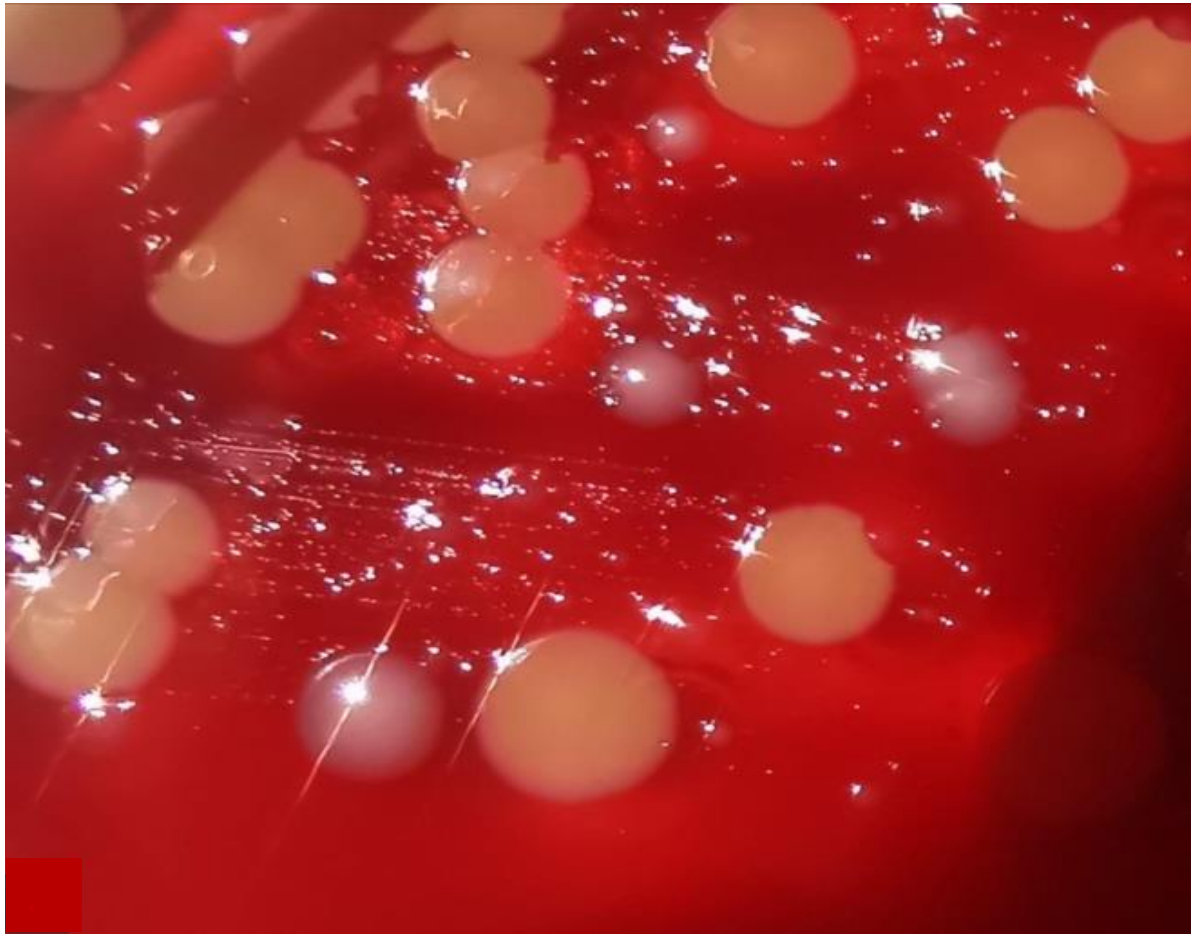
Riemergenza della **DIFTERITE** e **RESISTENZA** correlata ai *sequence type*

Simona Barnini
AOUP

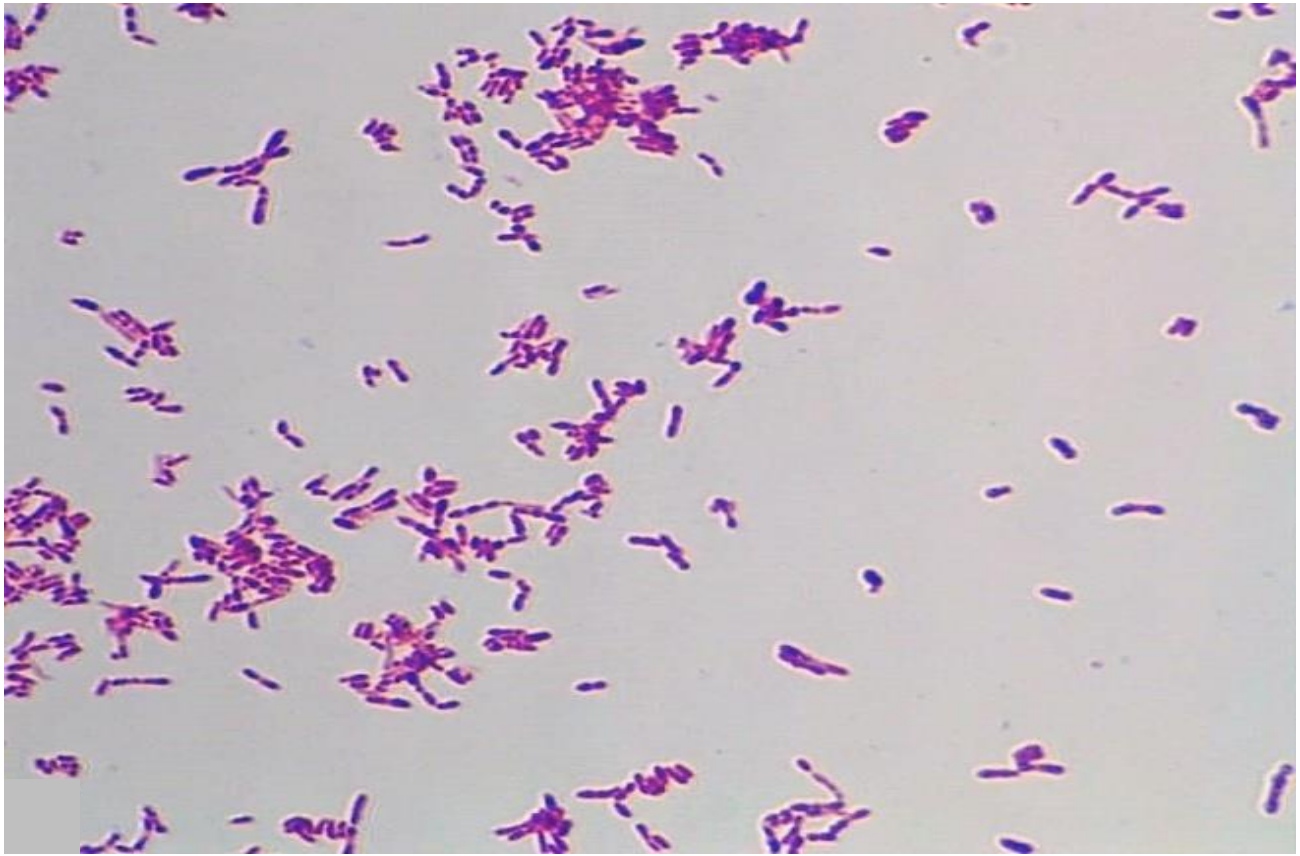
L'eclettismo dell'antibiotico-resistenza, Firenze 7 giugno 2023



L’eclettismo dell’antibiotico-resistenza, Firenze 7 giugno 2023


48 h
24 h


20
14
31
9
22
18
29
7
28
12
35
3
1-18

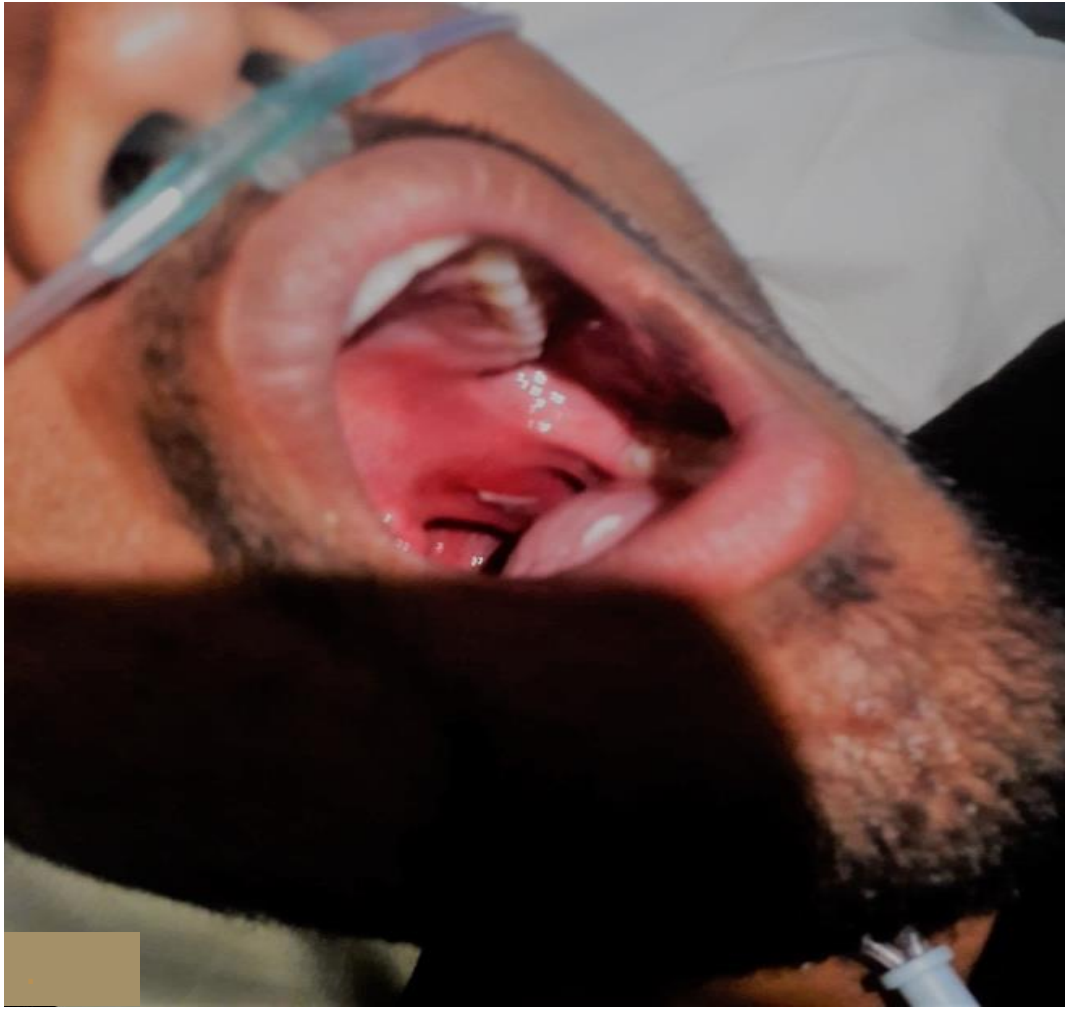


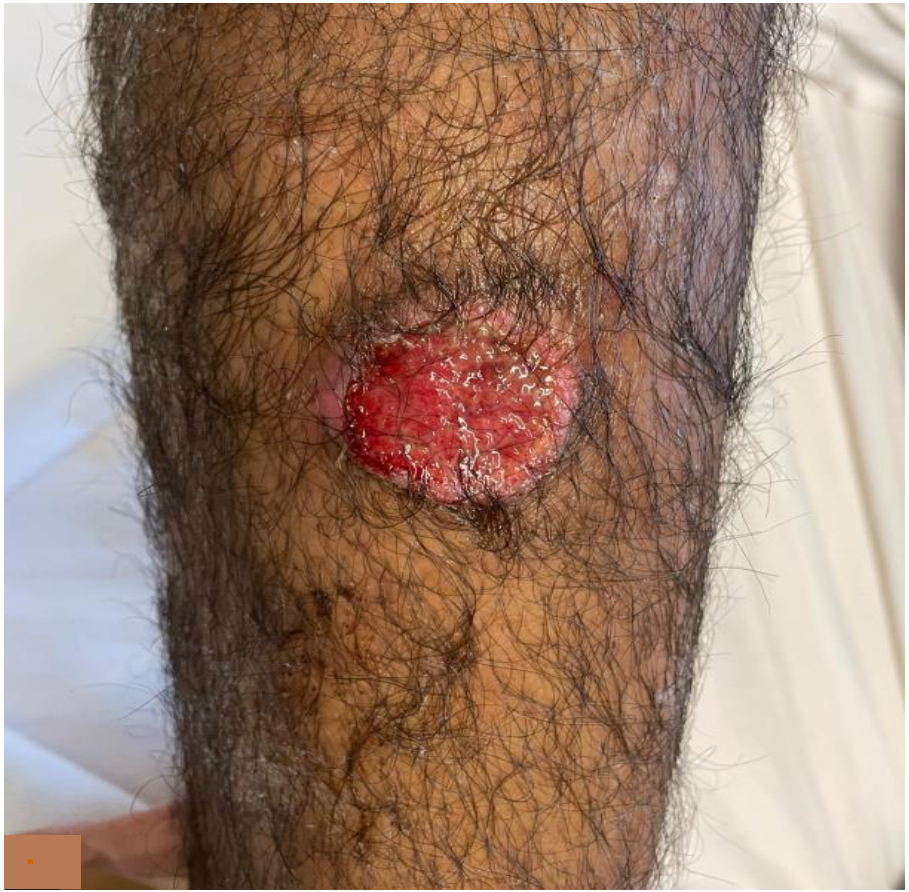
8506756504#3	0	C1	Standard	

Score	Detected Species	
2.35	Corynebacterium diphtheriae DSM	
2.32	Corynebacterium diphtheriae DSM	
2.28	Corynebacterium diphtheriae CCUG	
2.26	Corynebacterium diphtheriae ssp mitis	
2.23	Corynebacterium diphtheriae CCUG	
2.15	Corynebacterium diphtheriae	
2.02	Corynebacterium diphtheriae ssp	
1.75	Corynebacterium diphtheriae ssp	
1.69	Corynebacterium pseudotuberculosis	Le specie pseudotubercu
1.61	Corynebacterium ulcerans DSM	Le specie pseudotubercu

8506756504#3	0	C2	Standard	C

Score	Detected Species	
2.41	Corynebacterium diphtheriae DSM	
2.40	Corynebacterium diphtheriae DSM	
2.31	Corynebacterium diphtheriae CCUG	
2.28	Corynebacterium diphtheriae ssp mitis	
2.25	Corynebacterium diphtheriae CCUG	
2.15	Corynebacterium diphtheriae	
1.91	Corynebacterium diphtheriae ssp	
1.52	Corynebacterium diphtheriae ssp	
1.49	Corynebacterium ulcerans DSM	Le specie pseudotuberculo
1.42	Corynebacterium pseudotuberculosis	Le specie pseudotuberculo





Corynebacterium diphtheriae and *C. ulcerans*

Breakpoint EUCAST

Expert Rules and Expected Phenotypes

For abbreviations and explanations of breakpoints, see the Notes sheet

MIC determination (broth microdilution according to ISO standard 20776-1)
Medium: Mueller-Hinton broth + 5% lysed horse blood and 20 mg/L β-NAD (MH-F broth)
Inoculum: 5x10^5 CFU/mL
Incubation: Sealed panels, air, 35±1°C, 18±2h. Isolates with insufficient growth after 16-20h incubation are reincubated immediately and MICs read after a total of 40-44h incubation.
Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth. See "EUCAST Reading Guide for broth microdilution" for further information.
Quality control: *Streptococcus pneumoniae* ATCC 49619. For agents not covered by this strain, see EUCAST QC Tables.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO$_2$, 35±1°C, 18±2h. Isolates with insufficient growth after 16-20h incubation are reincubated immediately and inhibition zones read after a total of 40-44h incubation.
Reading: Unless otherwise stated, read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light. See "EUCAST Reading Guide for disk diffusion" for further information.
Quality control: *Streptococcus pneumoniae* ATCC 49619. For agents not covered by this strain, see EUCAST QC Tables.

Penicillins	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.125	1		1 unit	28	24		1/A. Isolates "susceptible, increased exposure" (I) to benzylpenicillin can be reported susceptible to amoxicillin. Isolates resistant to benzylpenicillin should be tested for susceptibility to amoxicillin or reported resistant.
Amoxicillin	1[1]	1[1]			Note[A]	Note[A]		

Cephalosporins	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefotaxime	0.064[1]	2[1]		5	28[1]	24[1]		1/A. Susceptibility to cefotaxime can be inferred from benzylpenicillin.

Carbapenems	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Meropenem	0.25	0.25[1]		10	28[1]	28[1]		1/A. Isolates "susceptible, increased exposure" (I) to benzylpenicillin can be reported susceptible to meropenem. Isolates resistant to benzylpenicillin should be tested for susceptibility to meropenem or reported resistant.

Fluoroquinolones	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.001	0.5		5	30	26		

Macrolides and lincosamides	MIC breakpoints			Disk content (µg)	Zone diameter breakpoints (mm)			Notes

ECDC
Communicable Disease Threats Report C. diphtheriae among migrants – Europe –2022
Week 43, 23 - 29 October 2022

Overview:

Summary: As of 26 October 2022, and since the last update on 19 October 2022, two confirmed cases of diphtheria in migrants have been reported by the Netherlands, and three cases by Italy. New cases were also reported by Austria (5), Belgium (5), France (8) and the United Kingdom (9).
On 20 October 2022, Dutch health authorities reported that the first case of cutaneous diphtheria was detected on 12 October 2022. The disease was caused by Corynebacterium diphtheriae (C. diphtheriae) as confirmed by PCR. However, the ELEK test was negative. The case did not show any respiratory symptoms and throat swabs were negative for C. diphtheriae. The case's vaccination status is unknown. A second case of toxigenic C. diphtheriae was detected on 21 October 2022, however, the laboratory method of confirmation is not known at this stage. The case did not show any respiratory symptoms, but results of the throat swab are pending. The case was not vaccinated. Both cases concern individuals who arrived in the Netherlands from Syria at the end of September 2022 and resided in refugee centres. Both cases developed symptoms prior to their arrival in the Netherlands and while travelling through Greece or North Macedonia.
On 20 October 2022, Italian authorities reported three cases of toxigenic C. diphtheriae. Of these, two are of cutaneous form and one presented with both cutaneous and respiratory disease. All cases were admitted to hospital with skin lesions and/or wounds, and one case additionally presented with fever and acute pharyngitis without pseudomembrane formation. The cases are among male refugees aged 35–44 years, arriving in Italy between August and October from Bangladesh, Pakistan and Turkey. The cases' vaccination status is unknown. PCR tests were positive for all cases, ELEK tests were positive for two cases and one result is still pending.
SURVEILLANCE REPORT Weekly Communicable Disease Threats Report, Week 43, 23 - 29 October 2022

Background: Since the beginning of 2022, and as of 26 October 2022, there have been 90 cases of diphtheria among migrants reported by eight EU/EEA countries: Austria (24), Belgium (8), France (14), Germany (31), Italy (3), the Netherlands (2), Norway (7) and Spain (1). Cases have also been reported in Switzerland (25) and the United Kingdom (14), bringing the overall number for Europe to 129.
Among these cases, the majority presented with the cutaneous form of the disease (n=100), 19 cases had respiratory diphtheria, and for 10 cases this information was missing. All cases were caused by toxigenic C. diphtheriae and were detected in male migrants aged 8 to 44 years.

NTCD

Le infezioni da *C. diphtheriae* non tossigeno sono in aumento (non prevenibili con il vaccino); spesso si innescano su lesioni cutanee, ma possono progredire in batteriemia, artrite settica ed endocardite.

I gruppi di popolazione particolarmente a rischio sono i rifugiati, i viaggiatori internazionali, chi fa uso intravenoso di droghe, i senzadimora nelle aree metropolitane..

Sequence type e tracciamento

MLST per *C. diphtheriae* include più di 600 ST; benché sia utile per tracciare gli outbreak, ha poca risoluzione per evidenziare le relazioni genetiche tra batteri, specie nei casi in cui si ha un singolo ST; inoltre non serve a discernere l'acquisizione di geni di antibiotico resistenza o per fattori di virulenza. WGS-SNPs (l'analisi dei polimorfismi a livello di singolo nucleotide dell'intero genoma) è invece efficace: gli isolati nei cluster epidemici differiscono per meno di 150 SNPs, mentre i casi isolati per più di 30000.

Questi cluster, come quello descritto nel 2018-2019 nella King County (USA)* sono in genere dovuti a forme cutanee da *C. diphtheriae* non tossigeno ma dotato di determinanti di virulenza, come i sistemi di acquisizione di ferro ed eme, assemblaggio del pilo per l'adesione, etc., ed evidenziano come, una volta guadagnato anche il batteriofago che codifica per la tossina, possano costituire un serbatoio potenziale per la forma respiratoria di difterite.

*Xiaoli et al. Microbial Genomics, 2020:6 DOI 10.1099/mgen.0.000467

Outbreak-associated clones**

Molecular epidemiological investigations suggest the **existence of outbreak associated clones** with multiple genotypes circulating around the world. In several investigations, **ribotyping and MLST data show an overall dominance of certain clones in a specific geographical area**. Existence of unique clones in these investigations demonstrates that **the genome of *C. diphtheriae* is constantly changing in many regions**. Outbreak analysis of >1000 diphtherial cases, mostly with cutaneous lesions from Seattle during 1972–1982, indicated involvement of the intermedius, mitis and gravis biovars, and molecular analysis using restriction fragment length polymorphism with three different probes revealed that the intermedius and gravis biotypes were of clonal origin. Outbreak-associated strains of *C. diphtheriae* during the 1990s in Russia and the NIS exhibited considerable genetic diversity in ribotyping, multilocus enzyme electrophoresis and PCR single-strand conformation polymorphism analysis of tox and its regulatory element diphtheria toxin repressor (encoded by dtxR)

** Sharma, N.C. *et al*. Diphtheria. *Nat Rev Dis Primers* **5**, 81 (2019). https://doi.org/10.1038/s41572-019-0131-y

In diphtheria-endemic countries, shifts in the strains of *C. diphtheriae* are typified by changes in the predominance of certain biotypes and ribotypes. The Russian epidemics between the 1950s and 1960s are first represented by *C. diphtheriae* strains of the gravis biotype, ribotype M11, followed by the mitis biotype, closely related ribotypes M1 and M1v. In the early 1990s, the ribotype provisionally designated D11 was documented amongst strains isolated in the United Kingdom, Russia, Germany, Romania, Italy and Sweden, whereas ribotype D75 has only been reported in the United Kingdom. In Russia, ribotypes G1 and G4 were predominantly found between 1991 and 1997. In outbreak-affected areas within the NIS, the *C. diphtheriae* gravis biotype was predominant during 1996–2000 with ribotype Sankt-Peterburg. During 2001–2005, this was replaced by the mitis biotype and ribotype Rossija. **The MLST STs of *C. diphtheriae* isolates seem to be country specific, suggesting that the same isolates have been prevailing for many years.** Different STs of outbreak-related *C. diphtheriae* were reported from Belarus (ST-8, in the 1990s), Algeria (ST-116, between 1992 and 2005), Thailand (ST-243, in 2012), the United Kingdom (ST-10, between 2007 and 2013), South Africa (ST-378, in 2015) and Malaysia (ST-453, between 1981 and 2016). A diphtheria outbreak during 2015 in South Africa indicated the prevalence of another ST (ST-395), which has been spreading within the country for >30 years. **WGS analysis** results have shown close genetic relatedness among toxigenic *C. diphtheriae* isolated from infected wounds of refugees from Northeast Africa and Syria in Europe; circulation of genetically related strains in Malaysia; novel lineages in South Africa; several NTCD outbreak clusters with ST-8, originating from Hamburg and Berlin, Germany; relevance of pilins, adhesion factors and iron utilization in infections caused by NTCD; and the presence of different genetic backgrounds of DT-mediated pathogenicity in *C. diphtheriae*, *C. pseudotuberculosis* and *C. ulcerans*

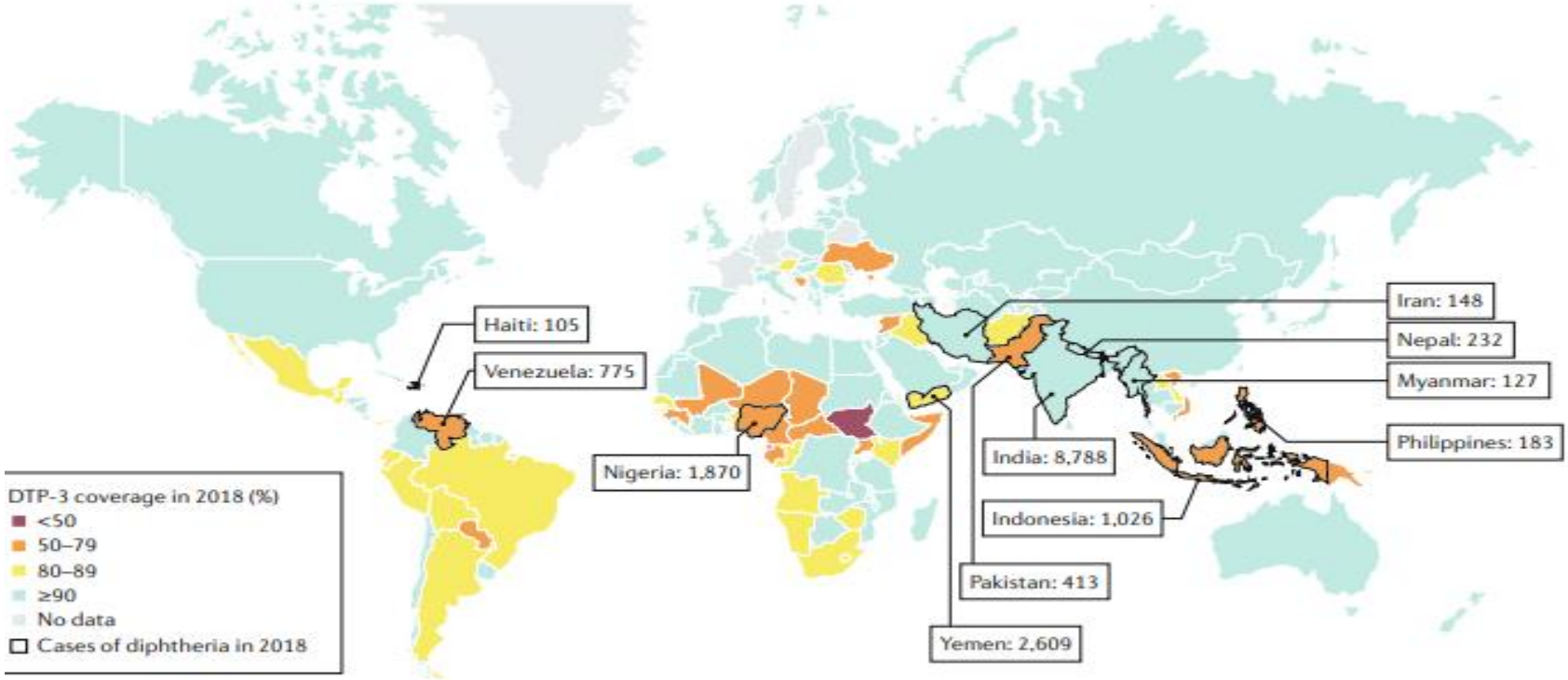


Fig. 2 | **Global DTP vaccine coverage and number of cases of diphtheria.** The map shows the coverage of the third dose of the vaccine for diphtheria, tetanus and pertussis (DTP-3) in 2018. The number of cases of diphtheria reported in the same year is shown for countries with >100 reported cases. DTP, diphtheria, tetanus and pertussis. Data from WHO Reported estimates of DTP-3 coverage and Diphtheria reported cases.

Antibiotic resistance

Antimicrobial resistance in toxigenic *C. diphtheriae* has not been a major problem in the treatment of diphtheria, except sporadic reports from a few countries. However, the other species, *C. ulcerans* and *C. pseudotuberculosis*, are reportedly resistant to many drugs, including penicillin. Multidrug-resistant *C. pseudotuberculosis* and *C. ulcerans* were reported to cause nosocomial infections (India). Resistance to daptomycin among non *C. diphtheriae* isolates and penicillin-resistant and cephalosporin-resistant cutaneous *C. diphtheriae* are increasingly detected in clinical cases (USA, Canada)

The genetic characteristics of area-specific *C. diphtheriae* variants are influenced by several factors, including antibiotic pressure, as unique trends in resistance prevail in certain geographical regions, for example, resistance to tetracycline in Indonesia, erythromycin in Vietnam and rifampin in France.

C. striatum causes serious infections primarily in immunocompromised patients, such as those in the terminal stage of cancer and presenting with other critical conditions[4]. In addition, invasive diagnostic and therapeutic procedures, long-term use of broad-spectrum antibiotics and prolonged hospitalization were also identified as relevant risk factors for *C. striatum* infections. This systematic review included 42 studies that analyzed 85 individual cases with various invasive infections caused by *C. striatum*. More than one isolate of *C. striatum* exhibited 100% susceptibility to vancomycin, linezolid, teicoplanin, piperacillin-tazobactam, amoxicillin-clavulanate and cefuroxime. On the other hand, some strains of this bacterium showed a high degree of resistance to fluoroquinolones, to the majority majority of β-lactams, aminoglycosides, macrolides, lincosamides and cotrimoxazole. Despite the antibiotic treatment, fatal outcomes were reported in almost 20% of the patients included in this study.

Milosavljevic MN, Milosavljevic JZ, Kocovic AG, Stefanovic SM, Jankovic SM, Djesevic M, Milentijevic MN. Antimicrobial treatment of Corynebacterium striatum invasive infections: a systematic review. Rev Inst Med Trop Sao Paulo. 2021 Jun 18;63:e49. doi: 10.1590/S1678-9946202163049. PMID: 34161555; PMCID: PMC8216692.

Corynebacterium striatum
Corynebacterium urealyticum
Corynebacterium amycolatum
Corynebacterium jeikeium
Corynebacterium glucuronolyticum
Corynebacterium mucifaciens
Corynebacterium tuberculostearicum
Corynebacterium lipophiloflavum
Corynebacterium propinquum
Corynebacterium minutissimum
Corynebacterium aurimucosum
Corynebacterium pseudodiphtheriticum
Corynebacterium confusum
Corynebacterium accolens
Corynebacterium species
Corynebacterium afermentans
Corynebacterium macginleyi
Corynebacterium xerosis
Corynebacterium singulare
Corynebacterium kroppenstedtii
Corynebacterium argentoratense
Corynebacterium freneyi
Corynebacterium diphtheriae
Corynebacterium auris
Corynebacterium imitans

Dal 2021 a giugno 2023, in AOUP
730 *Corynebacterium* spp.
429 *Corynebacterium striatum*

Benzilpenicillina 78%R
Clindamicina 94%R
Linezolid 2,5%R
Fluorochinolonici…..
Daptomicina……..
Vancomicina 0%R

3 ° microrganismo: ***Corynebacterium diphtheriae***

Antibiotico	*MIC (µg/ml)*	*SIR*
Ampicillina	0,125	
Benzilpenicillina	0,125	
Cefotaxime	1	
Ceftriaxone	1	
Clindamicina	0,25	
Daptomicina	>1	
Doxiciclina	>2	
Eritromicina	<=0,0312	
Levofloxacina	<=0,25	
Linezolid	<=0,25	
Meropenem	0,0625	
Moxifloxacina	0,125	
Piperacillina/Tazobactam	4	
Teicoplanina	<=0,25	
Trimetoprim-sulfametossazolo	0,5	
Vancomicina	0,5	


grazie!