ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1979, p. 392-395
0066-4804/79/03-0392/04$02.00/0

Vol. 15, No. 3

In Vitro Activity of 39 Antimicrobial Agents Against *Treponema hyodysenteriae*

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Received for publication 27 December 1978

The in vitro activities of 39 antimicrobial agents against 23 isolates of *Treponema hyodysenteriae*, the majority of which were field isolates, were determined by the agar dilution technique. Quinoxalines, pleuromutilin, nitroimidazoles, and nitrofuran were the most active. Their activities ranged from ≤0.10 to 1.56 μg/ml. Lincomycin, penicillins, chloramphenicol, tetracyclines, cephalosporins, three peptides (virginiamycin, thiopeptin, and bacitracin), and one aminoglycoside (gentamicin) exhibited intermediate levels of activity ranging from 0.39 to 50 μg/ml. Four peptides (enduracidin, viomycin, bicyclomycin, and colistin), three aminoglycosides (kanamycin, streptomycin, and neomycin), polyene, and other agents, including novobiocin, vancomycin, rifampin, nalidixic acid, and *p*-arsanilic acid, displayed limited activities ranging from 12.5 to ≥100 μg/ml. Macrolides showed varying degrees of activity depending upon isolates.

Although a large spirochete, *Treponema hyodysenteriae*, was identified as the primary causative agent of swine dysentery (1, 8, 11, 27), the etiology of the disease complex is not fully understood. It has been known that a typical syndrome accompanied by severe bloody diarrhea, sometimes with death, can readily be induced by oral administration of infected colon and its contents. Therefore, drugs for prevention and treatment of the dysentery are commonly evaluated in vivo in pigs naturally infected with swine dysentery or in swine given contents of colon from pigs suffering from dysentery (2-4, 6, 9, 19-21; I. H. Sutherland, P. W. M. Van Adrichem, and W. H. D. Leaning, Proc. Int. Congr. Int. Pig Vet. Soc. 3rd, Lyons, France, Abstr. D26, p. 1-3, 1974). Since in vitro cultivation of this anaerobic spirochete became successful (1, 11, 16, 27), a limited number of compounds have been evaluated in vitro on solid blood agar medium (4, 20, 28; Sutherland et al., Proc. Int. Congr. Int. Pig Vet. Soc. 3rd, Abstr. D26, p. 1-3, 1974) and recently in a liquid medium (18). These studies indicated that compounds proved to be effective in vivo (3, 4, 6, 20, 21; Sutherland et al., Proc. Int. Congr. Int. Pig Vet. Soc. 3rd, Abstr. D26, p. 1-3, 1974) were also active against the spirochete in vitro (4, 18, 20, 28; Sutherland et al., Proc. Int. Congr. Int. Pig Vet. Soc. 3rd, Abstr. D26, p. 1-3, 1974). It seems, therefore, that susceptibility of the species in vitro is correlated to the effectiveness of antimicrobial agents in vivo.

The purpose of the present study was to assay the in vitro effects of drugs currently in medicinal and veterinary use, to determine the activities of newer agents, and to compare the results with those of earlier workers.

MATERIALS AND METHODS

Bacterial isolates. A total of 23 isolates of *T. hyodysenteriae* were tested. One isolate, S73/2, was kindly supplied by D. J. Taylor, University of Glasgow, Glasgow, Scotland. The others were individually isolated from mucosal scrapings of colons or diarrheal feces with mucus and blood from pigs naturally infected with swine dysentery during sporadic outbreaks since 1976 at different localities in Japan. Prophylactic and therapeutic regimens for the dysentery of the pigs from which isolates were obtained are unknown.

Media. An anaerobic diluent (15) was used for diluting mucosal scrapings or feces and preparing inocula for drug susceptibility tests. As a selective medium, Trypticase soy agar (TSA) (Baltimore Biological Laboratory [BBL], Cockeysville, Md.) incorporated with 400 μg of spectinomycin (supplied by the Upjohn Japan Co., Tokyo, Japan) or 200 U of polymyxin B sulfate (supplied by Taito Pfizer Co., Tokyo, Japan) per ml and supplemented with 5% defibrinated sheep blood was used for isolation of *T. hyodysenteriae* from colon or feces. This was prepared according to methods described previously (15, 24). TSA medium without spectinomycin or polymyxin B sulfate was used for maintaining pure cultures of the organisms and for antibiotic susceptibility tests.

Antimicrobial agents. The antimicrobial agents examined in this study and their sources are listed in Table 1. Stock solutions containing 1,000 μg of each

antimicrobial agent per ml, excepting trichomycin where 10,000 U/ml and penicillin G and bacitracin where 1,000 U/ml were used, were prepared fresh or kept frozen at −20°C until use.

Anaerobic systems. For agar cultures, an anaerobic atmosphere of approximately 80% hydrogen and 20% carbon dioxide was generated by evacuation and refilling in vented GasPak jars (BBL) with cold palladium catalysts.

Isolation and identification of organisms. To isolate the organisms, mucosal scrapings or feces were diluted with the anaerobic diluent and streaked onto the TSA selective medium. Plates were incubated anaerobically at 37 or 42°C for 3 to 6 days. Organisms were transferred from the selective medium by removing a few small plugs of typical β-hemolytic areas with a loop 3 mm in diameter and by streaking this material across TSA medium. The isolated organisms were identified as *T. hyodysenteriae* after microscopic observations for morphology, cytochrome *c* oxidase, catalase and hemolytic tests, and production of acetic acid from glucose, as described previously (7, 10). Pure cultures of 23 isolates were maintained routinely by transferring them to non-antibiotic-containing TSA medium.

MIC determinations. Minimal inhibitory concentrations (MICs) for 39 antimicrobial agents were determined by agar dilution methods (5, 26). Plates were prepared with TSA medium containing serial twofold dilutions of each antimicrobial agent from 100 to 0.1 μg or U per ml. After excess surface moisture was evaporated, the plates were held anaerobically for 24 h before use.

To prepare inocula, five or six loopfuls of test isolates were taken from pure culture, transferred onto TSA medium, and incubated anaerobically at 37°C for 3 days. Colonies of each isolate were picked and suspended in the anaerobic diluent. The inoculum was adjusted to a no. 1 MacFarland turbidity standard. The final inocula ranged from 10^6 to 10^7 colony-forming units per ml. A 0.01-ml amount of the suspension was spot-inoculated on each plate containing an antimicrobial agent with a device similar to the Steers replicator (25). Plates were incubated anaerobically at 37°C for 3 days. Plates without antimicrobial agents were incubated as a control for viability of the test organisms. Growth of *T. hyodysenteriae* is characterized by β-hemolysis with a discrete and sharply defined edge which is readily apparent after 2 to 4 days of incubation (17). MICs were read as the lowest concentration of antimicrobial agent which completely prevented hemolysis. All MICs were read independently by two persons. When the two readings varied by more than two concentrations, the test was repeated. Each MIC presented is the median value of three experiments.

RESULTS

The ranges of MICs and the values that inhibited 90% or more of the isolates are summarized in Table 1. Of 39 antimicrobial agents tested, quinoxalines, plueuromutilin, nitroimidazoles, and nitrofuran were the most active compounds, inhibiting all 23 isolates at ≤1.56 μg/ml. Carbadox displayed the highest degree of activity. Antibiotics that exhibited intermediate levels of activity ranging from 0.39 to 50 μg/ml were lincomycin, penicillins, chloramphenicol, tetracyclines, cephalosporins, three peptides (virginiamycin, thiopeptin, and bacitracin), and one aminoglycoside (gentamicin). Lincomycin activity varied from 0.20 to 50 μg/ml. Only 8 isolates were susceptible to this antibiotic at ≤1.56 μg/ml. Four peptides (enduracidin, viomycin, bicyclomycin, and colistin), three aminoglycosides (kanamycin, streptomycin, and neomycin), polyene, and other agents, including novobiocin, vancomycin, rifampin, nalidixic acid, and *p*-arsanilic acid, showed limited activities. Their activities ranged from 12.5 to ≥100 μg/ml. Only 8 isolates were unsusceptible to macrolides. For susceptible isolates, the MICs for leucomycin, erythromycin, and tylosin were 6.25, 12.5, and 25 μg/ml, respectively. The remaining isolates were not susceptible. Only 5 isolates were susceptible to oleandomycin at 50 μg/ml. Spiramycin was inactive.

DISCUSSION

Carbadox inhibited all 23 isolates at ≤0.1 μg/ml. These findings are comparable to those of Williams and Babcock (28), who tested carbadox against 10 isolates in ox blood agar at concentrations as low as 0.005 μg/ml. The MIC of tiamutilin for one pathogenic strain of *T. hyodysenteriae* evaluated in a liquid medium was reported to be 0.025 μg/ml (18). Tiamutilin at 0.1 μg/ml, the lowest concentration tested in this study, inhibited 22 isolates. Activities of ipronidazole (20), ronidazole (Sutherland et al., Proc. Int. Congr. Int. Pig Vet. Soc. 3rd, Abstr. D26, p. 1–3, 1974), lincomycin (4, 18), and virginiamycin (28) have been reported, and the results are in agreement with the present findings. Macrolide antibiotics displayed different degrees of activity depending upon isolates in this study. One pathogenic strain tested by Laber (18) was resistant to macrolides. The majority of isolates tested by Williams and Babcock (28) were not susceptible to tylosin.

Carbadox (3), nithiamide (19), ronidazole (Sutherland et al., Proc. Int. Congr. Int. Pig Vet. Soc. 3rd, Abstr. D26, p. 1–3, 1974), dimetridazole (2), ipronidazole (20), lincomycin (4), virginiamycin (21), tylosin (6), and gentamicin (9) are effective in preventing or treating dysentery in pigs. These in vivo and in vitro studies indicate that there is a correlation between susceptibility in vitro and effectiveness in vivo. Williams and Babcock (28) observed that tylosin was effective in pigs infected with susceptible isolates but failed to control infection in pigs with resistant isolates.

TABLE 1. *Susceptibility of T. hyodysenteriae to antimicrobial agents*

Antimicrobial agent	MIC (μg/ml)	
	Range	For ≥90% of the isolates
Quinoxalines		
Carbadox[a]	≤0.10	≤0.10
Olaquindox[b]	0.20–0.39	0.39
Pleuromutilin		
Tiamutilin[c]	≤0.10–0.20	0.10
Nitroimidazoles		
Nithiamide[d]	≤0.10–0.39	0.20
Metronidazole[e]	≤0.10–0.39	0.39
Ronidazole[e]	≤0.10–0.78	0.39
Dimetridazole[e]	≤0.10–0.78	0.39
Nitrofuran		
Furazolidone[f]	≤0.10–1.56	0.78
Lincomycin		
Lincomycin[g]	0.20–50	25
Penicillins		
Penicillin G[h]	0.78–1.56[i]	0.78[i]
Ampicillin[j]	1.56–6.25	3.13
Chloramphenicol		
Chloramphenicol[c]	1.56–3.13	3.13
Tetracyclines		
Tetracycline[d]	0.39–50	25
Oxytetracycline[a]	0.78–25	12.5
Chlortetracycline[h]	6.25–>100	100
Cephalosporins		
Cephaloridine[h]	3.13–25	25
Cefazolin[j]	6.25–50	50
Peptides		
Virginiamycin[j]	0.78–6.25	3.13
Thiopeptin (22)[j]	6.25–25	12.5
Bacitracin[h]	12.5–50[i]	50[i]
Enduracidin (13)[k]	50–100	100
Viomycin[c]	100–>100	>100
Bicyclomycin (23)[j]	100–>100	>100
Colistin[f]	>100	>100
Macrolides		
Leucomycin (12)[l]	3.13–>100	>100
Tylosin[h]	3.13–>100	>100
Erythromycin[h]	6.25–>100	>100
Oleandomycin[a]	12.5–>100	>100
Spiramycin[h]	>100	>100
Aminoglycosides		
Gentamicin[h]	3.13–25	12.5
Kanamycin[m]	12.5–>100	100
Streptomycin[m]	12.5–>100	>100
Neomycin[h]	50–>100	>100
Polyene		
Trichomycin (14)[j]	>100[i, n]	>100[i, n]
Others		
Novobiocin[g]	12.5–>100	>100
Vancomycin[o]	>100	>100
Rifampin[p]	>100	>100
Nalidixic acid[q]	100–>100	>100
p-Arsanilic acid[r]	100–>100	>100

[a] Taito Pfizer Co., Tokyo.
[b] Nippon Tokushu Noyaku Co., Tokyo.
[c] Sankyo Co., Tokyo.
[d] Lederle Japan Ltd., Tokyo.
[e] Nippon Merck Sharp and Dohme, Osaka.
[f] Kokin Chemical Co., Tokyo.
[g] The Upjohn Japan Co., Tokyo.
[h] National Veterinary Assay Laboratories, Tokyo.
[i] Units per milliliter.
[j] Fujisawa Pharmaceutical Co., Ltd., Osaka.
[k] Takeda Chemical Industries, Osaka.
[l] Toyo Jozo Co., Tokyo.
[m] Meiji Seika Co., Tokyo.
[n] Resistant to 1,000 μg/ml.
[o] Eli Lilly & Co., Indianapolis, Ind.
[p] Lepetit, Italy.
[q] Daiichi Seiyaku Co., Tokyo.
[r] Tokyo Chemical Industries, Tokyo.

Our study and those of earlier workers indicate that the in vitro assay of compounds is a useful tool for selection of a therapeutic drug, particularly when susceptibility of *T. hyodysenteriae* varies depending upon its origin.

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