus* and to see whether cultures from presumably more anaerobic infection sites might demonstrate a greater increase in MIC under anaerobic conditions. The agar shake tube technique used in these studies (3) made it possible to determine anaerobic and aerobic MICs simultaneously in the same series of tubes.

Fifty recently isolated strains of *S. aureus* from different clinical sources were obtained for this study. The standard reference strain of *S. aureus*, ATCC 25923, was included in each experiment as a control. All strains were examined for colony morphology, Gram-stain reaction, catalase, coagulase, deoxyribonuclease, growth on mannitol-salt agar, and anaerobic fermentation of glucose. All cultures reacted as typical *S. aureus* in this series of tests.

Six aminoglycoside antibiotics (amikacin, gentamicin, kanamycin, netilmicin, sisomicin, and tobramycin) were tested. The agar shake tube technique (3) was used for all experiments. If the MIC for the control culture (ATCC 25923) deviated more than one twofold dilution from the normal value (a relatively rare occurrence), the experiment was repeated.

In 96% of the culture-antibiotic combinations, the anaerobic MIC was at least 4-fold greater than the aerobic MIC, and in the majority of cases it was at least 10-fold greater. Table 1 summarizes these results as the average of anaerobic/aerobic MIC ratios for the different antibiotics against the cultures from different clinical sources. The results indicate that the osteomyelitis cultures had the greatest increase in resistance as a result of anaerobiosis when amikacin, kanamycin, or netilmicin was the antibiotic tested. However, the isolates from blood were most affected by anaerobiosis when gentamicin, sisomicin, or tobramycin was tested. The average values summarize the overall effect of anaerobiosis on the effectiveness of the different antibiotics. The effectiveness of amikacin was reduced considerably more by anaerobiosis than that of the other aminoglycosides. Sisomicin activity was least affected. The standard reference strain (ATCC 25923) was more affected by anaerobiosis than was the average clinical isolate.

These studies indicate one reason why patients being treated with an aminoglycoside for gram-negative infections may suddenly develop a staphylococcal suprainfection, perhaps originating from sites having a low redox potential. They also may explain the lack of effectiveness of aminoglycoside antibiotics against osteomyelitis and some other staphylococcal infections that may occur in anaerobic sites. It would appear that amikacin is more prone to such problems, because its activity generally is most af-
affected by anaerobiosis. However, when data from individual cultures are examined, the anaerobic MICs are far more consistent than are the aerobic/anaerobic MIC ratios, because the aerobic MICs show considerably more variation between strains. Therefore, generalizations based on these ratios, and particularly averages of ratios, have little predictive value when applied to an individual patient. However, the MICs under anaerobic conditions were consistently higher than those based on the usual aerobic methods.

We have not investigated the metabolic basis for this general increase in resistance under anaerobic conditions. However, it has been suggested (2, 7) that transport of the antibiotic into the bacterial cell is impaired by anaerobic conditions. The level of resistance here is probably not as high as would be conferred by plasmid-borne resistance factors (14).

Of considerable practical importance is the occurrence of variants with increased resistance under aerobic conditions noted after exposure to aminoglycosides. Lacey (8) noted that dwarf colony variants had undergone mutations that blocked their aerobic respiratory metabolism and, hence, they were dependent on their fermentative pathway of energy metabolism and had aminoglycoside resistance equivalent to anaerobic conditions. Other workers also have described variant colonies after antibiotic therapy (4, 8-10, 16). In the present study, isolated colonies developed in the aerobic zone of many of the tubes at concentrations above the aerobic MIC of the antibiotic. We have picked some of these colonies and found that when subcultured they have an aerobic MIC close to the anaerobic MIC of the parent strain and the anaerobic MIC is usually unchanged. These cultures had no apparent impairment in aerobic respiration, and the basis for their increased resistance has not yet been determined. It could result from an increased enzymatic capability to inactivate the aminoglycosides or from an impaired system for aminoglycoside transport. Such variants may be of considerable practical importance as the source of resistant strains in patients receiving aminoglycoside therapy.

This study again demonstrates the importance of determining the anaerobic MIC of aminoglycosides for facultatively anaerobic bacteria that may be encountered in a clinical situation. It also illustrates the diversity of information that is provided by the agar shake tube technique for determining MIC levels.

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**LITERATURE CITED**


