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Genotyping of the Resistance Determinant of *Neisseria Gonorrhoeae* with Reduced Susceptibility to Ceftriaxone in Manaus-AM-Brazil

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Abstract

Gonorrhea is the second most prevalent sexually transmitted infection worldwide, with an estimated 78.3 million new cases. At the Alfredo da Matta foundation, *gonorrhea* as the main cause of urethral discharge with prevalence of 16.8%. *Gonococci* have developed resistance to all the antibiotics leaving cephalosporins as the last option for treatment. In this report, we genotype the determinants of resistance to Extended Spectrum Cephalosporins, such as *penA, ponA, porB, mtrR, pilQ,* of a *Neisseria gonorrhoeae* strain, isolated from a male patient with urethral discharge. The ST1901was identified by MLST protocol and genotyping of the *penA*, showed mutations on regions F505L, A511V, A517G, N542H e P522S which confirmed the presence of *gonococcus* with reduced susceptibility to ceftriaxone in the region.

Keywords: Genotyping; MLST; *N. Gonorrhoeae*; Cephalosporin; Resistance

Text

In last few decades, gonococci have developed resistance to all the antibiotics used as first line of treatment for gonococcal infections, leaving Extended- Spectrum Cephalosporins (ESCs) as the last remaining option for gonorrhea [1,2]. With reports of reduced susceptibility or resistance to ESCs from different regions, and due to therapeutic limitations, the infection has become a serious health problem to the point that disease complications can no longer be treated in the near future, besides the possibility of the gonococcus to evolve into "superbug" [3-7].

Gonorrhea is the second most prevalent sexually transmitted infection worldwide, with an estimated 78.3 million new cases in 2012 [8]. At the Alfredo da Matta foundation (Manaus-Brazil), gonorrhea appears as the main cause of urethral discharge, with an average of 513 cases in 20 years and prevalence of 16.8%. In this report, we describe the molecular characteristics of the *N. gonorrhoeae* strain NgFUAM84, isolated from the urethral discharge of a male patient, with MIC of 0.064µg/mL for ceftriaxone in E-test (AB Biodisk, Solna, Sweden) [9]. The determinants of resistance to ESCs: *penA, ponA, porB, mtrR and pilQ* were amplified by PCR (Proflex PCR System-Applied Biosystems) using primers previously described [10,11]. The sequencing of amplicons was performed on the ABI 3130 Genetic Analyzer (Applied Biosystems). The substitutions in the residues were analyzed using the software Geneious v.10.0.10 and identified by comparison with the sequences deposited in GenBank (Figure 1).

The molecular epidemiology was determined by Multi Locus Sequence Typing, performed according to the guidelines described in (http://pubmlst.org/neisseria). Clinical aspects, phenotypic characteristics, antimicrobial susceptibility test, analysis of the genes *gyrA* and *parC*, and identification of the ST225 for the NG-MAST (http://www.ng-mast.net)was performed as described earlier [9]. The NgFUAM84 was no beta-lactamase producer and were resistant to Ciprofloxacin (> 32 g/mL), Chloramphenicol (3 g/mL), Ofloxacin (> 32 g/mL), reduced susceptibility to Penicillin (0.75 g/mL) and Tetracycline (0.75 g/mL) [9]. The genotyping by MLST identified ST1901; clone associated with reduced sensibility and resistance to ECSs and is predominant worldwide [1].

Analysis of the gene *ponA* showed a single mutation at the L421P position, whereas in the gene *mtrR*, a single deletion of adenine (A) in the inverted position of the promoter region was identified. The NgFUAM84 also presented resistance determinant *penB* with substitutions at position G120K, A121D of loop 3 of PorB1b. These mutations have been associated with reduced sensitivity and resistance of gonococci to ECSs [6,14]. A single substitution on position G554D was identified in PilQ (Table 1). Analysis of the *penA* made possible identification of substitutions at positions F505L, A511V, A517G and P552S. The PBP2 protein showed similarity of 99.3% with the XXIV allele (GenBank

accession number: FJ465093 and 99.7% and with strain NJ5 GenBank accession number: KF576657) [3,6]. Changes in the susceptibility of gonococcus to ceftriaxone were previously detected in Manaus however; genotyping of those strains was not performed. Recent studies (unpublished) show that gonococcus circulating in the region are still sensitive to the ESCs, however, elevated MICs to ceftriaxone has been detected [15]. Other authors have associated the presence of PBP2 mosaic alleles and mutations at A501V and A501T positions with reduced sensitivity and resistance to cefixime and ceftriaxone [5,7,14]. We did not identify these substitutions in NgFUAM84, however, the MIC of 0.064µg/mL reinforces the possibility that non-mosaic *penA* mutations can increase the MICs of ESCs similar to those mediated by mosaic allele [3].

The mutations observed in the resistance determinants of ESCs in NgFUAM84 strain as well the identification of STs 225 and 1901, confirm the presence of gonococci with reduced susceptibility to ceftriaxone in the region and reinforces the need for monitoring of the susceptibility of gonococcus to these antibiotics and extensive research for better understanding of the resistance's mechanisms in order to maximize the effectiveness of ESCs in the treatment of gonorrhea. [4].

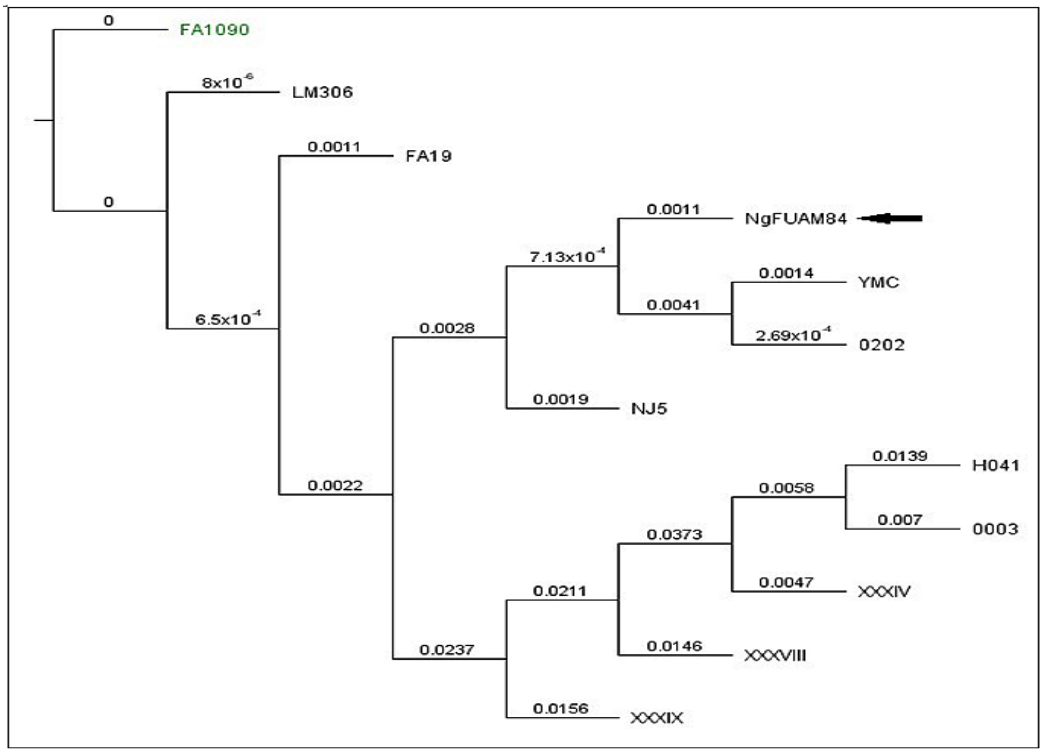

Figure 1: Cladogram of full-length of FUAM84's *penA* sequence with reference FA1090 (NC002946) and others: NgLM306 (NGOPENA-M32091.0) ; NgFA19; (NZ_AKCG00000000.1)WT ; NgYMC/NG02/37 (FJ465093.1) Lee[3] ; Ng0202 (AB511946) Ohnishi[13] ; NgNJ-5 (KF576657.1) Li[6] ; NgH041 (AB546858) Ohnishi[5] ; Ng0003 (AB511945) Ohnishi[13] ; (HQ204552) NgXXXIV and (HQ204565) NgXXXVIII Allen[11] ; (JF893455.1) NgXXXIX Martin unpublished. The arrow represents the rate of the sample of the study.

Table 1: Genotype characteristics - NgFUAM84

Gene	Mutations	GenBank Access n°
penA•	F505L, A511V, A517G and P552S	MF048800
ponA	L421P	MF062527
porB	G120K, A121D	MF048801
mtrR	Deleção A	MF095076
pilQ	G554D	MF095077

•Presence of the extra codon on positions 346 of NgFUAM84 PBP2

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