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Comparison of Antimicrobial Susceptibilities of *Corynebacterium* Species by Broth Microdilution and Disk Diffusion Methods

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Corynebacterium **species are increasingly being implicated in foreign-body infections and in immunocompromised-host infections. However, there are no specific recommendations on the method or the criteria to use in order to determine the in vitro activities of the antibiotics commonly used to treat *Corynebacterium* infections. The first aim of our study was to compare the susceptibilities of various species of *Corynebacterium* to vancomycin, erythromycin, and penicillin by using a broth microdilution method and a disk diffusion method. Second, the activity of penicillin against our isolates was assessed by using the interpretative criteria recommended by the National Committee for Clinical Laboratory Standards for the determination of the susceptibility of streptococci and *Listeria monocytogenes* to penicillin. Overall, 100% of the isolates were susceptible to vancomycin, while considerable variations in the activities of erythromycin and penicillin were noted for the different species tested, including the non-*Corynebacterium jeikeium* species. A good correlation in the susceptibilities of vancomycin and erythromycin between the disk diffusion and the microdilution methods was observed. However, a 5% rate of major or very major errors was detected with the *Listeria* criteria, while a high rate of minor errors (18%) was noted when the streptococcus criteria were used. Our findings indicate considerable variations in the activities of erythromycin and penicillin against the various species of *Corynebacterium*. Because of the absence of definite recommendations, important discrepancies were observed between the methods and the interpretations of the penicillin activity.**

For many years, *Corynebacterium* spp. were considered part of the normal skin flora and thought to have limited potential for pathogenicity. However, they are now causing more infections (2, 7), especially intravascular catheter-related infections and bacteremias (2, 4, 5). They also often act as opportunistic pathogens in immunocompromised hosts and in heavily instrumented patients.

The taxonomy of the genus *Corynebacterium* is rapidly changing, and with the help of cellular fatty acid patterns and cell wall characteristics, many species have lately been renamed (5, 8). Some species remain in the genus *Corynebacterium*, whereas others, such as "*Corynebacterium aquaticum*" or CDC group A, are now classified as coryneform groups (5, 9). There are about 30 different species of *Corynebacterium*. Some, such as *C. jeikeium*, are considered quite virulent and are usually resistant to multiple antibiotics. Others, such as *C. pseudodiphtheriticum* or *C. xerosis*, are considered much less harmful, although they have been implicated in cases of endocarditis and meningitis (2). Emergence of antimicrobial resistance among non-*C. jeikeium* strains has been reported (21).

Although there have been a few published studies of the antimicrobial susceptibilities of *Corynebacterium* spp. (11, 12, 16, 19), there are presently no definite recommendations on the method or the criteria to use in order to determine the in vitro activities of the antibiotics commonly used to treat *Corynebacterium* infections. Some authors have suggested use of the National Committee for Clinical Laboratory Standards (NCCLS) criteria recommended for determination of the susceptibility of staphylococci to penicillin (10). The first aim of our study was to compare the susceptibilities of various species of *Corynebacterium* and species classified as coryneform groups to vancomycin, erythromycin, and penicillin by using two antimicrobial susceptibility methods: a broth microdilution method and a disk diffusion method. We also assessed and compared the activities of penicillin against our isolates by using the interpretative criteria recommended by the NCCLS for the determination of the susceptibility of streptococci and *Listeria monocytogenes* to penicillin.

(This study was presented in part at the 95th General Meeting of the American Society for Microbiology [20a]).

MATERIALS AND METHODS

Organisms. The *Corynebacterium* and coryneform bacteria included in this study were 101 single-patient clinical isolates obtained from blood cultures or intravascular catheter cultures, processed in our microbiology laboratory over a 24-month period. The following were included: 27 *C. jeikeium*, 15 *C. pseudodiphtheriticum*, 11 *C. minutissimum*, 8 *C. striatum*, 8 *C. xerosis*, 6 CDC group G1, 5 CDC group G2, 5 *C. afermentans*, 4 *C. aquaticum*, 2 *C. urealyticum*, 2 *C. diphtheriae*, 2 CDC group A, and 2 CDC group I2 strains and 1 strain each of *C. matruchotii*, *C. pseudotuberculosis*, *C. ulcerans*, and CDC group F1. The identification of isolates was based on colonial morphology and color, Gram stain, motility, a positive catalase reaction, and API-Coryne system (Bio Mérieux, Marcy l'Etoile, France) identification. Some strains, for which the API-Coryne identification was occasionally nonconclusive (probability, <90%), were sent to the Quebec Public Health Laboratory (St. Anne de Bellevue, Quebec, Canada) for final identification by conventional methods. This problem occurred occasionally for *C. xerosis* and CDC groups F1, G1, and G2. Although certain species such as *C. ulcerans* or *C. aquaticum* are no longer proper names for pure taxonomic purposes, we retain the usual denomination used in the medical literature (5, 8).

Quality control organisms *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were included in each test run. Expected MICs were obtained for these strains.

Antimicrobial agents. The most commonly used or usually active antibiotics against *Corynebacterium* spp. were selected. Penicillin, erythromycin, and vancomycin were thus included in our panel. For the disk diffusion method, disks with penicillin (10 U), vancomycin (30 μg), and erythromycin (15 μg) (Remel, Lenexa, Kans.) were used. Stock and working solutions were prepared from sterile standardized antibiotic powders purchased from Nucrotechnics, Scarborough, Ontario, Canada.

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TABLE 1. Percentages of strains found to be susceptible by two methods

Organism (no. of strains)	% of strains susceptible to[a]:							
	ERY		VAN		PEN (L)		PEN (S)	
	DD	MD	DD	MD	DD	MD	DD	MD
C. jeikeium (27)	14.8	14.8	100	100	18.5	18.5	18.5	18.5
C. pseudodiphtheriticum (15)	66.6	66.6	100	100	100	100	100	100
C. minutissimum (11)	18.1	27.2	100	100	90.9	90.9	72.7	27.2
C. striatum (8)	87.5	87.5	100	100	87.5	87.5	25	0
C. xerosis (8)	25	12.5	100	100	62.5	75	25	12.5
CDC group G1 (6)	66.6	66.6	100	100	100	100	100	100
CDC group G2 (5)	20	20	100	100	20	40	20	20
C. afermentans (5)	20	20	100	100	80	80	80	40
C. aquaticum (4)	75	75	100	100	50	75	25	25
Other[b] (12)	75	75	100	100	66.6	75	66.6	58.3
Total (101)	42.5	42.5	100	100	62.3	66.3	51.4	40.5

[a] ERY, erythromycin; VAN, vancomycin; PEN, penicillin; L, *Listeria* criteria; S, streptococcus criteria; DD, disk diffusion method; MD, microdilution method.
[b] See Table 2, footnote *a*.

Susceptibility tests. Broth microdilution tests were performed as outlined by the NCCLS (15), using a cation-adjusted Mueller-Hinton (CAMH) broth supplemented with 4.5% lysed horse blood (Quélab Inc., Montreal, Canada). Because of lipid-dependent CDC coryneform groups, 6.6% rabbit serum (Quélab Inc.) was added to the broth. The disk diffusion test was also performed as described by the NCCLS (14) with a CAMH agar supplemented with 5% sheep blood. All media and plates used for both methods were prepared in-house.

Inocula were prepared for both tests from an overnight blood agar plate by suspending the growth in Mueller-Hinton broth. The inocula were adjusted to a 0.5 McFarland standard. For the broth microdilution technique, the inoculum was further diluted 1/100 in CAMH broth supplemented with lysed horse blood and rabbit serum in order to obtain a final inoculum of approximately 5×10^5 CFU/ml in each well.

We had 11 strains (9 *C. jeikeium* strains, 1 *C. matruchotii* strain, and 1 *C. afermentans* strain) for which we encountered some difficulty in obtaining a smooth suspension. This represented only about 10% of all strains. These strains had a granular aspect in suspension. We dealt with this problem by crushing the suspended colonies with a cotton swab, vigorously vortexing the tubes in order to obtain a homogeneous suspension (0.5 McFarland standard), and immediately inoculating the different tubes with the vortexed solution. Moreover, because we obtained MICs which were either very high for *C. jeikeium*, as expected, or very low for the two other species, we did not have an inoculum-related MIC interpretation problem. All plates were incubated for 24 and 48 h at 35°C in an ambient atmosphere.

Reading and definitions. The agar and broth microdilution plates were both read at 24 and 48 h according to the currently published parameters of the NCCLS (14, 15). The 48-h readings were necessitated mainly by suboptimal growth at 24 h of many slowly growing species. All results were recorded for the 48-h mark. Penicillin susceptibility was assessed by using the criteria for streptococci and *Listeria* as suggested by the NCCLS (14, 15).

Discrepancies were classified as minor, major, or very major errors. The broth microdilution method was used as the "gold standard." A minor error was defined as identification of a strain as susceptible or resistant by the disk diffusion method but intermediate by the microdilution technique. A strain categorized as intermediate by disk diffusion and as either susceptible or resistant by broth microdilution was also included in this definition. A major error was defined as identification of a strain as resistant by the disk diffusion method but susceptible by the microdilution method. A very major error was defined as categorization of a strain as susceptible by the disk diffusion method but resistant by the microdilution method.

RESULTS AND DISCUSSION

All strains were found to be susceptible to vancomycin by both methods (Table 1). We also noted a perfect agreement between the two susceptibility methods for this antibiotic. For all species but one, the MICs were always ≤0.5 mg/liter, except for *C. aquaticum*, for two strains of which the MIC was 4 mg/liter (Table 2). Although no longer a *Corynebacterium* species per se, *C. aquaticum* is still classified as a coryneform bacterium pending assignment to a new genus (5). This bacterium was implicated in dialysis-associated peritonitis (3, 13) and seems to be less susceptible than *Corynebacterium* spp. to

TABLE 2. MICs of three antimicrobial agents for *Corynebacterium* species and other coryneforms

Organisms (no. of isolates)	Antimicrobial agent	MIC (mg/liter)		
		Range	50%	90%
C. jeikeium (27)	Penicillin	≤0.03–≥64	≥64	≥64
	Erythromycin	≤0.03–≥64	≥64	≥64
	Vancomycin	0.125–0.25	0.25	0.25
C. pseudodiphtheriticum (15)	Penicillin	≤0.03	≤0.03	≤0.03
	Erythromycin	≤0.03–≥64	≤0.03	≥64
	Vancomycin	0.125	0.125	0.125
C. minutissimum (11)	Penicillin	0.06–4	0.25	0.5
	Erythromycin	≤0.03–≥64	4	≥64
	Vancomycin	0.06–0.25	0.125	0.25
C. striatum (8)	Penicillin	0.25–32	0.25	
	Erythromycin	≤0.03–≥64	≤0.03	
	Vancomycin	0.06–0.25	0.125	
C. xerosis (8)	Penicillin	≤0.03–16	1	
	Erythromycin	≤0.03–≥64	16	
	Vancomycin	≤0.03–0.25	0.125	
CDC group G1 (6)	Penicillin	0.06–0.125	0.125	
	Erythromycin	≤0.03–≥64	≤0.03	
	Vancomycin	≤0.03–0.25	0.25	
CDC group G2 (5)	Penicillin	0.125–≥64	≥64	
	Erythromycin	≤0.03–≥64	≥64	
	Vancomycin	≤0.03–0.25	0.25	
C. afermentans (5)	Penicillin	≤0.03–8	0.25	
	Erythromycin	≤0.03–≥64	≤0.03	
	Vancomycin	≤0.03–0.125	0.06	
C. aquaticum (4)	Penicillin	0.125–32	0.25	
	Erythromycin	≤0.03–8	≤0.03	
	Vancomycin	0.125–4	0.5	
Other[a] (12)	Penicillin	≤0.03–≥64	0.125	
	Erythromycin	≤0.03–≥64	≤0.03	
	Vancomycin	0.06–0.5	0.25	

[a] *C. urealyticum* ($n = 2$), *C. diphtheriae* ($n = 2$), CDC group A ($n = 2$), CDC group I2 ($n = 2$), *C. ulcerans* ($n = 1$), *C. matruchotii* ($n = 1$), *C. pseudotuberculosis* ($n = 1$), and CDC group F ($n = 1$).

TABLE 3. Percent agreement between the two susceptibility methods and number of errors per species[a]

Organism (no. of strains)	Erythromycin				Penicillin (L)[b]				Penicillin (S)[b]			
	AG	Min	M	VM	AG	Min	M	VM	AG	Min	M	VM
C. jeikeium (27)	96.5	1	0	0	100	0	0	0	92.6	2	0	0
C. pseudodiphtheriticum (15)	100	0	0	0	100	0	0	0	100	0	0	0
C. minutissimum (11)	81.8	2	0	0	100	0	0	0	63.6	4	0	0
C. striatum (8)	100	0	0	0	100	0	0	0	75	2	0	0
C. xerosis (8)	87.5	1	0	0	75	0	1	1	50	4	0	0
CDC group G1 (6)	83.3	1	0	0	100	0	0	0	100	0	0	0
CDC group G2 (5)	100	0	0	0	80	0	1	0	80	1	0	0
C. afermentans (5)	100	0	0	0	100	0	0	0	60	2	0	0
C. aquaticum (4)	100	0	0	0	75	0	1	0	50	2	0	0
Other[c] (12)	100	0	0	0	91.6	0	1	0	91.6	1	0	0
Total (101)	95	5	0	0	95	0	4	1	82.1	18	0	0

[a] AG, percent agreement; Min, minor error; M, Major error; VM, very major error.
[b] L, *Listeria* criteria; S, streptococcus criteria.
[c] Includes the 12 strains listed in Table 2, footnote *a*.

antibiotics which are usually active against such species, including vancomycin (13, 21). Until definitive results of susceptibility testing are available, vancomycin will continue to often be the recommended treatment for severe *Corynebacterium* sp. infections (19, 21). Vancomycin-resistant coryneforms *C. aquaticum* and *Brevibacterium* spp. (formerly CDC groups B1 and B3) have been rarely reported. In a study reported by Williams et al. (21), only 2 of 254 strains were resistant to vancomycin.

Overall, erythromycin had moderate activity against our isolates of *Corynebacterium* (Tables 1 and 2). Of interest was the poor activity of erythromycin against *C. jeikeium*, with MICs at which 50 and 90% of the isolates are inhibited (MIC_{50} and MIC_{90}, respectively) of ≥64 mg/liter. Similar susceptibility patterns have been reported by others (11, 18, 21). Nonetheless, a very good correlation in the susceptibility of *Corynebacterium* spp. to erythromycin between the microdilution and the disk diffusion methods was found (Tables 1 and 3). Only five minor errors were recorded. Erythromycin would not have been used on these occasions. A minor error was observed once for an isolate of *C. jeikeium*, for which erythromycin is usually not recommended. Minor errors were also noted for two strains of *C. minutissimum*. According to a previous study and a case report, erythromycin has poor antimicrobial activity against *C. minutissimum* (4, 21). As for the two other strains (*C. xerosis* and CDC group G1), erythromycin is poorly efficient against *C. xerosis*, with only 12.5% of the isolates being susceptible (Table 1). This observation was also made in two other studies. In one of them, the MIC_{50} was 256 mg/liter (19), and in the other, only 11% of the isolates were susceptible to erythromycin (21). In the last case (CDC group G1), other antibiotics (penicillin and vancomycin) could be used instead of erythromycin in severe infections, as this species seems to be very susceptible to these agents (Table 1). One interesting observation was the big difference in susceptibility to erythromycin between *C. xerosis* and *C. striatum* (Table 1). Since identification of these strains by the API-Coryne system can sometimes be difficult (6), this characteristic could be useful in differentiating the two species. It appears that the disk diffusion method could therefore be used as a reliable and easy method for evaluating the susceptibility of *Corynebacterium* spp. to erythromycin.

With the exception of *C. jeikeium* or *C. urealyticum*, penicillin for a long time has been considered the antibiotic of choice for treating *Corynebacterium* infections. In the last few years, some authors have reported a decreasing activity of this antibiotic against *Corynebacterium* spp. (19, 21). However, no specific interpretative criteria have been suggested for determining the susceptibility of this microorganism to penicillin. We used and compared two criteria, those suggested for determination of the susceptibility of streptococci and *L. monocytogenes* to penicillin. The *Listeria* criteria were chosen because they are the only established criteria for gram-positive rods (14, 15). The streptococcus criteria were used because of their more stringent values and the presence of an intermediate category acting as a buffer zone in the interpretation of the results. On a technical point, because of the 48-h reading time, penicillin lability was not a problem, as the quality control strains performed as expected. Our results confirmed the fact that although penicillin retains good activity, only 48% of the *Corynebacterium* isolates other than *C. jeikeium* were found to be susceptible by the broth microdilution method using the more stringent streptococcus criteria. There were considerable variations in penicillin activity against the different species. Some species were always susceptible (e.g., *C. pseudodiphtheriticum*, CDC group G1, and *C. diphtheriae*), whereas for others, such as *C. jeikeium*, *C. striatum*, *C. xerosis*, and *C. urealyticum*, penicillin had poor activity (Table 2). CDC groups G1 and G2 are thought to belong to the same taxa (8); however, our results show that the latter is much more resistant to penicillin, whatever criteria are used (Table 1). This was also noted for ampicillin in another study (19). This finding may have a significant clinical impact, since CDC group G2 is more often associated with endocarditis, whereas CDC group G1 is often implicated in eye pathologies (5, 17).

Depending on the interpretative criteria used to assess the activity of penicillin, important discrepancies between the microdilution and the disk diffusion methods were noted (Table 3). Overall, four major errors were detected when the *Listeria* criteria were used. One very major error was even observed for *C. xerosis*. These important discrepancies were nonexistent when the streptococcus susceptibility criteria were used. A high rate of minor errors (18%) was noted, however. Important discrepancies related to species have previously been reported by others. Zapardiel et al. observed very major errors with *C. xerosis* when comparing the activities of ampicillin as determined by an agar dilution method and the E-test (22). The borderline penicillin MICs ranging from 0.25 to 1 mg/liter frequently observed for *C. xerosis* and *C. aquaticum* might explain these species-related discrepancies.

Our findings suggest that in order to avoid either major or

very major errors, it would be advisable to use the streptococcus instead of the *Listeria* interpretative criteria when assessing the activity of penicillin against *Corynebacterium* spp. This is true in spite of an increased number of strains which will be reported as intermediate rather than susceptible. The concept that penicillin is the antibiotic of choice for treatment of *Corynebacterium* infections other than those caused by *C. jeikeium* or *C. urealyticum* may not be true anymore, and a certain discernment has to be applied, depending on the species involved. This has some important implications in clinical situations such as septicemia, endocarditis, or abscess, for which appropriate treatment has to be rapidly instituted (1, 20). Our results may be useful for prescription of antibiotics for *Corynebacterium* infections. More studies will have to be performed in order to establish the appropriate interpretative criteria necessary to accurately determine the in vitro activities of antibiotics against *Corynebacterium* spp., which are now considered increasingly important pathogens.

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