ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1977, p. 566-568

Vol. 11, No. 3
Printed in U.S.A.

Antimicrobial Susceptibilities of *Yersinia enterocolitica* Biotype 4, Serotype O:3

S. HAMMERBERG, S. SORGER, AND M. I. MARKS[1] *

McGill University-Montreal Children's Hospital Research Institute and Departments of Pediatrics (Infectious Disease) and Microbiology, Montreal, Quebec H3H 1P3 Canada

Received for publication 29 October 1976

Agar dilution antimicrobial susceptibility tests were carried out against recent clinical isolates of *Yersinia enterocolitica* biotype 4, serotype O:3. Aminoglycosides and co-trimoxazole were the most active drugs. All isolates were resistant to ampicillin, carbenicillin, cloxacillin, and erythromycin.

Yersinia enterocolitica is being isolated with increasing frequency from children with gastroenteritis. At the present time there are few data pertaining to antimicrobial chemotherapy and the quantitative in vitro susceptibilities of this organism to antimicrobial agents (2, 3, 5). The present study was undertaken to expand upon the few existing reports of other groups (2, 3, 5). The comparative in vitro activity of 16 single antimicrobials and 1 drug combination of these were studied against recent clinical isolates from ill children; the effects of different media and inocula on the agar dilution minimum inhibitory concentrations (MIC) for this organism were also examined briefly.

Twenty-three isolates of *Y. enterocolitica* were obtained from 22 ill children at the Montreal Children's Hospital from 1974 to 1976. These strains were all cultured from stool specimens with the exception of a swab from an appendix. Identification was carried out using the differential characteristics defined by Sonnenwirth (6). Biological and serological typing were performed by S. Toma (Public Health Laboratories, Toronto). All isolates were biotype 4, serotype O:3, the predominant serotype associated with clinical infection in humans in Canada (8).

The agar dilution replicator technique of Steers et al. (7) was used for MIC determinations. Mueller-Hinton agar (MH; Difco) adjusted to pH 7.4 was used for all drugs, and additional sets of sulfamethoxazole (SMZ)-trimethoprim (TMP) (alone and in combination) plates were prepared with Diagnostic Sensitivity Test (DST) agar (Oxoid DST broth with 12 g of agar [Difco] per liter) for comparative purposes (4). A stock solution of each drug was prepared and serial twofold dilutions in distilled water (0.5 ml) were added to 9.5 ml of agar for each dilution plate. The effects of two inocula, an undiluted overnight growth in MH broth (approximately 10^8 colony-forming units [CFU]/ml by colony count) and a 10^{-3} dilution of this suspension, were compared. After inoculation, plates were incubated for 16 to 18 h at 37°C and the results were recorded. The MIC of all drugs except SMZ alone was defined as the lowest drug concentration at which no growth was visible on the surface of the agar. The MIC of SMZ was defined as the lowest concentration at which there was an 80% or sudden reduction in growth.

Disk diffusion susceptibility tests (1) were carried out with the following antibiotics (antibiotic concentration of disk in parentheses): ampicillin (10 μg), kanamycin (30 μg), gentamicin (10 μg), cephalothin (30 μg), SMZ/TMP (23.75/1.25 μg), sisomicin (10 μg), amikacin (10 μg), and netilmicin (10 μg).

The agar dilution test results are illustrated in Table 1 and Fig. 1. When one considers the higher achievable serum concentrations of kanamycin and amikacin, all strains had similar susceptibilities to the six aminoglycosides tested: gentamicin, kanamycin, netilmicin, sisomicin, amikacin, and tobramycin. Chloramphenicol was less active than the aminoglycosides and similar to tetracycline and rifampin.

All isolates were resistant to ampicillin, carbenicillin, cloxacillin, and erythromycin. Susceptibility to cephalothin ranged from moderate resistance (MIC, 25 μg/ml) to complete resistance (MIC >100 μg/ml).

The above results were similar with both inocula tested (10^8 and 10^5 CFU/ml); however, the lighter inoculum produced more clear-cut end points than the undiluted, overnight growth.

TMP and SMZ were examined alone and in combination on both MH and DST agar with undiluted and diluted inocula. The clearest re-

[1] Address reprint requests to: Montreal Children's Hospital, Montreal, Quebec, H3H 1P3, Canada.

sults were obtained with the 10^5-CFU/ml inoculum and are illustrated in Fig. 1. The inoculum effect for SMZ MICs performed on both DST and MH agar was extreme. All isolates had an MIC of >400 μg of SMZ per ml when the inoculum was from an undiluted overnight growth but 3.12 to 6.25 μg/ml with the 10^5 inoculum. MH agar proved to be superior to DST agar for TMP susceptibilities as clearly defined end points were obtained only on this medium and only with the 10^5-CFU/ml inoculum. The inoculum effect was sufficient to alter the susceptibility pattern from susceptible to resistant as in the case of SMZ. When SMZ and TMP were combined in a 20:1 ratio, marked synergism resulted, reducing the MIC of the former at least fourfold and the latter at least eightfold. All isolates were susceptible to a ratio of 1.56 μg of SMZ per ml and 0.078 μg of TMP per ml.

TABLE 1. *Susceptibilities of 23 isolates of Y. enterocolitica to 14 antibiotics (agar dilution, inoculum-replicating method, 10^5-CFU/ml inoculum)*

Antimicrobial	Range of MIC (μg/ml)
Gentamicin	0.78–1.56
Netilmicin	0.78–1.56
Sisomicin	0.78–1.56
Tobramycin	0.78–3.12
Amikacin	1.56–6.25
Kanamycin	3.12–6.25
Tetracycline	6.25
Chloramphenicol	6.25
Rifampin	6.25
Erythromycin	25
Cloxacillin	50–100
Ampicillin	25–100
Cephalothin	25–>100
Carbenicillin	200

Disk susceptibility tests carried out on 8 of 17 drugs gave results corresponding to those agar dilution MICs where an inoculum of 10^5 CFU/ml was used.

The results of this study demonstrate that aminoglycosides and co-trimoxazole are the most active antimicrobials in vitro against. *Y. enterocolitica*. Although the isolates tested in this study were slightly more resistant to TMP alone and more susceptible to SMZ than reported by Gutman et al., the SMZ-TMP combination results were similar (2). Our results with cloxacillin, tetracycline, kanamycin, gentamicin and ampicillin correlated well with those reported by Hausnerová et al. (3) who also studied *Y. enterocolitica* biotype 4 strains. In contrast to their report where 12% were susceptible to cephalothin (MIC, <10 μg/ml), none of our strains were susceptible to this drug; our strains were also more resistant to carbenicillin. This inconsistency could be attributed to the fact that their strains were isolated from 1967 to 1972 when these two β-lactam drugs were relatively new. The isolates from our hospital appear to be extremely homogeneous in their antibiotic susceptibility pattern as well as biotype and serotype. No other biotype or serotype has been isolated in this hospital up to the present time.

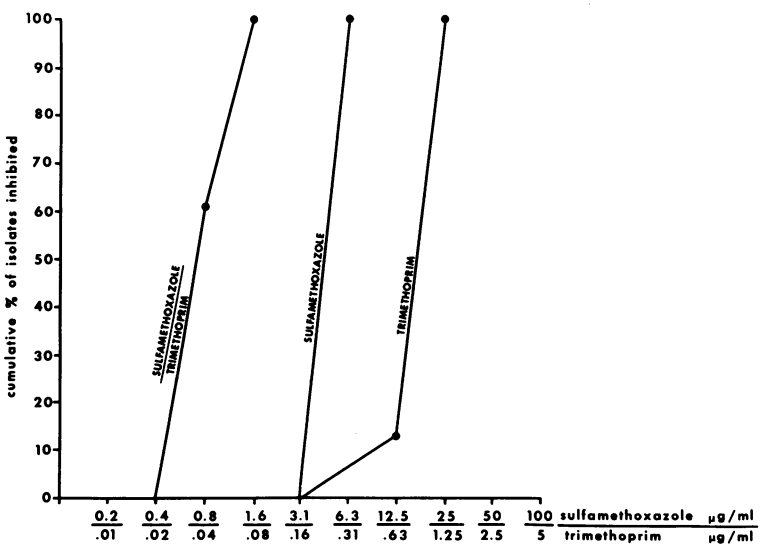

FIG. 1. *Susceptibilities of 23 clinical isolates of Y. enterocolitica to SMZ and TMP, alone and in combination. (Method as in Table 1).*

We recommend the use of MH agar and a 10^5-CFU/ml inoculum (a 10^{-3} dilution of an overnight growth in MH broth) for agar dilution MICs with the drugs tested; a Steers replicator delivers approximately 0.002 ml, thus the inoculum deposited on the agar surface is 2×10^2 CFU. A standardized disk diffusion test correlates well with the quantitative test. These in vitro results suggest co-trimoxazole or aminoglycoside antibiotics may have clinical usefulness in the therapy of *Y. enterocolitica* infections.

LITERATURE CITED

1. **Barry, A. L., F. Garcia, and L. D. Thrupp.** 1970. An improved single-disk method for testing the antibiotic susceptibility of rapidly-growing pathogens. Am. J. Clin. Pathol. 53:149–158.
2. **Gutman, L. T., C. M. Wilfert, and T. Quan.** 1973. Susceptibility of *Yersinia* enterocolitica to trimethoprim-sulfamethoxazole. J. Infect. Dis. 128(Suppl): 538.
3. **Hausnerová, S., O. Hausner, and V. Paučkova.** 1973. Antibiotic sensitivity of *Yersinia enterocolitica* strains isolated in two regions of Czechoslovakia, p. 76–80. *In* Contributions to microbiology and immunology, vol. 2. Yersinia, Pasteurella and Francisella. Karger, Basel.
4. **Marks, M. I., and G. Weinmaster.** 1975. Influence of media and inocula on the in vitro susceptibility of *Hemophilus influenzae* to co-trimoxazole, ampicillin, penicillin and chloramphenicol. Antimicrob. Agents Chemother. 8:657–663.
5. **Nilehn, B.** 1967. Studies on *Yersinia enterocolitica*. Acta Pathol. Microbiol. Scand. 69:83–91.
6. **Sonnenwirth, A. C.** 1974. *Yersinia*, p. 222–229. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
7. **Steers, E., E. L. Foltz, and B. S. Graves.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. 9:307–311.
8. **Toma, S., and L. Lafleur.** 1974. Survey on the incidence of *Yersinia enterocolitica* infection in Canada. Appl. Microbiol. 28:469–473.