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MINIREVIEW

The Genus *Corynebacterium* and Other Medically Relevant Coryneform-Like Bacteria

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Catalase-positive Gram-positive bacilli, commonly called "diphtheroids" or "coryneform" bacteria were historically nearly always dismissed as contaminants when recovered from patients, but increasingly have been implicated as the cause of significant infections. These taxa have been underreported, and the taxa were taxonomically confusing. The mechanisms of pathogenesis, especially for newly described taxa, were rarely studied. Antibiotic susceptibility data were relatively scant. In this minireview, clinical relevance, phenotypic and genetic identification methods, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) evaluations, and antimicrobial susceptibility testing involving species in the genus *Corynebacterium* and other medically relevant Gram-positive rods, collectively called coryneforms, are described.

This minireview will be limited to "club-shaped" Gram-positive, medically relevant species of the genus *Corynebacterium* and of the "irregular" Gram-positive coryneforms mostly but not exclusively from the suborder *Micrococcineae*. Those coryneforms were selected because they most closely resemble *Corynebacterium* species *sensu stricto* phenotypically and so can be difficult to differentiate from members of that genus. Coryneforms described here are rare, opportunistic human pathogens, and they are Gram-positive bacilli or coccobacilli. Nearly all are catalase positive, and they express a range of pigments and metabolic processes.

Clinical relevance. These coryneform bacteria are increasingly being recognized as causing opportunistic disease under specific circumstances, such as in patients who are immunocompromised, have prosthetic devices, or have been in hospitals/nursing homes for long-term periods of time (20). The most significant pathogen of this group remains *Corynebacterium diphtheriae*, the primary cause of diphtheria, a disease which has essentially disappeared from developed countries after implementation of universal vaccination that targets the primary virulence factor, the diphtheria toxin (DT) (52). Comprehensive reviews outline epidemiology, the diseases caused by *C. diphtheriae*, vaccine recommendations, immune response to the vaccine at population levels, and the function and pathogenesis of DT (20, 52), with other manifestations shown in Table 1.

The zoonotic agents *Corynebacterium pseudotuberculosis* (the cause of caseous lymphadenitis primarily in sheep and goats) (3), transmitted to humans by contact with diseased animals (38) and *C. ulcerans*, historically thought to cause disease in humans after contact with contaminated milk or farm animals, but more recently linked to transmission between humans and their companion pets such as cats and dogs (13, 51), are also able to produce diphtheria toxin and cause diphtheria-like disease in humans. It is therefore recommended that those in frequent contact with animals (veterinarians, animal care technologists, or farm workers) ensure that they maintain adequate vaccine coverage against diphtheria. *Corynebacterium* species that have caused disease in humans with some also considered as zoonotic agents are reviewed in Table 1. Infections or case reports involving coryneforms are outlined in Table 2.

Determining clinical relevancy: when to identify to genus and species. Studies have demonstrated that *Corynebacterium* species and various coryneforms, particularly those taxa found as part of normal skin flora, are prominent contaminants of clinical materials, although occasionally it is difficult to correctly decide in a timely fashion if recovery of such bacteria implies contamination or has clinical relevance (53). Therefore, it is recommended that clinicians should attempt to identify any of the taxa described in this minireview to the genus and species level if the organism is isolated (i) from normally sterile body sites, e.g., blood culture (with the exception if recovered from only one of multiple specimens taken becoming positive), (ii) from adequately collected clinical material where *Corynebacterium* species or coryneforms are the predominant organism, and (iii) if recovered from urine specimens, e.g., *C. urealyticum*, is the sole bacterium encountered with a bacterial count of $>10^4$/ml or if it is the predominant organism recovered and the total bacterial count is $>10^5$/ml (19, 53).

Taxonomy and habitat. The genus *Corynebacterium* consists of 88 validly published species, including 3 species in press or published since the *Bergey's Manual* chapter was compiled (6). Of these, 53 species are occasional or extremely rare causes of infection in humans or are transmitted to humans by zoonotic contact, with the remaining 35 species having been recovered solely from animals or birds, the environment, water, foodstuffs or synthetic materials. Many medically relevant species are thought to be part of human skin flora, including *C. amycolatum*, *C. jeikeium*, as well as some of the other lipophilic species, many of which have been found to be resistant to multiple drug classes and can cause significant and occasionally fatal disease, particularly in immunocompromised patients (37). Over the past 30 years, all *Corynebacterium* species that had genetic and chemotaxonomic features inconsistent with those currently attributed to this genus have

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TABLE 1 Medically relevant *Corynebacterium* species[a]

Taxon[b]	Infection or site recovered in humans or animals[c]
C. accolens (lipophile)	Blood, osteomyelitis, abscess
C. afermentans subsp. *afermentans* (FR)	Blood, abscess (MDR)
C. afermentans subsp. *lipophilum* (FR) (lipophile)	Blood, abscess
C. ammoniagenes	Feces
C. amycolatum [zoonotic] (FR)	Blood, variety of sterile sites, cellulitis, wounds, sepsis endocarditis, peritonitis
C. appendicis (lipophile)	Abscess
C. argentoratense	Throat, respiratory tract, blood culture
C. atypicum	Unknown
C. aurimucosum	Blood culture, urogenital sites, complications of pregnancy
C. auris	Healthy and diseased ears
C. bovis [zoonotic] (lipophile) (FR)	Blood culture (human); mastitis (cows)
C. canis [zoonotic]	Wound (human) after dog bite
C. confusum	Abscess, bone, blood culture
C. coyleae	Blood cultures, abscess, sepsis, ulcer
C. diphtheriae [zoonotic], biovar mitis, gravis, belfanti; biovar intermedium (lipophile) (FR)	Diphtheria, throat, respiratory specimens, cutaneous lesions, blood culture, abscesses, sequelae from diphtheria toxin (humans, animals)
C. durum	Throat, respiratory specimens, blood cultures
C. falsenii [zoonotic]	Blood culture, CSF, mouth of eagle
C. freiburgense [zoonotic]	Wound (human) after dog bite
C. freneyi	Wound, abscess, ulcer, sperm, female genital tract, blood
C. glucuronolyticum [zoonotic]	Prostitis, urethritis; male urogenital tract, semen, blood, peritoneal fluid, disk fluid (humans); urogenital tract (pigs)
C. hansenii	Pus
C. imitans	Pharyngeal infection, throat, blood culture
C. jeikeium [zoonotic] (lipophile) (FR)	Endocarditis, sepsis, prosthetic device infections; blood cultures, heart valves, bone marrow, bile, other
C. kroppenstedtii (lipophile)	Granulomatous mastitis, breast abscess; otitis externa
C. kutscheri [zoonotic]	Soft tissue, skin infection (human) after rat bite; found in mice and rats
C. lipophiloflavum (lipophile)	Vaginal secretion
C. macginleyi (lipophile) (FR)	Conjunctivitis, ocular infections, bacteremia, endocarditis, aortic abscess, urine, tracheostomy site
C. massiliense	Hip fluid from orthopedic prosthesis
C. mastitidis-like [zoonotic] (lipophile)	Cataract; diabetic retinopathy, ocular sites
C. matruchotii	Oral cavity
C. minutissimum (FR)	Bacteremia, meningitis, endocarditis, cellulitis, abscesses, peritonitis, pyelonephritis
C. mucifaciens	Bacteremia, wound, joint fluid, abscess, peritoneal fluid, tissue biopsy, cavitary pneumonia
C. mycetoides	Skin ulcer
C. pilbarense	Ankle aspirate
C. propinquum	Blood cultures, respiratory specimens, endocarditis, osteosynthesis aspirate, pleural effusion
C. pseudodiphtheriticum (FR)	Respiratory specimens after pneumonia; exudative pharyngitis; blood, wound, keratitis, conjunctivitis, urine, peritoneal fluid, cervical necrosis, ear, synovial fluid
C. pseudotuberculosis [zoonotic] (FR)	Lymphadenitis (human); potential for diphtheria-like disease (humans); caseous lymphadenitis (sheep, goats, other animals)
C. pyruviciproducens	Groin abscess, blood culture, synovial fluid
C. resistans (lipophile)	Blood cultures, bronchial aspirates, cellulitis (MDR)
C. riegelii	UTI, urosepsis, blood culture
C. simulans	Abscess, boil, axillar lymph node, blood, bile
C. singulare	Semen, blood culture
C. sputi (lipophile)	Pneumonia-sputum
C. stationis	Blood cultures, stool, also seawater
C. striatum (FR)	Bacteremia, pneumonia, bronchitis, endocarditis, osteomyelitis, necrotic fasciitis, abscess, wound (MDR)
C. sundsvallense	Blood culture, intrauterine device, draining sinus
C. thomsenii	Pleural fluid, also from air sample
C. timonense	Blood culture, endocarditis
C. tuberculostearicum (lipophile) (includes most CDC group G-2 strains)	Blood culture, lymph node, urethra, skin, urine, peritoneum/peritonitis, urogenital tract, abscess, CSF, Hickman catheter site, synovial fluid
C. tuscaniense	Blood culture/endocarditis
C. ulcerans [zoonotic] (FR)	Diphtheria-like disease; pharyngitis, sinusitis, tonsillitis, pulmonary nodules, skin ulcers (humans); bovine mastitis; otherwise infections in cats, dogs, monkeys, squirrels, otters, orcas, camels, lions, pigs, and goats

(Continued on following page)

TABLE 1 (Continued)

Taxon[b]	Infection or site recovered in humans or animals[c]
C. urealyticum (lipophile) [zoonotic] (FR)	Urinary tract infections especially in patients with underlying genitourinary disorders; also blood cultures, endocarditis, respiratory specimens associated with pneumonia; soft tissue infection; cause of UTIs in cats, dogs, and other animals (MDR)
C. ureicelerivorans (lipophile)	Blood culture; ascitic fluid
C. xerosis [zoonotic] (FR)	Ear, brain abscess, osteomyelitis; cause of abscess, arthritis, mastitis, abortion, septicaemia, osteomyelitis in goats, pigs, cows, and sheep

[a] The *Corynebacterium* species shown here are infrequently described pathogens with the exception of the frequently reported (FR) pathogens. Data in this table are from references 4, 6, and 19.
[b] The taxon and whether the species is described as being lipophilic or zoonotic are shown. FR, frequently reported, defined as >10 cases reported in the literature for the species or provisional name.
[c] MDR, described as usually multidrug resistant; CSF, cerebral spinal fluid; UTI, urinary tract infection.

been reassigned to other genera, or conversely, relevant taxa assigned to other genera and those with *Corynebacterium*-like features, have been added to the genus (6). Centers for Disease Control and Prevention had published identification schemes and methods for *Corynebacterium* species and coryneforms, based on phenotypic features of strains from their culture collection and gave *Corynebacterium*-like taxa provisional "group names" (24, 25). Nearly all have since been assigned to validly named genera and species, but occasionally, case reports or GenBank accession deposits that use these provisional names are published. The provisional "group names" relevant to the genus *Corynebacterium* include the following (provisional name shown first, and the current species name shown in parentheses after the provisional name): CDC group 6 (*C. accolens*), CDC group ANF-1 (*C. afermentans*), CDC group ANF-3 (*C. propinquum*), CDC group D-1 (*C. pseudodiphtheriticum*), CDC group D-2 (*C. urealyticum*), CDC group F-2 (*C. amycolatum*), CDC group G-1 (*C. macginleyi*), CDC group G-2 (assignable to *C. tuberculostearicum*), CDC group I-1 (assignable to *C. striatum*), CDC group I-2 (*C. amycolatum*), and CDC group J-K (*C. jeikeium*). Some strains of CDC group F-1 from recognized culture collections with a presence on the Internet have not yet been assigned to a species. The provisional "group names" relevant to catalase-positive coryneforms include the following: CDC group A-1, A-2 (*Oerskovia* or *Cellulosimicrobium* spp.), CDC groups A-3, A-4 (*Cellulomonas* spp.), CDC groups A-4, A-5 (*Microbacterium* spp.), CDC group B-1 and B-3 (primarily *Brevibacterium casei*), and CDC groups 3 and 5 (*Dermabacter hominis*) (6, 8, 12, 19, 20).

TABLE 2 Medically relevant coryneforms

Family[a]	Genus (no. of species in genus[b])	Medically relevant species
Brevibacteriaceae	*Brevibacterium* (26)	*B. casei*, *B. massiliense*, *B. mcbrellneri*, *B. otitidis*, *B. paucivorans*, *B. ravenspurgense*, *B. sanguinis*
Cellulomonadaceae	*Cellulomonas* (18)	*C. hominis*, *C. denverensis*
Dermabacteriaceae	*Brachybacterium* (14)	*Brachybacterium* species
	Dermabacter (1)	*D. hominis*
	Helcobacillus (1)	*H. massiliensis*
Intrasporangiaceae	*Janibacter* (6)	*J. melonis*, *Janibacter* species
	Knoellia (3)	*Knoellia* species
Microbacteriaceae	*Curtobacterium* (9)	*Curtobacterium* species
	Leifsonia (15)	*L. aquatica*
	Microbacterium (73)	*M. aurum*, *M. binotii*, *M. esteraromaticum*, *M. foliorum*, *M. hominis*, *M. hydrocarbonoxydans*, *M. lacticum*, *M. laeveniformis*, *M. oxydans*, *M. paraoxydans*, *M. phyllosphaerae*, *M. resistans*, *M. schleiferi*, *M. trichothecenolyticum*, *M. testaceum*, *M. thalassium*
	Pseudoclavibacter (3)	*Pseudoclavibacter* species[c]
Micrococcaceae	*Arthrobacter* (67)	*A. albus*, *A*, *aurescens*, *A. creatinolyticus*, *A. cumminsii*, *A. luteolus*, *A. oxydans*, *A. protophormiae*, *A. sanguinis*, *A. schlerome*, *A. woluwensis*
	Rothia (6)	*R. aeria*, *R. dentocariosa*, *R. muciloginosa*
Promicromonosporaceae	*Cellulosimicrobium* (3)	*C. cellulans*, *C. funkei*
Unassigned[a]	*Exiguobacterium* (13)	*E. acetylicum*, *E. auranticum*

[a] Families are from the phylum *Actinobacteria* suborder *Micrococcineae*, except for the genus *Exiguobacterium* (phylum *Firmicutes*, order *Bacillales*, unassigned *Bacillales* family XII genus *incertae sedis*).
[b] Numbers of species are derived from the List of Prokaryotic names with Standing in Nomenclature (LPSN) found at http://www.bacterio.cict.fr/ on 20 January 2012. The genus *Zimmermannella* is considered a later synonym of the genus *Pseudoclavibacter*. *Cellulosimicrobium cellulans* and *C. funkei* contain strains formerly identified as *Oerskovia xanthineolytica* and *O. turbata*, respectively. Data were derived from references 8, 9, 12, 15, 18, 23, 27, 30, 31, 32, 33, 34, 36, 39, 40, 42, 44, and 55.
[c] Human cases caused by *Pseudoclavibacter*-like bacteria could not be assigned to existing species (31, 33).

Recovery of *Corynebacterium* spp. and coryneforms from clinical material. Diphtheria is very rarely detected in developed countries, so laboratory expertise in the recovery of the toxin-producing species has declined in recent years (35). Those laboratories that lack recent experience in recovering diphtheria toxin-producing species should carefully review selective procedures such as using cystine-tellurite blood or freshly prepared Tinsdale agars or consider sending the specimen to a reference center. Selection for other species in the genus has been enhanced by using fosfomycin-enriched blood agar or by incorporating 0.1 to 1% Tween 80 to blood agar for lipophilic species, but otherwise, *Corynebacterium* species usually grow well under standard growth conditions. The coryneforms described here should grow well on usual laboratory media and standard 37°C growth conditions with the possible exception of some coryneforms, which may grow better at 30°C or under 5% CO_2 (*Rothia*) (19).

Identification methods. Three identification approaches are reviewed, with the caveat that occasionally isolates will be encountered that cannot be readily assigned to a validly named genus and species, no matter which method(s) is used, and such strains should be studied further at a reference center. Approaches such as cellular fatty acid composition analysis are useful to discern *Corynebacterium* species *sensu stricto* from coryneforms (5), and other cell wall components (such as menaquinones, phospholipids, or mycolates) used to determine genus level features (6) are otherwise not described here, as such work is done only at specific reference centers. Detection of genes associated with diphtheria toxin production and expression of DT are done by PCR-based methods and the Elek test, respectively (14, 16).

(i) Phenotype-based identification methods: usefulness and limitations. Generally, identification of these taxa is initiated by describing Gram stain observations, details of the colony at 24 h (or later for slower growing species), and catalase and oxidase reactions and establishing whether the isolate is lipophilic (4, 19, 20). Lipophilic *Corynebacterium* species grow poorly in broth or as tiny pinpoint colonies in 24 h on standard plate agar but grow more luxuriantly if the agar or broth is supplemented with an exogenous lipid such as sterile Tween 80. Lipophilia, or fatty acid auxotrophy, appears to be linked to the absence of a microbial type I fatty acid synthase gene (45). Lipophilic *Corynebacterium* species may grow poorly in manual or automated phenotypic test systems and so give rise to poorly interpretable results. No medically relevant coryneform described in Table 2 has demonstrated lipophilic characteristics.

Corynebacterium species and coryneforms exhibit a variety of metabolic processes, that is, they can be fermentative, oxidative, assimilative, or nonreactive to most or all substrates on a species-specific basis. Biochemical reactions highlighting such properties have been outlined in brief (6, 19). Utilization of sugars using macrovolume substrate testing are now only very rarely used, primarily by reference centers and are particularly effective for those species that are slowly reactive in substrates or require lipid supplementation (4). These incur greater preparation and quality control costs with inherently slower turn-around times; the results are then compared with the results of standard identification schemes (6, 19). Biochemical testing is otherwise performed using commercially available manual identification test strips, such as API Coryne (for *Corynebacterium* spp. and some coryneforms), API CH50 plus API 20E (bioMérieux, for some coryneforms) or the RapID CB Plus method (Remel). Detection of enzymes incorporated into the API Coryne or API ZYM (bioMérieux) panels are usually included as part of the *species nova* descriptions for many of these taxa. Identification is obtained by comparing reactions to identification schemes manually or to proprietary databases such as API WEB (bioMérieux). Automated systems used to characterize these bacteria include Vitek 2 (bioMérieux) (41) and the Biolog systems (17) coupled to interpretation software. Only 2 evaluations of these products or platforms have been published in the past 10 years, that is, for the Vitek 2 ANC (41) and the API Coryne panels (2). Both studies describe significant problems in correctly identifying rarer species from Table 1 and for most taxa in Table 2.

Limitations for using phenotypic testing as the sole identification method for these taxa include the following. (i) The bacteria are misidentified or not identified compared to identification using genetically based methods (1, 29), with >30% of isolates being incorrectly identified without ancillary testing or not identified at all (2). (ii) There are a limited number of substrates in the panels, which does not provide a sufficient number of unique features to differentiate among all relevant taxa. This limitation is exacerbated for taxa of bacteria that are metabolically inert or slow growing or have similar expression of phenotypic results, such as *C. amycolatum* and *C. xerosis.* (iii) The underlying databases have only been infrequently updated and so lack entries for newly described species. Panels, whether manual or automated, generally lack data to identify most taxa in Table 2 correctly with or without the use of ancillary tests (2). (iv) The panels are expensive, with cost increasing with the number of substrates found in a panel. (v) The results for some systems are obtained in days, rather than hours. In summary, phenotypic testing as the sole identification approach is reasonably satisfactory for common *Corynebacterium* species that grow well in 24 to 48 h and are reactive (not inert), with respect to substrates found in commonly used test systems. It is recommended that when definitive identification of these species is required, that strains be analyzed using molecular identification methods, where biochemical test results corroborate genetic characterization compared with commonly used identification algorithms (6, 19).

(ii) Sequence-based identification methods. 16S rRNA gene sequencing is recommended as a starting point for characterization of all of the taxa described in this minireview (6). 16S rRNA gene sequencing discerns among most species in the genus *Corynebacterium*, as these are separated by 1.3% or greater variance (when comparing the whole gene sequence) if criteria of >98.7% identity (for members of the same species) is used (43). Exceptions include the following *Corynebacterium* species with ≤2% variance to each other: *C. afermentans*, *C. coyleae*, and *C. mucifaciens* (<2%); *C. aurimucosum*, *C. minutissimum*, and *C. singulare* (<2%); *C. sundsvallense* and *C. thomssenii* (<1.5%); *C. ulcerans* and *C. pseudotuberculosis* (<1% to each other, both <2% to *C. diphtheriae*); *C. propinquum* and *C. pseudodiphtheriticum* (<2%); *C. xerosis*, *C. freneyi*, and *C. hansenii* (<2%); and *C. macginleyi* and *C. accolens* (<2%) (19). Definitive identification can usually be resolved by sequencing of a secondary gene target when 16S rRNA gene sequencing provides ambiguous answers. Partial or full sequencing of the *rpoB* gene is the most widely used approach for characterizing *Corynebacterium* species, as data for this gene have been deposited for most but not all type strains (6, 28). All coryneforms described in Table 2 have 16S rRNA gene sequence data for at least the type strains deposited in public do-

main databases such as GenBank, but secondary gene targets have not been comprehensively studied.

(iii) Identification using the MALDI-TOF approach. Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analysis is rapidly becoming a routinely used tool in many microbiology laboratories, whereby proteins liberated from bacteria are ionized and detected by a mass spectrometer (MS), the spectrum is analyzed, and its pattern is compared to entries found in a database, giving rise to a degree of match (1, 29). This technology is touted as being revolutionary, because it does not require extensive training or expertise in MS or chemistry to use and the cost for consumables is described as less than US$0.50 per sample, excluding the cost of the MALDI-TOF instrument (21). Protein fingerprints are deemed to provide identification to genus and species with an accuracy which approaches that of genetic methods (1, 29). There is limited data evaluating the use of this tool for the identification of *Corynebacterium* spp. and coryneforms, and these studies have been done mostly using the Bruker system and its proprietary database, Biotyper (Bruker), rather than the Shimadzu system and the Saramis database (bioMérieux). Konrad et al. (29) found that 99.1% of *C. diphtheriae*, *C. ulcerans*, or *C. pseudotuberculosis* isolates were correctly identified with high scores by the MALDI-TOF system, with 100% positive and negative predictive values for the 3 diphtheria toxin-producing species. Alatoom et al. found that 87% of 2 *Corynebacterium*-like strains previously identified using *rpoB* or 16S rRNA gene sequencing as the gold standard methods were correctly identified by the Biotyper system, although in some instances, they had high scores to more than one closely related species, such as *C. propinquum* and *C. pseudodiphtheriticum*, or were correctly identified but had lower-than-expected scores (1). Identification of coryneforms described in Table 2 has not as yet been rigorously evaluated with respect to the MALDI-TOF method, although many of those species can be found in the Biotyper version 3.2.1 database.

Other: full genome sequencing. A number of *Corynebacterium* species have had complete genomes sequenced. Salient highlights from completed genome projects include the following. (i) Virulence genes associated with the production of neuraminidase and phospholipase D in *C. ulcerans* and *C. pseudotuberculosis* were elucidated (48, 50). (ii) The lack of mycolates in species may be due to the loss of a condensase gene cluster and mycolate reductase gene (46). (iii) Genes located on the pET44827 plasmid are linked to production of a black pigment, which is thought to provide protection in the high hydrogen peroxide concentration environment of the vagina, in pigmented strains of *C. aurimucosum* (49). (iv) A specialized urease locus in *C. urealyticum* facilitates alkalization of urine and formation of struvite stones (47). (v) An intact, toxin-producing strain of *C. diphtheriae* has 11 more pathogenicity islands and 37 additional regions, compared with C7(−), a nontoxigenic strain of that species (26). Among medically relevant coryneforms, stress-responsive systems that regulated the biofilm phenotype have been characterized from a mucoid strain of *Rothia mucilaginosa* (54).

Typing systems. A variety of typing methods have been used to study *C. diphtheriae*. The typing methods used include amplified fragment length polymorphisms (AFLP), whole-genome restriction fragment length polymorphism (RFLP) analysis, random amplification of polymorphic DNA (RAPD), ribotyping (the preferred method for many years), pulsed-field gel electrophoresis (PFGE), multilocus enzyme electrophoresis (MEE), spoligotyping or PCR single-strand conformational polymorphism (PCR-SSCP) as reviewed previously (6). More recently, a standardized MLST (multilocus sequence typing) method based on analysis of short sequences derived from 7 housekeeping alleles has been described, whereby data, sent by Internet to http://pubmlst.org/perl/mlstdbnet/mlstdbnet.pl?file=cd_isolates.xml is assigned a sequence type and can be compared with a global collection of diphtheria strains (7). Molecular typing schemes have not been widely used for the medically relevant coryneforms shown in Table 2.

Serologic tests. Antibodies directed against diphtheria toxin and detected primarily by enzyme immune assays are used in serologic tests. Targeted antibody levels of ≥0.1 IU/ml serum are thought to confer protection, whereas levels of <0.01 IU/ml indicate a susceptible host and levels of 0.01 to 0.1 IU/ml indicate partially immune individuals; elderly populations in particular are at risk for waning immunity toward diphtheria (22). This can pose a potentially significant public health risk and is one underlying reason for reemergence and massive outbreak of this disease in Russia and its satellite states in the 1990s (52). Booster doses of toxoid should be administered at 10-year intervals after childhood immunization is completed.

Antimicrobial susceptibility testing. The Clinical and Laboratory Standards Institute (CLSI) has published test conditions and interpretative criteria for susceptibility testing of *Corynebacterium* spp. using a broth microdilution method (10, 11). That method uses bacterial suspensions equivalent to a 0.5 McFarland standard incubated in cation-adjusted Mueller-Hinton broth with 2 to 5% (vol/vol) lysed horse blood at 35°C in ambient air for up to 48 h. Interpretative categories for the MICs obtained are presently available for 16 antimicrobial agents. There are limited articles describing use of the disk diffusion method, but the Etest method (bioMérieux) has been used to evaluate MICs for these bacteria, including with direct comparison to broth microdilution, as recently reviewed (6). *Corynebacterium* species that have expressed multidrug resistance for most or some clinical strains tested include *C. amycolatum*, *C. argentoratense*, *C. auris*, *C. coyleae*, *C. glucuronolyticum*, *C. jeikeium*, *C. minutissimum*, *C. pseudodiphtheriticum*, *C. resistens*, *C. striatum*, *C. tuberculostearicum*, *C. urealyticum*, *C. ureicelerivorans*, as well as representatives of *C. afermentans* and *C. aurimucosum* (6, 37). Otherwise, multidrug resistance among the infrequently recovered *Corynebacterium* species is rarely observed. Various mechanisms of resistance, such as mutations in the *gyrA* gene linked to quinolone resistance in *C. striatum*, *C. amycolatum*, and *C. macginleyi* have been reviewed (6). The presence of the *ermX* methylase gene has been definitively linked to the resistance phenotype macrolide-lincosamide-streptogramin B (MLSb), expressed as resistance to erythromycin and clindamycin and associated with resistance to other antimicrobial agents, including chloramphenicol and trimethoprim-sulfamethoxazole (37). To date, daptomycin resistance has been reported in only one strain of *C. jeikeium*, and so far, no resistance has been reported for any *Corynebacterium* strains tested for linezolid and vancomycin (6). Antimicrobial susceptibility in the coryneforms described here is not predictable based on identification to genus and species. There were no specific guidelines for these taxa, but CLSI methods for *Corynebacterium* species were extended to *Brevibacterium*, *Cellulomonas*, *Dermabacter*, *Leifsonia*, *Microbacterium*, *Oerskovia*, *Rothia*, and *Turicella* as well as *Arcanobacterium* (10). Vancomycin-resistant coryneform bacteria or coryneform bacteria with elevated MICs

have been reported, and it is deemed inappropriate to recommend glycopeptides as first-line drugs for the treatment of infections, with some coryneform bacteria (e.g., *Microbacterium resistens*) being intrinsically vancomycin resistant (19).

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