



Virtual Training 15: Focus on the Agents of Diphtheria

Diphtheria surveillance, and its global resurgence

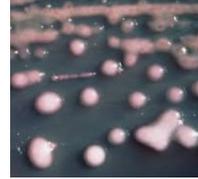
Pr Sylvain Brisse, Institut Pasteur, Biodiversity and Epidemiology of Bacterial Pathogens Unit, National Reference Center for Diphtheria; Paris, France; European Reference Laboratory for diphtheria and pertussis

sylvain.brisse@pasteur.fr ; coryne@pasteur.fr

07 March 2025

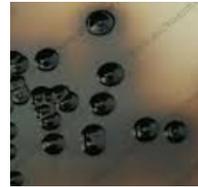
Emergence and evolution (of bacterial strains)

- Multidrug resistance
- Vaccine-escape
- Epidemiological surveillance
- One Health
- Links genotype-phenotype



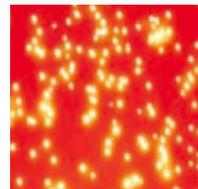
Klebsiella pneumoniae

Multidrug resistance



Corynebacterium diphtheriae

Multidrug resistance



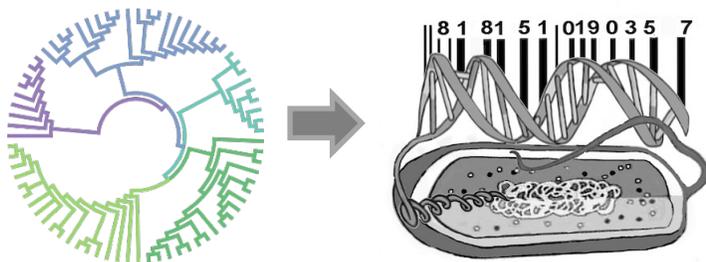
Bordetella pertussis

Vaccine-driven evolution



National Reference Center

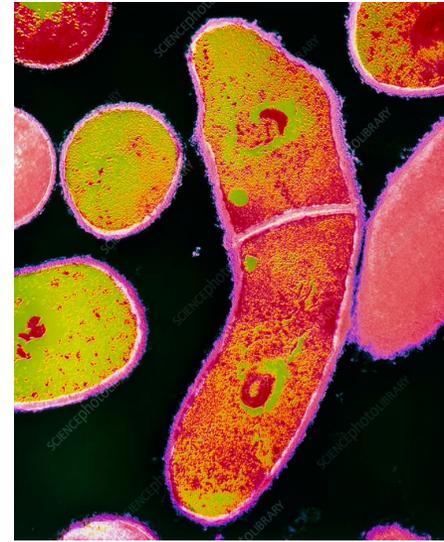
Genomic taxonomies of strains



Intended Learning Objectives

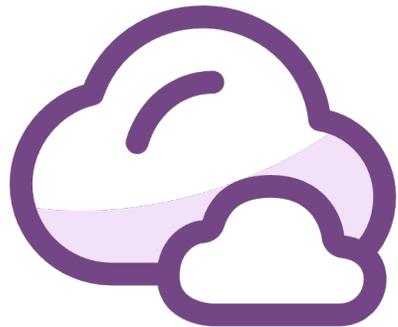
Specific objectives of this session:

1. Diphtheria surveillance in France
2. Diphtheria definitions and toxigenicity
3. Classical diphtheria resurgence *versus* microbiology-driven surveillance
4. Recent diphtheria epidemiology in France
5. European Reference Laboratory for Diphtheria and Pertussis



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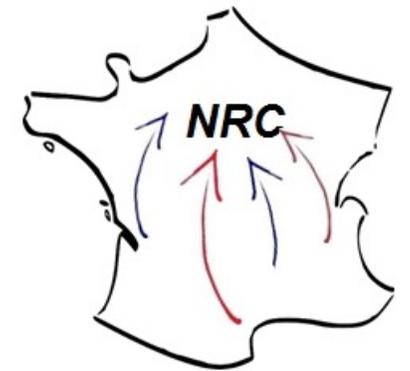


What country do you work in?

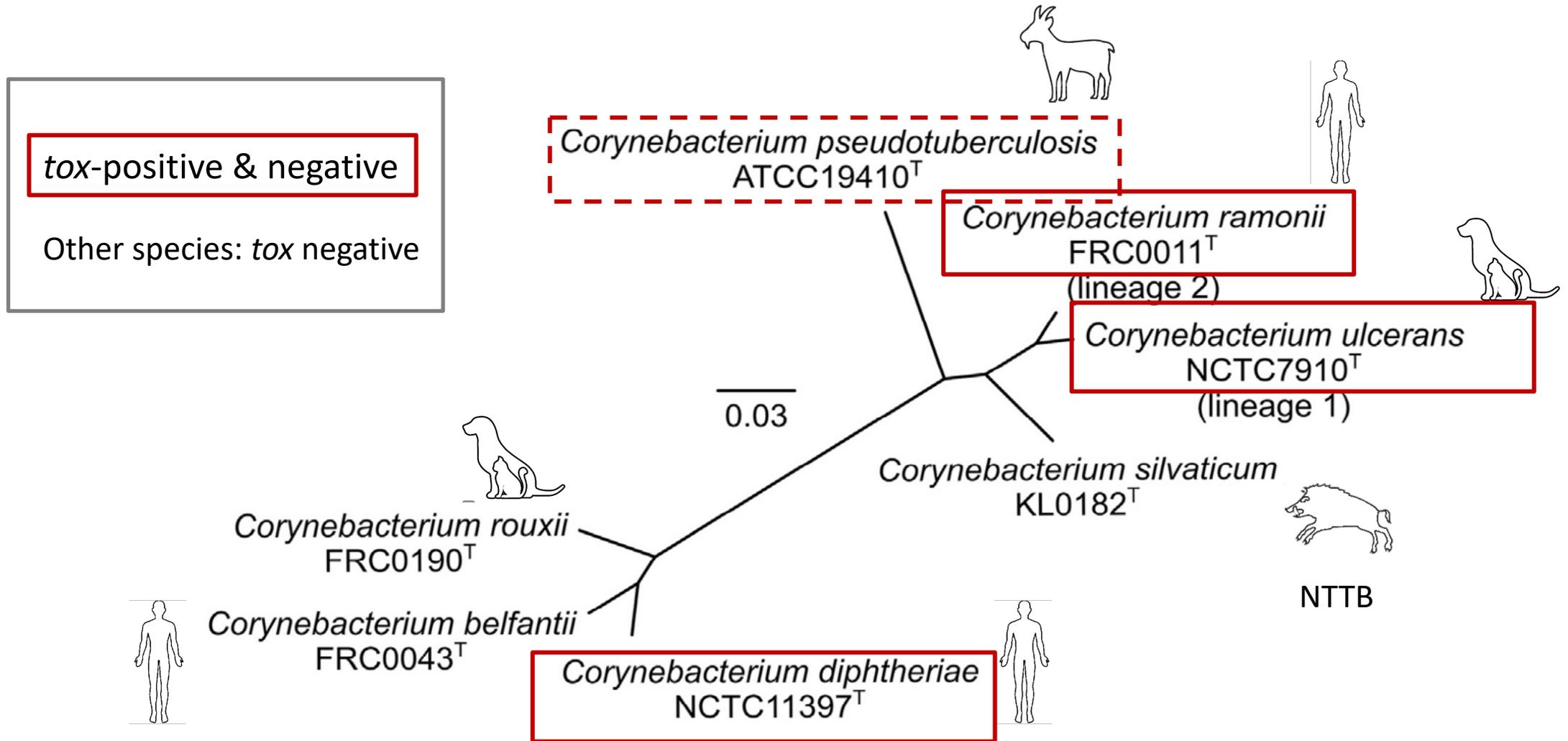
① Start presenting to display the poll results on this slide.

Diphtheria surveillance, France

- Mandatory notifiable disease
- Public health France
 - Agency of Ministry of Health
 - ECDC's National Focal point
- Supported by a dedicated National Reference Center
 - NRC for *Corynebacteria* of the *diphtheriae* complex
 - Designated for 5 years mandates, renewable
- Four main missions of NRC
 - Microbiological diagnostic and expertise
 - Advice (authorities, health professionals)
 - Surveillance (yearly reporting); humans and animal sectors
 - Alert (any exceptional event)



Corynebacterium diphtheriae Species Complex (CdSC)*



* CdSC: Phylogenetic group of taxonomic species

Identification of diphtheria agents

MALDI-TOF

Distinguishes three groups:

C. diphtheriae / *belfantii* / *C. rouxii* (*C. rouxii* peaks not in DB)

C. ulcerans / *C. ramonii* (*C. ramonii* peaks not in DB)

C. pseudotuberculosis



qPCR Multiplex (Badell et al., 2018)

Distinguishes two groups:

C. diphtheriae / *C. belfantii* / *C. rouxii*

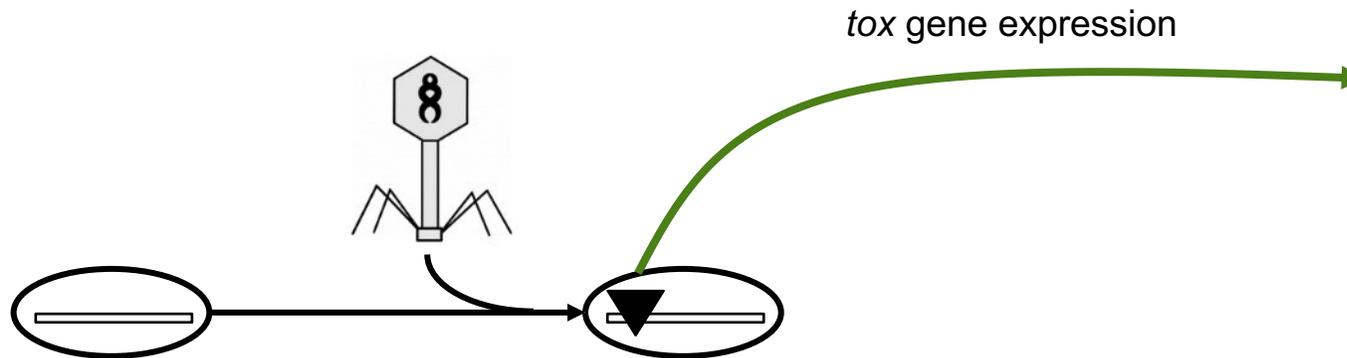
C. ulcerans / *C. ramonii* / *C. pseudotuberculosis*

Combines *rpoB* identification and *tox* gene detection

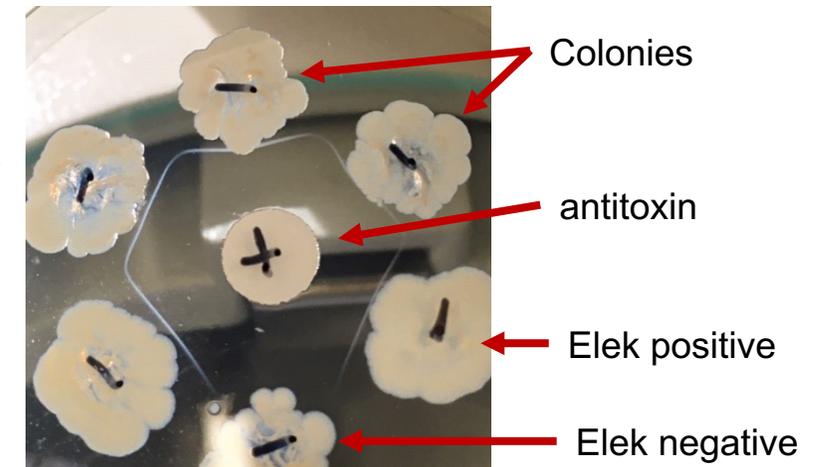


WHO definition of diphtheria

« Isolation of *Corynebacterium diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis* and Elek-positive test (*in vitro* toxin production)



Elek test (immunoprecipitation)
(Engler et al., 1997)



- (Mainly in reference labs, e.g., NRCs and WHO collaborative centers)
- Takes 24 to 48 hours

Genetic basis of diphtheria surveillance

e.g., used in France (HCSP, Conduite à Tenir, 2011)

« Isolation of *Corynebacterium diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis*

and

Detection of *tox* gene, which codes for diphtheria toxin,

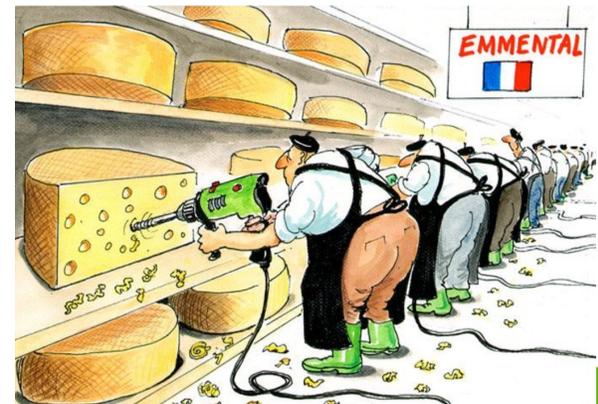
irrespective of Elek test result (*in vitro* diphtheria toxin production)



Faster diagnostic

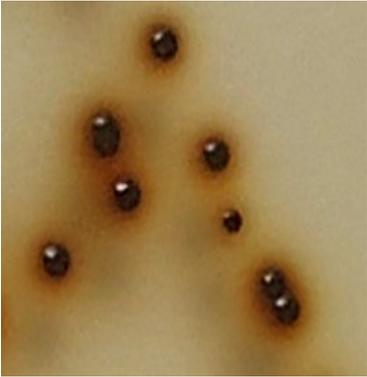


Prone to false positives (non-toxigenic, *tox*-bearing isolates)

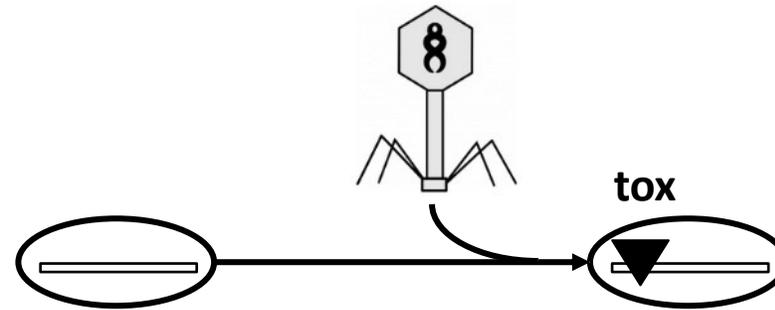


Tox be or not Tox be: that's the question

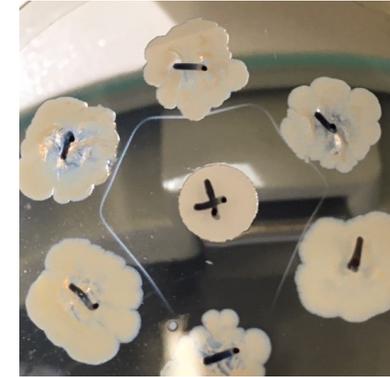
Isolates of the *C. diphtheriae* species complex



Corynephage



Elek test



- *tox*-positive: carries the *tox* gene, coding for diphtheria toxin (assessed by PCR or qPCR)
- Toxigenic: produces to diphtheria toxin (*tox* gene expressed; assessed by Elek's test)
- **Non-toxigenic, *tox* gene-bearing (NTTB)**: carries the *tox* gene but is Elek-negative
 - Disrupting mutations in the *tox* gene: frameshift, IS insertion
 - Mutations in regulatory sequences? (yet to be discovered)
- 10% to 15% non-redundant *C. diphtheriae* are NTTB

Diphtheria vaccination

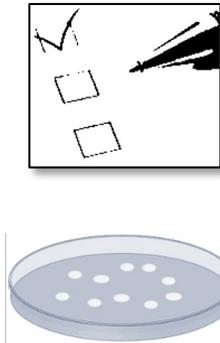
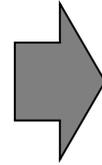
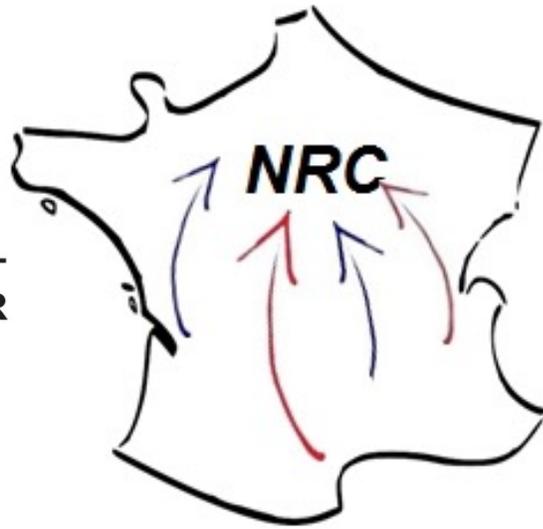


- Vaccination schedule (France, 2014 -> .):

Months (year 1)			Years								
2	4	11	6	11 to 13	25	45	65	75	85	95	

- Mandatory for infants (since 2018); Y1 coverage >98%
- Strongly recommended for health professionals

NRC for *Corynebacteria* of the *diphtheriae* complex



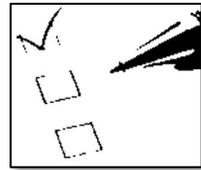
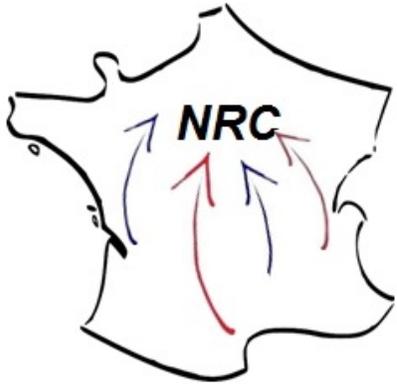
Isolates bio-collection
& attached source data

- **Rapid diagnosis:** real-time qPCR (**tox** & species: *C. diphtheriae*, *C. ulcerans*, ...)
- **Isolation** (Tinsdale medium)
- **Toxin production** (Elek test) & **biotyping** (biovar Gravis, Mitis, ...)
- **Antimicrobial susceptibility testing**
- **Genomic sequencing and genotyping**

Integrated diphtheria surveillance and research



NRC: Strong funding support from Public Health France



Julie Toubiana



Edgar Badell-Ocando



Christèle Auguste



Sylvie Bremont



Marion Barbet

Isolates bio-collection & attached source data

NRC activities: Expertise for rapid diagnosis: real-time qPCR (*tox* & species: *C. diphtheriae*, *C. ulcerans*, ...); surveillance, alert and counseling

Research at Institut Pasteur's hosting unit: Population biology and structure, antimicrobial resistance, *tox* phage biology, global spatial spread and evolution, diagnostic developments, genotyping tools



Nora Zidane



Chiara Crestani



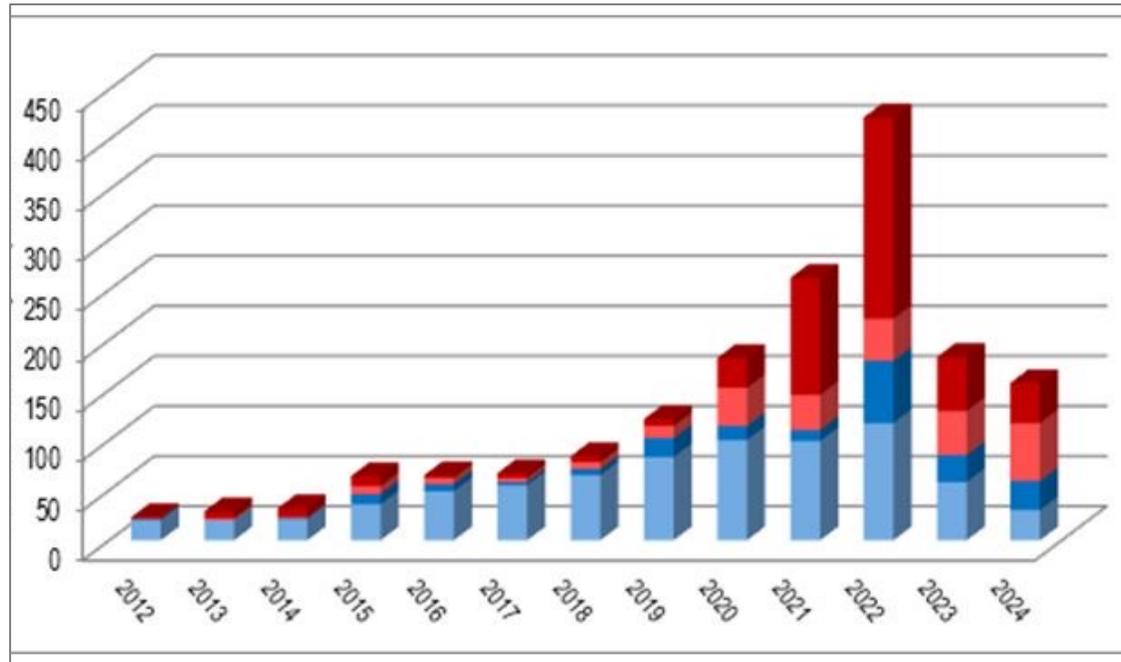
Virginie Passet



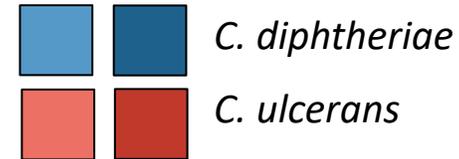
José F. Delgado-Blas

Diphtheria isolates received at NRC over time, France

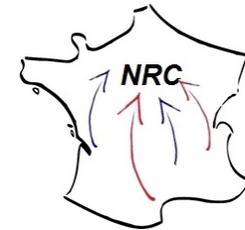
1990-2001:
No case



- +



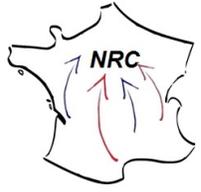
Data: SpF, CNR & HCSP, 2021



Drivers (& biases) of increase in referred isolates:

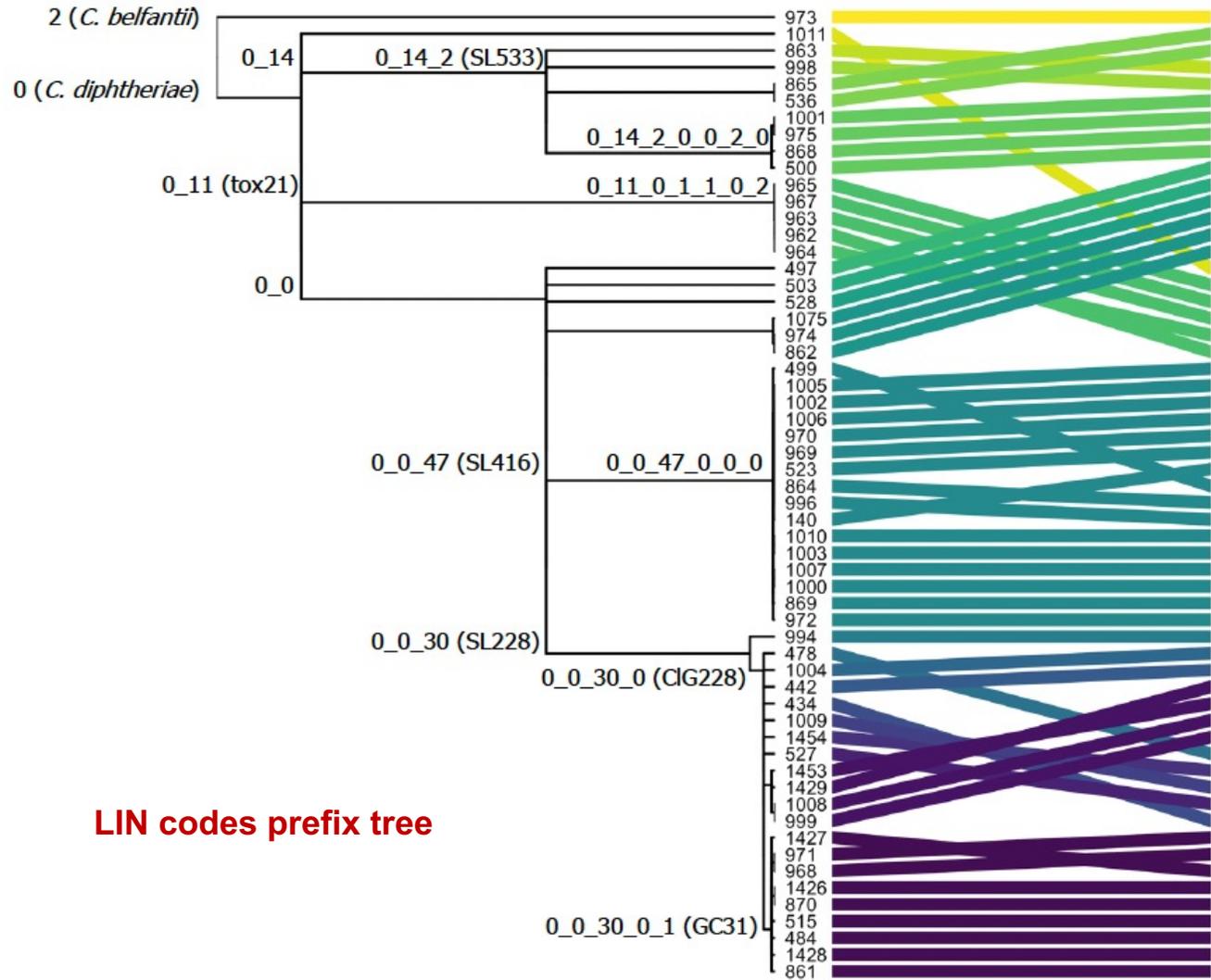
- 2003: broadened definition (*C. ulcerans* et *C. pseudotuberculosis*)
- Improved identification methods (MALDI-TOF): lab-driven detection (wounds)
- Insufficient vaccination in multiple country, combined with travel

NRC isolates, 2023: species and geography



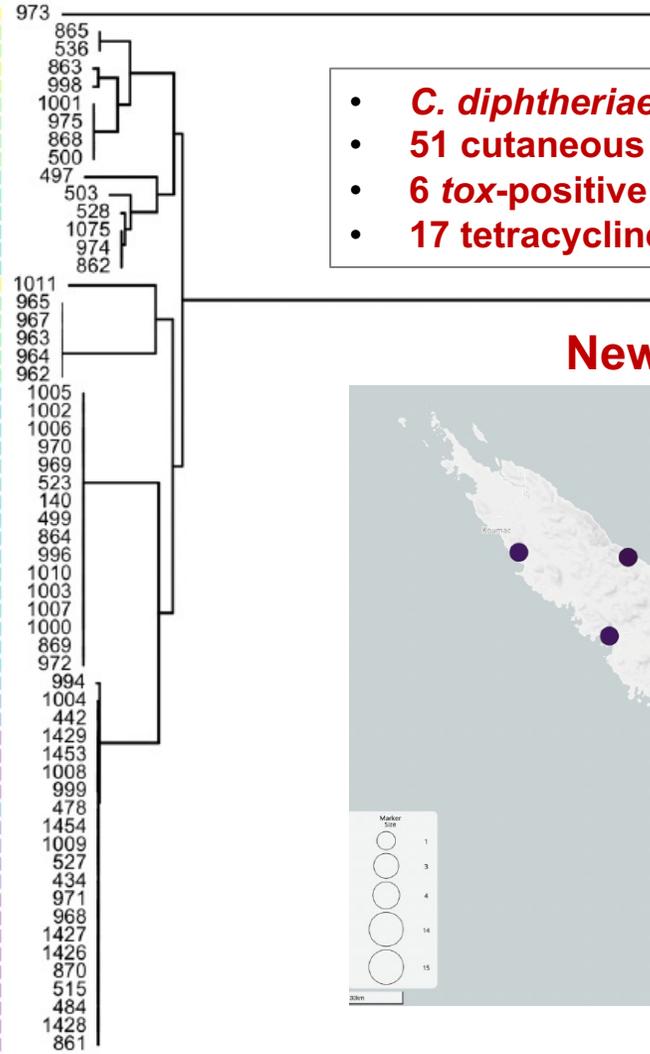
	Mainland (human)	Mainland (animal)	French Guiana (human)	La Réunion (human)	Mayotte (human)	New Caledonia (human)	Polynesia (human)	Total
A. tox positive isolates								
<i>C. diphtheriae</i>	21	4	0	1	1	0	0	27
<i>C. ulcerans</i>	18	37	0	1	0	0	0	56
<i>C. belfantii</i>	0	0	0	0	0	0	0	0
<i>C. rouxii</i>	0	0	0	0	0	0	0	0
<i>C. pseudotuberculosis</i>	0	0	0	0	0	0	0	0
Total	39	41	0	2	1	0	0	83
B. tox negative isolates								
<i>C. diphtheriae</i>	34	9	5	0	7	0	3	58
<i>C. ulcerans</i>	7	36	1	0	0	0	0	44
<i>C. belfantii</i>	7	0	0	0	0	0	0	7
<i>C. rouxii</i>	2	13	0	1 (animal)	0	0	0	16
<i>C. pseudotuberculosis</i>	2	5	0	0	0	0	0	7
Total	52	63	6	1	7	0	3	132
TOTAL (A + B)	91	104	6	3	8	0	3	215

C. diphtheriae endemicity (New Caledonia)



LIN codes prefix tree

1220 Sp. 1035 L 500 SL 5525 CGGC



- **C. diphtheriae** isolates, 2015-2019
- **51 cutaneous** out of 58
- **6 tox-positive**
- **17 tetracycline-R; 13 cgMLST Sublineages**

New Caledonia



0.00 0.01 0.02 0.03 0.04

Cutaneous case series, Indian Ocean, 2015-2018

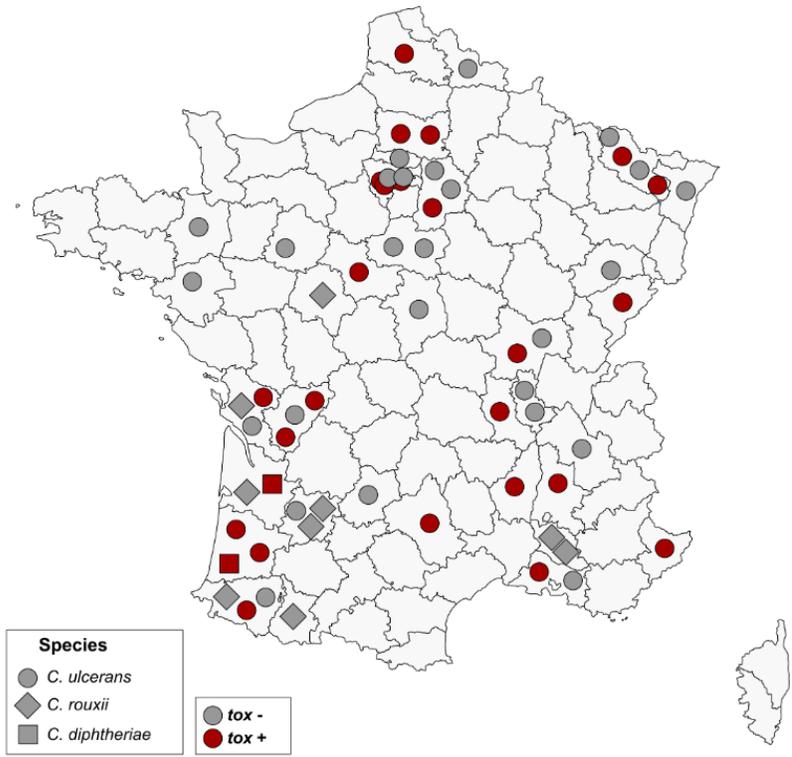
Alberto, ..., Bagny, 2020, Ann Dermatol Venereol



- Between December 2015 and June 2018, 13 cutaneous cases: eleven patients (Nos. 1—11) were diagnosed in Reunion Island, and 2 patients (Nos. 12—13) in Lyon.
- Recent travel from Mayotte (4), Madagascar (7)
- Patients aged 4 to 77 years
- **5** of 13 produced toxin
- Painful and rapidly progressive round ulcerations of the legs, that were superficial and in some cases purulent, with anerythematous-purple border covered with greyish membrane.
- Only 6 patients had been properly immunized over the preceding 5 years
- Polymicrobial -> The clinician should order specific screening for *C. diphtheriae* from the bacteriologist

Animal case series: 64 CdSC cases

- Testing of ~ 18,000 diseased animals, during 2 years
- Identification of 64 isolates of the *C. diphtheriae* species complex



- 24 *C. ulcerans* tox +
- 27 *C. ulcerans* tox -
- 11 *C. rouxii* tox -
- 2 *C. diphtheriae* tox +
- Monoinfections -> imputability (e.g., cat rhinitis)

Diphtheria wave, France and Europe, 2022

- **France**

- 101 *C. diphtheriae* isolates, 45 *tox*-positives (30 in metropolitan France)
- Global dataset (976 non-redundant genomes)

(Hennart *et al.*, Peer Comm J, 2024)

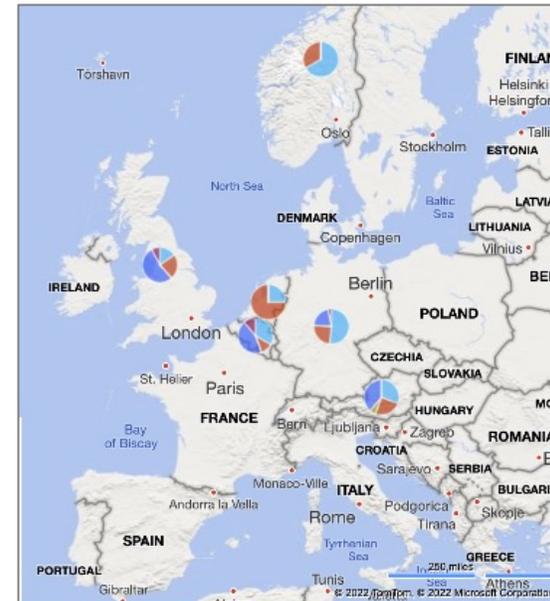


Peer Community Journal

- **Europe**

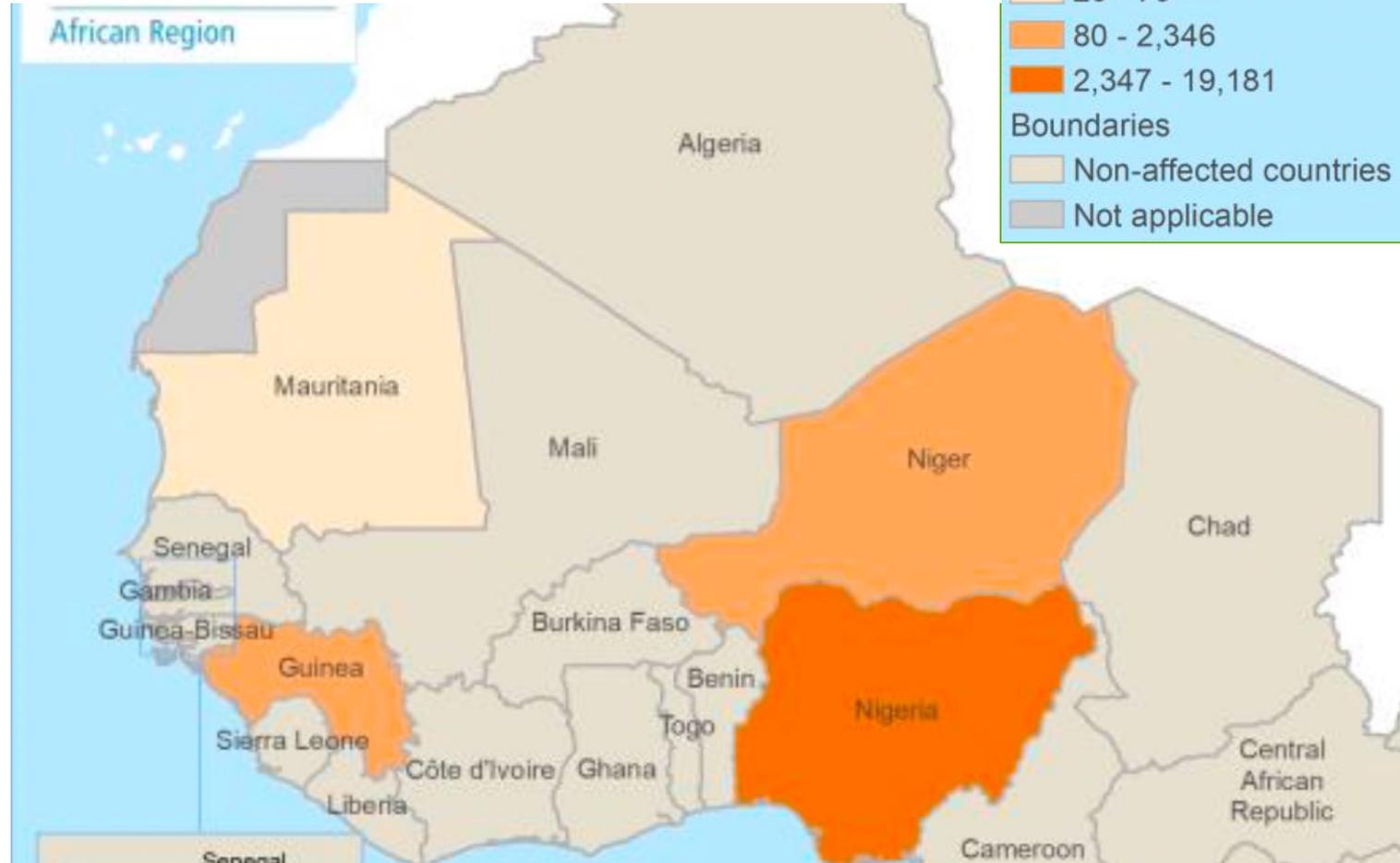
- 363 *tox*-positive *C. diphtheriae*
- Germany (118 cases), Switzerland (52), UK (59), Austria (69), France (30), Belgium (21), Norway (8), The Netherlands (5), Italy (3), and Spain (1)

(Hoefler *et al.*, medRxiv, 2024)



medRxiv
THE PREPRINT SERVER FOR HEALTH SCIENCES

WHO African Region Health Emergency Situation Report



Nov 26th, 2023:

22842 cases, 779 deaths (3.4%)

Unvaccinated: 56% cases

57% under 15 years old

60% female

Classical diphtheria: respiratory signs, pseudomembrane

May 2024:

> 41 000 cases

- Reports that penicillin is not working
- Low vaccine coverage, catch-up difficult (procurement, ...)
- DAT stockpile low, procurement
- Contact tracing & screening not appropriately conducted

See also:

Ibrahim *et al.*; Mahamadou *et al.*; Samarasekera; 2024

Diphtheria: Africa-imported cases, France, 2023

France, 2023: travel from West Africa

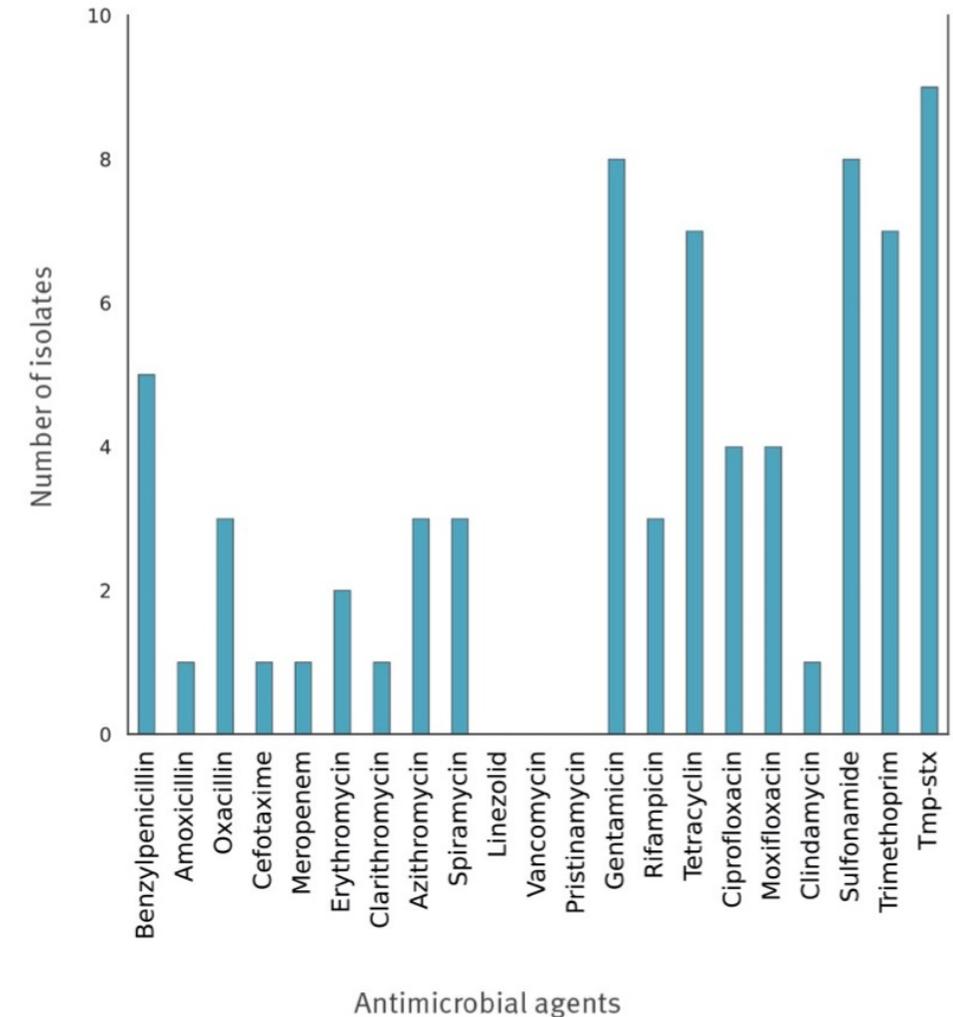
- 10 unlinked imported cases
- 9 cutaneous, 1 respiratory
- 4 tox-positives, toxigenic
- 7 multidrug-resistant
- Genetically diverse
- The toxigenic respiratory isolate had high-level resistance to macrolides and beta-lactams (incl. amoxicilline)

RAPID COMMUNICATION EuroSurveillance, 2023

Multidrug-resistant *Corynebacterium diphtheriae* in people with travel history from West Africa to France, March to September 2023

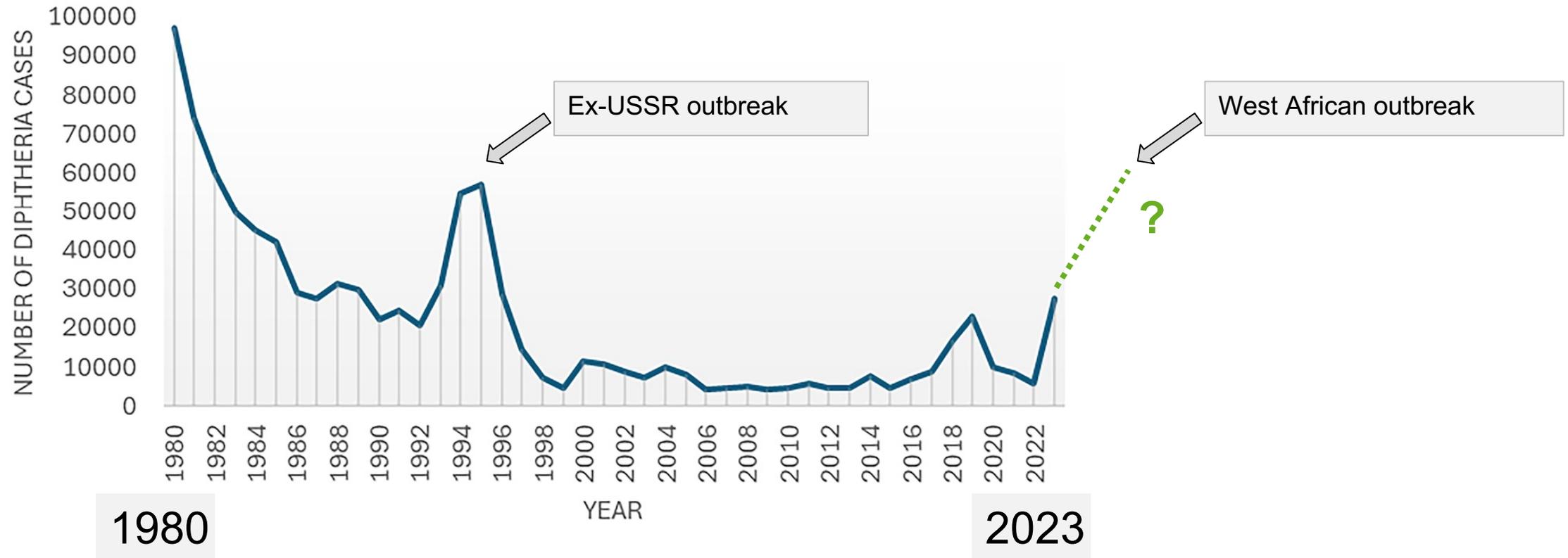
Sylvie Brémont^{1,2}, Virginie Passet¹, Mélanie Hennart¹, Laure Fonteneau³, Julie Toubiana^{1,2,4}, Edgar Badell^{1,2}, Sylvain Brisse^{1,2}

A. Resistance to antimicrobial agents



Diphtheria, Global, 2024

Global annual cases of diphtheria,
reported to the WHO, from 1980 to 2023



1980

2023

Diphtheria: clinical forms

Case fatality rate: 5 – 20%

Clinical presentations:

❖ Respiratory (classical):

- Pharyngeal infection (angina)
- Pseudomembranes
- Lymphadenitis (bull's neck)
- Laryngeal obstruction (croup)

❖ Cutaneous infection

- Ulcer (non-healing, membranes)

❖ Invasive infection (*tox-negative*)



Systemic manifestations (diphtheria toxin)

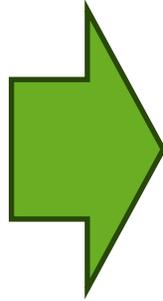
- Cardiac
- Neurological
- Renal

Diphtheria surveillance: clinical *versus* isolate-driven

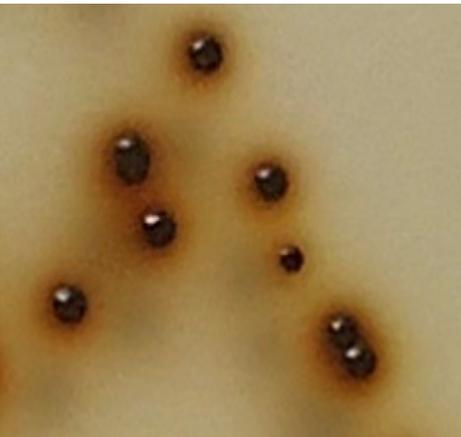
Inclusion criteria



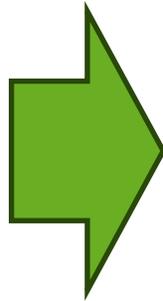
Clinical



- Mostly respiratory
- *C. diphtheriae*
- Toxigenic
- Epidemic (some clonality)



Microbiological



- Mostly cutaneous (reservoir?)
 - *C. diphtheriae*, *C. ulcerans*
 - Toxigenic and non-toxigenic
 - Endemic (clonal diversity)
-
- 'Microbiology-first effect'

Classical diphtheria

- Respiratory infection
- *Corynebacterium diphtheriae*
- Diphtheria toxin-producing (= toxigenic) strains
- Human-to-human transmission
- Outbreaks
- High case fatality rate (5 – 20%), mostly in children < 5 years old



- *Excludes non-toxigenic strains, cutaneous infections, and other species*
- *'Diphtheria in the strict sense'*

EURL PH DIPE (2025 -- 2031)



European Reference Laboratory for Diphtheria and Pertussis



- Qiushui He, Alex-Mikael Barkoff, *et al.* University of Turku (UTU), Finland
(coordination)
- Andreas Sing, Anja Berger, *et al.* Bavarian health and food safety authority (LGL), Germany
- Eveline Van Honacker, Helena Martini, *et al.* Universitair Ziekenhuis Brussel (UZB), Belgium
- Isabelle Desombere, Leen Vandermosten, *et al.* Sciensano (SCI), Belgium
- Sylvain Brisse, Chira Crestani, Carla Rodrigues, *et al.* Institut Pasteur (IP), France

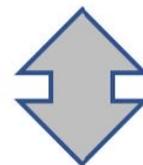


EURL PH DIPE (2025 -- 2031)

EURL network

EU/ECDC

Other stakeholders



WORK PLAN OF THE EURL-EUDIPE

WP1: Coordination

T1.1 Coordination of the network

T1.2 Coordination with other EURLs

T1.3 Communication & dissemination

T1.4 Network meetings

WP2: Protocols

T2.1 Laboratory handbook

WP3: EQA

T3.1 EQA rounds for pertussis

T3.2 EQA rounds for diphtheria

WP4: Support

T4.1 Advice and technical support to network members

T4.2 Advice and support to ECDC

T4.3 Support to ECDC in outbreaks

T4.4 WGS task force

WP5: Seroprevalence

T5.1 Seroprevalence study

WP6: Gaps

T6.1 Diphtheria gaps analysis

WP7: Training

T7.1 Wet-lab training

T7.2 Twinning visits

T7.3 Scientific webinars

Acknowledgements

Institut Pasteur, Paris

National Reference Center for Corynebacteria of the *diphtheriae* complex

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Federica PALMA, Melanie HENNART, Virginie PASSET, Chiara CRESTANI

Public Health France

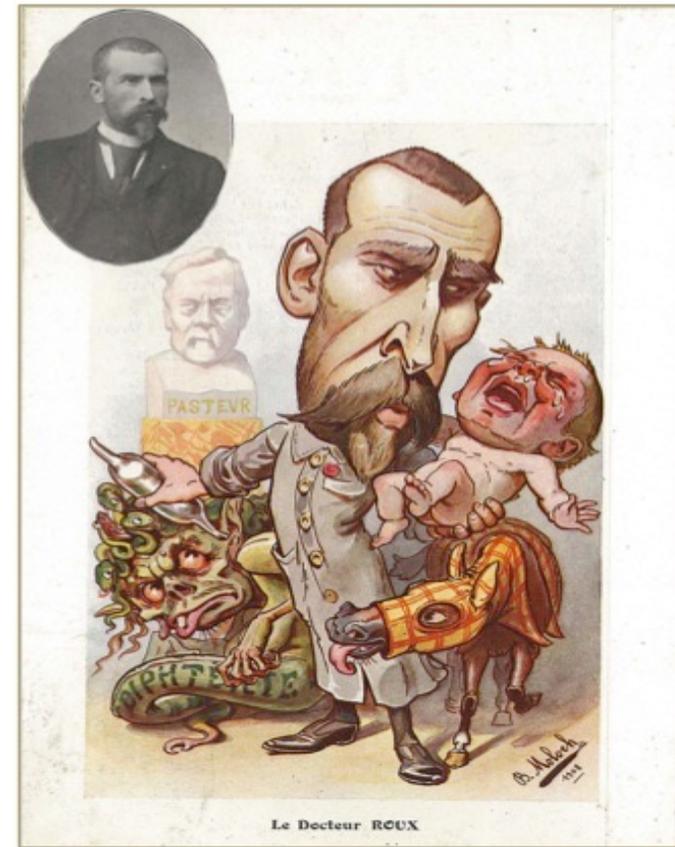
Laure FONTENEAU, Sophie VAUX, Isabelle PARENT DU CHATELET

European Reference Laboratory diphtheria

Qiushui HE, Alex BARKOFF (Turku U, Finland), and colleagues in Belgium, Germany, and France

2022 European diphtheria event consortium

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Funding



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**Santé
publique
France**

slido

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How is diphtheria surveillance organized in your country?

① Start presenting to display the poll results on this slide.

Acknowledgements

The creation of this training material was commissioned by ECDC to Institut Pasteur with the direct involvement of Pr Sylvain BRISSE, Institut Pasteur