

## Efficacy of Ampicillin versus Trimethoprim-Sulfamethoxazole in a Mouse Model of Lethal Enterococcal Peritonitis

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**Lethal enterococcal peritonitis in mice was used to compare trimethoprim-sulfamethoxazole (TMP-SMX) therapy with ampicillin therapy. Peritoneal fluid showed a  $10^3$ -CFU decrease in enterococci with ampicillin compared with TMP-SMX. Mortality of the untreated mice was 100%, compared with 40% for ampicillin and 95% for TMP-SMX, despite adequately measured levels in serum and peritoneal fluid.**

Enterococci are becoming increasingly important nosocomial pathogens (5). The increasing prevalence of highly aminoglycoside-resistant enterococci (14), as well as the emergence of  $\beta$ -lactamase-producing (8) and vancomycin-resistant (7) strains, has presented a need to evaluate alternate therapies. The role of trimethoprim (TMP)-sulfamethoxazole (SMX) in treating enterococcal infections remains controversial. Early studies showed in vitro susceptibility of enterococci to TMP-SMX (2), but other studies have emphasized the unreliability of in vitro susceptibility testing because of the ability of enterococci to utilize exogenous folates (15). There are reports of treatment failures with TMP-SMX used in enterococcal infections (4), but other in vivo data on the efficacy of TMP-SMX in serious enterococcal infections are lacking. We used a model of lethal enterococcal peritonitis in mice modified from previous studies (6) to determine the effectiveness of monotherapy of TMP-SMX compared with ampicillin.

*Enterococcus faecalis* JH2-2 carrying plasmid pAD<sub>1</sub>, conferring hemolysin activity, was grown overnight in brain heart infusion broth (Difco Laboratories, Detroit, Mich.), centrifuged, and suspended in sterile saline. Female 4- to 6-week-old CD-1 mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were injected intraperitoneally (i.p.) with 1 ml of the sterile saline containing a final concentration of  $10^9$  organisms per ml with 50% sterile rat fecal extract. Rat fecal extracts were prepared as previously described (3). One hour prior to inoculation, mice ( $n = 20$ ) were injected intramuscularly (i.m.) with ampicillin (150 mg/kg of body weight; Bristol Laboratories, Evansville, Ind.) or TMP-SMX (15 mg of TMP per kg; Elkins-Sinn, Cherry Hill, N.J.). The ratio of TMP-SMX in this solution was 1:5. Control mice ( $n = 10$ ) received i.p. enterococci with 50% sterile fecal extracts and no antibiotics or sterile 50% rat fecal extracts alone ( $n = 5$ ). Antibiotics were continued every 6 h in survivors, and mortality was assessed hourly.

Additional mice were injected with enterococci and antibiotics as described above, and two or three mice were sacrificed at 1, 3, and 6 h for determination of peritoneal antibiotic levels and quantitative culture of peritoneal fluid. Samples (0.1 ml each) of peritoneal fluid were serially diluted 100-fold in sterile normal saline. Samples of 0.1 ml were plated on brain heart infusion agar, and colonies were reported as CFU per milliliter. Antibiotics were also injected

i.m. into three or four healthy mice, and serum was obtained by cardiac or venocaval puncture at 1 and 6 h for antibiotic levels. Levels of ampicillin in serum and peritoneal fluid were measured by agar diffusion assay with *Bacillus subtilis*, as previously described (11). TMP levels were determined by spectrofluorimetry (12), and SMX levels were measured by using high-performance liquid chromatography (13). Antimicrobial susceptibility of the enterococcus to ampicillin and TMP-SMX was tested by a standardized broth dilution microtiter technique as previously described (10). MIC and MBC were also tested in standard medium supplemented with 50% sterile mouse serum. Results were analyzed by using  $\chi^2$  analysis or Student's *t* test when appropriate.

Mortality rates for mice treated with ampicillin or TMP-SMX versus those for controls are shown in Fig. 1. The mortality rate increased sharply in both the TMP-SMX and control groups beginning at 3 h after infection with enterococci, reaching 100% mortality in the control group. There was no significant difference between the TMP-SMX-treated mice and controls at any time. In contrast, deaths in the ampicillin group were delayed until 7 h, and total mortality reached 40% at 24 h ( $P < 0.005$  after 7 h by  $\chi^2$  analysis). Mice given only 50% sterile rat fecal extracts i.p. had no deaths. Quantitative peritoneal cultures showed a  $10^3$ -CFU difference in viable bacterial counts between the TMP-SMX- and ampicillin-treated groups by 6 h after injection of antibiotics (Fig. 2). Enterococcal counts increased in TMP-SMX-treated animals to a level of  $9.7 \times 10^9$  CFU/ml, while ampicillin-treated animals had a decline to  $7.4 \times 10^6$  CFU/ml ( $P < 0.0005$  by Student's *t* test).

Peak levels of antibiotics in serum after injection of ampicillin or TMP-SMX i.m. into healthy mice are summarized in Table 1. Trough levels in serum for ampicillin at 6 h showed no detectable bioactivity, while TMP remained measurable at 2.9  $\mu$ g/ml (standard error  $\pm 0.35$ ). The peritoneal concentrations of antibiotics after i.m. injection of antibiotics followed by i.p. injection of enterococci at 1 h are also shown. Ampicillin levels were 5.6  $\mu$ g/ml ( $\pm 0.10$   $\mu$ g/ml standard error) while TMP levels were 1.7  $\mu$ g/ml ( $\pm 0.19$   $\mu$ g/ml standard error) in peritoneal fluid 1 h after antibiotic injection. The MICs for the enterococcus were 0.4 and 0.3  $\mu$ g/ml for ampicillin and TMP-SMX, respectively. The MBC of both drugs was 1.6  $\mu$ g/ml. When 50% mouse serum was added to test wells, the enterococcus was no longer inhibited at  $>100$   $\mu$ g of TMP-SMX per ml, while the ampicillin MIC and MBC were unchanged.

The efficacy of TMP-SMX for the treatment of serious

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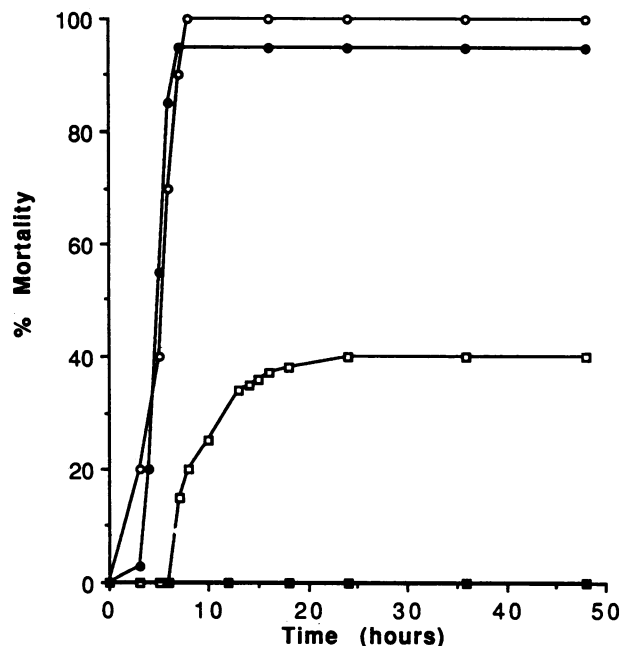


FIG. 1. Percent mortality over time of mice ( $n = 20$ ) given an inoculum of  $10^9$  enterococci mixed with 50% sterile rat fecal extracts. Test mice were treated 1 h prior to inoculation with ampicillin ( $\square$ ), TMP-SMX ( $\bullet$ ), or no antibiotics ( $\circ$ ). Five control mice ( $\blacksquare$ ) received only i.p. sterile rat fecal extract with no antibiotics.

enterococcal infections has remained controversial. When tested in thymidine- or thymine-free medium, enterococci can be readily inhibited by low concentrations of TMP-SMX (4, 15). However, studies have shown that enterococci

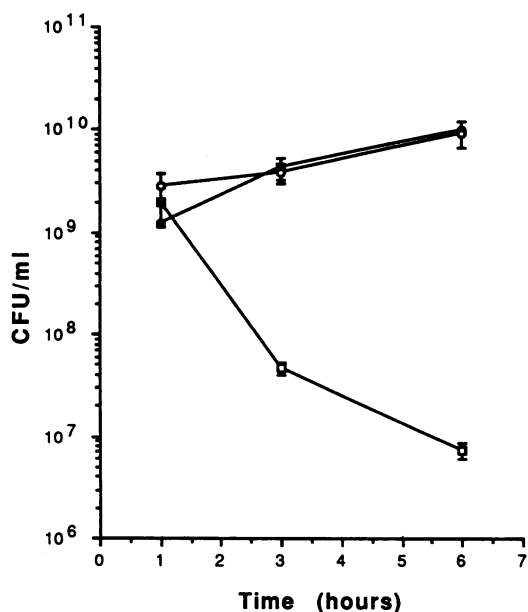


FIG. 2. Quantitative peritoneal cultures performed on mice ( $n = 2$  or 3) treated with ampicillin ( $\square$ ), TMP-SMX ( $\bullet$ ), or no antibiotics ( $\circ$ ) at various times after antibiotic injection. An i.p. inoculum of  $10^9$  enterococci with 50% sterile rat fecal extracts was given 1 h after antibiotic injection. Error bars represent standard errors of the mean.

TABLE 1. Levels of antibiotics in serum and peritoneal fluid after i.m. injection

Sample and antimicrobial agent	Dose (mg/kg)	Mean level of drug ( $\mu\text{g/ml} \pm \text{SE}$ )		
		1 h	3 h	6 h
<b>Serum<sup>a</sup></b>				
Ampicillin	150	$9.7 \pm 1.0$	NT <sup>b</sup>	0
TMP	15	$5.5 \pm 0.22$	NT	$2.9 \pm 0.35$
SMX		$94.8 \pm 8.7$	NT	$14.4 \pm 1.5$
<b>Peritoneal<sup>c</sup></b>				
Ampicillin	150	$5.6 \pm 0.10$	$3.0 \pm 0.25$	$1.9 \pm 0.26$
TMP	15	$1.7 \pm 0.19$	$0.6 \pm 0.14$	NT
SMX		$47.9^d$	$38.2 \pm 2.4$	$26.4 \pm 3.4$

<sup>a</sup> Determinations of levels in serum were performed on three or four healthy mice.

<sup>b</sup> NT, Not tested.

<sup>c</sup> Determinations of levels in peritoneal fluid were performed on two or three test mice.

<sup>d</sup> Represents level in a single mouse.

become more resistant to TMP by incorporating exogenous folates in the test medium (1, 15). Zervos and Schaberg found the mean MIC of TMP-SMX for 21 clinical isolates of *E. faecalis* to be  $0.13 \mu\text{g/ml}$ ; however, that rose to  $3.3 \mu\text{g/ml}$  when medium was supplemented with  $1 \mu\text{g}$  of folic acid per ml (15). Further studies reveal that in vitro bactericidal assays are also method dependent, with results varying with the inoculum and length of incubation after subculturing (9). Although there have been reports documenting treatment failure for two patients with uncomplicated urinary tract infections who developed bacteremia on TMP-SMX therapy (4), many factors could contribute to treatment failure.

Our study again confirms that by using standard microtiter dilution assays, the MIC of TMP-SMX for our enterococcus was low ( $\text{MIC} = 0.3 \mu\text{g/ml}$ ). Despite assuring appropriate levels of TMP and SMX in serum at the time of injection of enterococci i.p., TMP-SMX offered no therapeutic advantage compared with no antibiotics. By 8 h after injection of enterococci, 100% of control mice and 95% of TMP-SMX-treated mice had died. The single survivor in the TMP-SMX-treated group showed no evidence of illness, suggesting that the inoculum was injected intraluminally in the gastrointestinal tract. However, ampicillin alone protected mice with a decreased mortality of 40% at 24 h. Although peritoneal levels of TMP were low compared with those of ampicillin, the levels obtained at 1 h, at the time of injection of enterococci, still exceeded the MIC for the enterococcus strain by threefold.

Our findings add in vivo evidence to support the fact that in vitro testing of the susceptibility of enterococci to TMP-SMX is misleading and does not reliably predict in vivo susceptibility. On the basis of these and previous studies, interpretation of in vitro susceptibility tests should be done with skepticism and caution should be used in treating enterococcal infection with TMP-SMX. On the basis of this animal model, we do not recommend the use of TMP-SMX in the treatment of serious enterococcal infection.

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