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SUSCEPTIBILITY

In Vitro Antibiotic Susceptibility Pattern of Non-*diphtheriae Corynebacterium* Isolates in Ontario, Canada, from 2011 to 2016

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ABSTRACT Non-*diphtheriae Corynebacterium*-associated disease has been increasingly observed and often presents a conundrum to the treating physician. Analysis of antibiotic susceptibility testing data for 1,970 clinical *Corynebacterium* isolates received between 2011 and 2016 revealed that empirical drug treatment options are limited to vancomycin and linezolid. *Corynebacterium striatum* was the most frequently observed species during this study period, along with *C. amycolatum* and *C. pseudodiphtheriticum/C. propinquum*. Low levels of susceptibility to penicillin (14.5%), erythromycin (15.1%), and clindamycin (8.7%) were observed for non-*diphtheriae Corynebacterium* species, while 3.0% of isolates were not susceptible to daptomycin. Similarly, 26.9% and 38.1% of *Corynebacterium* isolates were susceptible to ciprofloxacin and trimethoprim-sulfamethoxazole, respectively. Our data show much lower susceptibility to penicillin than previously reported in the literature and an increasing number of isolates resistant to daptomycin, highlighting the need for continued antibiotic surveillance studies for appropriate patient management and treatment success.

KEYWORDS *Corynebacterium*, antibiotic resistance, penicillin resistance, susceptibility testing

Corynebacterium species are Gram-positive catalase-positive rod-shaped bacteria often referred to as "diphtheroids." Effective vaccination programs have resulted in a decrease in *Corynebacterium diphtheriae*-related infection cases in the last 50 years. However, in the past 2 decades, there has been a rise in disease due to non-*diphtheriae Corynebacterium* species, with a variety of infections reported, including skin and soft tissue infections, prosthetic joint infections, bacteremia, respiratory infections, pneumonia, meningitis, surgical site infections, urinary tract infections, peritonitis, and endocarditis (1–3). Of additional concern, some of the non-*diphtheriae Corynebacterium* species have been shown to be resistant to multiple classes of antibiotics, thus potentially limiting effective empirical treatment (1–4).

This study presents the *in vitro* susceptibility profiles of 1,970 isolates of non-*diphtheriae Corynebacterium* species to 19 different antibiotics that were tested at the Public Health Ontario Laboratory (PHOL; Ontario, Canada) from 2011 to 2016.

RESULTS AND DISCUSSION

This study evaluated the antibiotic susceptibility test results of 1,970 isolates of nondiphtherial corynebacteria that were submitted to the PHOL from 2011 to 2016. Within this group, a total of 42 different *Corynebacterium* species were represented, as well as 24 isolates that could only be identified to the genus level and were reported as *Corynebacterium* species. The majority of the isolates belonged to *C. striatum* (47%), followed by *C. pseudodiphtheriticum/C. propinquum* (10.96%), *C. amycolatum* (9.64%), *C.*

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afermentans subsp. *afermentans* (5%), *C. minutissimum* (*4%*), *C. jeikeium* (4%), *C. coyleae* (3%), and *C. urealyticum* (3%). Other *Corynebacterium* species tested during this time period included *C. resistens*, *Corynebacterium* CDC group G, *C. accolens*, *C. glucuronolyticum*, and others (see Table S1 in the supplemental material). In our study, *C. striatum* was the most commonly submitted species for identification and susceptibility testing, whereas other reports have noted *C. amycolatum* as the most prevalent nondiphtherial corynebacteria reported (1, 5). Despite being the most commonly submitted species, there was no significant difference in the proportions of *C. striatum* isolates submitted over the years ($P = 0.45$) (data not shown). Additionally, no significant trend was observed for the top five commonly encountered corynebacterial species in terms of the proportion submitted for identification and susceptibility testing over time.

Isolates were predominantly cultured from blood ($n = 479$ [24.3%]), followed by sputum ($n = 382$ [19.4%]) (Table S2). A number of isolates were also recovered from bone ($n = 97$ [4.9%]) and synovial fluid ($n = 41$ [2.0%]) (Table S2).

Between 2011 and 2016, 28 different corynebacterial species were isolated and identified from blood, with *C. striatum* as the most frequent (26.2%), followed by *C. afermentans* (19.8%), *C. coyleae* (9.8%), and *C. minutissimum* (7.7%). Blood culture was the most common source for *C. mucifaciens* (92% [$n = 13$]), *C. afermentans* (83% [$n = 89$]), and *C. coyleae* (81% [$n = 58$]). The most frequently recovered species from sputum specimens were *C. striatum* (63%) and *C. pseudodiphtheriticum/C. propinquum* (35%). For *C. striatum*, the chief specimen sources were sputum (26%), blood (14%), tissue (11%), bone (9%), wounds (6%), bronchoalveolar lavage (BAL) and/or pleural fluid (3%), and peritoneal fluid (2%).

Of the 1,970 isolates submitted for susceptibility testing, 1,010 isolates were recovered from male patients (51.3%), 739 isolates were from female patients (37.5%), and 221 specimens were from a patient of unknown sex. While 15 (0.7%) specimens were recovered from patients less than 1 year of age, 24 (1.2%) specimens were from patients age 1 to 17 years, 844 (42.8%) specimens were from patients age 18 to 64 years, and 1,070 (54.3%) specimens were from patients age 65 or older; the remaining 17 isolates did not have the patient's age on the submission form.

The susceptibility profiles of the 1,970 nondiphtherial corynebacterial isolates showed that they were universally susceptible to vancomycin and linezolid (Table 1; see also Data Set S1 in the supplemental material). The majority of isolates (>90%) were susceptible to rifampin, gentamicin, and quinupristin-dalfopristin. Interestingly, 45 out of the 1,959 isolates tested for daptomycin had an MIC of ≥2 mg/liter, which is considered "nonsusceptible" based on the current CLSI interpretative criteria (6). Overall as a group, the nondiphtherial corynebacteria in this study demonstrated low rates of susceptibility to penicillin (14.5%), erythromycin (15.12%), clindamycin (8.7%), and ciprofloxacin (26.9%). Based on the EUCAST clinical breakpoint (30), 27.7% of nondiphtherial corynebacterial isolates were susceptible to moxifloxacin.

Among the nondiphtherial corynebacteria in this study, *C. pseudodiphtheriticum/C. propinquum* isolates showed the highest susceptibility to penicillin (95.8%), whereas *C. amycolatum* (10.5%), *C. afermentans* subsp. *afermentans* (1.1%), *C. minutissimum* (3.9%), *C. coyleae* (1.7%), *C. urealyticum* (3.8%), and *C. aurimucosum* (5.9%) isolates were the least susceptible to penicillin (Table 1). Only a single isolate of *C. striatum* (1/931 isolates) and none of the *C. jeikeium* ($n = 76$) or *C. resistens* ($n = 19$) isolates were susceptible to penicillin. Susceptibility to penicillin and other beta-lactams has reduced in the last 2 decades among certain nondiphtherial corynebacteria, including *C. striatum* (7–10). Consistent with our findings, a study from Japan showed that none of the *C. striatum* ($n = 22$) isolates recovered from blood specimens were susceptible to penicillin (9). However, a recent Canadian study (4) reported the susceptibility rate of penicillin for the *Corynebacterium* genus to be 77%, which is much higher than the susceptibility rate (14.5%) found in our study. The difference in the susceptibility rate may be partially explained because Bernard et al. (4) used CLSI 2010 interpretative criterion (susceptibility [S], ≤1 mg/liter) versus the revised CLSI 2015 interpretative criterion (S ≤ 0.12 mg/liter) for penicillin that was used in our study. Our study also did

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TABLE 1 *In vitro* susceptibilities of the *Corynebacterium* isolates received at the PHOL from 2011 to 2016

Antimicrobial agent by organism	No. of isolates	MIC range	MIC_{50}	MIC_{90}	% S[a]
All *Corynebacterium* spp.	1,970				
Penicillin	1,970	≤0.06 to >8	4	>8	14.5
Ampicillin	1,969	≤0.12 to >8	2	>8	ND
Oxacillin	1,969	≤0.25 to >4	>4	>4	ND
Erythromycin	1,970	≤0.25 to >4	>4	>4	15.1
Clindamycin	1,958	≤0.5 to >2	>2	>2	8.7
Ciprofloxacin	1,959	≤1 to >2	>2	>2	26.9
Levofloxacin	1,968	≤0.25 to >4	>4	>4	ND
Moxifloxacin[b]	1,968	≤0.25 to >4	>4	>4	27.7
Tetracycline	1,969	≤2 to >16	≤2	>16	64.2
Vancomycin	1,970	≤0.25 to 2	0.5	0.5	100
Trimethoprim-sulfamethoxazole	1,967	≤0.5 to >4	4	>4	38.1
Tigecycline	1,967	≤0.03 to >0.5	0.12	0.25	ND
Rifampin	1,968	≤0.5 to>4	≤0.5	≤0.5	91.4
Quinupristin-dalfopristin	1,969	≤0.5 to >4	≤0.5	1	93.3
Linezolid	1,969	≤1 to 2	≤1	≤1	100
Gentamicin	1,970	≤2 to >16	≤2	4	93.3
Daptomycin	1,959	≤0.5 to >4	≤0.5	≤0.5	97.7
Chloramphenicol	1,960	≤2 to >16	4	>16	ND
Nitrofurantoin	1,969	≤32 to >64	>64	>64	ND
C. striatum	931				
Penicillin	931	≤0.06 to >8	8	>8	0.1
Ampicillin	930	≤0.12 to >8	4	>8	ND
Oxacillin	930	≤0.25 to >4	>4	>4	ND
Erythromycin	931	≤0.25to >4	>4	>4	14.6
Clindamycin	922	≤0.5 to >2	>2	>2	5.4
Ciprofloxacin	926	≤1 to >2	>2	>2	4.6
Levofloxacin	930	≤0.25 to >4	>4	>4	ND
Moxifloxacin[b]	929	≤0.25 to >4	>4	>4	5.2
Tetracycline	930	≤2 to >16	>16	>16	34.7
Vancomycin	931	≤0.25 to 1	0.5	0.5	100
Trimethoprim-sulfamethoxazole	928	≤0.5 to >4	>4	>4	11.5
Tigecycline	929	≤0.03 to >0.5	0.12	0.25	ND
Rifampin	930	≤0.5 to >4	≤0.5	>4	85.6
Quinupristin-dalfopristin	930	≤0.5 to 2	≤0.5	≤0.5	99.4
Linezolid	930	≤1	≤1	≤1	100
Gentamicin	931	≤2 to >16	≤2	4	92.8
Daptomycin	924	≤0.5 to>4	≤0.5	≤0.5	98
Chloramphenicol	924	≤2 to >16	≤2	16	ND
Nitrofurantoin	930	≤32 to >64	>64	>64	ND
C. amycolatum	190				
Penicillin	190	≤0.06 to >8	1	>8	10.5
Ampicillin	190	≤0.12 to >8	1	>8	ND
Oxacillin	190	≤0.25 to >4	>4	>4	ND
Erythromycin	190	≤0.25 to >4	>4	>4	15.8
Clindamycin	189	≤0.5 to>2	>2	>2	20.5
Ciprofloxacin	189	≤1 to >2	>2	>2	26.8
Levofloxacin	190	≤0.25 to >4	>4	>4	ND
Moxifloxacin[b]	190	≤0.25 to >4	4	>4	27.4
Tetracycline	190	≤2 to >16	≤2	16	85.8
Vancomycin	190	≤0.25 to 1	0.5	0.5	100
Trimethoprim-sulfamethoxazole	190	≤0.5 to >4	1	>4	66.8
Tigecycline	189	≤0.03 to >0.5	0.12	0.25	ND
Rifampin	190	≤0.5 to >4	≤0.5	≤0.5	98.4
Quinupristin-dalfopristin	190	≤0.5 to 2	≤0.5	≤0.5	99.5
Linezolid	190	≤1	≤1	≤1	100
Gentamicin	190	≤2 to >16	≤2	4	93.2
Daptomycin	190	≤0.5 to 2	≤0.5	≤0.5	99.5
Chloramphenicol	189	≤2 to >16	≤2	16	ND
Nitrofurantoin	190	≤32 to >64	>64	>64	ND

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TABLE 1 (Continued)

Antimicrobial agent by organism	No. of isolates	MIC range	MIC_{50}	MIC_{90}	% S[a]
C. pseudodiphtheriticum/C. propinquum	216				
Penicillin	216	≤0.06 to 4	≤0.06	≤0.06	95.8
Ampicillin	216	≤0.12 to 4	≤0.12	≤0.12	ND
Oxacillin	216	≤0.25 to >4	0.5	1	ND
Erythromycin	216	≤0.25 to >4	>4	>4	21.3
Clindamycin	216	≤0.5 to >2	>2	>2	20.8
Ciprofloxacin	216	≤1 to >2	≤1	>2	78.7
Levofloxacin	216	≤0.25 to >4	1	>4	ND
Moxifloxacin[b]	216	≤0.25 to >4	≤0.25	>4	79.2
Tetracycline	216	≤2 to >16	≤2	≤2	98.6
Vancomycin	216	≤0.25 to 2	0.5	0.5	100
Trimethoprim-sulfamethoxazole	216	≤0.5 to >4	≤0.5	4	87.5
Tigecycline	216	≤0.03 to >0.5	0.06	0.25	ND
Rifampin	216	≤0.5 to 1	≤0.5	≤0.5	100
Quinupristin-dalfopristin	216	≤0.5 to 1	≤0.5	≤0.5	100
Linezolid	216	≤1 to 2	≤1	≤1	100
Gentamicin	216	≤2	≤2	≤2	100
Daptomycin	216	≤0.5 to >4	≤0.5	≤0.5	99.5
Chloramphenicol	216	≤2 to >16	4	>16	ND
Nitrofurantoin	216	≤32 to >64	>64	>64	ND
C. afermentans subsp. *afermentans*	89				
Penicillin	89	≤0.06 to >8	4	>8	1.12
Ampicillin	89	≤0.12 to >8	4	8	ND
Oxacillin	89	≤0.25 to >4	>4	>4	ND
Erythromycin	89	2 to >4	>4	>4	0
Clindamycin	89	1 to >2	>2	>2	0
Ciprofloxacin	89	≤1 to >2	>2	>2	30.3
Levofloxacin	89	≤0.25 to >4	>4	>4	ND
Moxifloxacin[b]	89	≤0.25 to >4	>4	>4	31.5
Tetracycline	89	≤2 to >16	≤2	8	87.6
Vancomycin	89	≤0.25 to 1	≤0.25	0.50	100
Trimethoprim-sulfamethoxazole	89	≤0.5 to>4	2	>4	55.1
Tigecycline	89	≤0.03 to >0.5	0.12	0.25	ND
Rifampin	89	≤0.5 to >4	≤0.5	≤0.5	94.4
Quinupristin-dalfopristin	89	≤0.5 to >4	1	4	51.7
Linezolid	89	≤1 to 2	≤1	≤1	100
Gentamicin	89	≤2 to >16	≤2	≤2	98.9
Daptomycin	89	≤0.5 to 4	≤0.5	≤0.5	97.8
Chloramphenicol	89	≤2 to >16	8	>16	ND
Nitrofurantoin	89	≤32 to >64	>64	>64	ND
C. jeikeium	76				
Penicillin	76	0.5 to >8	>8	>8	0
Ampicillin	76	≤0.12 to >8	>8	>8	ND
Oxacillin	76	0.5 to >4	>4	>4	ND
Erythromycin	76	≤0.25 to >4	>4	>4	11.8
Clindamycin	76	≤0.5 to >2	>2	>2	2.6
Ciprofloxacin	75	≤1 to >2	>2	>2	35.5
Levofloxacin	75	≤0.25 to >4	>4	>4	ND
Moxifloxacin[b]	76	≤0.25 to >4	4	>4	35.5
Tetracycline	76	≤2 to >16	≤2	≤2	92.1
Vancomycin	76	≤0.25 to 2	0.50	1	100
Trimethoprim-sulfamethoxazole	76	≤0.5 to >4	>4	>4	18.4
Tigecycline	76	≤0.03 to >0.5	0.12	0.25	ND
Rifampin	76	≤0.5 to >4	≤0.5	≤0.5	97.4
Quinupristin-dalfopristin	76	≤0.5 to 4	≤0.5	2	89.5
Linezolid	76	≤1	≤1	≤1	100
Gentamicin	76	≤2 to >16	≤2	>16	81.6
Daptomycin	75	≤0.5 to >4	≤0.5	1	89.5
Chloramphenicol	75	≤2 to >16	4	16	ND
Nitrofurantoin	76	≤32 to >64	>64	>64	ND

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TABLE 1 (Continued)

Antimicrobial agent by organism	No. of isolates	MIC range	MIC_{50}	MIC_{90}	% S[a]
C. minutissimum	76				
Penicillin	76	≤0.06 to >8	1	8	3.9
Ampicillin	76	≤0.12 to >8	1	4	ND
Oxacillin	76	2 to >4	>4	>4	ND
Erythromycin	76	≤0.25 to >4	>4	>4	19.7
Clindamycin	76	≤0.5 to >2	>2	>2	1.3
Ciprofloxacin	74	≤1 to >2	≤1	>2	55.3
Levofloxacin	76	≤0.25 to >4	≤0.25	>4	ND
Moxifloxacin[b]	76	≤0.25 to >4	≤0.25	>4	61.8
Tetracycline	76	≤2 to >16	≤2	4	92.1
Vancomycin	76	≤0.25 to 1	0.50	0.50	100
Trimethoprim-sulfamethoxazole	76	≤0.5 to >4	2	>4	61.8
Tigecycline	76	≤0.03 to >0.5	0.06	0.25	ND
Rifampin	75	≤0.5 to >4	≤0.5	≤0.5	96.1
Quinupristin-dalfopristin	76	≤0.5 to 4	≤0.5	1	93.4
Linezolid	76	≤1 to >8	≤1	≤1	98.7
Gentamicin	76	≤2 to >16	≤2	≤2	96.1
Daptomycin	75	≤0.5 to >4	≤0.5	1	92.1
Chloramphenicol	75	≤2 to >16	4	8	ND
Nitrofurantoin	76	≤32 to >64	64	>64	ND
C. coyleae	58				
Penicillin	58	≤0.06 to >8	4	>8	1.7
Ampicillin	58	≤0.12 to >8	4	8	ND
Oxacillin	58	2 to >4	>4	>4	ND
Erythromycin	58	≤0.25 to >4	>4	>4	3.5
Clindamycin	58	≤0.5 to >2	>2	>2	3.5
Ciprofloxacin	57	≤1 to >2	>2	>2	27.6
Levofloxacin	58	≤0.25 to >4	>4	>4	ND
Moxifloxacin[b]	58	≤0.25 to >4	4	>4	29.3
Tetracycline	58	≤2 to >16	≤2	≤2	93.1
Vancomycin	58	≤0.25 to 2	0.5	0.50	100
Trimethoprim-sulfamethoxazole	58	≤0.5 to >4	4	>4	48.3
Tigecycline	58	≤0.03 to >0.5	0.06	0.25	ND
Rifampin	58	≤0.5 to >4	≤0.5	≤0.5	91.4
Quinupristin-dalfopristin	58	≤0.5 to 4	2	4	43.1
Linezolid	58	≤1 to 2	≤1	≤1	100
Gentamicin	58	≤2	≤2	≤2	100
Daptomycin	57	≤0.5 to >4	≤0.5	≤0.5	93
Chloramphenicol	58	≤2 to >16	4	>16	ND
Nitrofurantoin	58	≤32 to >64	>64	>64	ND
C. urealyticum	52				
Penicillin	52	0.12 to >8	>8	>8	3.8
Ampicillin	52	≤0.12 to >8	>8	>8	ND
Oxacillin	52	1 to >4	>4	>4	ND
Erythromycin	52	≤0.25 to >4	>4	>4	1.9
Clindamycin	51	≤0.5 to >2	>2	>2	3.8
Ciprofloxacin	51	≤1 to >2	>2	>2	3.8
Levofloxacin	52	≤0.25 to >4	>4	>4	ND
Moxifloxacin[b]	52	≤0.25 to >4	>4	>4	3.8
Tetracycline	52	≤2 to >16	≤2	≤2	92.3
Vancomycin	52	≤0.25 to 2	0.50	1	100
Trimethoprim-sulfamethoxazole	52	≤0.5 to >4	>4	>4	1.9
Tigecycline	52	≤0.03 to >0.5	0.06	0.12	ND
Rifampin	52	≤0.5 to >4	≤0.5	≤0.5	92.3
Quinupristin-dalfopristin	52	≤0.5 to 1	≤0.5	≤0.5	100
Linezolid	52	≤1	≤1	≤1	100
Gentamicin	52	≤2 to >16	≤2	>16	55.7
Daptomycin	51	≤0.5	≤0.5	≤0.5	100
Chloramphenicol	52	≤2 to >16	8	>16	ND
Nitrofurantoin	52	>64	>64	>64	ND

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TABLE 1 (Continued)

Antimicrobial agent by organism	No. of isolates	MIC range	MIC_{50}	MIC_{90}	% S[a]
C. aurimucosum	51				
Penicillin	51	0.12 to >8	1	>8	5.8
Ampicillin	51	0.25 to >8	1	8	ND
Oxacillin	51	2 to >4	>4	>4	ND
Erythromycin	51	≤0.25 to >4	>4	>4	11.7
Clindamycin	51	1 to >2	>2	>2	0
Ciprofloxacin	51	≤1 to >2	>2	>2	43.1
Levofloxacin	51	≤0.25 to >4	4	>4	ND
Moxifloxacin[b]	51	≤0.25 to >4	2	>4	43.1
Tetracycline	51	≤2 to >16	≤2	4	90.2
Vancomycin	51	≤0.25 to 1	0.50	0.50	100
Trimethoprim-sulfamethoxazole	51	≤0.5 to >4	2	>4	62.7
Tigecycline	51	≤0.03 to 0.25	0.06	0.12	ND
Rifampin	51	≤0.5	≤0.5	≤0.5	100
Quinupristin-dalfopristin	51	≤0.5 to 4	≤0.5	1	92.2
Linezolid	51	≤1	≤1	≤1	100
Gentamicin	51	≤2 to 4	≤2	≤2	100
Daptomycin	51	≤0.5 to 3	≤0.5	≤0.5	98
Chloramphenicol	51	≤2 to >16	4	8	ND
Nitrofurantoin	51	≤32 to >64	64	>64	ND

[a]% S, percentage of susceptible isolates; ND, not determined.
[b]EUCAST breakpoints were applied for moxifloxacin (≤0.5 mg/liter, susceptible; >0.5 mg/liter, resistant).

not include any *C. diphtheriae* isolates, which constituted 35% of the 595 isolates tested in the Bernard et al. study. Another explanation could be differences in geographical distribution of various clones of nondiphtherial corynebacteria. For example, our data demonstrate that *C. pseudodiphtheriticum/C. propinquum* was mostly susceptible to beta-lactams (95%); however, this differs from a report from Brazil in which high rates of penicillin resistance were observed (11). In our study, the MIC_{50} and MIC_{90} for ampicillin among *C. striatum* were 4 mg/liter and >8 mg/liter, respectively. On the other hand, Soriano et al. (8) reported the MIC_{50} and MIC_{90} values of ampicillin for *C. striatum* to be 0.5 mg/liter and 2 mg/liter, respectively, in a study conducted on isolates collected in Spain. Similarly, Martínez-Martínez et al. (12) reported the MIC_{50} and MIC_{90} of ampicillin for *C. striatum* ($n = 86$) of 1 mg/liter and 2 mg/liter, respectively, on isolates collected in Spain.

Susceptibility to erythromycin varied among *Corynebacterium* spp., with none of the *C. afermentans* subsp. *afermentans* isolates displaying susceptibility ($n = 89$), while only 1.9% of *C. urealyticum* and 21.2% of *C. pseudodiphtheriticum/C. propinquum* isolates were found to be susceptible (Table 1). Similarly, clindamycin susceptibility ranged from 0% to 20.8% among nondiphtherial corynebacteria. Resistance to erythromycin among nondiphtherial corynebacteria has been reported previously (1, 4, 7, 8) and is mainly attributed to the presence of the *ermX* gene (3). The erythromycin and clindamycin susceptibilities in this study were lower than those recently reported by Bernard et al. (4).

Among non-*diphtheriae Corynebacterium* species in this study, 26.9% of isolates were susceptible to ciprofloxacin. Ciprofloxacin had the lowest activity against *C. striatum* (4.6%) and *C. urealyticum* (3.8%) and the highest activity against *C. pseudodiphtheriticum/C. propinquum* (Table 1). The activities of moxifloxacin and levofloxacin were similar to that of ciprofloxacin for all nondiphtherial corynebacteria. *C. urealyticum* has been known to cause acute and chronic urinary tract infections (UTIs) and may lead to bacteremia. Consistent with our findings, a previous study had reported that 96.1% of *C. urealyticum* isolates were found to be nonsusceptible to ciprofloxacin (13). Fluoroquinolones are extensively used to treat UTIs (14); however, isolates of *C. urealyticum* in this study displayed resistance to several major drug classes, including ciprofloxacin. Ciprofloxacin resistance has also been reported among *C. glucuronolyticum* (15). In our study, 73% of the *C. glucuronolyticum* ($n = 15$) isolates were susceptible to ciprofloxacin.

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The recent study by Bernard et al. (4) reported the rates of susceptibility to ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole to be 69.9%, 87.1%, and 73.1%, respectively, for isolates identified as *Corynebacterium* since the 1990s (4). However, in our study, the susceptibilities among all the *Corynebacterium* isolates are lower for ciprofloxacin, tetracycline, and trimethoprim, with susceptibility rates of 27.5%, 64%, and 38%, respectively. The differences in the percentage of susceptible isolates to ciprofloxacin seen here are in line with what has been observed in other recent studies by Hahn et al. (2) and Soriano et al. (16) and may due to differences in the number of isolates and, in particular, the years of collection.

Resistance to gentamicin has been reported previously in *C. striatum* and is associated with the presence of the *aac(3)-XI* gene encoding AAC(3)-XI, a new aminoglycoside 3-*N*-acetyltransferase (17). In our study, 92.8% of *C. striatum* isolates were found to be susceptible to gentamicin. Among all species of *Corynebacterium*, *C. urealyticum* (55.8%), *C. jeikeium* (81.2%), and *Corynebacterium* CDC group G (85.7%) showed the lowest susceptibility against gentamicin.

As stated earlier, 45 (3.0%) out of 1,959 *Corynebacterium* isolates tested for daptomycin showed an MIC of ≥2 mg/liter and are considered nonsusceptible isolates. Daptomycin has been increasingly suggested as an important therapeutic alternative for multidrug-resistant *Corynebacterium* species, including *C. striatum* and *C. jeikeium* (18). However, daptomycin-nonsusceptible isolates of *C. striatum* ($n = 16$), *C. minutissimum* ($n = 5$), *C. jeikeium* ($n = 7$), *C. coyleae* ($n = 3$), and a single isolate each of *C. amycolatum*, *C. aurimucosum*, *Corynebacterium* CDC group G, *C. durum*, *C. imitans*, *C. pseudodiphtheriticum*, *C. singular*, *C. simulans*, *C. riegelii*, *C. resistens*, and other *Corynebacterium* species were identified in this study. Daptomycin resistance has been previously described in *C. jeikeium* (19), as well as in patients with *C. striatum* infection receiving daptomycin treatment (20–23). Significantly, this study shows that daptomycin nonsusceptibility is not limited to *C. striatum* and *C. jeikeium* but is present in other nondiphtherial corynebacteria as well. Details regarding treatment were not available to us; therefore, it cannot be concluded if these isolates developed resistance while the patients had received treatment with daptomycin.

Tigecycline is another antibiotic that has been suggested in recent reports to be a therapeutic alternative for the treatment of complicated infections caused by these corynebacteria (24, 25). There are no breakpoints for resistance as defined by CLSI or EUCAST for tigecycline. The MIC_{50} and MIC_{90} were 0.12 mg/liter and 0.25 mg/liter, respectively, for all the corynebacteria in this study.

Antibiotic susceptibility testing data showed that 24 *Corynebacterium* species within the genus were completely susceptible to quinupristin-dalfopristin, and more than 90% of *C. striatum*, *C. riegelii*, *C. accolens*, *C. jeikeium*, *C. minutissimum*, *C. imitans*, *C. aurimucosum*, *Corynebacterium* CDC group G, and *C. amycolatum* isolates were susceptible. However, none of the *C. glycinophilum* ($n = 3$) isolates were susceptible to quinupristin-dalfopristin, and lower rates of susceptible isolates were found among *C. coyleae* (43%), *C. kroppenstedtii* (50%), *C. afermentans* (50%), *C. auris* (67%), and *C. tuberculostearicum* (67%).

C. minutissimum was previously considered to be susceptible to many different classes of antibiotics (1, 7, 9, 26), but most of the isolates in this study were resistant to more than four drug classes, including penicillin, erythromycin, gentamicin, and ciprofloxacin. Similarly, *C. striatum* was found to be highly multidrug resistant, with varied percentages of resistance to penicillin, erythromycin, gentamicin, ciprofloxacin, and daptomycin. Other frequently reported *Corynebacterium* species that were resistant to more than four drug classes included *C. amycolatum*, *C. aurimucosum*, *C. afermentans*, *C. jeikeium*, *C. urealyticum*, and *C. coyleae*. *C. pseudodiphtheriticum/C. propinquum* isolates were mostly susceptible to beta-lactams (95%), vancomycin, linezolid, tetracycline, and gentamicin, though the susceptibility to macrolides and lincosamides was greatly reduced.

Our findings demonstrate that there are differences in antibiotic susceptibility among *Corynebacterium* species and that there is considerable antibiotic resistance in

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some species. Laboratories should identify clinically relevant *Corynebacterium* isolates to the species level, which may provide additional clues regarding antibiotic susceptibility. The increased use of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification systems in clinical laboratories will greatly enhance front-line laboratory capacity and capability in identifying *Corynebacterium* organisms to the species level.

One of the limitations of our study is that it is not known whether *Corynebacterium* isolates recovered from specimens were truly the causative agents of the infection. The clinical relevance of nondiphtherial corynebacteria, including *C. striatum*, has been well documented since their identification (27), and *Corynebacterium* species, such as *C. striatum*, have been linked to multiple outbreaks in hospitals and nosocomial environments (28, 29). Therefore, although not often considered pathogenic, it is now clear that under the right circumstances, nondiphtherial corynebacteria can be the causative agents of infection. Therefore, in the absence of other organisms, the isolation of *Corynebacterium* spp. from sterile sites and/or from immunocompromised patients could be clinically relevant, and clinicians will need to decide whether to treat the infection based on clinical presentation as well as other findings. As nondiphtherial corynebacteria exhibit varied susceptibilities to many antibiotics, empirical treatment options are limited to vancomycin and linezolid. Multidrug resistance is on the rise among the nondiphtherial corynebacteria, with a decrease in susceptibility rates among species, such as *C. striatum* and *C. minutissimum*, which were previously reported to have low or no resistance (10, 26). As susceptibility varies among different nondiphtherial corynebacteria, and many commonly encountered *Corynebacterium* spp. are multidrug resistant, it is important to perform susceptibility testing particularly for those isolates that are recovered from sterile sites in order to manage patients appropriately. However, identification and susceptibility testing on these isolates can be time-consuming; therefore, regular surveillance studies may be useful in monitoring changes in trends of resistance and providing better empirical treatment options to patients.

MATERIALS AND METHODS

In Ontario, hospital and community laboratories generally identify *Corynebacterium* to the genus level and refer isolates that are deemed clinically significant for species-level identification and susceptibility testing to the PHOL. All isolates of nondiphtherial corynebacteria recovered from specimens received by the PHOL between 2011 and 2016 were included in the study. Since the data were deidentified prior to analysis, it is not known what the proportion of isolates is from the same patients. From 2011 to 2014, bacterial identification was primarily done by biochemical tests (3, 5), and when a definitive identification could not be made, 16S rRNA gene sequence analysis was performed. From 2015 onward, MALDI-TOF MS (MALDI BioTyper; Bruker) was primarily used for identification combined with select biochemical tests and 16S rRNA sequencing for definitive identification when needed. Prior to the implementation of MALDI-TOF MS for the identification of *Corynebacterium* spp., biochemical testing and 16S rRNA gene sequencing were unable to reliably differentiate between *C. pseudodiphtheriticum* and *C. propinquum*. As a result, these isolates were reported as *C. pseudodiphtheriticum/C. propinquum*. When a definitive species-level identification could not be made, organisms were reported out as *Corynebacterium* species (not *Corynebacterium diphtheriae*).

Antibiotic susceptibility testing was performed using commercial broth microdilution Sensititre GPALL1F plates (Trek Diagnostic Systems, Thermo Fisher Scientific), and the susceptibility results for each antibiotic were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (6). MIC testing was performed for the following antibiotics: erythromycin (0.25 to 4 mg/liter), penicillin (0.06 to 8 mg/liter), vancomycin (0.25 to 32 mg/liter), gentamicin (2 to 16 mg/liter), daptomycin (0.5 to 4 mg/liter), ciprofloxacin (1 to 2 mg/liter), moxifloxacin (0.25 to 4 mg/liter), levofloxacin (0.25 to 4 mg/liter), ampicillin (0.125 to 8 mg/liter), oxacillin (0.25 to 4 mg/liter), nitrofurantoin (32 to 64 mg/liter), rifampin (0.5 to 4 mg/liter), tigecycline (0.03 to 0.5 mg/liter), clindamycin (0.5 to 2 mg/liter), tetracycline (2 to 16 mg/liter), chloramphenicol (2 to 16 mg/liter), trimethoprim-sulfamethoxazole (0.5 to 4 mg/liter), quinupristin-dalfopristin (0.5 to 4 mg/liter), and linezolid (1 to 8 mg/liter). The CLSI does not have interpretative criteria for moxifloxacin, and therefore, clinical breakpoints for moxifloxacin were used from the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (30).

The number of isolates susceptible to each antibiotic was determined per year. The Cochran-Armitage test was used to measure trends in antimicrobial resistance over time, with a P value of less than 0.05 considered statistically significant. The Mann-Kendall test at a 5% significance level was used to analyze trends of *Corynebacterium* species submitted over time. Statistical analyses were done using R version 3.3.2.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01776-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.

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