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ORIGINAL ARTICLE

Changes in the six most common sequence types of *Neisseria gonorrhoeae*, including ST4378, identified by surveillance of antimicrobial resistance in northern Taiwan from 2006 to 2013

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Background: There has been no longitudinal study of drug susceptibility in *Neisseria gonorrhoeae* in Taiwan since 2006.
Methods: We collected 1090 gonococcal isolates from Taipei City Hospital, Taiwan from April 2006 to August 2013. We used a disk diffusion assay to determine the susceptibility to five antibiotics and an E-test to determine the minimum inhibitory concentrations for cefixime and ceftriaxone in isolates with resistance. *Neisseria gonorrhoeae*-multi Antigen Sequence Typing and DNA sequencing of the *por* and *tbpB* genes were used to identify sequence types.
Results: Among the 1090 isolates, the resistances to penicillin, ciprofloxacin, cefpodoxime, cefixime, and ceftriaxone were 61.01%, 83.39%, 9.63%, 6.70%, and 2.39%, respectively. The highest minimum inhibitory concentrations of cefixime and ceftriaxone were 0.19 mg/L and 0.50 mg/L, respectively. There were 327 sequence types. The four most common sequence types in homosexuals were ST4378, ST359, ST4654, and ST547; the two most common sequence types in heterosexuals were ST421 and ST419. Each of these sequence types had more than 25

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isolates. There were significant differences in the sequence types in patients with different sexual orientations ($p < 0.001$).
Conclusion: Oral cefixime or ceftriaxone injections were used as first-line drugs for the treatment of gonorrhea from 2006 to 2013 because gonorrhea isolates had low minimum inhibitory concentrations for these two drugs. The abrupt emergence of ST4378 (closely related to the notorious ST1407) since 2009 is a cause for alarm. Changes in sexual behavior, including an increase in sexual activity without the use of condoms, may have contributed to the peak in gonorrhea in 2010. Further molecular epidemiological investigations are required.
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Introduction

Gonorrhea is a sexually transmitted infection caused by *Neisseria gonorrhoea*. Along with chlamydia, syphilis, and trichomoniasis, gonorrhea is among the most common sexually transmitted infections and is a significant public health problem worldwide.[1] Research in the late 1990s indicated that gonorrhea and genital ulcer diseases can increase the spread of sexually transmitted HIV.[2,3] Recent research has continued to focus on the role of gonorrhea in the spread of HIV.[4,5] In particular, a study in 2005 indicated that the rate of HIV transmission in patients with gonorrhea can be up to five times greater than in those without gonorrhea.[6]

Gonorrhea can also increase the risk of developing other acute infections and complications. An effective treatment for gonorrhea is urgently needed, but treatment options are diminishing because *N. gonorrhoeae* has developed resistance to several commonly used antimicrobial drugs, such as the sulfonamides, penicillin, tetracyclines, and quinolones.[4]

The World Health Organization estimated in 1999 that approximately 340 million new cases of chlamydia, gonorrhea, syphilis, and trichomoniasis occur every year and that most new cases occur in developing countries, including Southeast Asia, sub-Saharan Africa, Latin America, and the Caribbean.[7] The diagnosis and treatment of gonorrhea can be problematic because *N. gonorrhoeae* is difficult to culture and has developed resistance to many oral antibiotics. This has led to the inadequate control of gonorrhea in many parts of the world.

Surveillance of antimicrobial resistance in *N. gonorrhoeae* can help to identify new resistant strains and changes in the pattern of resistance, ultimately assisting in disease control. Current treatment guidelines recommend the use of single-dose injectable or oral third-generation cephalosporin (ceftriaxone or cefixime). The emergence and spread of cephalosporin-resistant and multidrug-resistant *N. gonorrhoeae* strains is a worrying trend, indicating the need for careful monitoring and investigation. Thus clinical laboratories must remain alert for the early detection of resistant strains so they can implement more appropriate control strategies. The treatment strategy and prevention of gonorrhea require regular review and revision.

The Gonococcal Resistance to Antimicrobials Surveillance Program of the Health Protection Agency reported in 2010 that 17.4% of *N. gonorrhoeae* isolates in the UK had cefixime minimum inhibitory concentration (MIC) values of $\geq$0.125 mg/L and that 6.3% of isolates had cefixime MIC values $\geq$0.25 mg/L. Thus the Health Protection Agency announced that cefixime was no longer a suitable first-line treatment for gonorrhea as the percentage of *N. gonorrhoeae* isolates with a cefixime MIC $\geq$0.25 mg/L has exceeded 5%.[8]

There were 2265 cases of gonorrhea in Taiwan in 2010 and the annual incidence was 9.79 per 100,000 population, higher than in 2009 (9.26 per 100,000) and the highest reported incidence since 2006.[3] In addition, disk diffusion tests indicated that 9% of gonococci were resistant to cefixime in 2003, but that resistance to cefixime and cefpodoxime (another third-generation cephalosporin) were 16.4% and 21.2%, respectively, during 2006–2007.[8,9] In 2006, a new surveillance program, the Gonococci-National Isolate Collection for Epidemiology (G-NICE), began the collection of gonococcal isolates from hospitals and clinics to monitor changes in drug resistance and the molecular epidemiology of gonococci in Taiwan. The G-NICE presented data for 2009 and 2010 in the *Taiwan Epidemiology Bulletin* of 2010 and 2011.[10,11] However, there has been no longitudinal study of gonococcal resistance following the establishment of G-NICE in 2006.

The results presented here provide the most recent information on the emergence and transmission of drug-resistant gonococci in high-risk sexual networks in Taiwan.

Materials and methods

Collection of gonococcal isolates

We collected 1111 gonococcal isolates at our Kun-Ming Branch of Taipei City Hospital, Taiwan from April 2006 to August 2013. Our Kun-Ming Branch is a specialized clinic for sexually transmitted diseases in Taipei City. Twelve isolates had incomplete data and nine isolates could not be cultured for definitive identification. Thus 1090 isolates were used for analysis.

According to the Taiwan Centers for Disease Control (CDC) annual report, there were about 1500 confirmed cases of gonorrhea per year in Taiwan after 2003 and about 500 of these cases were from Taipei City (23% of all cases in Taiwan). Our hospital in Taipei City collected about 80–200 isolates of gonorrhea each year from 2006 to 2012.

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