Implementation of genotypic resistance testing for *Neisseria gonorrhoeae* in a multiplex-PCR

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Background

Neisseria gonorrhoeae causes the sexually transmitted disease gonorrhoea. Treatment options for this infection are limited by the increasing emergence of resistance to antimicrobial agents. For this reason, it is important to treat the infection according to the resistance situation. We have developed a multiplex PCR for the rapid identification of SNP mutations in *Neisseria gonorrhoeae* resistance-relevantfor ciprofloxacin, ceftriaxone, cefixime and penicillin, which enables timely and resistance-adapted therapy.

Methods

Colonies of Neisseria gonorrhoeae cultured from patients with known resistance combinations (epsilometer test MICs assessed according to EUCAST criteria) were used for establishing the method. Genotypic resistance was identified by SNP-specific multiplex real-time PCR: gyrA and parC (Ciprofloxacin resistance) according to Giles et al. (2004), PPNG (penicillinase-producing *Neisseria gonorrhoeae)* according to Goire N et al. (2011) and PBP-2 (penicillin-binding protein) according to Ochiai S et al. (2008). The probes were labelled for multiplex PCR with the colours Yakima Yellow, Texas Red, Cy5.5 and FAM. Extraction of bacterial DNA was performed with the STARMag 96 Universal Cartridge Kit (Seegene), the PCR operated with the TaqPath™ ProAmp™ multiplex master mix (Applied Biosystems) on the CFX96 cycler (Biorad). Phocine herpesvirus 1 (PhHV-1, TIB Molbiol) served as internal PCR control. The sensitivity of multiplex PCR was determined in dilution series, and the minimal copy number to detect mutations was calculated. The PCR was tested in 29 routine PCR swab samples, from which no culture could be established due to the medium in the PCR swabs.

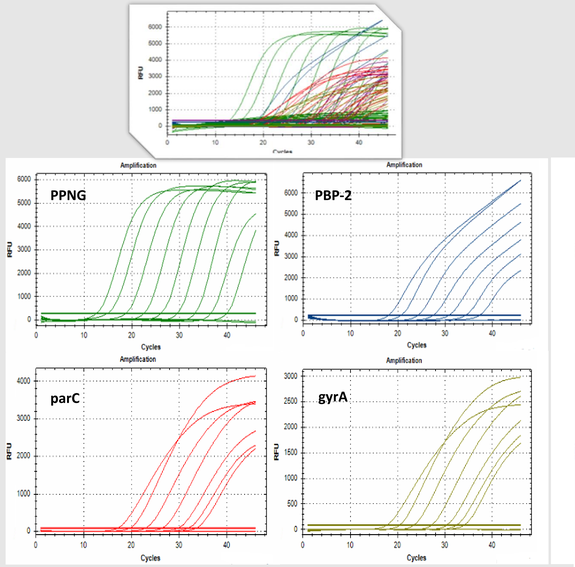

Fig. 1: gyrA-PCR (olive green), *parC*-PCR (red), PBP-2 PCR (blue), PPNG-PCR (bilious green) and IC (purple). PCR for serial dilutions.

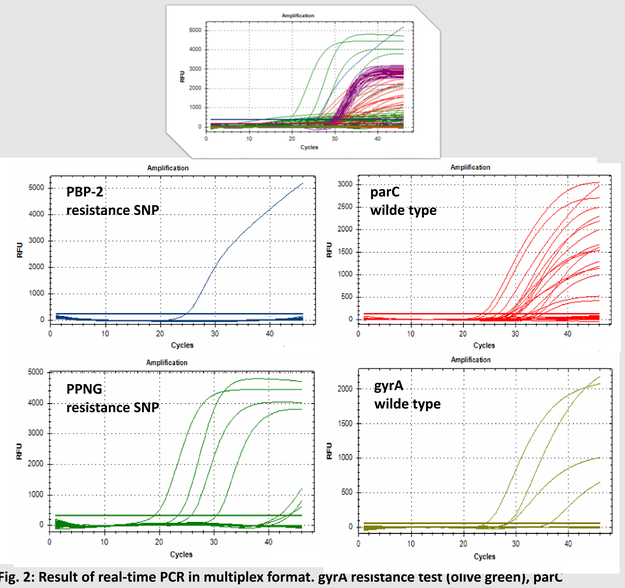

Fig. 2: Result of real-time PCR in multiplex format. gyrA resistance test (olive green), parC resistance test (red), PBP-2 resistance test (blue), PPNG resistance test (poison green) and IC (purple). PCR result of the individual targets for testing on routine samples

Tab.1 : minimal copy number per reaction

Target	copies per reaction
gyrA	90
parC	1095
PBP-2	80
PPNG	5

CONCLUSIONS

The method developed in this work can detect genotypic resistance in less than 4 hours, and routine use in the laboratory is possible. The phenotypic relevance of the positively tested *Neisseria gonorrhoeae* should still be confirmed with further samples. The correlation between Ct values and measured MICs showed partly larger differences, this should be further investigated. The significance of Ct values above 38 has not yet been clearly clarified; further investigations will follow.

Results

The probes for the *gyrA* and *parC* genes were designed in a way that a fluorescent signal appears only when the wild type is amplified. No signal is a hint for a possible resistance mutation. A positive PCR result for PPNG and PBP-2 indicates a resistance-associated SNP. Here a negative PCR result means that no SNP and thus no resistance was present. Multiplex PCR showed high sensitivity for the individual SNPs without mutual interference of the results (Fig. 1). The detection limits for *gyrA; parC*; PBP-2 and PPNG were 90; 1095; 80 and 5 genome copies per reaction, respectively. Resistance detected in classical culture was confirmed in multiplex PCR (Fig. 3). The reaction for *parC* resistance was positive in two additional samples, one sample showed a very weak resistance to ciprofloxacin in culture, the second showed no resistance in culture at all. One sample had a phenotypic high-level penicillin resistance in culture but only a Ct value of 36. In addition, a sample with multiple resistances was tested and the PCR of the single resistances was not affected.

Of the 29 routine samples (Fig. 2) tested, 13 had a SNP in the *parC* gene, 25 samples were positive for a *gyrA* resistance. Four samples showed a penicillin resistance mediated by PPNG. Additionally three Ct values >38 for PPNG were observed. The prevalence of PBP-2 in *Neisseria gonorrhoeae* was very low as only one sample tested positive. The data show that over a wide range of pathogen concentrations (Ct), the detection of resistance is possible.

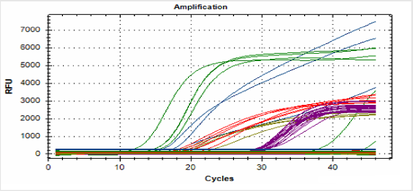

Fig. 3: Results of the multiplex format of different colonized samples: gyrA PCR (olive green), parC PCR (red), PBP-2 PCR (blue), PPNG PCR (poison green) and IC (purple).