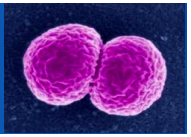
P0977 β-lactams plasmid-mediated resistance in *Neisseria gonorrhoeae* in France



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Introduction

Acquired resistance to β-lactams in *Neisseria gonorrhoeae* (NG) is mainly related to:

- ✓ target modification (PLP2 mosaicism)
- ✓ efflux or decrease in permeability
- ✓ β-lactamase production, especially TEM-1 β-lactamase.

In 2012, 9% of NG strains isolated in France was β-lactamase producers (1). Although class A TEM-1 β-lactamase was classically described for NG, a recent emergence of TEM-135 has been described in Asia (2).

Objective

The **objective** of this work was to explore the β-lactamase enzymes and their genetic support for NG-producing β-lactamase clinical isolates from France.

Methods

From December 2010 to October 2012, 177 penicillinase-producing *N. gonorrhoeae* (PPNG) strains were isolated in the French Reference National Center.

Penicillinase production was detected by performing the nitrocefin reference test (cefinase, Biomerieux). MICs were determined using the E-test method. DNA was extracted by using Instagene Matrix (Biorad®) and the entire bla_{TEM} gene was amplified and sequenced using OT3/OT4 and two additional internal primers.

The complete sequence of 22 plasmids carrying $bla_{TEM-135}$ and bla_{TEM-1} were determined. Plasmid DNA extraction was performed using the QIAprep Spin Miniprep kit (Qiagen®). Plasmid sequences were obtained by PCR mapping, sequencing and alignment on Bioedit software.

Molecular epidemiology typing was performed by the reference Multi-Antigen Sequence Typing (NG-MAST) method (3). Each strain ST was determined using the NG-MAST database (4).

Results and discussion

- For the 177 penicillinase-producing *N. gonorrhoeae* clinical isolates, MICs to ceftriaxone varied from 0.002 to 0.032 mg/L and MICs to cefixime varied from <0.016 to 0.064 mg/L.
- All the 177 PPNG isolates harboured the bla_{TEM} genes encoding several variants of the TEM enzymes:
  - ✓ 152 (86%) TEM-1
  - ✓ 14 (8%) TEM-135 (mutation M182T)
  - ✓ 11 (6%) other TEM variants not yet described (cf. Table)
- Complete sequencing of 22 plasmids of interest (cf. Table) found that:
  - the $bla_{TEM-135}$ genes were carried on two different plasmids quite identical to the pJD4 or pJD7 plasmid previously described (5).
  - the other bla_{TEM} alleles were carried by the pJD5 plasmid.
- According to NG-MAST Sequence Type (ST), 10 different ST were observed for NG expressing the TEM-135 enzyme suggesting that the diffusion of this variant is not linked to a clonal diffusion. Additionally, these ST were different than those found in Asia (2)

Table: Characteristics of 22 NG isolates expressing the TEM-135 and TEM-1 variants Tet: Tetracycline, Cip: ciprofloxacin, ND: not determined, *: 99% homology)

NG isolates	Mutations (TEM variant)	Plasmids	MIC ceftriaxone (mg/L)	MIC cefixime (mg/L)	Coresistance	ST types (NG-MAST)
S3	M182T (TEM-135)	pJD7 Toronto / Rio (5.2 Kb)	0.032	0.064	$Tet^{K}Cip^{K}$	STND-A
S19			0.004	0.016	$Tet^{K}Cip^{K}$	ST5060
S51			0.002	0.016	$Tet^{I}Cip^{K}$	ST5624
S93			0.003	0.016	$Tet^{R}Cip^{R}$	ST758
S188			0.002	0.016	$Tet^{ND}Cip^{R}$	ST1737 *
S111, S113	M182T (TEM-135	pJD4 Asian (7.4 Kb)	0.003	0.016	$Tet^{R}Cip^{R}$	ST4995
S36			0.023	0.047	$Tet^{I}Cip^{R}$	ST2485
S63			0.016	0.016	$Tet^{I}Cip^{R}$	ST3873
S104, S182			0.002	0.016	$Tet^{ND}Cip^{R}$	STND-B
S119. S20			0.002	0.016	$Tet^{R}Cip^{R}$	STND-C
S18, S49	P14S (TEM-1)	pJD5 African (5.5 Kb)	0.003	0.016	Tet^{R}	ST5268
S54	Q269K (TEM-1)		0.006	0.016	$Tet^{K}Cip^{K}$	ST3109
S172	G228S (TEM-1)		0.016	0.032	$Tet^{NU}Cip^{K}$	STND-D
S177	P14L (TEM-1)		0.003	0.016	Tet^{NU}	ST4915 *
S2	WT (TEM-1)		0.032	0.064	$Tet^{K}Cip^{K}$	ST5526 *
S4			0.023	0.023	$Tet^{R}Cip^{R}$	ST3307
S11			0.003	0.016	$Tet^{R}Cip^{R}$	STND-E
S1			0.023	0.023	$Tet^{R}Cip^{R}$	ST1288

Conclusions

This work illustrates the recent evolution of plasmid-encoded bla_{TEM} found in *N. gonorrhoeae* from France and highlights their localisation in several genetic backgrounds.

As the mutation M182T has been described as a stabilizing mutation (6), this study suggests a possible emergence of extended spectrum β-lactamase-producing NG in the future.

References

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