PHENOTYPIC AND GENOTYPIC CHARACTERISATION OF *NEISSERIA GONORRHOEAE* ISOLATES FROM NEW ZEALAND WITH REDUCED SUSCEPTIBILITY TO CEFTRIAXONE

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ABSTRACT

Objectives

Currently, ceftriaxone is the last remaining drug recommended for empirical treatment of gonorrhoea. *Neisseria gonorrhoeae* with reduced susceptibility to ceftriaxone have been isolated worldwide in countries such as Japan, France, Spain, Slovenia, Australia and Sweden. These have led to treatment failures and the emergence of ceftriaxone-resistant *N. gonorrhoeae*. Various mutations in *penA* (mosaic and nonmosaic), which encodes the penicillin-binding protein 2 (PBP2), have been reported to be the primary reason for reduced ceftriaxone susceptibility, but it can be reduced further by mutations in *mtrR*, $porB_{IB}$ and *ponA*. In this study, we aimed to determine the antimicrobial resistance patterns of New Zealand isolates of *N. gonorrhoeae* with reduced susceptibility to ceftriaxone and to characterise the *penA*, *mtrR,* $porB_{IB}$ and *ponA* in the isolates.

Methods

A total of 28 *N. gonorrhoeae* isolates with elevated ceftriaxone MIC (0.03 to 0.12 mg/L), collected from 2012 to 2015 and obtained from the Institute of Environmental Science and Research (ESR), were examined in this study. Samples came from laboratories in Auckland (26), Wellington (1) and Taranaki (1). The antimicrobial resistance of penicillin G, tetracycline, ciprofloxacin, azithromycin and ceftriaxone were determined through antimicrobial susceptibility test, using minimum inhibitory concentration (MIC) test strips. Polymerase chain reactions (PCRs) and sequencing to identify specific mutations in *penA*, *mtrR*, $porB_{IB}$ and *ponA,* that are associated with elevated minimum inhibitory concentrations (MICs) to ceftriaxone, were undertaken. The association between the phenotypic and genotypic results was investigated by comparing the presence of the number of mutated genes and the MIC level of ceftriaxone.

Results

Based on the AST results using MIC test strips and interpreted using The European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, 23 out of 28 isolates (82%) showed reduced susceptibility to ceftriaxone, with MICs of 0.03 to 0.06 mg/L. All of the isolates were resistant to ciprofloxacin, while 36%, 25% and 7% were resistant to penicillin G, tetracycline and azithromycin, respectively. Two azithromycin-resistant *N. gonorrhoeae* isolates were observed, and isolate 264 (azithromycin MIC: 4mg/L) also exhibited reduced susceptibility to ceftriaxone (MIC: 0.03 mg/L). A total of 21% (6/28) of the isolates produced β-lactamase. The 23 isolates that conveyed reduced ceftriaxone susceptibility were found to harbour three or four mutated genes (*penA, mtrR and/or porB$_{IB}$ and ponA*). Reduced susceptibility to ceftriaxone among *N. gonorrhoeae* isolates in this study was associated with mosaic PBP2 (encoded by *penA*) with G545S/A501V mutations, with nonmosaic PBP2 with an A501V mutation, plus the presence of mutation in *mtrR* promoter with G120 and A121 alterations in PorBIB. A total of 65% (15/23) of the *N. gonorrhoeae* isolates with reduced susceptibility to ceftriaxone harboured mosaic PBP2 XXXIV, a pattern found in *N. gonorrhoeae* associated with ceftriaxone treatment failures in Europe and Australia. The current study also revealed that the partial sequences of four mosaic PBP2 (M-2, M-3, M-4, M-5) were different from the common mosaic PBP2 sequences reported in various studies.

Conclusion

There is an association between the phenotypic and genotypic character of *N. gonorrhoeae* isolates expressing reduced susceptibility to ceftriaxone in this study population. Furthermore, the presence of important mosaic PBP2 that link to ceftriaxone treatment failure might be circulating among *N. gonorrhoeae* isolates in New Zealand .

Keywords: *Neisseria gonorrhoeae,* ceftriaxone, reduced susceptibility, New Zealand

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CHAPTER ONE: INTRODUCTION

APPENDIX G

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
ATCC	American type culture collection
BHI	Brain Heart Infusion
BLAST	Basic Local Alignment Search Tool
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
CLSI	Clinical Laboratory Standards Institute
CMRNG	Chromosomally-mediated resistant *Neisseria gonorrhoeae*
CO_2	Carbon dioxide
CRO	Ceftriaxone
DDBJ	DNA Data Bank of Japan
DHB	District health board
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
ESCs	Extended-Spectrum Cephalosporins
ESR	Institute of Environmental Science and Research
EtBr	Ethidium Bromide
EUCAST	The European Committee on Antimicrobial Testing
FDA	Food and Drug Administration
GISP	Gonococcal Isolate Surveillance Project
HIV	Human Immunodeficiency Virus
HLR	High-level resistance
HTH	Helix-turn-helix
IM	Intramuscular injection
MDR	Multidrug-resistant
$MgCl_2$	Magnesium Chloride
MGS	Massey Genome Service
MIC	Minimum Inhibitory Concentration
MLST	Multi-locus sequence typing

MSM	Men who have sex with men
mtr	Multiple transferable system
MUHEC	Massey University Human Ethics Committee
NAAT	Nucleic Acid Amplification Test
NCBI	National Center for Biotechnology Information
NETs	Neutrophil Extracellular Traps
NG-MAST	*Neisseria gonorrhoeae* Multi-antigen Sequence Typing
NPV	Negative predictive value
NZSHS	The New Zealand Sexual Health Society Incorporation
PBP	Penicillin Binding Protein
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PEN	Penicillin
PHE	Public Health England
PID	Pelvic Inflammatory Disease
PMNs	Polymorphonuclear leukocytes
PPNG	Penicillinase-producing *Neisseria gonorrhoeae*
PPV	Positive predictive value
qPCR	Quantitative Polymerase Chain Reaction
STIs	Sexually transmitted infections
TMP-SMX	Trimethoprim/sulfamethoxazole
TAE	Tris-Acetate-EDTA
TrisHCL	Tris hydrochloride
TRNG	Tetracycline-resistant *Neisseria gonorrhoeae*
WHO	World Health Organization
XDR	Extensively drug resistant

Nucleotides Abbreviations

A	Adenine
G	Guanine
C	Cytosine
U	Uracil
T	Thymine

Amino Acids Abbreviations

A	Alanine
R	Arginine
N	Asparagine
D	Aspartic Acid
C	Cysteine
E	Glutamic Acid
Q	Glutamine
G	Glycine
H	Histidine
I	Isoleucine
L	Leucine
K	Lysine
M	Methionine
F	Phenylalanine
P	Proline
T	Threonine
V	Valine
Y	Tyrosine
S	Serine
W	Tryptophan