Characterization and Comparison of Invasive Corynebacterium diphtheriae Isolates from France and Poland

E. Farfour, A. Badell, A. Zasada, H. Hotzel, H. Tomaso, S. Guillot, and N. Guiso

Institut Pasteur, Unité Prévention et Thérapies Moléculaires des Maladies Humaines, National Reference Centre of Toxigenic Corynebacteria, CNRS-URA 3012, Paris, France; National Institute of Public Health-National Institute of Hygiene, Department of Bacteriology, Warsaw, Poland; and Friedrich Loeffler Institute, Institute of Bacterial Infections and Zoonoses, Jena, Germany

Corynebacterium diphtheriae, the agent of diphtheria, is rarely responsible for bacteremia. However, high numbers of bacteremia have been reported in countries with extensive immunization coverage. Here, we used molecular and phenotypic tools to characterize and compare 42 invasive isolates collected in France (including New Caledonia) and Poland over a 23-year period.

Forty-two isolates were analyzed: 13 were from Poland, 5 were from New Caledonia (a French overseas territory), and 24 were from mainland France. Biotyping using the API Coryne strip demonstrated that 50% were of the mitis biotype, 47.6% were of the gravis biotype, and 2.4% were of the belfanti biotype. All isolates were nontoxicogenic. All were susceptible to erythromycin, but 6 of them had reduced susceptibility to penicillin G (MIC range, 0.38 to 0.5 mg/liter), 1 was resistant to tetracycline (MIC, 24 mg/liter), and 9 were resistant to rifampin (MIC, >24 mg/liter) (Table 1).

The 42 isolates were distributed among 11 sequence types (STs) (Table 1), 4 of which were new (ST193, ST194, ST195, and ST196). As the 11 different STs shared at the most only five alleles, they did not belong to the same clonal complex (Fig. 1). Three STs included 34 isolates (81%). A predominant ST was found to be localized in each region: ST8 in Poland, ST82 in New Caledonia, and ST130 in mainland France.

All 13 Polish isolates were collected after 2004. They were all of the gravis biotype and ST8, but 6 of them had reduced susceptibility to penicillin G. Interestingly, ST8 belongs to a clonal complex associated with the former Soviet Union (FSU) epidemic. However, isolates from the FSU epidemic carried the tox gene whereas invasive Polish isolates did not. The FSU epidemic was caused by low vaccination coverage, the introduction of a new clone, and the socioeconomic changes that followed the breakup of the Soviet Union. Poland was spared from this diphtheria epidemic by higher immunization coverage than that of the FSU.

In New Caledonia, all isolates were of the gravis biotype. Four out of 5 isolates were ST82 and all of these were collected between 2002 and 2006. A single isolate collected in 1991 was ST39. Notably, ST39 was also recovered in 1999 in the United States (in California and Maine), where it caused at least two other bacteremias, one case of arthritis and one upper respiratory tract infection.

The 24 isolates from mainland France were distributed among 8 STs. This high diversity can be explained by the larger number of isolates from this region. Most isolates were ST130, including all 17 isolates that were collected between 1991 and 1993 and isolates resistant to rifampin, which suggests a clonal diffusion of the re-
sistance to this antibiotic. ST128 (gravis biotype isolate collected in 1987) was also recovered in Brazil (a nontoxigenic gravis biotype isolate collected from a blood culture in 2003) (11). This isolate forms a clonal complex with ST80, which was previously assigned to a nontoxigenic gravis biotype isolate that caused a respiratory infection in Brazil (Rio de Janeiro) in 1999 and to another isolate from Canada (11). To our knowledge, ST153 and ST156 have not been previously described elsewhere.

Consistent with previous reports, invasive isolates were of the gravis or mitis biotype and were nontoxigenic. Two previous analyses using ribotyping were performed on 29 invasive isolates from France and Poland (8, 13). MLST results are in accordance with ribotyping results, which identified a predominant ribotype in each region. Ribotyping had previously been the “gold standard” for molecular characterization of C. diphtheriae (3). However, this method is long and laborious, interpreting its results is subjective, and the data are difficult to transfer. MLST could therefore replace ribotyping as the method of choice for characterizing C. diphtheriae isolates.

Our data demonstrate that (i) a predominant ST could be implicated in unrelated invasive infections in each geographic area, (ii) isolates of the predominant ST were able to cause bacteremia for a limited period (e.g., ST130 between 1991 and 1993) or for a long period of time (e.g., ST5 in Poland since 2004), (iii) some STs were located in geographically remote regions, reflecting their ability to spread (e.g., ST8 and ST39), and (iv) isolates causing bacteremia can be responsible for noninvasive diseases, including cases of diphtheria (e.g., ST8 isolates).

Although diphtheria is rare in countries with high rates of immunization, it is still necessary to monitor infections due to tox-
genic and nontoxigenic \textit{C. diphtheriae}. New typing tools such as MLST can provide a better understanding of the diversity of this pathogen.

**ACKNOWLEDGMENTS**

We thank P. Riegel (Hôpitaux universitaires de Strasbourg, Strasbourg, France), A. Berlioz, B. Garin, and P. Coudene (Institut Pasteur, Nouvelle-Caledonie, France), O. Patey (Centre hospitalier de Villeneuve Saint-George, Villeneuve Saint-Georges, France), and A. Lefèche (Institut Pasteur Paris, France) for providing some of the isolates for this study.

This work was supported by the Institut Pasteur Foundation (Paris, France), the CNRS-URA 3012, and the Institut national de Veille Sanitaire (Saint Maurice, France).

**REFERENCES**

1. Bolt F, et al. 2010. Multilocus sequence typing identifies evidence for recombination and two distinct lineages of \textit{Corynebacterium diphtheriae}. J. Clin. Microbiol. 48:4177–4185.

2. Efstratiou A, George RC. 1999. Laboratory guidelines for the diagnosis of infections caused by \textit{Corynebacterium diphtheriae} and \textit{C. ulcerans}. World Health Organization. Commun. Dis. Public Health 2:250–257.

3. Grimont PA, et al. 2004. International nomenclature for \textit{Corynebacterium diphtheriae} ribotypes. Res. Microbiol. 155:162–166.

4. Gubler J, Huber-Schneider C, Gruner E, Altwegg M. 1998. An outbreak of nontoxigenic \textit{Corynebacterium diphtheriae} infection: single bacterial clone causing invasive infection among Swiss drug users. Clin. Infect. Dis. 27:1295–1298.

5. Hauser D, Popoff MR, Kiredjian M, Boquet P, Bimet F. 1993. Polymerase chain reaction assay for diagnosis of potentially toxigenic \textit{Corynebacterium diphtheriae} strains: correlation with ADP-ribosylation activity assay. J. Clin. Microbiol. 31:2720–2723.

6. Kakis A, et al. 2006. Cluster of invasive infections, including endocarditis, caused by nontoxigenic \textit{Corynebacterium diphtheriae}. South Med. J. 99:1144–1145.

7. Mishra B, Dignan RJ, Hughes CF, Hendel N. 2005. \textit{Corynebacterium diphtheriae} endocarditis—surgery for some but not all! Asian Cardiovasc. Thorac. Ann. 13:119–126.

8. Patey O, et al. 1997. Clinical and molecular study of \textit{Corynebacterium diphtheriae} systemic infections in France. Coryne Study Group. J. Clin. Microbiol. 35:441–445.

9. Pimenta FP, et al. 2008. A PCR for dtxR gene: application to diagnosis of non-toxigenic and toxigenic \textit{Corynebacterium diphtheriae}. Mol. Cell. Probes 22:189–192.

10. Romney MG, et al. 2006. Emergence of an invasive clone of nontoxigenic \textit{Corynebacterium diphtheriae} in the urban poor population of Vancouver, Canada. J. Clin. Microbiol. 44:1625–1629.

11. Viguetti SZ, et al. 2011. Multilocus sequence types of invasive \textit{Corynebacterium diphtheriae} isolated in the Rio de Janeiro urban area, Brazil. Epidemiol. Infect. [Epub ahead of print.] doi:10.17/50950268811000963.

12. Walory J, Grzesiowski J, Hryniewicz W. 2001. The prevalence of diphtheria immunity in healthy population in Poland. Epidemiol. Infect. 126:225–230.

13. Zasada AA, Baczewska-Rej M, Wardak S. 2010. An increase in nontoxigenic \textit{Corynebacterium diphtheriae} infections in Poland—molecular epidemiology and antimicrobial susceptibility of strains isolated from past outbreaks and those currently circulating in Poland. Int. J. Infect. Dis. 14:e907–e912.

**FIG 1** Minimum spanning tree of the MLSTs of the 42 \textit{C. diphtheriae} isolates. Each circle corresponds to an ST. The area of each circle corresponds to the number of isolates. Each ST is color coded according to its corresponding geographical origin. The relationships between strains are indicated by the connections between the isolates and the lengths of the branches linking them. Black lines connecting pairs of STs indicate that they differ in two or three alleles (thick lines), four alleles (dashed), and five or six alleles (thin).