WORLD 2015 STI & HIV CONGRESS
BRISBANE AUSTRALIA 13 - 16 SEPTEMBER

Session: Detecting antimicrobial resistance and treatment failure
14.09.2015

Multiplex real-time PCR with High Resolution Melting analysis for detecting resistance mechanisms in *Neisseria gonorrhoeae*

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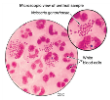
Background



- **Culture** ⟹ gold standard; antimicrobial susceptibility testing possible, but requires at least **2 days**
- **NAATs** ⟹ sensitive and rapid, but **do not provide** information about **antimicrobial susceptibility**

Antibiotics	1998-2001		2010-2012	
	% non-S	MIC_{50}	% non-S	MIC_{50}
Penicillin	42.3	3	85.3	16
Spectinomycin	0.0	12	0.0	8
Ciprofloxacin	7.7	0.006	73.5	≥32
Cefoxime	0.0	≤0.016	8.8	0.125
Ceftriaxone	0.0	0.004	0.0	0.047
Azithromycin	11.5	0.25	23.6	0.38

Endimiani *et al.*, 2014

Antimicrobial resistance determinants

16S rRNA	C1192T
5S rRNA	Thr24Pro
GyrA	Ser91Phe
PBP2 (*penA*)	Mosaic (i.e., Gly545Ser), Ala501Pro/Val/Thr
23S rRNA	A2059G - C2611T

Objectives/Methods

Development of a **rapid diagnostic test** for the identification of ***N. gonorrhoeae*** and of potential **antimicrobial resistance** determinants directly from **clinical specimens**

⇩

SybrGreen-based real-time PCR with **High Resolution Melting** analysis of gDNA after direct extraction from eSwabs

Methods

2 detection + 6 antimicrobial resistance targets

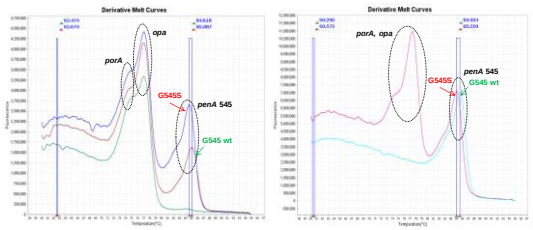
Triplex	***opa*, *porA*, *penA* Gly545Ser**
Duplex #1	**GyrA Ser91Phe and 23S rRNA A2059G**
Duplex #2	**23S rRNA C2611T and *penA* 501**
Duplex #3	**16S rRNA C1192T and 5S rRNA Thr24Pro**

Control strains used for method validation:

- **REFERENCE STRAINS:**
  - **Ceftriaxone-R** (mosaic *penA* XXXIV, Ala501Pro)
  - **Azithromycin-R** (23S rRNA A2059; 23S rRNA C2611T)
  - **Spectinomycin-R** (16S rRNA C1192T; 5S rRNA)
  - **Other WHO, ATCC strains**
- **FULLY CHARACTERIZED ISOLATES:**
  - **GyrA Ser91Phe** (n=22)
  - ***penA* mosaics** (XXXIV, n=7) and non-mosaics
- **NON-GONOCOCCAL SPECIES** (n=10)

Results: Triplex
opa + *porA* + *penA* 545

Neisseria gonorrhoeae | ***Neisseria* spp.**

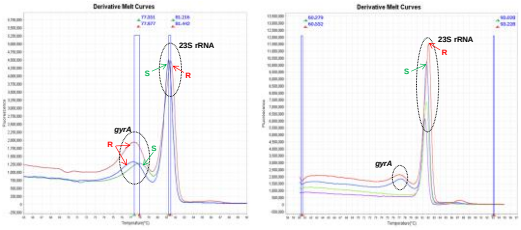


Blue: mosaic *penA* pattern X, XXXIV
Red: *penA* pattern XIX
Green: *penA* pattern V, II, XII

Pink: *N. gonorrhoeae penA* pattern XXXIV
Light blue: *N. mucosa, N. flavescens*

Results: Duplex #1
GyrA Ser91Phe + 23S rRNA A2059G

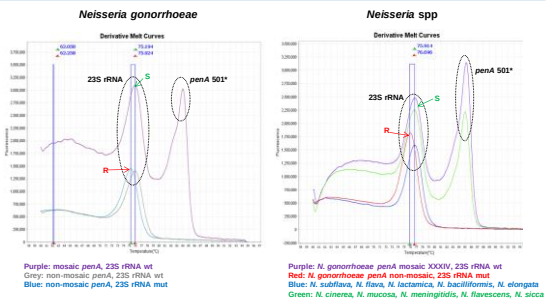
Neisseria gonorrhoeae | ***Neisseria* spp**



Red: *gyrA* mut, 23S rRNA mut
Blue: *gyrA* mut, 23S rRNA wt
Green: *gyrA* wt, 23S rRNA wt

Red: *N. gonorrhoeae* 23S rRNA mut
Blue: *N. gonorrhoeae* 23S rRNA wt
Green: *N. meningitidis, N. lactamica, N. bacilliformis*
Pink: *N. cinerea, N. mucosa, N. subflava, N. flava, N. elongata, N. flavescens, N. sicca*

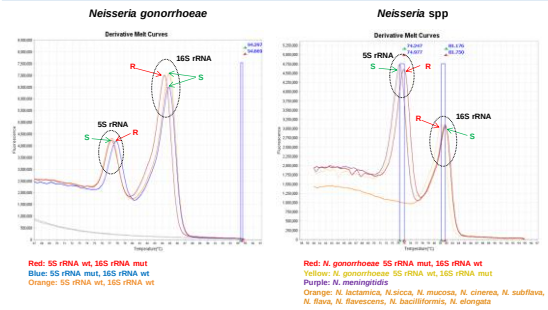
Results: Duplex #2
23S rRNA C2611T + *penA* 501



Purple: mosaic *penA*, 23S rRNA wt
Grey: non-mosaic *penA*, 23S rRNA wt
Blue: non-mosaic *penA*, 23S rRNA mut

Purple: *N. gonorrhoeae penA* mosaic XXXIV, 23S rRNA wt
Red: *N. gonorrhoeae penA* non-mosaic, 23S rRNA mut
Blue: *N. subflava, N. flava, N. lactamica, N. bacilliformis, N. elongata*
Green: *N. cinerea, N. mucosa, N. meningitidis, N. flavescens, N. sicca*

Results: Duplex #3
5S rRNA Thr24Pro + 16S rRNA C1192T



Red: 5S rRNA wt, 16S rRNA mut
Blue: 5S rRNA mut, 16S rRNA wt
Orange: 5S rRNA wt, 16S rRNA wt

Red: *N. gonorrhoeae* 5S rRNA mut, 16S rRNA wt
Yellow: *N. gonorrhoeae* 5S rRNA wt, 16S rRNA mut
Purple: *N. meningitidis*
Orange: *N. lactamica, N.sicca, N. mucosa, N. cinerea, N. subflava, N. flava, N. flavescens, N. bacilliformis, N. elongata*

Results:

Control strains
(Gonococcus, n=40; *Neisseria* spp., n=20)

Molecular target	sensitivity	specificity
opa	100%	100%
porA	100%	100%
GyrA Ser91Phe	100%	100%
23S rRNA A2059G	100%	100%
23S rRNA C2611T	100%	100%
Mosaic *penA*	100%	100%
16S rRNA C1192T	100%	100%
5S rRNA Thr24Pro	100%	100%

Gold standard: PCR/Seq

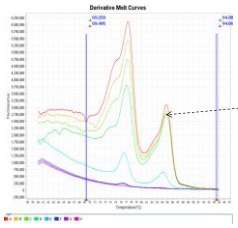
Clinical isolates
(n=99)

Identification/AMR	sensitivity	specificity
Detection of NG	100% (99/99)	NA
Ciprofloxacin	100% (20/20)	100% (79/79)
Azithromycin	2.5%* (1/40)	100% (59/59)
Cephalosporins	NA	99%* (98/99)
Spectinomycin	NA	100% (99/99)

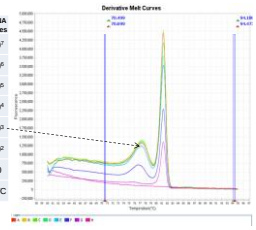
Gold standard: MALDI-TOF MS/MIC (Etest)
(EUCAST 2015)

Results:
Limit of detection (LOD)

***opa* + *porA* + *penA* 545**



GyrA Ser91Phe + 23S rRNA A2059G



LOD for proper high resolution melting analysis is ≥10^3-10^4 gDNA copies/reaction

Conclusions

Advantages:

- **Rapid** (1.5 h) and **cheap**
- **Multiplex** 2 or 3 reactions/well
- **Good sensitivity** and **specificity** when testing main resistance targets in **isolated cultures**

Disadvantages:

- **Technical issues** (i.e., G/C SNPs)
- **Needs high limit of detection** for proper high resolution melting analysis
- **Cross-reaction** with *Neisseria* spp.

Suitable for screening of isolated cultures but not for clinical specimen with low target gDNA (e.g., pharyngeal samples)

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