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

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***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.**

Corynebacterium diphtheriae is the etiologic agent of diphtheria, a health-threatening infection that affects the upper respiratory tract and the skin. *C. diphtheriae* infection was once endemic worldwide, but since the universal vaccination of children and adolescents was introduced, the frequency of this infection has fallen dramatically in countries that practice immunization. However, the emergence of invasive infections due to nontoxigenic *C. diphtheriae* isolates has been reported in several countries with high vaccination coverage (4, 6, 10). These infections have a high mortality rate (up to 40%), which can be explained by the presence of comorbidity (cardiac valvulopathy) (7) and predisposing conditions (intravenous drug use, skin lesions, and socioeconomic disadvantage) (4, 10). Several studies using ribotyping have suggested the invasive isolates to be clonal (4, 6, 10) whereas others have found multiple types of *C. diphtheriae* bacteria (11).

We characterized and investigated retrospectively the relatedness of invasive *C. diphtheriae* isolates causing bacteremia in France (including New Caledonia) and Poland. All invasive isolates collected from blood culture or vascular thrombus and received at the Polish and French reference laboratories between 1987 and 2010 were included in this study. Microbiological identification was performed using amplification of the *dtxR* gene (9). The isolates were biotyped using the API Coryne strip test (2) (product no. 20900; bioMérieux, Marcy l'Etoile, France). The toxigenic status was evaluated by amplification of the *tox* gene (5). Antibiotic susceptibility was evaluated for 41 isolates with the disc diffusion or the Etest methods and interpreted using the recommendations of French Microbiology Society (CA-SFM) criteria for 11 antibiotics. Molecular characterization was performed using multilocus sequence typing (MLST) as described by Bolt et al. (1). We assessed the clonal relationships between invasive isolates using the eBurst version 3 program (http://eburst.mlst.net/). Isolates were assigned as members of an eBurst group if they shared six out of seven MLST alleles.

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 nontoxigenic. 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 (12). As diphtheria vaccine includes diphtheria toxoid only, it has only prevented the toxigenic isolates from spreading from the FSU into Poland.

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 (11).

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-

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TABLE 1 Microbiological characteristics and results of the MLST analysis (ST) for the 42 invasive *C. diphtheriae* isolates

Isolate no. (reference)	Year	Origin	Antibiotic resistance[a]	Biotype	ST	Allelic profile
280.87 (8)	1987	Mainland France	0	Gravis	128	2-1-47-19-13-3-6
337.87 (8)	1987	Mainland France	0	Mitis	153	1-3-3-36-32-3-2
033.90 (8)	1990	Mainland France	0	Mitis	156	2-5-6-19-5-3-20
389.90 (8)	1990	Mainland France	0	Belfanti	193	6-7-8-37-9-7-15
680.90 (8)	1990	Mainland France	Tet	Mitis	196	2-27-4-1-5-3-20
757.90 (8)	1990	Mainland France	0	Gravis	194	13-1-21-37-14-11
064.91 (8)	1991	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
203.91 (8)	1991	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
290.91 (8)	1991	Mainland France	0	Mitis	130	2-10-9-1-3-27-2
457.91 (8)	1991	Mainland France	0	Mitis	130	2-10-9-1-3-27-2
666.91 (8)	1991	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
678.91 (8)	1991	Mainland France	0	Mitis	130	2-10-9-1-3-27-2
875.91 (8)	1991	Mainland France	0	Mitis	130	2-10-9-1-3-27-2
247.92 (8)	1992	Mainland France	0	Mitis	130	2-10-9-1-3-27-2
450.92 (8)	1992	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
620.92 (8)	1992	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
846.92 (8)	1992	Mainland France	0	Mitis	130	2-10-9-1-3-27-2
088.93 (8)	1993	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
157.93 (8)	1993	Mainland France	0	Mitis	130	2-10-9-1-3-27-2
207.93 (8)	1993	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
538.93 (8)	1993	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
657.93 (8)	1993	Mainland France	Ri	Mitis	130	2-10-9-1-3-27-2
840.93 (8)	1993	Mainland France	0	Mitis	130	2-10-9-1-3-27-2
2002.4591	2002	Mainland France	0	Mitis	195	3-1-49-19-3-16-25
862.91 (8)	1991	New Caledonia	0	Gravis	39	13-1-8-4-14-11-2
2002.0322	2002	New Caledonia	0	Gravis	82	2-1-33-28-11-2-2
2002.0338	2002	New Caledonia	0	Gravis	82	2-1-33-28-11-2-2
2003.1641	2003	New Caledonie	0	Gravis	82	2-1-33-28-11-2-2
2006.1569	2006	New Caledonia	0	Gravis	82	2-1-33-28-11-2-2
(SJ)493/K/04 (1, 13)	2004	Poland	0	Gravis	8	3-5-6-5-3-3-6
14176/06 (13)	2004	Poland	0	Gravis	8	3-5-6-5-3-3-6
(LM)2861/04 (13)	2006	Poland	0	Gravis	8	3-5-6-5-3-3-6
(RD)8713/07 (13)	2007	Poland	0	Gravis	8	3-5-6-5-3-3-6
(PD)10185/LEB/08	2007	Poland	PG	Gravis	8	3-5-6-5-3-3-6
(PS)804320 (13)	2008	Poland	0	Gravis	8	3-5-6-5-3-3-6
(PW)21708/KR	2008	Poland	PG	Gravis	8	3-5-6-5-3-3-6
(DC)38057/09	2008	Poland	PG	Gravis	8	3-5-6-5-3-3-6
(LP)8874-1	2009	Poland	nt	Gravis	8	3-5-6-5-3-3-6
(MK)BA/I/3188	2009	Poland	PG	Gravis	8	3-5-6-5-3-3-6
7308(JP)	2009	Poland	PG	Gravis	8	3-5-6-5-3-3-6
8947/LEB	2010	Poland	PG	Gravis	8	3-5-6-5-3-3-6
9500/LEB	2010	Poland	0	Gravis	8	3-5-6-5-3-3-6

[a] Intermediate or resistant; Tet, tetracycline; Ri, rifampin; PG, penicillin G; nt, not tested.

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., ST8 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 toxi-

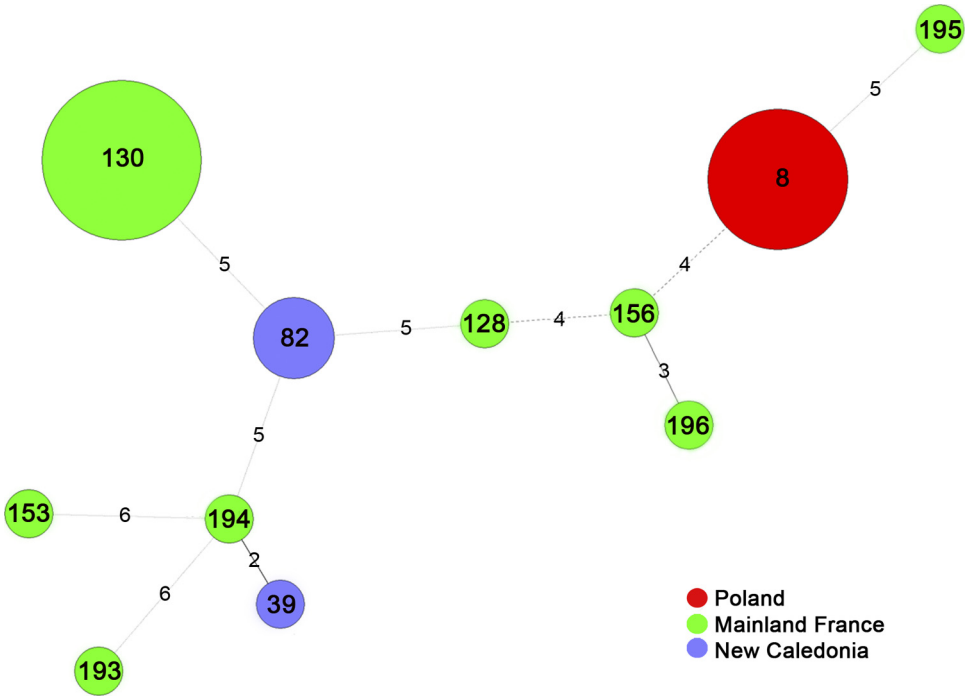

FIG 1 Minimum spanning tree of the MLSTs of the 42 *C. diphtheriae* isolates. Each circle corresponds to an ST. The area of each circle corresponds to the number of isolates. Each ST is color coded according to its corresponding geographical origin. The relationships between strains are indicated by 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).

genic and nontoxigenic *C. diphtheriae*. New typing tools such as MLST can provide a better understanding of the diversity of this pathogen.

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