Por Types and Mutations Associated with Antimicrobial Resistance in *N. gonorrhoeae* Isolates

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Introduction

• *N. gonorrhoeae porB* genes (*porB1a* and *porB1b*) encode PorB proteins, PIA and PIB, respectively.

• PorB is a transmembrane protein comprising 8 highly variable loops.

•Mutatons on PorB protein are associated with antimicrobial resistance.

•Shanghai, China, with a population of more than 17 million, is the trade capital of China. The STI rate in Shanghai is increasing and is the highest in China, with a gonorrhea prevalence rate of ~75 to 107 per 100 thousand population.

http://www.maps-of-china.com/china-country.shtml

•We reported the relationship between gonococcal *porB* types and the antimicrobial resistance of the isolates from Shanghai.

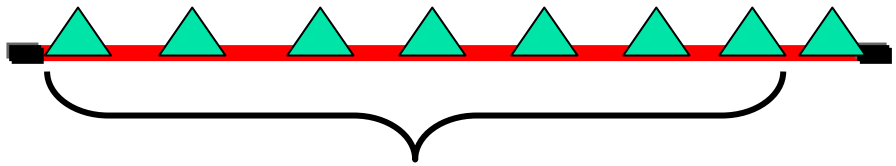
Methods

• **143 *N. gonorrhoeae* isolates** from male patients were consecutively collected at the Shanghai Skin Disease and STD Hospital, Shanghai, China, between November 2004 and May 2005.

• **The Minimum Inhibitory Concentrations** (MICs) to penicillin, tetracycline, ciprofloxacin, spectinomycin and ceftriaxone were determined by the agar dilution method, with interpretive criteria as described by the NCCLS.

• ***porB* DNA sequencing:** The entire *N. gonorrhoeae porB* was amplified from chromosomal DNA by PCR, and both strands of the PCR products were analyzed by DNA sequencing.

• **Sequence editing**: Each set of sequences was aligned using CLUSTAL W and edited using Jalview Alignment Editor (http://www.ebi.ac.uk/clustalW). The unambiguous sequences spanned ~85% of *porB* and contained 7 polymorphic areas encoding the surface-exposed loops (Loop I-VII) as well as the interspacing regions II-VII.



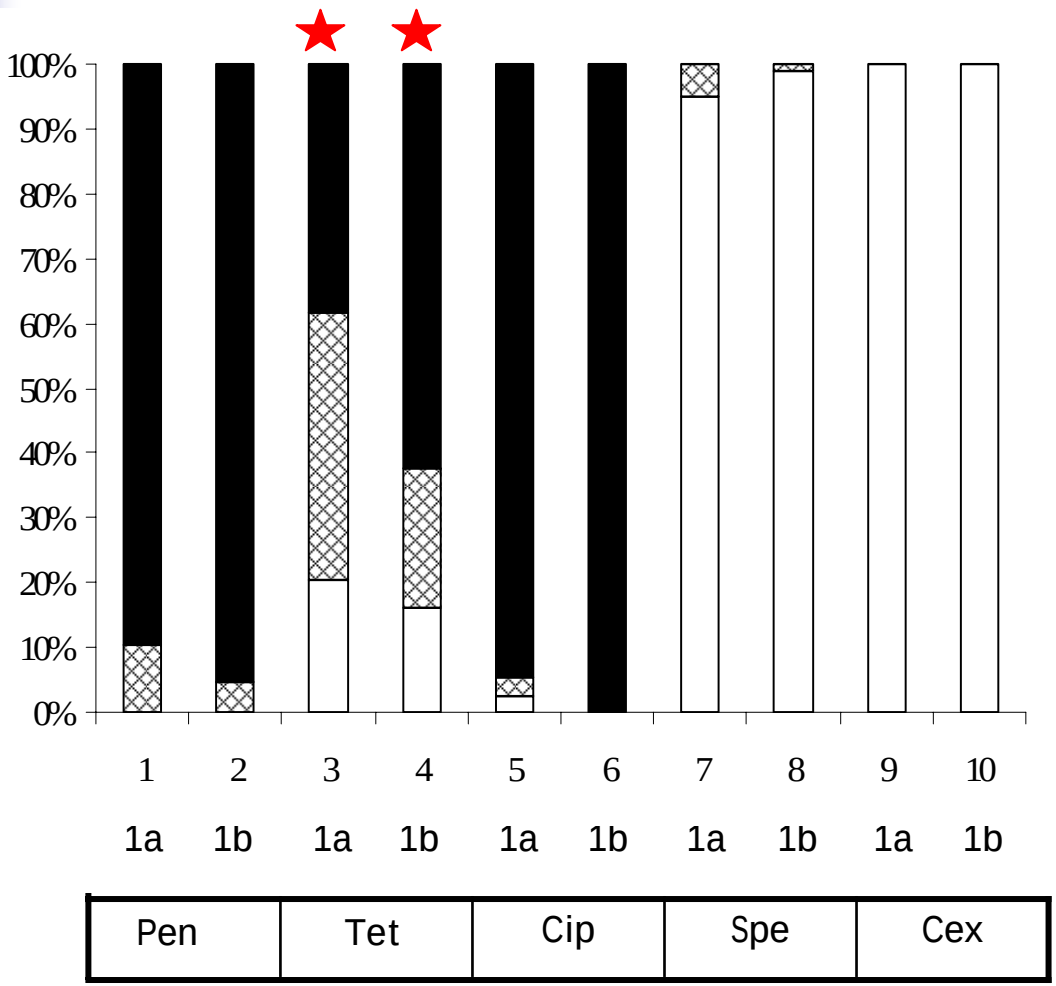
• ***porB* genotypes** were assigned based on the presence or absence of two nucleotide fragments in the loop V coding region. *porB*1a isolates were characterized by a deletion of ~60 nucleotides corresponding to positions nt681-705 and nt718-736 of *porB*1b.

• *porB* DNA sequences were translated into amino acid (**AA**) sequences using Proteomics and Sequence Tools of ExPasy Proteomics Server (http://ca.expasy.org).

• **Alignments** of the deduced protein sequences were carried out using Clustal W. *porB* sequences from strain MS11 (*porB*1a, GeneBank accession #J03029) or FA1090 (*porB*1b, GeneBank accession #J03017) were used as prototypes.

• **Statistical analysis:** Chi-squared analysis was performed to determine the significance of differences among the enumeration data sets.

Association of antimicrobial susceptibility and *porB* genetic types of *N. gonorrhoeae*

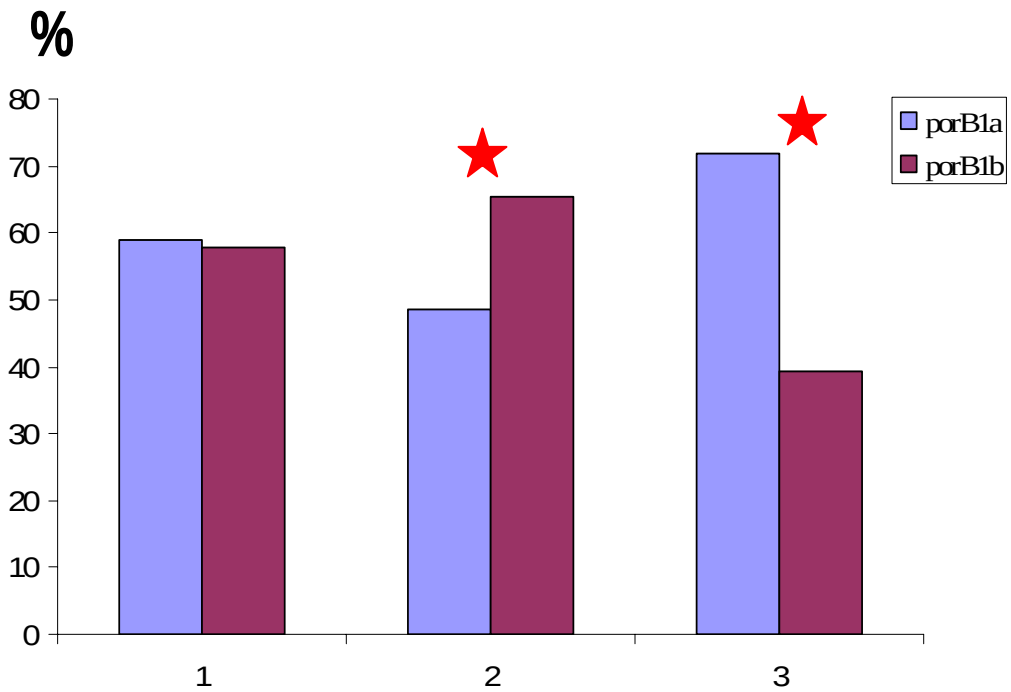


Resistance
Intermediate
Susceptible

Pen: penicillin;
Tet: tetracycline;
Cip: ciprofloxacin;
Spe: spectinomycin;
Cex: ceftriaxone.
Stars indicate that the percentages were significantly different.

porB1a, n=39
porB1b, n=104

Antimicrobial resistant phenotypes of gonococcal *porB*1a and *porB*1b isolates.



porB1a, n=39
porB1b, n=104

PM: Plasmid-mediated resistant
CM: Chromosomally mediated resistant
I: Intermediate susceptible
S: Susceptible

Antimicrobial resistant phenotypes of *porB*1a and *porB*1b *N. gonorrhoeae* isolates

Antimicrobial Susceptibility	*porB*1a, n=39 (%)	*porB*1b, n=104 (%)	P value[a]
PPNG	12 (30.8)	42 (40.4)	>0.05
PP/TRNG	6 (15.4)	14 (13.5)	>0.05
TRNG	5 (12.8)	4 (3.8)	≤0.05
CMPR	15 (38.5)	21 (20.2)	≤0.025
CMTR	2 (5.1)	25 (24.0)	≤0.025
CMRNG	2 (5.1)	22 (21.2)	≤0.025
Penicillin I[b]	4 (10.3)	5 (4.8)	>0.05
Penicillin S[b]	0	0	Not available
Tetracycline I[b]	8 (20.5)	21 (20.2)	>0.05
Tetracycline S[b]	16 (41.0)	18 (17.3)	≤0.01

a. P values were determined by Chi-square tests.
b. Susceptible isolates (S) or isolates with intermediate levels of MICs (I)

Mutations of residues G120 and A121 of *porB* in *N. gonorrhoeae* isolates from Shanghai

	*porB*1b, n=104 (%)	*porB*1a, n=39 (%)
G120K/A121D	**74 (71.1)**	NA
G120K/A121G	**7 (6.7)**	NA
G120K/A121N	**6 (5.8)**	NA
G120R/A121D	**1 (1.0)**	NA
G120N/A121N	**1 (2.0)**	NA
G120D/A121A	**12 (11.5)**	NA
G120G/A121D	**1 (1.0)**	NA
G120G/A121A	**2 (1.9)**	NA
G120D/A121G	NA	32 (82.1)
G120G/A121G	NA	7 (17.9)
Total	**104 (100.0)**	**39 (100.0)**

Conclusions

- The burden of antimicrobial resistance of *N. gonorrhoeae* to penicillin, tetracycline and ciprofloxacin in Shanghai was high. Decreasing levels of susceptibility to spectinomycin were noted in some isolates.
- *porB*1b isolates were less susceptible to antibiotics with significantly higher ratio of chromosomally-mediated resistance.
- All *porB*1a isolates having either G120D/A121G or a single mutation A121G.
- High percentages of *porB*1b isolates carried G120K/A121D double mutations.
- Novel spontaneous mutations in *porB*1b isolates included G120R/D, or A121G/N.

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