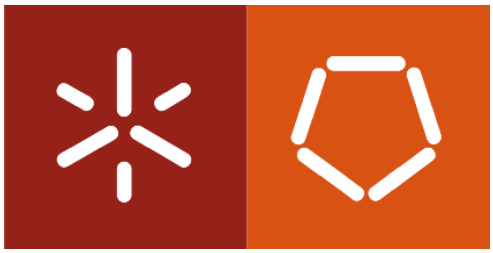

Universidade do Minho

Escola de Engenharia

Neisseria gonorrhoeae

Algorithms for the analysis of biological sequences / Laboratory of Bioinformatics

MSc in Bioinformatics

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NEISSERIA GONORRHOEAE

Adaptation from "*Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea"* and *"Gonorrhea in Men Sex Men and Heterosexual Men"*

1. General description

Gonorrhea is a sexually transmitted infectious disease known since ancient times, with biblical references. The etiologic agent is *Neisseria gonorrhoeae,* commonly called gonococcus. *N. gonorrhoeae* has accumulated mechanisms of antimicrobial resistance so that from the year 2007 it joined the list of multi-resistant, informally called "Superbugs" (*Shafer in Neisseria Molecular Mechanisms of Pathogenesis, 2010*).

Infections caused by *Neisseria gonorrhoeae* are a major public health problem globally. In 2008, the World Health Organization (WHO) estimated 106 million new cases of gonorrhea among adults worldwide. This places gonorrhea as the most common bacterial sexually transmitted infection (STI), that is, together with *Chlamydia trachomatis* infections also estimated to 106 million cases.

Neisseria gonorrhoeae was described by Neisser in 1879 and first cultivated in 1882 by Leistikow and Loeffler. Currently, members of the genus *Neisseria* are classified in the family *Neisseriaceae* with the genera *Kingella*, *Eikenella*, *Simonsiella*, and *Alysiella*. This family is now placed in the β-subgroup of the Phylum *Proteobacteria* (*Janda and Gaydos in Manual of Clinical Microbiology, 2007*).

Figure 1. Urethral exudate containing *Neisseria gonorrhoeae. (http://pt.wikipedia.org/wiki/Neisseria_gonorrhoeae#mediaviewer/File: Neisseria_gonorrhoeae_PHIL_3693_lores.jpg)*

N. gonorrhoeae is a gram-negative diplococci with adjacent sides flattened, don't form spores, oxidizes dimethyl or tetramethyl-phenylenediamine (oxidase test positive) and catalase positive (superoxol test with 30% H_2O_2). It may grow optimally at 35-37ºC, but it's unable to grow at low temperature (22ºC). Most of the gonococcus have an obligate requirement for CO_2 (5%) and humidity of 70-80% and it doesn't tolerate drying out. The essential amino acid for their growth is cysteine. Some strains have specific requirements of certain amino acids, pyrimidines and purines as a result of defective or different biosynthetic pathways. These particular nutritional requirements form the basis for a typing method for gonococci called auxotyping.

N. gonorrhoeae differs from other species of the genus by its capacity of oxidizing glucose and do not use maltose, sucrose, lactose or fructose in cysteine-tryptic digest semisolid agar-base medium (CTA) containing 1% carbohydrate and a phenol red pH indicator, or rapid carbohydrate test; not to reduce nitrites and their inability to grow at low temperatures (22ºC).

2. Transmission and clinical manifestations

N. gonorrhoeae is an exclusive human pathogen and this is their only reservoir. The transmission is from person to person through sexual intercourse, so the primary infection in adults is installed in the genital area, anal and/ or pharynx.

Gonococcal infection is more common among young persons, particularly those aged 15-24 years, persons with lower socio-economic status, men who have sex with men (MSM), illicit drug users, commercial workers an racial/ethnic minority groups *(Barry, 2009).*

In males, *N. gonorrhoeae* causes an acute urethritis with dysuria and urethral discharge. The incubation period averages 2 to 7 days. About 2.5 to 5 % of men are asymptomatic. In women, gonococcal infections cause cervicitis, only approximately half of which occur with symptoms and which can go on to cause pelvic inflammatory disease,

ectopic pregnancies, and infertility. In addition, in both men and women exposed orally or anally, gonococcal infections can cause a predominantly asymptomatic pharyngitis or proctitis. Less commonly, *N. gonorrhoeae* can cause conjunctivitis, endocarditis, tenosynovitis, arthritis, meningitis, inflammation of the liver capsule and disseminated blood stream infections *(Barry, 2009).*

Gonococcal infection may facilitate transmission of human immunodeficiency virus (HIV), increasing the number of target cells for HIV in the inflammatory exudate present in symptomatic patients *(Bala, 2010).*

3. Antimicrobial resistance and clinic impact

N. gonorrhoeae has developed resistance to all antimicrobials previously recommended as first-line treatment of gonorrhea, e.g. penicillins, tetracyclines and fluoroquinolones, as well as macrolides such as erythromycin and azithromycin.

The mechanisms of resistance to penicillin are the production of β-lactamase (penicillinase), chromosomal resistance by altering PBP1 and 2, reduced income and increased efflux. The penicillin introduction by the end of 1940s led to the eradication of *N. gonorrhoeae* sulfonamides resistant and it was the antimicrobial agent that kept its effectiveness for nearly forty years, although it required increasing the dose in response to the emergence of chromosomal resistance and the use of alternative drugs for the treatment of isolates penicillinase producers (PPNG), since its appearance in 1976 *(Ashford, 1976).* In the mid of 1980s the prevalence of these strains exceeded 40% for what should no longer be used for empiric treatment of gonorrhea. At that time, the percentage of isolates tetracycline resistant was high, greater than 50% *(Famiglietti, 2001).* To the high prevalence of isolates with chromosomal resistance to tetracycline (CMRNG), originated in 1970, joined the plasmid resistance (TRNG) which is mediated by the *tetM* determinant in conjugative plasmid that confers high level resistance *(Morse, 1986).* Plasmid tetracycline resistance is mediated by the *tetM* determinant while chromosome type resistance involves the

modification of ribosomal protein *(Shaffer in Neisseria Molecular mechanisms of Pathogenesis, 2010).*

The market introduction of fluoroquinolones in the middle of 1980s, was the ideal replacement of penicillin, not only be used as a single-dose and would be highly effective, but also has advantage on the route of administration of drug and fewer adverse effects.

At the last years, the emergence of *N. gonorrhoeae* resistant to fluoroquinolones reinstated the global problem of the treatment and control of gonorrhea *(Update CDC, 2007).*

About macrolides, since its introduction in 1952, erythromycin was used for the treatment of various infections with an acceptable degree of adverse effects. In 1975 it was recommended as treatment for gonorrhea in pregnant women allergic to penicillin *(U.S. Public Health Service, 1975)*. In 1977, it was observed a decline in the drug effectiveness *(Brown, 1977).*

Given that *N. gonorrhoeae*, easily develops resistance to antimicrobial agents and its rapid spread, the WHO and CDC recommend changing the treatment regime when the prevalence of antimicrobial resistance is> 5% *(WHO, 2003).*

ANALYSIS OF THE SEQUENCE AND FEATURES PRESENT IN THE NCBI

The *get_sequence_file* function has as input variables the beginning and end of the gene sequence, in this case, 246001 and 468400 and the name that you pretend to assign to the file. The purpose of this function is to get a GenBank® type file with the record of the desired sequence. Finally this function will return the desired file.

In the case of the GenBank® file has already been created, the *read_sequence_file* function will take as input parameter the file name to read.

The *get_info* function will receive as an input parameter the SeqIO.read of the desired GenBank® file and will get all the information contained therein like the CDS_proteinID, GeneID, CDS_GI, LocusTag, CDS_ECnumber, Gene_name, dadostraducao, dadosfuncoes,

CDS_note and size. This information is obtained by the use of attributes in the file, for example, features and qualifiers, and then stored in lists, where the user can choose to save like a *.txt file type. This option is enabled by the function save, which receives as input the list that you want to save.

The *table* function, receives as input the table name to read and create a list of table rows.

The *compara* function receives as input the list of rows in the table created in the previous function, and the start and end of the sequence containing the proteins to be compared by adding to a list of lines that lie between the boundaries of the sequence.

The *valida* function, receives the previously created list and the list with the ID of the proteins obtained from *get_info* function, and compares the protein. If the proteins are present in the table, a list of the protein ID where is said to be valid is created.

Finally, the main function, allows the users to choose which features they want to run and what data they want to save.

HOMOLOGY ANALYSIS USING BLAST

The *Blast* function takes as input a GI of a gene and keep the BLAST results obtained in a file whose name is up to the user to choose.

The *analisa* function receives as a parameter the name of a type *.xml file that contains the BLAST of a gene and returns the results that have an e-value less than 0.05, i.e., good results.

Thus, it is possible to make a BLAST for each protein individually. The user can choose the protein and the database and then analyze the BLAST automatically.

ANALYSIS TOOLS OF PROTEIN PROPERTIES

The review of the proteins was done manually and individually in the UniProt database. Therefore it was possible to determine if the proteins of records were healed or not.

In order to automate and determine the ID used for each gene in the GeneOntology database the *Gene_Ontology* script was used. The *Gene_Ontology*, which contains the GO function, allows searching the GeneOntology number of each gene through UniProt IDs in the data input function.

The method used to determine the cellular localization of each protein was manually through the UniProt database.

CONCLUSION AND FINAL TABLE

Finally, with all the data from the databases we were able to characterize almost all the genes and proteins. All the data is compiled in the final table annexed.

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