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Molecular characterization of *Neisseria meningitidis* strains causing non-invasive disease in Portugal from 2012-2024

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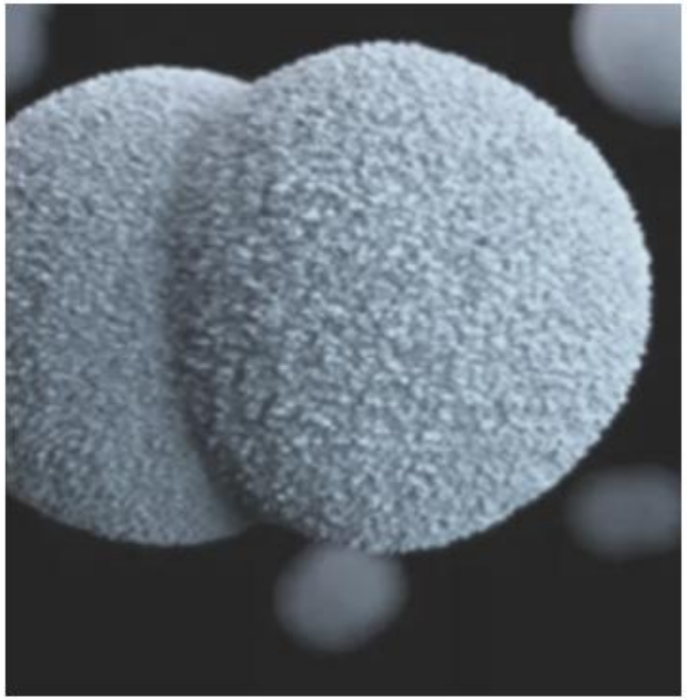
Background & Aim

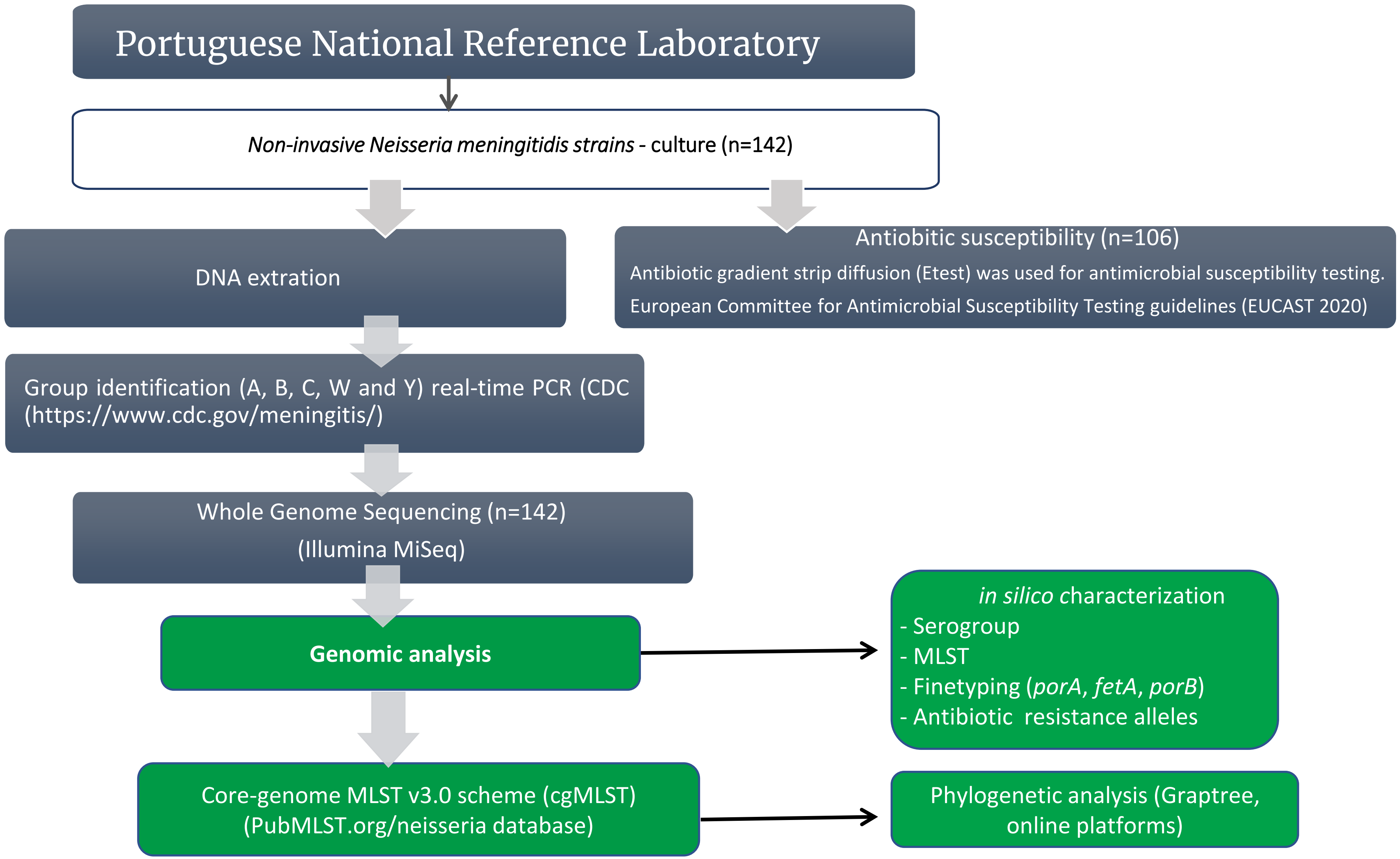


Neisseria meningitidis, often present in the human nasopharynx, is also an important pathogen, responsible for invasive and non-invasive infections.

Non-invasive meningococcal disease (NIMD) is not notifiable, and the prevalence of serogroups and antimicrobial resistance (AMR) profiles are unknown.

The aim of this study is to investigate the genetic diversity of non-invasive isolates identified in Portugal (2012-2024), as well as to identify AMR profiles comparing the results with contemporary invasive isolate populations.

Material and Methods



Portuguese National Reference Laboratory

Non-invasive Neisseria meningitidis strains - culture (n=142)

DNA extration

Group identification (A, B, C, W and Y) real-time PCR (CDC (https://www.cdc.gov/meningitis/)

Whole Genome Sequencing (n=142)

(Illumina MiSeq)

Genomic analysis

in silico characterization

- Serogroup
- MLST
- Finetyping (*porA*, *fetA*, *porB*)
- Antibiotic resistance alleles

Core-genome MLST v3.0 scheme (cgMLST) (PubMLST.org/neisseria database)

Phylogenetic analysis (Graptree, online platforms)

Antiobitic susceptibility (n=106)

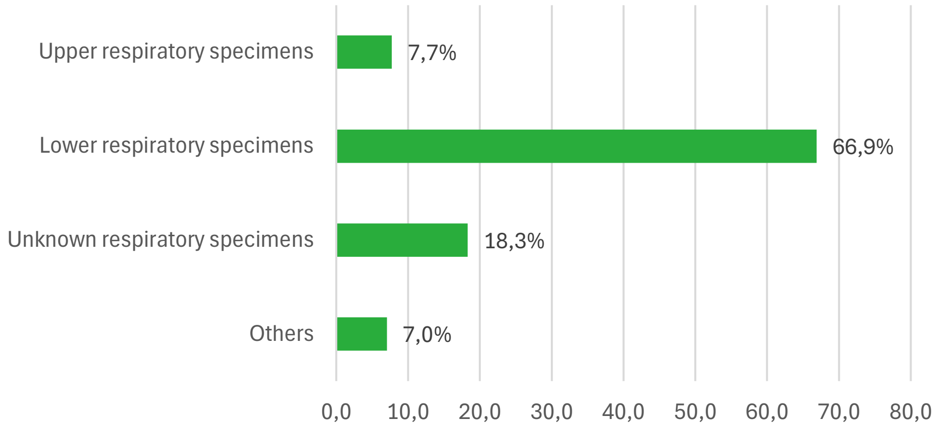
Antibiotic gradient strip diffusion (Etest) was used for antimicrobial susceptibility testing.

European Committee for Antimicrobial Susceptibility Testing guidelines (EUCAST 2020)

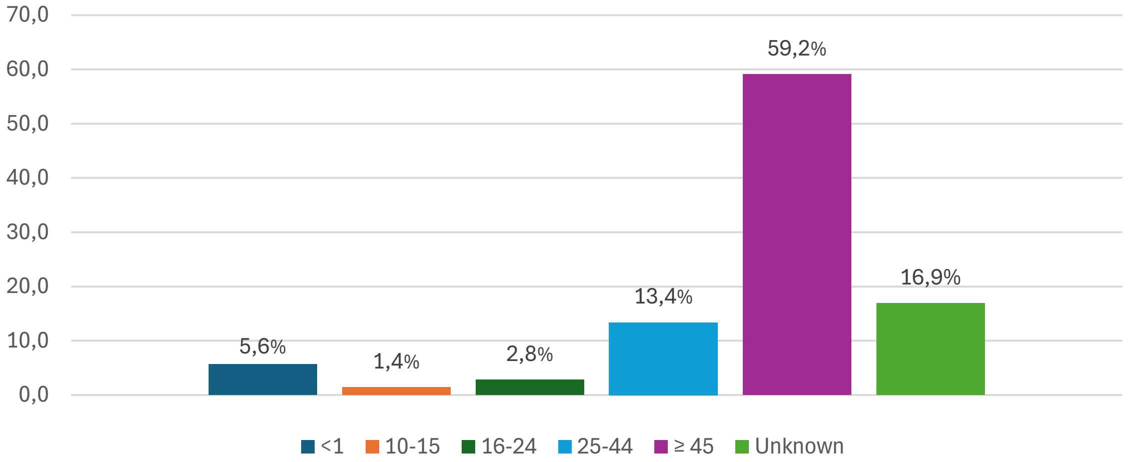
Results

Characterization of non-invasive *Neisseria meningitidis* isolates (n=141), 2012-2024, Portugal

Source of non-invasive *N. meningitidis* isolates

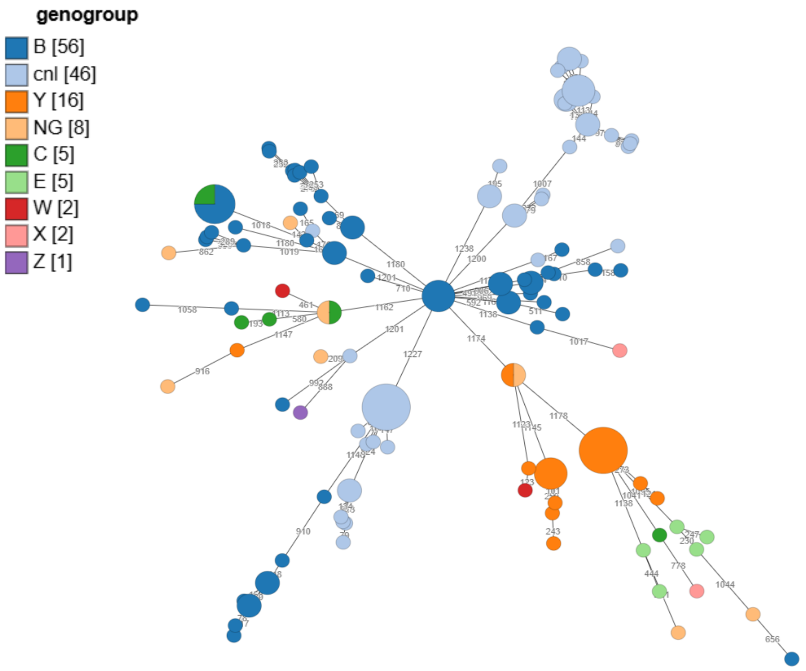


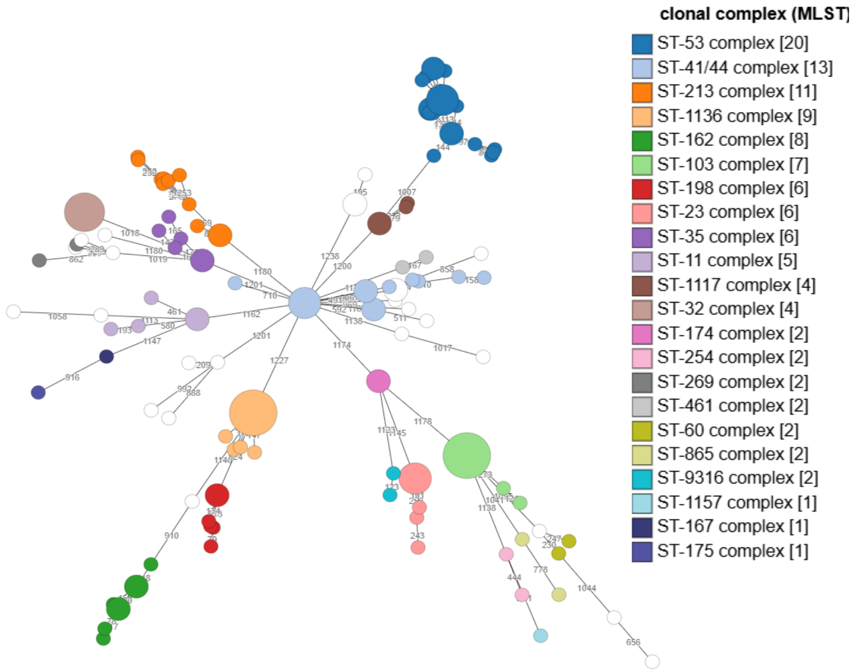
Distribution of non-invasive *N. meningitidis* isolates by age

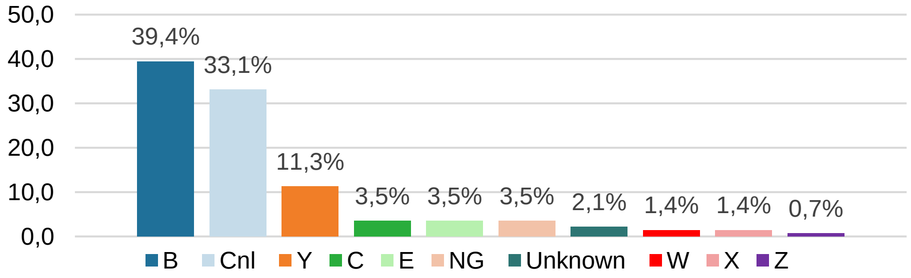


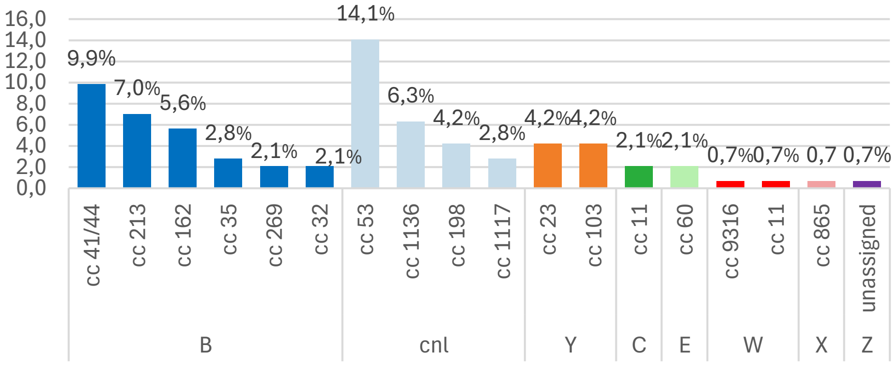
Genetic diversity of Portuguese non-invasive *N. meningitidis* isolates 2012-2024

Genetic clusters identified among non-invasive Portuguese *N. meningitidis* isolates **(n=141)**. The genetic diversity among isolates was evaluated by a gene-by-gene analysis using the *N. meningitidis* cgMLST schema v3, that includes 1329 core *loci.*









Antimicrobial susceptibility, MIC50, MIC90 and MIC range of *N. meningitidis isolates* from non-invasive and invasive disease cases, Portugal, 2012-2024

Number of clinical isolates and antibiotics	% of susceptible isolates (n)	% of intermediate isolates (n)	% of resistant isolates (n)	MIC (g/mL)		
				MIC50	MIC90	Interval
Non-invasive isolates (n=106)						
Penicillin	30.2 (32)	44.3 (47)	25.5 (27)	0.125	0.50	<0.016-1.0
Rifampicin	96.2 (102)	-	3.8 (4)	0.032	0.125	0.002-0.050
Ciprofloxacin	97.2 (103)	-	2.8 (3)	0.004	0.006	<0.002 ->32
Ceftriaxone	100 (106)	-	0	<0.002	<0.002	<0.002 -0.016
Invasive isolates (n=337)						
Penicillin	39.8 (134)	45.7 (154)	14.5 (49)	0.094	0.38	<0.016-1.0
Rifampicin	98.8(333)	-	1.2 (4)	0.016	0.064	<0.002 ->32
Ciprofloxacin	99.1 (334)	-	0.9 (3)	0.006	0.008	<0.002- >32
Ceftriaxone	100 (337)	-	0	0.002	0.016	<0.002-0.023

Penicillin susceptible, MIC ≤0.06 mg/L; intermediate, MIC 0.12-0.25 mg/L; resistant, MIC ≥0.5 mg/L
Ciprofloxacin susceptible, MIC≤0.03 mg/L; R= resistant MIC>0.03 mg/L
Rifampicin susceptible, MIC≤0.25 mg/L; R= resistant MIC>0.25 mg/L
Ceftriaxone susceptible MIC≤0.125 mg/L; R= resistant MIC>0.125 mg/L

Genetic mutations identified in the *penA*, *rpoB* and *gyrA* genes responsible for resistance to penicillin, rifampicin and ciprofloxacin, respectively, from non-invasive *Neisseria meningitidis* isolates, Portugal, 2012-2024

- 106 isolates characterised by WGS were tested for antibiotic susceptibility

Resistance Phenotype	**Genetic mutations**
Penicillin Reduced Susceptibility + Resistant (n=73)	***penA* gene**
	F504L, A510V, I515V, H541N, I566 (**76.7%**, 55/73)
Rifampicin Resistant (n=4)	***rpoB* gene**
	no mutations
Ciprofloxacin Resistant (n=3)	***gyrA* gene**
	T91I (n=2)

- 76.7% of the isolates not susceptible to penicillin had the five well-characterised non-synonymous mutations (F504L, A510V, I515V, H541N and I566V)

- *penA9* (18.8%, n=10), *penA7* and *penA11* (14.5%, each, n=8)), and *penA19* (12.7%, n=7), were the most frequent alleles with alterations in the PBP2 protein.

Conclusions

- Isolates belonging to serogroup B (cc41/44 and cc213) and cnl (cc53 and cc1136) were responsible for the highest number of non-invasive meningococcal cases.

- Differences in resistance levels between invasive and non-invasive populations may be associated with horizontal gene transfer between bacteria, the presence of specific mutations or the use of antimicrobials to treat other infections.

- In addition, the results of this study have some limitations, namely the small sample size and the lack of additional information on the patient's condition or treatment.

In conclusion, no resistance to ceftriaxone was observed and resistance rates to rifampicin and ciprofloxacin remained low, showing that these drugs are effective against meningococcal infections.