ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1984, p. 398–399
0066-4804/84/030398-02$02.00/0

Vol. 25, No. 3

Ribosomal Resistance of Clinical Enterococcal to Streptomycin Isolates

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Received 1 August 1983/Accepted 28 November 1983

The mechanism of high-level resistance to streptomycin was studied in 12 clinical isolates of *Streptococcus faecalis*. Six strains produced streptomycin-modifying enzymes. Each of three enzyme-negative strains tested demonstrated ribosomal resistance to streptomycin. Lack of ribosomal susceptibility is a significant cause of high-level streptomycin resistance among clinical enterococcal isolates.

By the late 1970s, ca. 50% of clinical enterococcal isolates in Boston demonstrated high-level resistance to streptomycin, defined by MICs > 2,000 μg/ml (2). High-level streptomycin resistance correlates with lack of bactericidal synergism between penicillin and streptomycin against such strains (2). That high-level resistance to streptomycin could result from the lack of susceptibility to the drug at the 30S ribosomal subunit level was shown in a laboratory mutant strain of *Streptococcus faecalis* (15), but demonstration that aminoglycoside-modifying enzymes are common in enterococci shed doubt on the importance of ribosomal resistance to streptomycin among clinical isolates (7). This study was undertaken to determine whether ribosomal resistance to streptomycin occurs among clinical strains of *S. faecalis* and, if so, to assess the relative importance of this resistance mechanism.

Enterococci used in this study were clinical isolates recovered and screened for high-level resistance to streptomycin and kanamycin between 1970 and 1974 (2, 11). Strains selected for further examination were identified as *S. faecalis* by the method of Facklam (4). Susceptibility to streptomycin (Eli Lilly & Co., Indianapolis, Ind., and Sigma Chemical Co., St. Louis, Mo.) was determined by agar dilution on glucose phosphate broth medium (GIBCO Diagnostics, Madison, Wis.) solidified with 1.5% agar. Inocula of ca. 10^3 CFU were prepared by dilution of overnight broth cultures and applied with a 32-prong inoculator. *S. faecalis* E1 (streptomycin MIC = 400 μg/ml) was used in studies of ribosomal protein synthesis. Ribosomes from this strain are normally susceptible to streptomycin (15).

Aminoglycoside-modifying enzyme assays were performed by the method of Benveniste and Davies (1) with minor modifications (7). Ribosomal protein synthesis in crude 30S cell extracts was determined by the method of Nirenberg (13), except that the magnesium concentration was adjusted to 17 mM. Polyuridylic acid-directed incorporation of [^{14}C]phenylalanine into polypeptide was studied in the presence or absence of streptomycin or gentamicin. Samples were run in duplicate, and the results (net counts per minute after subtraction of nonspecific binding) were averaged.

Twelve strains of *S. faecalis* with high-level resistance to streptomycin but not kanamycin were arbitrarily selected for further study. Strains highly resistant to streptomycin and kanamycin were excluded to avoid those most likely to possess plasmid-mediated aminoglycoside-modifying enzymes (3, 7). Of these 12 strains, 6 were found to produce streptomycin adenylyltransferase (Table 1), which most likely adenylylates at the 6 position of the streptidine ring (7). The remaining strains demonstrated no adenylylating, phosphorylating, or acetylating activity against streptomycin. Streptomycin MICs against enzyme-producing strains were ≤16,000 μg/ml. In contrast, enzyme-negative strains were resistant to streptomycin at concentrations of 128,000 μg/ml.

Three enzyme-negative strains were examined for evidence of ribosomal resistance to streptomycin. Results of a typical experiment are shown in Fig. 1. Streptomycin, in concentrations as low as 1.0 μg/ml, resulted in 90% inhibition of [^{14}C]phenylalanine incorporation by ribosomes derived from *S. faecalis* E1. With ribosomes from strain 4927, there was negligible inhibition by streptomycin at concentrations up to 100 μg/ml. This concentration of streptomycin also did not inhibit polypeptide synthesis by ribosomes of strain 1379 and resulted in only a 34% decrease in [^{14}C]phenylalanine incorporation with ribosomes of strain 7621. In contrast, gentamicin at concentrations as low as 1.0 μg/ml inhibited polypeptide synthesis by at least 70% from levels obtained in the absence of antibiotic. Although ribosomal resistance was examined in only three strains, that the other enzyme-negative strains are likely to be ribosomally resistant also was suggested by the fact that these strains were resistant to 128,000 μg of streptomycin per ml, whereas MICs for enzyme-producing strains were considerably lower. This observation is consistent with the hypothesis that enzyme inactivation can be overcome at high streptomycin concentrations, whereas ribosomal mechanisms confer "absolute" resistance to the drug. Growth on media containing streptomycin at concentrations of 64,000 to 128,000 μg/ml may be useful in screening for enterococcal isolates which possess high-level streptomycin (but not kanamycin) resistance by ribosomal mechanisms.

Ribosomal resistance to streptomycin in laboratory mutants has been extensively studied (6), and it is widely assumed that alteration of ribosomal target sites is a potential mechanism of streptomycin resistance among clinical strains. Nevertheless, ribosomal resistance to streptomycin has been described in only a small number of clinical isolates of *Staphylococcus aureus* (8), *Neisseria gonorrhoeae* (9),

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TABLE 1. Streptomycin adenylyltransferase activity in and MICs for *S. faecalis* isolates with high-level resistance to streptomycin

Strain	ANT[a]	MIC (μg/ml)
END 6	+	16,000
END 11	+	8,000
END 27	+	4,000
U 1	+	8,000
W 58	+	4,000
EBC 28	+	4,000
1310	0	128,000
1379[b]	0	128,000
4927[b]	0	128,000
7621[b]	0	128,000
U 22	0	128,000
END 16	0	128,000

[a] ANT, Streptomycin adenylyltransferase.
[b] Strains tested for ribosomal resistance to streptomycin.

Pseudomonas aeruginosa (14), and, recently, viridans streptococci (5). Our results confirm the occurrence of ribosomal resistance to streptomycin in clinical enterococcal isolates and suggest that this resistance mechanism is not rare.

Data collected in the United States (2, 10, 11), Chile, and Thailand (12) reveal that ca. 10% of enterococcal isolates are highly resistant to streptomycin but not kanamycin. Our results suggest that perhaps one-half of these or as many as 5% of clinical isolates may be ribosomally resistant to streptomycin. This figure may underestimate the true prevalence of ribosomal resistance, since this potential resistance mechanism was not investigated in enzyme-producing strains because of previous observations in our laboratory that aminoglycoside modification occurring under conditions of our protein synthesis assay may result in false-positive determination of ribosomal resistance. We conclude that ribosomal resistance to streptomycin is a significant cause of high-level resistance to this antibiotic among clinical enterococcal isolates.

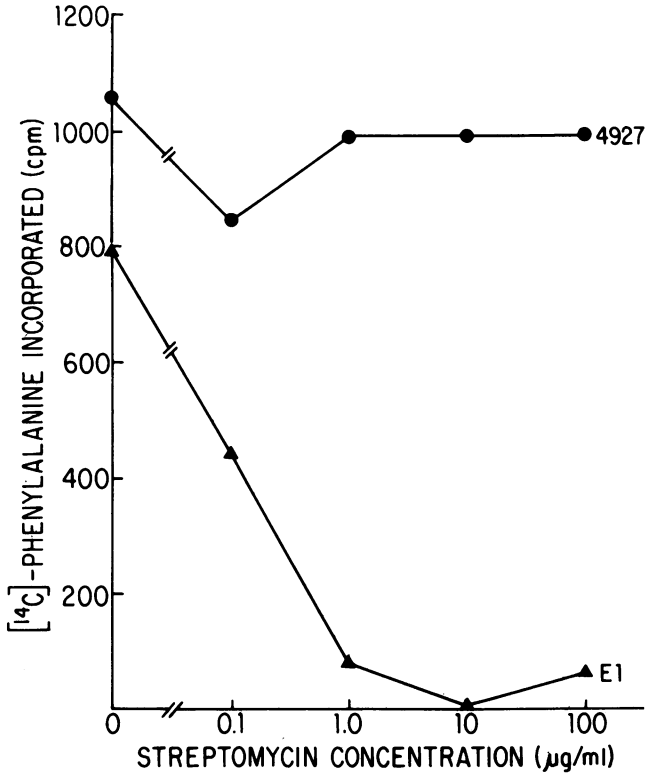

FIG. 1. Effect of streptomycin on the incorporation of [^{14}C]phenylalanine into trichloroacetic acid-precipitable polypeptide by cell extracts of *S. faecalis* strains 4927 and E1.

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