Reduced susceptibility to extended-spectrum cephalosporins (ESC^{rS}) in *Neisseria gonorrhoeae* from Ontario, Canada

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V. ALLEN[1,2], A. REBBAPRAGADA[1,2], D. FARRELL[1,2], N. TIJET[1], S. PERUSINI[1], J. TAN[1], L. TOWNS[1], S. LO[1], D. LOW[1,2,3], and R. G. MELANO[1,2,3‡]

[1]Ontario Agency for Health Protection and Promotion, Public Health Laboratories; [2]Department of Laboratory Medicine and Pathobiology, University of Toronto; [3]Mount Sinai Hospital, Toronto, ON, Canada

‡ Corresponding author
roberto.melano@oahpp.ca

ABSTRACT

Objectives: to determine the level of susceptibility to ESC (cefixime -CFM- and ceftriaxone -CRO-) in *N. gonorrhoeae* (*Ng*) isolates from ON, Canada, and characterize the molecular mechanisms underlying ESC^{rS}.

Methods: 149 consecutive non-redundant *Ng* isolates were studied. MICs were determined according to CLSI guidelines. PCR and sequencing of targets affecting ß-lactam susceptibility were performed in all the isolates displaying ESC^{rS}. The sequence type (ST) of ESC^{rS} isolates was determined by multi antigen sequence typing (NG-MAST).

Results: ESC^{rS} were observed in 14 isolates (9.5%, MICs 0.125-0.25 µg/ml to CFM and 0.032-0.125 µg/ml to CRO) as well as resistance to CIP (n=14), TET (n=13), PEN (n=4; 10 PEN^{I}), and non-susceptibility to ERY (MICs ≥1 µg/ml, n=11). No mutations in the *pilQ* gene were found. bla_{TEM-1} was not detected. A single amino acid change in PBP 1 (L421P, n=6), single (in position A121, n=1) and double substitutions (positions G120 and A121, n=8) in porin PIB, a single adenine-deletion in the *mtrR* promoter gene (n=12) and an amino acid change in MtrR (G45D, n=1) were observed. Mutations affecting both MtrR repressor and *mtrR* promoter were observed in 1 isolate. Multiple substitutions in *penA* (encoding PBP 2 in a mosaic-like structure) resulting in new PBP 2 amino acid patterns, were found in all the strains. The most common was the sequence pattern XXXVIII (9 strains, 5 of them belonging to ST3158).

Conclusions: All ESC^{rS} isolates were associated with new PBP 2-mosaic structures. These alterations, in combination with mutations in other ß-lactam targets, were associated with, and presumably are responsible for, that phenotype. Detection of ESC^{rS} indicates a change in the epidemiology of *Ng* resistance and highlights the importance of a continued surveillance to ensure appropriate therapy in light of the limited antimicrobial options available.

INTRODUCTION

Neisseria gonorrhoeae is one of the most common sexually transmissible infective agents. Antimicrobial resistance in *N. gonorrhoeae* has continued to emerge worldwide, limiting empiric treatment regimens for this disease. Extended-spectrum cephalosporins (ESC, ceftriaxone, CRO, and cefixime, CFM) are recommended as the first-line treatment of gonococcal infections in Canada and the US. While resistance of *N. gonorrhoeae* to ESC has not been described, reduced susceptibility to ESC (ESC^{rS}) has lead to considerable concern, particularly in Japan[1]. Strains with ESC^{rS} have mosaic structures in the transpeptidase-encoding domain of PBP 2. These mosaic PBP 2 are generally associated with mutations in *mtrR* promoter gene, porin PIB and PBP 1.

AIM

The goal of this study was to determine the level of susceptibility to ESC (CFM and CRO) in *N. gonorrhoeae* isolates from Ontario, Canada, and characterize the molecular mechanisms underlying ESC^{rS}.

MATERIALS & METHODS

Strains and antimicrobial susceptibility testing. All consecutive, non-duplicate *N. gonorrhoeae* isolates, received and confirmed during a one month period (Oct. 15 - Nov. 15, 2008) at the Ontario Public Health Laboratories, Toronto were included (N=149). MICs were determined by Etest and agar dilution method according to the Clinical and Laboratory Standards Institute guidelines[2]. *N. gonorrhoeae* ATCC 49226 was used as quality control strain. Presence of ß-lactamase activity was tested using nitrocefin.

Molecular assays. To characterize the molecular background of ESC^{rS}, all the isolates with reduced susceptibility to CRO and CFM were studied. Amino acid changes deduced from nucleotide sequences of *ponA* (PBP 1), *penA* (PBP 2), *porA/B* (outer membrane proteins PIA or B), *pilQ* (PilQ, a pilus secretin protein), *mtrR* (MtrR, a transcriptional repressor of the operon *mtrCDE* encoding an efflux pump), as well as mutations in the *mtrR* promoter were analyzed. The sequence type (ST) of ESC^{rS} isolates was determined by multi antigen sequence typing (NG-MAST)[3].

RESULTS

- **Antimicrobial resistance.** ESC^{rS} were observed in *14 isolates* (9.5%, MICs 0.125-0.25 µg/ml to CFM and 0.032-0.125 µg/ml to CRO). They were resistant to CIP (n=14), TET (n=13), PEN (n=4; 10 PEN^{I}), and non-susceptibility to ERY (MICs ≥2 µg/ml) was observed in 11 isolates.
- **Molecular patterns of the 14 ESC^{rS} isolates.** No mutations in the *pilQ* gene were found. bla_{TEM-1} was not detected. A single amino acid change in PBP 1 (L421P, n=6), single (in position A121, n=1) and double substitutions (positions G120 and A121, n=8) in porin PIB, a single adenine-deletion in the *mtrR* promoter gene (n=12) and an amino acid change in MtrR (G45D, n=1) were observed. Mutations affecting both MtrR repressor and *mtrR* promoter were observed in 1 isolate. Multiple substitutions in *penA* (encoding PBP 2 in a mosaic-like structure) resulting in 5 PBP 2 amino acid patterns, were found in all the strains. The most common was the sequence pattern XXXVIII (9 strains, 5 of them belonging to ST3158) (*Table 1* and *Fig. 1*).

Table 1. *N. gonorrhoeae* isolates with reduced susceptibility to CFM and CRO (n=14) classified by ß-lactam MICs, amino acid alterations in porin PIB, PBP 1 and 2, repressor MtrR and mutations in the *mtrR* promoter.

MIC (µg/ml)			Porin PIB	PBP-1	PBP-2	*mtrR*	MtrR	ST	No. of
PEN	CFM	CRO	(*porB*)	(*ponA*)	pattern	Promoter			isolates
2	0.25	0.125	G120K, A121D	L421P	XXXIV	Deletion A	WT	225	1
2	0.25	0.125	WT	L421P	XXXVII	Deletion A	G45D	299	1
1	0.25	0.125	G120K, A121N	WT	XXXVIII	Deletion A	WT	3158	3
1	0.125	0.125	WT	WT	XXXV	Deletion A	WT	546	1
0.5	0.125	0.125	G120K, A121N	WT	XXXVI	Deletion A	WT	3596	1
1	0.125	0.063	G120K, A121N	L421P	XXXVIII	Insertion T	WT	3158	1
1	0.125	0.063	G120K, A121N	L421P	XXXVIII	Deletion A	WT	3158	1
2	0.125	0.063	G120K, A121N	L421P	XXXVIII	Deletion A	WT	3570	1
2	0.125	0.063	A121S	L421P	XXXVIII	Deletion A	WT	3116	1
1	0.125	0.063	G120K, A121N	L421P	XXXVIII	Deletion A	WT	3563	1
1	0.125	0.063	WT	WT	XXXIV	WT	WT	51	1
0.5	0.125	0.032	WT	WT	XXXVIII	Deletion A	WT	3553	1

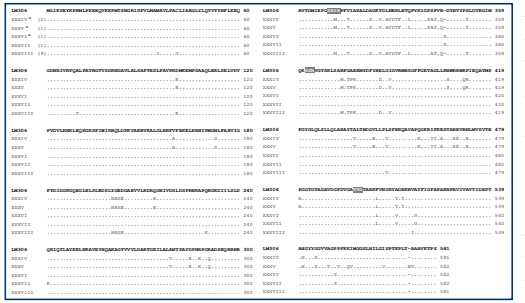

Fig. 1. Deduced amino acid sequences of PBP 2 from 14 ESC^{rS} *N. gonorrhoeae* found in this study, compared with the sequence from the wild type strain LM306 (GenBank accession no. M32091). Active sites are highlighted in grey. The numbers of isolates with each pattern are indicated in parentheses. Periods indicate amino acid residues identical to those of LM306; dashes are blanks.

[a] PBP 2 types previously described, GenBank accession numbers ADE22248 (XXXIV), ZP_04719537 (XXXV) and ZP_04723776 (XXXVI).

CONCLUSIONS

- 9.5% of *Neisseria gonorrhoeae* isolates demonstrated reduced susceptibility to extended spectrum cephalosporins.
- Most of the ESC^{rS} isolates were also multidrug resistant. This phenotype can complicate the empiric treatment regimens in case of ESC treatment failure.
- The 14 ESC^{rS} isolates with PBP 2-mosaic structure mostly showed mutations in *mtrR* promoter gene, porin PIB and PBP 1, supporting the association of all these mutations in the reduced susceptibility to CFM and CRO.
- The detection of ESC^{rS} indicates a change in the local epidemiology of this resistance and highlights the importance of a continued surveillance to preserve the last antimicrobial options available.

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