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Original article

Antimicrobial susceptibility and typing of *Neisseria gonorrhoeae* strains from Southern Spain, 2012–2014

Fernando Cobo*, Mª Teresa Cabezas-Fernández, Mª Isabel Cabeza-Barrera

Microbiology Section (Biotechnology Area), Hospital de Poniente, Almería, Spain

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ABSTRACT

Background: Gonorrhoea remains an important health problem worldwide. The latest European guidelines have recommended the introduction of dual antimicrobial therapy due to the increase in its resistance to antimicrobial agents.
Methods: In the present study, the susceptibility to some antibiotics was evaluated, and the typing of *Neisseria gonorrhoeae* strains was also performed. All *Neisseria gonorrhoeae* (NG) strains isolated from January 2012 to October 2014 were included in this work. Gonococcal isolates were tested for susceptibility according to the recommendations of both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). A total of 65 isolates were typed by the NG multi-antigen sequence types (NG-MAST) technique.
Results: The most frequent types found were ST 1407, ST 5405, ST 2992, and ST 5120. If CLSI and EUCAST criteria were applied, an ST 9807 type was found non-susceptible to ceftriaxone and cefixime (MIC 0.5 μg/mL). When only EUCAST breakpoints were taken into account, three strains were also resistant to cefixime (MIC 0.25 μg/mL) and two isolates were resistant to ceftriaxone (MIC 0.19 and 0.25 μg/mL, respectively). The majority of strains were resistant to ciprofloxacin, and all *Neisseria gonorrhoeae* strains were susceptible to spectinomycin; twenty-five percent of isolates were resistant to azithromycin.
Conclusions: The implementation of antimicrobial surveillance programs at regional level should be part of an overall gonococcal infection control strategy. Efforts should be made to perform antimicrobial susceptibility, a "cured test" in all gonorrhoea cases, and identify treatment failures to verify emerging resistance. Some types have been associated with decreased susceptibility to cephalosporins, making molecular typing a useful tool to predict antimicrobial resistance.

Sensibilidad a antimicrobianos y tipado de cepas de *Neisseria gonorrhoeae* procedentes del sur de España, 2012–2014

Palabras clave:
Neisseria gonorrhoeae
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Tipado molecular

RESUMEN

Antecedentes: La gonorrea sigue siendo un importante problema de salud pública a nivel mundial. Las últimas guías en Europa han recomendado la introducción de tratamiento antimicrobiano dual debido al incremento de las resistencias a los antimicrobianos.
Métodos: En este estudio fue evaluada la sensibilidad a algunos antibióticos y se realizó el tipado de cepas de *Neisseria gonorrhoeae*. Todas las cepas aisladas desde enero de 2012 hasta octubre de 2014 fueron incluidas en este trabajo. El estudio de la sensibilidad de las cepas de gonococo fue realizado según las recomendaciones del Clinical and Laboratory Standards Institute (CLSI) y del European Committee on Antimicrobial Susceptibility Testing (EUCAST). Un total de 65 cepas fueron tipadas mediante la técnica NG-multiantigen sequence types (NG-MAST).

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* Corresponding author.
E-mail address: fernando.cobo.sspa@juntadeandalucia.es (F. Cobo).

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Resultados: Los tipos encontrados más frecuentemente fueron el ST 1407, ST 5405, ST 2992 y el ST 5120. Al aplicar los criterios del CLSI y del EUCAST, un tipo ST 9807 resultó no sensible a ceftriaxona y cefixime (CMI 0,5 μg/mL). Al tener solo en cuenta los puntos de corte del EUCAST, 3 cepas más fueron también resistentes a cefixime (CMI 0,25 μgr/mL) y 2 más fueron resistentes a ceftriaxona (CMI 0,19 y 0,25 μg/mL, respectivamente). La mayoría de las cepas fueron resistentes a ciprofloxacino, y todas las cepas testadas fueron sensibles a espectinomicina; el 25% de los aislamientos fueron resistentes a azitromicina.

Conclusiones: La implementación de programas de vigilancia antimicrobiana a nivel regional debe ser parte de la estrategia de control para la infección gonocócica. Deben ser realizados esfuerzos para el estudio de la sensibilidad antimicrobiana, test de cura en todos los casos de gonorrea y la identificación de los fallos de tratamiento para verificar el aumento de resistencias. Algunos tipos han sido asociados con disminución de la sensibilidad a cefalosporinas, por lo que el tipado molecular puede ser una herramienta diagnóstica útil para predecir la resistencia a antibióticos.

Introduction

Gonorrhoea is the second most commonly reported bacteial disease causing sexually transmitted infections (STIs) worldwide.[1] The prevalence of this infection varies among populations but it continues to present a serious public health problem in many countries.[2] Thus, an appropriate diagnosis and an effective treatment of this infection are important factors contributing to public health control and to prevent serious complications.

Treatment is usually given empirically and it is based on national or regional guidelines. Currently, third generation cephalosporins such as ceftriaxone or cefixime are the treatments of choice for this infection. However, the increase of resistance to recommended treatments for gonorrhoea may seriously affect infection control.[3]

Recently, some authors have described emergence of isolates exhibiting decreased susceptibility and resistance to the third generation cephalosporins which have caused treatment failure in gonococcal infections.[4,5] In 2010, 9% of isolates from European countries showed decreased susceptibility to cefixime, increase in minimum inhibitory concentrations (MICs) for ceftriaxone, and a high prevalence of resistance to ciprofloxacin and azithromycin.

Due to these facts, antimicrobial surveillance programs are necessary to detect patterns of resistance not only at international and national levels, but also at regional level to ensure treatment efficacy against this infection. In this sense, molecular epidemiology surveillance can help to provide additional information on the emergence and dissemination of antimicrobial resistance. Molecular studies for epidemiological surveillance are necessary to detect association between genotype and antimicrobial resistance and to know how resistant strains emerge and disseminate.[6]

The objective of the present study was to analyze the antimicrobial susceptibility of *Neisseria gonorrhoeae* (NG) strains isolated from genital specimens in patients belonging to the health area of the Hospital of Poniente, as well as to perform the genotyping of these samples in order to detect epidemiological clusters of infection and possible modifications of the susceptibility patterns.

Methods

Collection of NG strains

All NG strains isolated from January 2012 to October 2014 were included in this study. The genital samples were obtained from patients with STIs belonging to the health area of the Hospital of Poniente (El Ejido, Almería, Spain), and were sent to the microbiology laboratory for culture. A total of 65 isolates were included for both antimicrobial susceptibility study and molecular typing. All samples were obtained from genital sites (urethral or endocervical/vaginal exudates); fifty-one samples were taken from men. All samples were cultured in VCA agar (BioMérieux, France). The identification of NG suspected strains was performed by means of Gram stain, oxidase and catalase production, and finally with both biochemical analysis by means of the API NH system (BioMérieux, France) and Maldi-tof technology (Vitek MS, bioMérieux, France). The identification of all strains was confirmed at the National Centre of Microbiology (Instituto de Salud Carlos III, Madrid, Spain).

Antimicrobial susceptibility testing

Gonococcal isolates were tested for susceptibility according to the recommendations of Clinical and Laboratory Standards Institute (CLSI)[7] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[8] All strains were tested for susceptibility to penicillin, ceftriaxone, cefixime, tetracycline, ciprofloxacin, azithromycin and spectinomycin by means of agar dilution tests. All isolates were also tested for penicillinase production using the Cefinase test (bioMérieux, Marcy-l'Etoile, France). MIC interpretation was performed according to both CLSI and EUCAST, and then compared.

Molecular typing

All NG strains ($n = 65$) were typed by the NG multi-antigen sequence types (NG-MAST) technique,[9] which differentiates strains on the basis of sequence variation in fragments of two hypervariable genes, the subunit B of the transferring binding protein (*tbpB*) and the porin Por B (*porB*). The typing was performed at the National Centre of Microbiology (Instituto de Salud Carlos III, Madrid, Spain). Allele numbers and STs were assigned using NG-MAST databases (www.ng-mast.net). Moreover, all NG strains were also serotyped at this center by means of the Phadebact® Monoclonal GC Test (MKL Diagnostics AB, Sollentuna, Sweden).

Results

The age of patients included in this study range from 21 to 47 years old (median age = 32 years old). Twenty-eight samples were obtained from immigrant population and 37 from indigenous patients. The majority of strains were obtained from urethral samples ($n = 55$) whereas 10 strains were obtained from vaginal or endocervical fluid. From the 65 typed strains, 34 different STs were obtained. The distribution of main types is shown in Table 1. The most frequent types found were STs 1407 ($n = 7$), STs 5405 ($n = 5$), STs 7192 ($n = 5$), STs 2992 ($n = 4$), and STs 5120 ($n = 4$). An