Lack of Homology of Enterococci Which Have High-Level Resistance to Trimethoprim with the *dfrA* Gene of *Staphylococcus aureus*

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Multiresistant enterococci were tested for susceptibility to trimethoprim (TMP). Although most enterococci are inhibited by ≤ 1.0 $\mu\text{g/ml}$, the MICs for 7 of 29 selected multiresistant isolates were ≥ 8 $\mu\text{g/ml}$, including for two β -lactamase positive (Bla^+) strains, for which the MICs of TMP were $> 1,000$ $\mu\text{g/ml}$, and for another Bla^+ strain, for which the MIC was 128 $\mu\text{g/ml}$. None of five isolates tested transferred TMP resistance and none of the resistant isolates hybridized to the *dfrA* gene of *Staphylococcus aureus*. Whether TMP resistance in enterococci is due to a mutation(s) or to acquisition of a new gene is not known. Acquisition of resistance to TMP is another example of the multiple antimicrobial resistance typically displayed by enterococci.

Members of the genus *Enterococcus* are the third most common isolates found in nosocomial infections (11). In recent years, these organisms have shown increasing resistance to antimicrobial agents, including high-level resistance to aminoglycosides, vancomycin, and penicillin (11). Since enterococci as a group also have inherent or acquired resistances to most other available antimicrobial agents, there has been renewed interest in compounds that are active against these organisms. Trimethoprim (TMP) and trimethoprim-sulfamethoxazole (TMP-SMX) have been suggested as possible antienterococcal agents (3, 8, 9). However, there is controversy regarding clinical use of TMP and TMP-SMX because of (i) reports of apparent clinical failures, (ii) the ability of enterococci to use preformed folates, resulting in the diminution of in vitro activity by concentrations of folates found in the body, (iii) elimination of an in vitro bactericidal effect by prolonged incubation and large inocula, and (iv) the presence of high-level resistance (5, 7, 9, 17, 24). In this study, we investigated the in vitro activity of TMP against multiresistant isolates of *Enterococcus faecalis* from five countries and tested the TMP-resistant strains for homology with the *dfrA* trimethoprim resistance gene found in staphylococci.

Bacterial strains and matings. Twenty-nine clinical isolates of *E. faecalis* that were obtained from different geographic locations and that have been previously described were used in this study (10, 13, 15, 16, 19, 20); 15 were Bla^+ , of which 14 were highly gentamicin resistant, and the others were highly resistant to gentamicin but did not produce β -lactamase (10, 13, 15). OG1, a standard enterococcal recipient cell, was used in conjugation as previously described (12).

Susceptibility testing. Because of the known difficulties encountered in testing organisms for susceptibility to trimethoprim, different methods were used to test the multiresistant enterococci. Disk diffusion susceptibility was determined by following the recommendations of the National Committee for Clinical Laboratory Standards (18) and by using TMP disks (5 μg) or combination TMP-SMX disks (1.25 and 23.75 μg , respectively) supplied by BBL, Cock-

eysville, Md. Agar dilution MICs were determined by using Mueller-Hinton agar with a final inoculum of 10^4 CFU per spot. Broth dilution susceptibility was determined in Iso-Sensitest broth (Oxoid Ltd., Basingstoke, England) with 0.05 U of thymine phosphorylase (Sigma, St. Louis, Mo.) per ml with an inoculum of 10^3 CFU/ml (6).

Hybridization. Colony lysates were prepared by inoculating bacteria from an overnight culture with a Steers replicator onto NitroPlus 2000 filters (Microns Separation, Inc., Westboro, Mass.) overlaid on brain heart infusion agar and incubated at 37°C overnight. Lysis was achieved by placing this filter (colony side up) on a Whatman quality 1 filter saturated with 10 mg of lysozyme per ml for 30 min at 37°C followed by 30 min on a filter saturated with 5 mg of lysostaphin per ml and by 10 min at room temperature on a filter saturated with 0.5 N NaOH. The filters were then placed on a Buchner funnel, vacuum dried, and washed three times, each time with a solution containing 30 ml of 1 M Tris (pH 7.6), 30 ml of 0.5 M Tris (pH 7.6)–1.5 M NaCl, 30 ml of CHCl_3 , and 30 ml of 95% ethanol. The filters were then air-dried and baked at 80°C for 1.5 to 2 h. Hybridization was performed under stringent conditions as previously described (14). A gene probe for the TMP resistance gene originally found in *Staphylococcus aureus* G1 was supplied on the recombinant plasmid pGO18 by Gordon Archer; the probe consists of an approximately 500-bp *HindIII*-*EcoRI* intragenic fragment of *dfrA* (1, 4). *S. aureus* G5 (1) and the recombinant plasmid pGO18 were included as controls. Because enterococci and staphylococci do not lyse as reliably as *Escherichia coli*, a staphylococcal β -lactamase (*Bla*) gene probe known to detect both staphylococcal and enterococcal β -lactamase (14) was used to verify that adequate lysis and preparation of the DNA was achieved. Bla^+ staphylococcal isolates 3711 and 3714 (23) and *E. faecalis* HH22 were used as Bla^+ controls.

The initial 15 isolates screened were those known to produce β -lactamase. Subsequent to the initiation of this study, the Argentinian isolates were shown by pulsed-field gel electrophoresis to represent minor variants of a single strain (15 and unpublished data). Similarly, the isolates from Delaware, Philadelphia, Pa., Houston, Tex., Pittsburgh, Pa., and Virginia also appear to represent a single clonal group

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TABLE 1. Susceptibility of β -lactamase-producing, highly gentamicin resistant enterococci to TMP

Strain(s)	Zone of inhibition (mm) ^a	MIC (μ g/ml) of TMP by:		Geographic origin of enterococcal isolates (reference)
		MH agar ^b	Iso-Sensitest	
BE1 and DEL	None	>1,000	>1,024	Beirut and Delaware (15)
WH245	None	128	64	West Haven, Conn. (19, 20)
WH571 and WH257	None	64	16	West Haven, Conn. (19, 20)
HH22	13	4	2	Houston, Tex. (13)
E47 and E48 ^c	11	8	2	Virginia (15)
CH570	16	8	NT ^d	Pittsburgh, Pa. (20)
HG4354, HG6049, HG6280, and HG9829; CH25; PA	≥ 24	≤ 0.5	NT	Argentina (10); Boston, Mass. (21); Philadelphia, Pa. (13)

^a On 5- μ g TMP disks.^b MH agar, Mueller-Hinton agar.^c Previously referred to as Virginia-1 and -2 (15).^d NT, not tested.

isolated over a 7-year period (15); these isolates differ in their susceptibilities to other antibiotics as well as to TMP. The isolates fell into three groups by TMP disk diffusion testing. Five isolates (one from Beirut, one from Delaware, and three from West Haven, Conn.) had no zone of inhibition with either TMP or TMP-SMX disks. Six isolates (four from Argentina, one from Philadelphia, Pa., and one from Boston, Mass.) had zone diameters around the TMP disks that were ≥ 24 mm and around TMP-SMX disks that were ≥ 32 mm. The remaining four isolates (two from Virginia and one each from Houston, Tex., and Pittsburgh, Pa.) had zones of inhibition of 11 to 16 mm with TMP disks and of 22 to 33 mm around TMP-SMX disks. The results of agar dilution susceptibility testing with TMP were in agreement with those of the disk diffusion tests (Table 1). Among the isolates with no zone of inhibition, the agar dilution MICs of TMP for two isolates (from Delaware and Beirut) were $>2,000$ μ g/ml, while the MICs for the three isolates from West Haven, Conn., were 64 to 128 μ g/ml. The MICs of TMP for isolates with the moderately susceptible zones (11 to 16 mm) were 4 (from Houston) or 8 (from Virginia and Pittsburgh) μ g/ml. The MICs for the other β -lactamase-producing isolates, which were determined to be susceptible to TMP by disk diffusion, were ≤ 0.5 μ g/ml. Eight of these isolates were also tested in Iso-Sensitest broth with thymidine phosphorylase and an inoculum of 10^3 CFU/ml. The MICs for the highly resistant strains (from Beirut and Delaware) were $>1,024$ μ g/ml. The MICs for other isolates tested in this system were 1 to 2 dilutions below those observed with agar dilution (Table 1); MICs for strains susceptible by other criteria were ≤ 0.125 μ g/ml.

Fourteen other highly gentamicin resistant isolates of *E. faecalis* from Chile and Bangkok, Thailand (16), isolated from 1979 to 1981 were also tested for resistance to TMP by agar dilution. The MIC for one isolate was 32 μ g/ml, while the MICs for the other isolates were 2 μ g/ml (one isolate), 1 μ g/ml (five isolates), 0.5 μ g/ml (two isolates), and ≤ 0.25 μ g/ml (five isolates).

The five isolates for which the Mueller-Hinton agar dilution MICs were ≥ 164 μ g/ml were tested for their ability to transfer TMP resistance to OG1. No transfer of resistance was achieved by using broth, filter, or cross-streak matings and 8 μ g of TMP per ml for selection. In addition, none of the enterococci hybridized with the staphylococcal TMP resistance gene probe; this probe hybridized to *S. aureus* G5 and to an *E. coli* strain containing pG018. The *Bla* probe hybridized to all the *Bla*⁺ enterococci, to G5 and to the two

other *Bla*⁺ staphylococci tested but did not hybridize to any of the *Bla*⁻ isolates.

High-level resistance to TMP among enterococci has been previously reported, including some isolates with transferrable resistance (9). Staphylococci are also known to have TMP resistance genes (4), and since enterococci and staphylococci share a large number of other resistance genes (11), it is not surprising that this trait appeared as well in members of the genus *Enterococcus*. However, the resistance seen in enterococci does not appear to be due to the presence of the dihydrofolate reductase encoded on the staphylococcal plasmids pG01 and pG05; this gene, *dhfrA*, also appears to be the one present in pSK1 from Australia (22). Whether TMP resistance in enterococci is due to the presence of another of the staphylococcal resistance genes, to another acquired gene, or to a mutation(s) remains to be seen. The finding of high-level TMP resistance, together with recent animal model studies showing failure of TMP-SMX to adequately treat endocarditis and pyelonephritis even with susceptible strains (2, 6), raises further concerns about the likelihood of efficacy of TMP or TMP-SMX for treatment of severe enterococcal infections caused by multiresistant strains.

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