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SHORT COMMUNICATION

Draft Genome Sequence of *Neisseria gonorrhoeae* Strain NG_869 with Penicillin, Tetracycline and Ciprofloxacin Resistance Determinants Isolated from Malaysia

Geik Yong Ang[1] · Choo Yee Yu[1] · Delicia Ann Yong[1] · Yuet Meng Cheong[2] · Wai-Fong Yin[1] · Kok-Gan Chan[1]

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Abstract Gonorrhea is a sexually transmitted infection caused by *Neisseria gonorrhoeae* and the increasing reports of multidrug-resistant gonococcal isolates are a global public health care concern. Herein, we report the genome sequence of *N. gonorrhoeae* strain NG_869 isolated from Malaysia which may provide insights into the drug resistance determinants in gonococcal bacteria.

Keywords Drug resistance · Genome sequence · *N. gonorrhoeae* · Sexual transmitted diseases · Gonorrhea

New cases of sexually transmitted infection caused by *Neisseria gonorrhoeae* were estimated to be a staggering 106.1 million in 2008 [1]. The emergence and dissemination of antimicrobial-resistant *N. gonorrhoeae* is undermining the management and control of gonococcal infections as effective therapeutic options continued to dwindle. Isolation of penicillinase-producing *N. gonorrhoeae* in Malaysia was first reported in 1977 followed by tetracycline- and quinolone-resistant strains in 1990 and 2001, respectively [2–4]. Although the resistance rate for penicillin amongst tested isolates from Malaysia has dropped from 61 % in 2007 to 23.5 % in 2010, resistance rate for quinolone remained above 80 % whilst tetracycline was between 35 and 70 % [1, 5].

The clinical isolate of *N. gonorrhoeae* strain NG_869 was obtained from a male patient in June 2014 and the genomic DNA was extracted using Epicenter MasterPure DNA Purification Kit (Madison, WI). The genome was sequenced using llumina HiSeq 2000 platform (San Diego, CA) with a 300-bp paired-end library template and a total of 3,251,582 reads with an average length of 98 bp were obtained. De novo assembly performed using CLC Genomics Workbench version 7.0 (Aarhus, Denmark) yielded 220 contigs with an average coverage of 218.13-fold. The contigs were annotated using Rapid Annotation using Subsystem Technology version 2.0 [6], Prokka [7] and NCBI Prokaryotic Genome Annotation Pipeline version 2.10 [8]. The genome was found to be 99.71 % complete when analyzed using CheckM [9]. The genome size of NG_869 is 2,124,678 bp out of which 2,072,962 bp (52.6 % G + C content) are chromosomal and the remaining 51,716 bp belong to three circular plasmids. A total of 2572 protein coding genes, 346 subsystems and 49 RNA genes (3 rRNA and 46 tRNA) were predicted. In the RAST-annotated genome, most of the genes were assigned into amino acids and derivatives (14.8 %) followed by protein metabolism (12.2 %) and cofactors, vitamins, prosthetic groups and pigment (10.7 %) subsystems (Table 1). Genes encoding iron acquisition system, multidrug resistance efflux pumps and type IV secretion system were also identified in the genome.

The 42,005-bp plasmid pNG869_1 (47.94 % G + C content) harboured the Dutch-type tetracycline resistance gene (*tetM*). Another tetracycline resistance gene which was chromosomally mediated was also found as Val-57-to-Met point mutation in the ribosomal protein S10 was identified [10, 11]. The $bla_{\text{TEM-1B}}$ gene was found within

✉ Geik Yong Ang
geikyong@gmail.com

✉ Kok-Gan Chan
kokgan@um.edu.my

[1] Division of Genetics and Molecular Biology, Faculty of Science, Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia

[2] Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, 46150 Selangor, Malaysia

Table 1 Subsystem features of *N. gonorrhoeae* strain NG_869 in RAST annotation system

Subsystem features	Counts
Cofactors, vitamins, prosthetic groups, pigments	167
Cell Wall and capsule	102
Virulence, disease and defense	43
Potassium metabolism	11
Phages, prophages, transposable elements, plasmids	4
Membrane transport	65
Iron acquisition and metabolism	20
RNA metabolism	97
Nucleosides and nucleotides	58
Protein metabolism	189
Cell division and cell cycle	31
Regulation and cell signaling	30
Secondary metabolism	4
DNA metabolism	105
Fatty acids, lipids, and isoprenoids	64
Nitrogen metabolism	30
Dormancy and sporulation	1
Respiration	75
Stress response	61
Metabolism of aromatic compounds	4
Amino acids and derivatives	230
Sulfur metabolism	3
Phosphorus metabolism	11
Carbohydrates	119
Miscellaneous	30

the 5600-bp African-type β-lactamase plasmid pNG869_2 (39.65 % G + C content) along with strong overlapping promoters (*Pa*/*Pb*) which are associated with an approximate 10-fold increase in β-lactamase transcriptional level [12, 13]. The third plasmid, pNG869_3 (51.54 % G + C content), harboured a virulence-associated protein D (*vapD*). Analysis of other genes involved in high-level penicillin resistance revealed that isolate NG_869 possessed the *penB* resistance determinant with double mutations of Gly-120-to-Asp and Ala-121-to-Gly but the *mtrR* resistance determinant required by *penB* porin mutants to exhibit an increased resistance to penicillin and tetracycline was absent [14]. Furthermore, penicillin-binding protein 2 was not encoded by a mosaic allele and Leu-421-to-Pro substitution was not observed in penicillin-binding protein 1. Mutations in the A subunit of DNA gyrase and ParC subunit of topoisomerase IV which confer resistance to ciprofloxacin were present whereby GyrA possessed the double mutations of Asp-95-to-Ala and Ser-91-to-Phe whilst an Asp-86-Asn mutation was found in ParC [15]. Multilocus sequence typing [16] and *N. gonorrhoeae* multi-antigen sequence typing [17] revealed that the isolate belonged to ST1587 and ST2575, respectively. To the best of our knowledge, both allelic profiles have not been reported for gonococcal isolates to date. The availability of this genome sequence may provide insights into *N. gonorrhoeae* vaccine development by facilitating the identification of potential vaccine candidates via reverse vaccinology approach [18] and information derived from the plasmids can be used for epidemiologic surveillance of plasmid-mediated antibiotic resistance among *N. gonorrhoeae* isolates [19]. In conclusion, whole-genome sequencing of more *N. gonorrhoeae* isolates would aid in the identification and tracking of resistant determinants in this species and further in-depth comparative genomic analysis will contribute towards understanding the evolution of this pathogen.

Nucleotide sequence accession numbers This Whole Genome Shotgun project has been deposited in GenBank under the Accession No. LFJW00000000. The version described in this paper is the first version, LFJW01000000.

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