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Antimicrobial Susceptibility and Species Identification of *Corynebacterium* spp. Strains Collected in Europe and USA Medical Centers (2006-2010)

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SENTRY ANTIMICROBIAL SURVEILLANCE

Abstract

Objectives: To evaluate the antimicrobial susceptibility of *Corynebacterium* spp. clinical strains collected from European and USA medical centers. The accuracy of species identifications performed at local labs was also assessed.

Methods: 121 *Corynebacterium* strains were collected from serious infections between 2006 and 2010 as part of the SENTRY Antimicrobial Surveillance Program from 15 and 18 medical centers in 9 European countries and USA, respectively. The strains were susceptibility (S) tested by the CLSI broth microdilution method in cation-adjusted Mueller-Hinton broth against numerous antimicrobial agents. MIC results were interpreted according to CLSI M45 breakpoint criteria. There are yet no criteria from EUCAST. Species identifications were performed by MALDI-TOF methodology using the Bruker Biotyper system.

Results: Isolates were from bacteremia (53.6%), skin and skin structure (17.3%), pneumonia (12.7%), among other infection sites. Vancomycin (100.0% S) and daptomycin (99.1% S) showed potent activity against *Corynebacterium* with $MIC_{50/90}$ of 0.25/0.5 and ≤0.06/0.25 mg/L, respectively. *C. jeikeium* and *C. urealyticum* species groups demonstrated lower S rates, 0.0% for penicillin and 36.8-40.0% for gentamicin, compared to other *Corynebacterium* species groups (see Table). 61 of 121 strains (50.4%) were identified to species level by the clinical laboratories and species identification was confirmed by the MALDI-TOF analysis on 47 (77.0%) of those strains. The remaining 14 strains had incorrect species (12) or genus (2 strains, *Brevibacterium* and *Microbacterium*). Among the 60 strains submitted as unspeciated *Corynebacterium*, 51 (85.0%) strains were confirmed as *Corynebacterium* and identified to species level, while the remaining 9 strains were identified to a different genus (*Brevibacterium* [3], *Microbacterium* [2], *Dermabacter* [1], *Rhodococcus* [1], *Staphylococcus* [1], *Turicella* [1]) by the MALDI-TOF analysis.

Conclusions: Antimicrobial resistance differences among *Corynebacterium* species were noted with higher rates among the *C. jeikeium* and *C. urealyticum* species groups. Identification to the species level in clinical laboratories for *Corynebacterium* remains a challenge; however, the MALDI-TOF method appears to generate more complete and accurate identifications to be compared to antimicrobial-S profiles.

Organism/species (no. tested)	MIC_{50} (mg/L) / % susceptible				
	DAP	ERY	GENT	PEN	VANC
All *Corynebacterium* spp. (110)	≤0.06/99.1	>2/24.6	≤2/80.9	1/51.8	0.25/100.0
C. amycolatum (33)	≤0.06/100.0	>2/27.3	≤2/84.9	1/63.3	0.5/100.0
C. jeikeium (19)	0.12/100.0	2/10.5	>8/36.8	>4/0.0	0.5/100.0
C. striatum (37)	≤0.06/99.3	>2/24.3	≤2/97.3	1/56.8	0.25/100.0
C. urealyticum (5)	≤0.06/100.0	>2/40.0	>8/40.0	>4/0.0	0.5/100.0
Other *Corynebacterium* spp. (16)	≤0.06/100.0	2/31.3	≤2/100.0	0.25/93.8	0.25/100.0

DAP = daptomycin, ERY = erythromycin, GENT = gentamicin, PEN = penicillin, VAN = vancomycin.

Introduction

The genus *Corynebacterium* is composed of at least 67 species of which many are medically important. When Gram's stained, corynebacteria are slightly curved Gram-positive rods with one end slightly wider giving the appearance of a club shape and clusters on the slide give a Chinese letter type appearance. Corynebacteria are aerobic, non-spore forming, catalase positive and usually non-motile.

Many species of corynebacteria are part of the normal flora of the skin which makes the correlation with clinical significance (cause of infection) difficult when they are isolated from specimens. Corynebacteria should be identified to species level only if isolated from normally sterile body sites or when they are alone or the predominant pathogen from an adequately collected clinical sample, especially when matched with a Gram's stain report. Many automated systems and biochemical reaction methods have been developed and optimized for the identification of clinically relevant *Corynebacterium* species.

One new method for the identification of bacterial species under development is the use of Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry to measure the unique molecular fingerprint of a tested organism and compare to the extensive stored database to determine the identity to the species level.

Materials and Methods

Bacterial isolates: 121 *Corynebacterium* strains were collected between 2006 and 2010 from serious infections and submitted to the SENTRY Antimicrobial Surveillance Program. Fifteen participant medical centers were located in Europe (Belgium, France, Germany, Ireland, Italy, Spain, Sweden, Switzerland and United Kingdom) and 18 were located in the United States (USA). The isolates were collected primarily from bloodstream (53.6%) followed by lower numbers from skin and skin structure infections (17.3%), pneumonia (12.7%), and other infection sites.

Identification: Local microbiology laboratories in each medical center performed species identifications on coryneform pathogens as per local SOP criteria and methods. Upon receipt at JMI Laboratories (North Liberty, Iowa, USA) each strain was reviewed for colony morphology and tested by Gram's stain and Vitek or Vitek 2 using ANI cards for identification, as necessary. Definitive identification was assigned from testing on the Bruker Biotyper system using the MALDI-TOF methodology.

Susceptibility testing: Antimicrobial susceptibility testing was performed by Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods in validated, panels manufactured by Thermo Fisher Scientific (formerly TREK Diagnostics Systems; Cleveland, Ohio, USA). The test medium was cation-adjusted Mueller-Hinton broth supplemented with 2.5-5.0% lysed horse blood with incubation at 37 ºC for 24 hours. CLSI interpretive criteria were used to categorize the isolates as susceptible, intermediate and resistant as described in the M45 document; European Committee on Antimicrobial Susceptibility Testing (EUCAST) has not yet defined interpretive criteria. The quality control organism *Streptococcus pneumoniae* ATCC 49619 was concurrently tested; all QC results were within published limits.

Results

- Among the collection of *Corynebacterium* strains assembled, only 61 of the 121 strains (50.4%) were identified to the species level by the clinical microbiology laboratories due to local standard operating procedures or by limited abilities of the automated/biochemical identification systems utilized in the monitored European or USA medical centers.
- Accuracy to species level among these identified isolates as confirmed by the MALDI-TOF method was 73.8% (45/61) agreement, 23.0% (14/61) had incorrect *Corynebacterium* species assigned and 3.3% (2/61) had an incorrect genus (Table 1).
- Most common incorrect species identifications observed were *C. jeikeium* → *C. amycolatum* (5), *C. xerosis* → *C. striatum* (3) and *C. striatum* → *C. amycolatum* (2). Incorrect genus identification was observed only twice; once each as *C. afermentans* → *Brevibacterium casei* and *C. jeikeium* → *Staphylococcus warneri* (Table 1).
- Among the 60 unspeciated *Corynebacterium* strains studied, 51 (85.0%) demonstrated a good identification to species level using the MALDI-TOF method, while 9 strains (15.0%) were confirmed as other Gram-positive bacilli including; *Brevibacterium* (3), *Microbacterium* (3), *Dermabacter*, *Rhodococcus* and *Turicella* (one each, Table 1).
- Greatest in vitro potency against the collection (110 *Corynebacterium* spp; Table 2) was observed with tigecycline ($MIC_{50/90}$, ≤0.03/0.06 mg/L), daptomycin ($MIC_{50/90}$, ≤0.06/0.25 mg/L), linezolid ($MIC_{50/90}$, 0.25/0.25 mg/L) and vancomycin ($MIC_{50/90}$, 0.25/0.5 mg/L).
- Based on CLSI M45 breakpoint criteria for *Corynebacterium*, highest resistance rates were observed for ciprofloxacin (73.7%), followed by trimethoprim/sulfamethoxazole (71.6%), erythromycin (65.5%) and ceftriaxone (63.6%; Table 2).
- Analysis by sub-groups showed *C. jeikeium* and *C. urealyticum* species groups had reduced susceptibility rates for penicillin (0.0%) and gentamicin (36.8-40.0%) compared to other *Corynebacterium* spp. groups.

Table 1. Summary of identification (ID) discords between MALDI-TOF and other methods for *Corynebacterium* species isolates from European and USA medical centers between 2006-2010.

Corynebacterium misidentifications			Genus misidentifications		
Site ID		MALDI-TOF ID (no.)	Site ID		MALDI-TOF ID (no.)
C. jeikeium	→	*C. afermentans*	*C. afermentans*	→	*Brevibacterium casei*
C. jeikeium	→	*C. amycolatum* (5)	*C. jeikeium*	→	*Staphylococcus warneri*
C. minutissimum	→	*C. aurimucosum*	Unspeciated *Corynebacterium*	→	*Brevibacterium casei* (2)
C. pseudodiptheriticum	→	*C. propinquum*	Unspeciated *Corynebacterium*	→	*Brevibacterium iodinum*
C. striatum	→	*C. amycolatum* (2)	Unspeciated *Corynebacterium*	→	*Dermobacter hominis*
C. striatum	→	*C. aurimucosum*	Unspeciated *Corynebacterium*	→	*Microbacterium maritypicum*
C. xerosis	→	*C. striatum* (3)	Unspeciated *Corynebacterium*	→	*Microbacterium oxydans*
			Unspeciated *Corynebacterium*	→	*Microbacterium* spp.
			Unspeciated *Corynebacterium*	→	*Rhodococcus erythropolis*
			Unspeciated *Corynebacterium*	→	*Turicella otitidis*

Table 2. Activity of 13 antimicrobial agents when tested against *Corynebacterium* species isolates from European and USA medical centers between 2006-2010.

Organism (no. tested)/ Antimicrobial agent	MIC (mg/L)		% susceptible / % resistant
	MIC_{50}	MIC_{90}	CLSI[a]
All *Corynebacterium* spp. (110)			
Ceftriaxone	4	>8	26.4 / 63.6
Ciprofloxacin	>4	>4	20.9 / 73.7
Clindamycin	>2	>2	- [b] / -
Daptomycin	≤0.06	0.25	99.1 / -
Erythromycin	>2	>2	24.6 / 65.5
Gentamicin	≤2	>8	80.9 / 14.6
Linezolid	0.25	0.25	100.0 / -
Penicillin	1	>4	51.8 / 41.8
Piperacillin/tazobactam	16	>64	- / -
Tetracycline	≤2	>8	75.5 / 23.6
Tigecycline	≤0.03	0.06	- / -
Trimethoprim/sulfamethoxazole	>2	>2	28.4 / 71.6
Vancomycin	0.25	0.5	100.0 / -
Corynebacterium amycolatum (33)			
Ceftriaxone	1	>8	57.6 / 30.3
Ciprofloxacin	>4	>4	15.2 / 75.8
Clindamycin	>2	>2	- / -
Daptomycin	≤0.06	0.12	100.0 / -
Erythromycin	>2	>2	27.3 / 66.7
Gentamicin	≤2	8	84.9 / 3.0
Linezolid	0.12	0.25	100.0 / -
Penicillin	1	>8	63.6 / 33.3
Piperacillin/tazobactam	≤0.5	>64	- / -
Tetracycline	≤2	≤2	93.9 / 6.1
Tigecycline	≤0.03	0.12	- / -
Trimethoprim/sulfamethoxazole	>2	>2	36.4 / 63.6
Vancomycin	0.5	0.5	100.0 / -
Corynebacterium jeikeium (19)			
Ceftriaxone	>8	>8	0.0 / 100.0
Ciprofloxacin	>4	>4	10.5 / 89.5
Clindamycin	>2	>2	- / -
Daptomycin	0.12	0.25	100.0 / -
Erythromycin	2	>2	10.5 / 63.2
Gentamicin	>8	>8	36.8 / 63.2
Linezolid	0.25	0.5	100.0 / -
Penicillin	>4	>4	0.0 / 94.8
Piperacillin/tazobactam	>64	>64	- / -
Tetracycline	≤2	>8	84.2 / 15.8
Tigecycline	≤0.03	0.06	- / -
Trimethoprim/sulfamethoxazole	>2	>2	0.0 / 100.0
Vancomycin	0.5	0.5	100.0 / -
Corynebacterium striatum (37)			
Ceftriaxone	8	>8	0.0 / 94.6
Ciprofloxacin	>4	>4	8.1 / 83.8
Clindamycin	>2	>2	- / -
Daptomycin	≤0.06	0.12	97.3 / -
Erythromycin	>2	>2	24.3 / 70.3
Gentamicin	≤2	4	97.3 / 0.0
Linezolid	0.25	0.25	100.0 / -
Penicillin	1	>4	56.8 / 29.7
Piperacillin/tazobactam	16	>64	- / -
Tetracycline	>8	>8	46.0 / 51.4
Tigecycline	≤0.03	0.06	- / -
Trimethoprim/sulfamethoxazole	>2	>2	13.5 / 86.5
Vancomycin	0.25	0.25	100.0 / -
Corynebacterium urealyticum (5)			
Ceftriaxone	4	-	40.0 / 60.0
Ciprofloxacin	>4	-	0.0 / 100.0
Clindamycin	>2	-	- / -
Daptomycin	≤0.06	-	100.0 / -
Erythromycin	>2	-	40.0 / 60.0
Gentamicin	>8	-	40.0 / 60.0
Linezolid	0.25	-	100.0 / -
Penicillin	>32	-	0.0 / 100.0
Piperacillin/tazobactam	>64	-	- / -
Tetracycline	≤2	-	100.0 / 0.0
Tigecycline	≤0.03	-	- / -
Trimethoprim/sulfamethoxazole	>2	-	0.0 / 100.0
Vancomycin	0.5	-	100.0 / -
Other *Corynebacterium* spp. (16)[c]			
Ceftriaxone	1	4	50.0 / 18.8
Ciprofloxacin	≤0.5	>4	81.3 / 27.8
Clindamycin	>2	>2	- / -
Daptomycin	≤0.06	0.12	100.0 / -
Erythromycin	2	>2	31.3 / 56.3
Gentamicin	≤2	≤2	100.0 / 0.0
Linezolid	0.25	0.5	100.0 / -
Penicillin	0.25	1	93.8 / 6.2
Piperacillin/tazobactam	1	16	- / -
Tetracycline	≤2	>8	87.5 / 12.5
Tigecycline	≤0.03	0.06	- / -
Trimethoprim/sulfamethoxazole	1	>2	87.5 / 12.5
Vancomycin	0.25	0.25	100.0 / -

a. Criteria as published by the FDA or CLSI [2012].
b. "-" = No criteria defined.
c. Includes *Corynebacterium afermentans* (3), *C. aurimucosum* (2), *C. glucuronolyticum* (1), *C. initans* (1), *C. minutissimum* (2), *C. propinquum* (3), *C. pseudodiptheriticum* (3), and *C. singulare* (1).

Conclusions

- Identification of *Corynebacterium* to the species level remains a challenge for clinical microbiology laboratories, due to the compromised accuracy of the automated/biochemical systems in use. The MALDI-TOF method appears to generate more complete and accurate identifications to the species level.
- This contemporary, well characterized collection of *Corynebacterium* strains can be used to predict effective empiric therapeutic choices and shows the higher rates of antimicrobial resistance for selected agents among the *C. jeikeium* and *C. urealyticum* species groups.

References

1. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition.* Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2010). *M45-A2. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria: second edition*. Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute (2012). *M100-S22. Performance standards for antimicrobial susceptibility testing: 22nd informational supplement.* Wayne, PA: CLSI.
4. Funke G, Bernard KA (2011). *Coryneform Gram-Positive Rods.* In *Manual of Clinical Microbiology.* Washington, D.C.: ASM Press.
5. Wolk DM, Dunne WM, Jr. (2011). New technologies in clinical microbiology. *J Clin Microbiol* 49: S62-S67.