ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1993, p. 2733–2735
0066-4804/93/122733-03$02.00/0

Vol. 37, No. 12

NOTES

Antimicrobial Susceptibilities of *Gardnerella vaginalis*

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Received 7 April 1993/Returned for modification 7 July 1993/Accepted 16 September 1993

The in vitro susceptibilities of 93 clinical isolates of *Gardnerella vaginalis* to 25 antimicrobial agents were determined by the agar dilution method. All isolates were susceptible to penicillin, ampicillin, erythromycin, clindamycin, chloramphenicol, and trimethoprim. Activity was poor for vancomycin, LY146032, the cephalosporins, ciprofloxacin, and imipenem. Some resistance was observed with tetracycline and minocycline. The MICs of metronidazole paralleled those of tinidazole, with the hydroxymetabolite of metronidazole being the most active. One strain was resistant to all three agents. Marked resistance to aztreonam, amikacin, and sulfamethoxazole was observed.

Gardnerella vaginalis, a gram-variable bacillus first described by Leopold in 1953 (10), has been implicated as the predominant organism in bacterial vaginosis (4). The name *Haemophilus vaginalis* was first proposed by Gardner and Dukes (4) because of the organism's colonial morphology and biochemical profile. Because the organism morphologically resembled diphtheroid bacilli in gram-stained preparations, it was subsequently named *Corynebacterium vaginale* (21). However, because of its variable reaction on Gram staining, being neither typically positive nor typically negative, it was subsequently placed in a unique genus called *Gardnerella* (5).

Besides being implicated in bacterial vaginosis, *G. vaginalis* has been associated with conditions such as chorioamnionitis, urinary tract infections (8), bacteremia in adults (16), and neonatal meningitis (1). Nitroimidazoles have been used successfully in the treatment of bacterial vaginosis, while a variety of other antimicrobial agents have been used to treat extravaginal invasive infections. There is therefore a need to test isolates of *G. vaginalis* to detect any emergence of resistance as well as to identify the appropriate therapeutic agents for use in the treatment of invasive diseases caused by *G. vaginalis*.

Ninety-three isolates of *G. vaginalis* obtained from the vaginas of women attending outpatient clinics between November 1987 and March 1988 at King Edward VIII Hospital, Durban, South Africa, were tested for their in vitro susceptibilities to 25 antimicrobial agents. All isolates were identified on the basis of characteristic diffuse beta-hemolysis on human blood agar, Gram staining variability, and positive α-glucosidase and negative β-glucosidase activities. MICs were determined by the agar dilution method. The antimicrobial agents tested were available as powders of the stated potencies for laboratory use supplied by the indicated pharmaceutical companies: metronidazole (Rhone-Poulenc, Essex, United Kingdom), 2-hydroxymetabolite of metronidazole RP20396 (Rhone-Poulenc), tinidazole (Sigma Chemical Co., St. Louis, Mo.), penicillin G (Glaxo Pharmaceuticals, Greenford, United Kingdom), ampicillin (Beecham Pharmaceuticals, Brentford, United Kingdom), cefamandole (Eli Lilly & Co., Indianapolis, Ind.), cefoxitin (MSD, Hoddesdon, United Kingdom), cefuroxime (Glaxo Pharmaceuticals), cefotaxime (Roussel, Paris, France), ceftriaxone (Roche, Basel, Switzerland), aztreonam (Squibb, Twickenham, United Kingdom), imipenem (MSD), tetracycline (The Upjohn Co., Kalamazoo, Mich.), minocycline (Lederle Laboratories), erythromycin (Abbott Laboratories, Johannesburg, South Africa), clindamycin (Upjohn Co.), vancomycin (Eli Lilly), LY 146032 (Eli Lilly), chloramphenicol (Parke Davis, Pontypool, United Kingdom), amikacin (Bristol Laboratories, Langley, United Kingdom), rifampin (CIBA-GEIGY, Corp., Summit, N.J.), ciprofloxacin (Bayer Miles, Haywards Health, United Kingdom), sulfamethoxazole (Wellcome, Berkhamstead, United Kingdom), trimethoprim (Wellcome), and co-trimoxazole (trimethoprim-sulfamethoxazole [1:19]). These agents were dissolved in appropriate solvents to prepare stock solutions and were then diluted in water and added to molten human blood agar to provide a final range of twofold concentrations from 0.001 to 128 μg/ml when tested. For testing of sulfamethoxazole, trimethoprim, and co-trimoxazole, saponin-lysed human blood was used in addition to whole human blood. All plates were kept at 4°C and were used within 24 h of preparation. Antibiotic-free plates were used as growth controls and to check for potential contamination. Three control strains were included with each test batch. These were reference cultures of *Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418, and *G. vaginalis* NCTC 10915.

Colonies of *G. vaginalis* were suspended in Mueller-Hinton broth, and the turbidity was adjusted to match that of a 0.5 McFarland barium sulfate standard. A 1 in 10 dilution of the inoculum was applied to agar plates by using a Cathra replicator (19), giving a final inoculum of approximately 10^5 CFU. All plates were incubated at 37°C in 6% CO_2 for 48 h, except those with metronidazole, tinidazole, and the 2-hydroxymetabolite of metronidazole, which were incubated anaerobically in GasPak jars (BBL) at 37°C. Growth on the antibiotic plates was compared with the growth on antibiotic-free plates; very fine growth or occasional single colonies

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TABLE 1. In vitro susceptibilities of 93 strains of *G. vaginalis* to 25 antimicrobial agents

Test agent	MIC (μg/ml)		
	Range	50%	90%
Metronidazole	2.0–128.0	8.0	16.0
2-Hydroxy[a]	0.25–16.0	1.0	4.0
Tinidazole	1.0–128.0	8.0	8.0
Penicillin G	0.015–0.5	0.12	0.5
Ampicillin	0.03–1.0	0.5	0.5
Cefamandole	0.12–2.0	1.0	2.0
Cefoxitin	0.06–4.0	1.0	1.0
Cefuroxime	0.06–4.0	1.0	4.0
Cefotaxime	0.25–4.0	2.0	2.0
Ceftriaxone	0.06–4.0	0.5	2.0
Aztreonam	4.0–32.0	32.0	32.0
Imipenem	0.06–1.0	0.25	1.0
Tetracycline	2.0–128.0	64.0	64.0
Minocycline	0.12–16.0	2.0	16.0
Erythromycin	0.007–0.06	0.03	0.06
Clindamycin	0.007–0.03	0.01	0.03
Vancomycin	0.12–0.5	0.25	0.5
LY146032	0.5–8.0	4.0	8.0
Chloramphenicol	0.5–2.0	1.0	2.0
Amikacin	8.0–128.0	32.0	128.0
Rifampin	0.5–0.5	1.0	2.0
Ciprofloxacin	1.0–4.0	1.0	2.0
Sulfamethoxazole	128.0–128.0	>128.0	>128.0
Trimethoprim	0.5–4.0	2.0	4.0
Co-trimoxazole[b]	4.0–64.0	64.0	64.0

[a] 2-Hydroxymetabolite of metronidazole [1-(2-hydroxyethyl)-2-hydroxymethyl-1,5-nitroimidazole].
[b] Sulfamethoxazole-trimethoprim in a 19:1 ratio.

were disregarded. The MIC of each antibiotic was defined as the lowest concentration which inhibited growth of the organism.

MICs for susceptibility were based on the interpretive standards of the National Committee for Clinical Laboratory Standards (13) for organisms other than *Haemophilus* spp. and *Neisseria gonorrhoeae*. The MIC ranges and the MICs for 50% (MIC_{50}s) and 90% (MIC_{90}s) of the 93 vaginal strains of *G. vaginalis* tested are shown in Table 1. The MICs of metronidazole were variable and paralleled those of tinidazole. The hydroxymetabolite of metronidazole was more active than both the parent compound and tinidazole, with MICs for the majority of strains being at least two dilutions less than those of metronidazole or tinidazole. Only one strain showed marked resistance to metronidazole, tinidazole, and the hydroxymetabolite (MIC, 128.0 μg/ml).

All strains were susceptible to penicillin (MIC_{90}, 0.5 μg/ml), ampicillin (MIC_{90}, 0.5 μg/ml), erythromycin (MIC_{90}, 0.06 μg/ml), clindamycin (MIC_{90}, 0.03 μg/ml), vancomycin (MIC_{90}, 0.5 μg/ml), and chloramphenicol (MIC_{90}, 2.0 μg/ml). LY146032, a cyclic lipopeptide antibiotic, showed limited activity against these strains (MIC_{90}, 8.0 μg/ml).

Tetracycline MICs were bimodal in distribution; for many strains, the tetracycline MIC was 2.0 to 4.0 μg/ml, while for the majority of the strains, tetracycline MICs were 64 μg/ml or greater. Sixty strains (64.5%) were susceptible to minocycline (MIC, <8.0 μg/ml), and for 33 (35.5%) strains, minocycline MICs were >16.0 μg/ml. For none of the strains tested were the MICs of the cephalosporins high. The MIC_{90}s of cefoxitin and cefuroxime were 1.0 and 4.0 μg/ml, respectively, whereas the MIC_{90}s of cefamandole, cefotaxime, and ceftriaxone were 2.0 μg/ml.

Ciprofloxacin and imipenem had MIC_{90}s of 2.0 and 1.0 μg/ml, respectively. Resistance to aztreonam and amikacin was marked, with MIC_{90}s of 32.0 and 128.0 μg/ml, respectively. All strains were susceptible to trimethoprim and resistant to sulfamethoxazole, and the combination of these two drugs did not demonstrate any marked synergistic activity. The growth on whole and lysed human blood agar plates containing sulfamethoxazole, trimethoprim, and co-trimoxazole was compared. There were no differences in the MICs that were obtained; however, the presence of growth on whole human blood agar plates was much easier to read because of diffuse beta-hemolysis.

Antimicrobial agents belonging to the nitroimidazole group are recommended as drugs of choice for the treatment of bacterial vaginosis. The majority of strains of *G. vaginalis* examined in the present study were susceptible to metronidazole and tinidazole; MICs for these strains were less than 16 μg/ml. Other studies (9, 15) have shown in vitro activity similar to those of both of these agents. Pheifer et al. (14) demonstrated good clinical results with metronidazole in treating women with bacterial vaginosis. Although the MICs for all of their isolates were not within the susceptible range, it was felt that the obligate anaerobic organisms present in vaginal secretions reduce metronidazole to its more active hydroxymetabolite form, thereby enabling clinical efficacy. We also showed better activity with the hydroxymetabolite of metronidazole, because MICs were at least two dilutions lower than that of the parent compound. It is notable that high-level resistance to metronidazole, tinidazole, and the hydroxymetabolite (MIC, >128 μg/ml) was encountered in only one strain.

The use of ampicillin for the treatment of bacterial vaginosis has often been associated with failure to eradicate *G. vaginalis* or bring about clinical cure (14). This is probably due to inactivation of ampicillin by the β-lactamases produced by vaginal anaerobes. However, this agent may have a role in treating *Gardnerella*-associated infections at extravaginal sites. We obtained penicillin and ampicillin MIC_{90}s of 0.5 μg/ml, designating the organisms as susceptible.

Erythromycin and clindamycin were the most active agents in the present study (MIC_{90}s, 0.06 and 0.03 μg/ml, respectively). The use of erythromycin may be limited in patients with bacterial vaginosis because of the acidic environment of the vagina (3). Clindamycin has been shown to be effective in treating bacterial vaginosis; it has been used both orally and as a local preparation (7). The activities of the cephalosporins ciprofloxacin and imipenem were relatively reduced compared with those which would be expected for most gentamicin-susceptible gram-negative bacteria. Limbert et al. (11) showed that *G. vaginalis* isolates are highly susceptible to cefoxitin, cefuroxime, and cefotaxime, with MIC_{90}s ranging from 0.06 to 0.15 μg/ml. This is in marked contrast to the results which we have obtained with the same cephalosporins. The monobactam aztreonam, which has activity only against gram-negative organisms, and amikacin demonstrated very little activity, with MICs of 4 μg/ml or greater.

The MICs of tetracycline showed a bimodal distribution, which is similar to the observations of McCarthy et al. (12). High-level resistance (MIC, >64 μg/ml) in *G. vaginalis* has been attributed to the TetM conjugative transposon located on the chromosome (18). Plasmid analyses were not performed in our study. The MICs of minocycline were more variable, in contrast to those of tetracycline, ranging from 0.12 to 32.0 μg/ml.

Gram-stained smears show that *G. vaginalis* is Gram

variable. It has been suggested that the organism is gram negative because of the presence of very little mucopeptide, the amino acid composition of the cell wall (2), and a "lipopolysaccharide-like" fraction (5); others suggest that it is gram positive on the basis of its pattern of septum formation (17), the absence of diaminopimelic acid (6), and its lipopolysaccharide (20). However, the results of the present susceptibility study are consistent with the notion that *G. vaginalis* is neither typically gram positive nor typically gram negative, since antimicrobial agents regarded as specifically active against gram-positive or gram-negative organisms showed relatively poor activity. The findings of the present study show that local isolates of *G. vaginalis* are susceptible to the nitroimidazole group of antimicrobial agents. The activities of erythromycin and clindamycin were also good in vitro, and therefore, these agents may have potential applications in the therapy of extravaginal invasive infections. The spectrum of activity of the other antimicrobial agents tested was variable, suggesting that their therapeutic potential is limited.

We thank C. Pillay for technical assistance and A. Pillay for typing the manuscript.

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