Properties of Silver Sulfadiazine-Resistant Enterobacter cloacae

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Two silver sulfadiazine-resistant isolates of Enterobacter cloacae obtained in a burns unit where the drug was in use were studied. These strains were resistant to elevated levels of the drug, and they were cross-resistant to silver benzoate, but not to silver nitrate. Growth of the strains in nutritionally poor defined media sensitized them to the inhibitory action of the drug. Exposure of the bacteria to penicillins rendered them susceptible to silver sulfadiazine. The resistant bacteria harbored episomes for resistance to carbenicillin and kanamycin; however, resistance to silver sulfadiazine could not be transferred by these episomes. Twenty-three strains of E. cloacae isolated in a general hospital were sensitive to much lower levels of the drug (≤50 µg/ml).

Silver sulfadiazine (AgSu), a topical antimicrobial agent, is of benefit in the treatment and prevention of burn infections (5, 6, 9–11, 17). More recently this antibacterial agent has also been used postoperatively to prevent wound infections. AgSu has a broad spectrum of action, and most microorganisms capable of infecting burns and wounds are susceptible to the antimicrobial action of the drug at levels which are readily obtainable topically (2, 19). The mechanism of action of AgSu was studied mainly in Pseudomonas aeruginosa where it affects primarily the external cell structure (3, 12, 14). Strains resistant to elevated levels of AgSu have not been isolated in the laboratory, nor has resistance to AgSu been a particularly bothersome clinical problem in spite of the widespread use of this drug. A partially-resistant strain of P. aeruginosa was isolated in a burns unit, but its growth was inhibited by slightly higher (and readily obtainable) AgSu concentrations (3). When we were informed that truly resistant Enterobacter cloacae had made their appearance in a burns unit in which AgSu was used, we decided to investigate the basis of this resistance and to compare the properties of these specimens to the E. cloacae strains recovered in a general hospital.

E. cloacae 7779 and 7780 were received from J. R. Lloyd of Children's Hospital of Michigan in Detroit. These strains were isolated in a burns unit in which AgSu was used. They probably represent two isolates of the same strain. The identity of these isolates was confirmed by the Clinical Microbiology Laboratory of this institution. Other strains of E. cloacae were clinical isolates from patients on various services of the Columbia Presbyterian Medical Center.

Unless otherwise indicated, minimal inhibitory concentrations (MIC) of AgSu were determined in Trypticase soy broth by standard procedures (1). Sensitivity to other antibiotics was determined by the disk plate procedure (1).

E. cloacae strains 7779 and 7780 were resistant to elevated levels of AgSu (Table 1), growing even in the presence of a suspension of 400 µg/ml. Contrariwise, 23 consecutive isolates of E. cloacae recovered from patients at this institution were all susceptible to much lower levels of AgSu (Table 1). In addition to their AgSu resistance, E. cloacae 7779 and 7780 also differed from the other E. cloacae strains by their resistance to carbenicillin and to kanamycin (Table 1).

The resistant strains were found to be cross-resistant to silver benzoate (MIC > 200 µg/ml), but not to organic mercurials (MIC for merthiolate, 0.78 µg/ml). The MIC of a sensitive strain (8060) to these chemicals was 12.5 and 0.78 µg/ml, respectively. E. cloacae 7779 and 7780 were not cross-resistant to silver nitrate (MIC of 0.78 µg/ml, the same as for E. cloacae 8060). Because of the insolubility of AgCl and the high chloride content of Trypticase soy broth, susceptibility to AgNO₃ had to be tested in a
synthetic medium (medium HA, ref. 13). In this medium, however, the resistance of strains 7779 and 7780 to AgSu was greatly decreased (MIC of 3.13 µg/ml versus 0.78 µg/ml for susceptible strain 8060). The increased susceptibility of the strains when grown in defined synthetic medium was not reversed by supplementation with p-aminobenzoic acid (17 µg/ml), thereby indicating that the increased resistance in Trypticase soy broth was not due to the presence of this vitamin therein. Nor could these bacteria be protected by simultaneous exposure to spermine (10^{-8} M), a chemical that stabilizes the bacterial cytoplasmic membrane (7, 18).

An attempt to assess the role of cell wall structure in resistance to AgSu was made by co-treatment of the AgSu-resistant bacteria with low levels of penicillins to induce some cell damage and thereby facilitate entry of AgSu. However both E. cloacae 7779 and 7780 are resistant to penicillins (penicillin G, carbenicillin, cephalothin, ampicillin, and oxacillin; see Table 1), which suggests the presence of a β-lactamase. Indeed the presence of penicillinase in these strains was demonstrated. To overcome this complication, the bacteria were treated with a mixture of ampicillin and oxacillin. The latter is a competitive inhibitor of penicillinase and may allow ampicillin to damage the cell wall.

Neither of these antibiotics (1,000 µg/ml) alone sensitized the bacteria to AgSu. However, when used together, it was found that when the oxacillin level was kept at 1,000 µg/ml and that of ampicillin at a minimum of 500 µg/ml, the MIC of AgSu required to suppress growth was reduced from >400 to 6.25 µg/ml. The amount of penicillins used was critical for even keeping the ratio 2:1, but decreasing their absolute concentrations did not sensitize the bacteria to AgSu. These results suggest that the structural basis of AgSu resistance involves the cell wall; this is supported by the finding that unlike normal E. cloacae, the electron microscopic
appearance of the cell wall of strains 7779 and 7780 is not altered after exposure to AgSu (unpublished data).

Strains 7779 and 7780 differed from the AgSu-susceptible isolates not only by being resistant to AgSu but also in exhibiting resistance to carbenicillin and kanamycin (Table 1). This suggested the possible presence of an R factor which carried the determinants for carbenicillin and kanamycin resistance and perhaps the AgSu marker as well. To determine this possibility, E. cloacae 7780 was mated with an F-

E. coli strain bearing a chromosomal mutation for resistance to nalidixic acid. By using selected media, transfer of resistance to carbenicillin and kanamycin to E. coli could be demonstrated, which indicates the episomal nature of these resistances. On the other hand, resistance to AgSu was not transferable under any of the experimental conditions used. Bacterial strains harboring R factors may also be resistant to heavy metals (e.g., Hg++) (16); however, strains with R factors or that are multiply resistant do not, in general, display an enhanced resistance to AgSu (2 and unpublished data). These findings, together with those described above, suggest that resistance to AgSu is not mediated by R factors.

The origin of the AgSu-resistant organisms is difficult to ascertain. Under laboratory conditions it was not possible to select, even after mutagenesis, bacteria with greatly increased AgSu resistance from normally susceptible E. cloacae. The highest level of resistance achieved was to 5 μg of AgSu per ml (unpublished data).

Although the basis of resistance to AgSu has not been clarified, it is interesting that resistant bacteria are cross-resistant to silver benzoate but not to silver nitrate and organic mercurials. Both of the organic silvers are not readily ionizable, and this may offer a clue as to the mechanism of action of AgSu (see also ref. 12 and 14).

Although AgSu-resistant E. cloacae were recovered, it is also significant that 23 strains of the same species (and 49 isolates of other Enterobacter species) recovered from patients in a general hospital were susceptible to the drug. In view of the role of Enterobacter species in opportunistic infections of burns and wounds (4, 8, 15) and the ability of AgSu to prevent such infections, the AgSu susceptibility of prevalent Enterobacter species is welcome.

The ability of penicillins to sensitize AgSu-resistant bacteria to the action of the drug may have some practical applications in future outbreaks of AgSu-resistant bacteria.

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