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In Vitro Activity of 79 Antimicrobial Agents against *Corynebacterium* Group D2

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***Corynebacterium* group D2 (CGD2) is involved in urinary tract infections in patients with underlying predisposing factors. This microorganism is highly resistant to a number of antimicrobial agents. We tested the activities of 79 antimicrobial agents against CGD2. β-Lactams, aminoglycosides, and macrolides were ineffective. Fluorinated quinolones showed irregular activities, ofloxacin being the most active one. Doxycycline, rifampin, and mainly glycopeptides (vancomycin and teicoplanin) were the most active antibiotics against CGD2.**

Corynebacterium group D2 (CGD2) is involved in the etiology of urinary tract infections (UTIs) in patients with underlying predisposing factors, such as urinary tract instrumentation, surgery, or malignancy (1). CGD2 has also been involved in the origin of phosphate-encrusted cystitis (10, 11) and in sepsis in compromised hosts (3). This group has been shown to be resistant to most antibiotics used for the treatment of UTIs (9), the utilization of glycopeptides being frequently necessary.

We studied the in vitro activities of 79 antimicrobial agents against CGD2 in order to find therapeutic alternatives useful for the treatment of UTIs caused by this microorganism.

The antibiotics tested, kindly provided by their manufacturers, were penicillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, azlocillin, mezlocillin, piperacillin, cloxacillin, mecillinam, temocillin, amoxicillin-clavulanic acid, sulbactam-ampicillin, piperacillin-tazobactam, ticarcillin-clavulanic acid, cefazolin, cefamandole, cefuroxime, cefotetan, cefonicid, cefaclor, cefoxitin, cefotaxime, cefsulodin, cefetamet, cefterame, latamoxef, ceftizoxime, cefoperazone, cefbuperazone, cefpirome, CGP-31608/WS (Ciba-Geigy), CGP-22495 (Ciba-Geigy), CGP-31523 (Ciba-Geigy), ICI-194008 (ICI-Farma), imipenem, meropenem, SCH-34343 (Schering), aztreonam, tigemonam, carumonam, nalidixic acid, norfloxacin, enoxacin, pefloxacin, ofloxacin, ciprofloxacin, difloxacin, temafloxacin, lomefloxacin, fleroxacin, irloxacin, E-3846 (Laboratories Esteve), CI-934 (Ciba-Geigy), sparfloxacin, gentamicin, tobramycin, amikacin, netilmicin, neomycin, tetracycline, doxycycline, fosfomycin, vancomycin, teicoplanin, erythromycin, clarytromycin, dirythromycin, roxythromycin, mydecamycin, josamycin, oleandomycin, trimethoprim plus sulfamethoxazole, clindamycin, lincomycin, chloramphenicol, rifampin, nitrofurantoin, and nitroxolin. Sixty-five clinical strains of CGD2 isolated from UTIs; CGD2 ATCC 43042, ATCC 43043, and ATCC 43044; *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; and *Staphylococcus aureus* ATCC 29213 were tested according approved standards (5) by using the methods recommended for the study of streptococci (Mueller-Hinton agar with 5% sheep blood, except when sulfonamides were tested, in which case Mueller-Hinton agar with 5% lysed horse blood was used). The inoculum was prepared in Mueller-Hinton broth from a 48-h growth on Mueller-Hinton 5% sheep blood agar, adjusted to a turbidity equivalent to that of a 0.5 McFarland standard, and diluted 1:10. The inoculation of the agar plates was performed by using a Steer's replicator, the final inoculum on the media being approximately 10^4 CFU per spot. Plates were incubated for 48 h at 35°C in room air, since after 24 h of incubation, the control plates (the same strains inoculated on the same blood agar without antibiotics) occasionally showed very faint growth or had no visible growth. The growth on these plates after 48 h was always satisfactory. The MIC was defined as the lowest concentration of antibiotic that suppressed visible bacterial growth. Single colonies, faint hazes, and skip plates were disregarded.

Results are shown in Table 1 and confirm the high resistance of CGD2 to a wide number of antimicrobial agents (6). CGD2 was resistant to all of the penicillins and combinations of penicillin and β-lactamase inhibitor tested. No strain was inhibited by any of these compounds and combinations at 128 μg/ml. To all of the cephalosporins, including the most recently developed ones and those active against other gram-positive bacteria resistant to cephalosporins, such as cefpirome, more than 90% of the strains showed resistance. The only exceptions were to cefoxitin and latamoxef, to which strains showed 74 and 88.9% resistance, respectively. As could be expected, monobactams were not active at all. Penems were also ineffective. CGD2 has been previously shown resistant to classical β-lactams, such as amoxicillin, older narrow-spectrum and expanded-spectrum cephalosporins, and ceftazidime (2, 7, 10, 12, 13). The only β-lactam–β-lactamase inhibitor combination and the only penem previously tested, amoxicillin-clavulanic acid and SCH 34343, respectively, were also shown to be ineffective against CGD2 (12, 13). Our results show that CGD2 is also resistant to all broad-spectrum cephalosporins, "fourth-generation" cephalosporins, and penems tested and shows a high percentage of resistance to latamoxef. This is an important fact, since one of the most notable characteristics of the "fourth generation" cephalosporins is the recovery of activity against gram-positive bacteria, lost to some extent in expanded- and broad-spectrum cephalosporins. There is scant knowledge about the mechanisms of resistance of these microorganisms, so we have no explanations for the

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TABLE 1. In vitro activity of 79 antimicrobial agents against 65 clinical isolates of CGD2

Antimicrobial agent	MIC (μg/ml)[a]			% of isolates resistant to agent	BP[b] (μg/ml)
	50	90	Range		
Penicillins[c]	>128	>128	>128	100	
β-Lactam–β-lactamase inhibitor combinations[d]	>128	>128	>128	100	
Narrow- and extended-spectrum cephalosporins[e]	>128	>128	>128	100	
Monobactams[f]	>128	>128	>128	100	
Cefoxitin	>128	>128	2–>128	74	≥32
Cefotaxime	>128	>128	0.5–>128	94.3	≥64
Cefsulodin	>128	>128	4–>128	91.2	≥64
Cefetamet	>128	>128	>128	100	≥64
Cefterame	>128	>128	>128	100	≥64
Latamoxef	>128	>128	32–>128	88.9	≥64
Ceftizoxime	>128	>128	>128	100	≥64
Cefoperazone	>128	>128	4–>128	95	≥64
Cefbuperazone	>128	>128	64–>128	100	≥64
Cefpirome	>128	>128	>128	100	≥64
CGP-31608/WS	>128	>128	64–>128	100	≥64
CGP-22495	>128	>128	2–>128	98.6	≥64
CGP-31523	>128	>128	>128	100	≥64
ICI-194008	>128	>128	>128	100	≥64
Imipenem	>128	>128	>128	100	≥16
SCH-34343	>128	>128	>128	100	≥16
Meropenem	>128	>128	2–>128	98.4	≥16
Nalidixic acid	128	>128	>128	100	≥32
Norfloxacin	128	>128	0.25–>128	76	≥16
Enoxacin	128	>128	2–>128	90	≥8
Pefloxacin	32	>128	0.25–>128	78	≥4
Ofloxacin	4	64	0.25–>128	53	≥8
Ciprofloxacin	16	128	0.06–>128	60	≥4
Difloxacin	8	32	0.06–64	68	≥4
Temafloxacin	4	32	0.25–>128	61	≥4
Lomefloxacin	32	>128	0.5–>128	84	≥4
Fleroxacin	8	32	0.5–>128	87	≥4
Irloxacin	64	>128	0.25–>128	71	≥4
E-3846	4	>128	0.25–>128	71	≥4
CI-934	8	32	0.125–32	67	≥4
Sparfloxacin	8	64	0.06–64	57	≥4
Gentamicin	>128	>128	0.25–>128	92	≥8
Tobramycin	>128	>128	0.5–>128	89	≥8
Amikacin	>128	>128	1–>128	90	≥32
Netilmicin	>128	>128	0.12–>128	90	≥32
Neomycin	>128	>128	4–>128	97	≥25
Tetracycline	16	64	0.5–128	64	≥16
Doxycycline	2	8	0.25–16	2	≥16
Fosfomycin	>128	>128	>128	100	≥128
Vancomycin	0.5	2	0.06–2	0	≥32
Teicoplanin	0.25	0.5	0.125–0.5	0	<32
Erythromycin	>128	>128	0.06–>128	90	≥8
Clarythromycin	>128	>128	2–>128	96	≥8
Dirythromycin	>128	>128	8–>128	100	≥8
Roxythromycin	>128	>128	8–>128	100	≥8
Midecamycin	>128	>128	8–>128	100	≥8
Josamycin	>128	>128	0.25–>128	98	≥8
Oleandomycin	>128	>128	0.5–>128	98	≥8
Trimethoprim-sulfamethoxazole	>128	>128	>128	100	≥8/152
Clindamycin	>128	>128	0.06–>128	92	≥4
Lincomycin	>128	>128	0.5–>128	92	≥4

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TABLE 1—*Continued*

Antimicrobial agent	MIC (μg/ml)[a]			% of isolates resistant to agent	BP[b] (μg/ml)
	50	90	Range		
Chloramphenicol	64	>128	16–>128	98	≥32
Rifampin	≤0.008	8	≤0.008–>128	26	≥4
Nitrofurantoin	>128	>128	>128	100	≥128
Nitroxolin	32	64	16–64	100	≥16

[a] 50 and 90, MIC for 50 and 90% of the strains, respectively.
[b] BP, break point.
[c] Penicillins include penicillin (BP ≥ 4 μg/ml), ampicillin (BP ≥ 4 μg/ml), amoxicillin (BP ≥ 4 μg/ml), carbenicillin (BP ≥ 64 μg/ml), ticarcillin (BP ≥ 128 μg/ml), piperacillin (BP ≥ 128 μg/ml), azlocillin (BP ≥ 128 μg/ml), mezlocillin (BP ≥ 128 μg/ml), cloxacillin (BP ≥ 4 μg/ml), mecillinam (BP ≥ 16 μg/ml), and temocillin (BP ≥ 64 μg/ml).
[d] β-Lactam–β-lactamase inhibitor combinations include amoxycillin-clavulanic acid (2:1) (BP ≥ 16/8 μg/ml), ampicillin-sulbactam (1:1) (BP ≥ 32/16 μg/ml), piperacillin-tazobactam (8:1) (BP ≥ 128/16 μg/ml), and ticarcillin-clavulanic acid (64:1) (BP ≥ 128/2 μg/ml).
[e] Narrow and extended-spectrum cephalosporins include cefazolin (BP ≥ 32 μg/ml), cefamandole (BP ≥ 32 μg/ml), cefuroxime (BP ≥ 32 μg/ml), cefotetan (BP ≥ 64 μg/ml), cefonicid (BP ≥ 32 μg/ml), and cefaclor (BP ≥ 32 μg/ml).
[f] Monobactams include aztreonam (BP ≥ 32 μg/ml), tigemonam (BP ≥ 32 μg/ml), and carumonam (BP ≥ 32 μg/ml).

lack of activity of β-lactams in general or for the activity of cefoxitin, which is scant but higher than that of other β-lactams.

Quinolones were shown to be more active than cephalosporins against CGD2. Ofloxacin, sparfloxacin, ciprofloxacin, and temafloxacin were the most active quinolones. Nevertheless, the activity of fluorinated quinolones was irregular. Proportions of resistance were between 53 and 90% and were higher than 50% for all the quinolones tested. On the whole, the activity of quinolones against CGD2 is lower than that previously reported (2, 4, 7, 8, 12, 13). As could be expected, the quinolones with the highest intrinsic activity and those more active against gram-positive bacteria (according other studies) were among the most effective against this group. Nevertheless, the most active quinolone was ofloxacin, that has an intrinsic activity lower than those of other fluorinated quinolones (ciprofloxacin, E-3846, and CI-934, etc.) and is not especially effective against other gram-positive bacteria.

For other groups, to aminoglycosides, macrolides, trimethoprim plus sufamethoxazole, lyncosamides, chloramphenicol, and sulfonamides, strains showed resistance of over 90%, similar to resistance percentages reported by other authors (2, 4, 7, 10, 12, 13). Tetracyclines showed irregular activity; 64% of strains were resistant to tetracycline, but doxycycline was effective against 98% of strains, and the MIC for the only resistant strain was two times the break point, a concentration that this antibiotic can easily reach in the urine. Nevertheless, their bacteriostatic activities may impair their usefulness in patients with infections caused by CGD2, who are frequently immunosuppressed, and the high urinary pH caused by this microorganism may also impair the activity of this group. Rifampin, although it showed a very wide range, was active against 74% of strains, but the use of this antibiotic alone is hindered by the possibility of development of one-step mutations leading to resistance. All strains were sensitive to glycopeptides, both vancomycin and teicoplanin showing similar activities. Similar results related to glycopeptides have been previously reported (2, 4, 7, 12, 13).

The great number of antibiotics from other groups developed in the last years does not bring valuable alternatives for the treatment of infections caused by CGD2, glycopeptides remaining the treatment of choice. Nevertheless, the pharmacokinetic characteristics of teicoplanin and the possibility of intramuscular administration can make it a valuable alternative to vancomycin for the treatment of UTIs caused by CGD2.

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