Tracing subsequent dissemination of a cluster of gonococcal infections caused by an ST1407-related clone harbouring mosaic penA alleles in Taiwan

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Objectives: Successful clones of Neisseria gonorrhoeae multiantigen sequence typing sequence type (ST) 1407 and ST1407-related genotypes have been reported to cause cefixime and ceftriaxone treatment failure in many countries. We characterized the 47 isolates of a strain cluster of ST4378, a genotype that differs in the porB sequence by only one nucleotide from ST1407, in Taiwan during April 2006 to June 2012.

Methods: We identified 47 ST4378 isolates among our 2357 total isolates from the Gonococci-National Isolate Collection for Epidemiology. The corresponding patients’ medical records were collected. The 47 isolates were further typed by multilocus sequence typing. Genes involved in β-lactam (penA), quinolone (gyrA and parC) and multidrug (mtrR, porB1b and pilQ) resistance were sequenced. Antimicrobial susceptibility was determined by the disc diffusion test and Etest.

Results: Cefixime MICs for the 47 isolates ranged from 0.016 to 0.19 mg/L and ceftriaxone MICs ranged from 0.012 to 0.094 mg/L. Forty-six of the 47 isolates had a mosaic penA allele type XXXIV and one had a new allele type XL, which appeared to be a recombinant of mosaic penA type XXXIV and non-mosaic penA type II. All of the isolates harboured nearly identical polymorphism in the penA, gyrA, parC, mtrR, porB1b and pilQ genes. Among the 33 patients with known medical records, 25 (76%) were men who have sex with men (MSM), 3 (9%) were bisexual and 5 (15%) were heterosexual. Fourteen (42%) of the 33 patients had HIV, 8 (24%) had syphilis and 7 (21%) had both infections.

Conclusions: This is the first report of a cluster of ST1407-related strains in Taiwan. ST4378 is a genotype that may develop to cause third-generation cephalosporin treatment failures. Our results showed that ST4378 strains primarily transmitted in a high-risk MSM/bisexual network. The potential of these strains to become untreatable and spread to other low-risk sexual networks should be closely monitored.

Keywords: antimicrobial resistance, cephalosporins, gonorrhoea, penicillin-binding proteins, PBPs, strain clusters

Introduction

Recently, the global transmission of Neisseria gonorrhoeae multidrug-resistant clones, especially those causing cephalosporin treatment failures, have caused worldwide concern. Since 2007, the strains of the successful global clones of N. gonorrhoeae multiantigen sequence typing (NG-MAST) sequence type (ST) 1407 and related STs have been reported to cause cefixime treatment failure in several countries, such as the UK and Canada, and ceftriaxone treatment failure in France and Sweden. These NG-MAST ST clones belonged to the same multilocus sequence typing (MLST) type, sequence type (ST) 1901. In these ST1407 and ST1407-related isolates, mosaic penA alleles related to genotype XXXIV have been recognized as a major cause of resistance to oral cephalosporins. In Taiwan, during April 2006 to June 2012, antimicrobial surveillance within the Gonococci-National Isolate Collection for Epidemiology (G-NICE) programme indicated that 4.2%, 5.8% and 0.8% of the 2357 isolates were resistant to cefixime, cefpodoxime and ceftriaxone (by using the disc diffusion test), respectively. Of the isolates, 2.5% had cefixime MICs ≥0.25 mg/L and none had a ceftriaxone MIC ≥0.25 mg/L. The maximum MICs were 0.5 and 0.19 mg/L for cefixime and ceftriaxone, respectively. In this
report, we describe a gonococcal cluster of 47 cases belonging to NG-MAST ST4378 and MLST ST1901 in Taiwan. ST4378 differs in the porB sequence by only one nucleotide from ST1407. Apart from the 47 ST4378 isolates in this study, only two ST1407 sporadic cases had been identified in Taiwan (in September 2008 and January 2011, respectively).

**Materials and methods**

**Collection of gonococcal isolates**

The Mycology and Sexually-Transmitted Diseases Laboratory in the Research and Diagnostic Center of Taiwan-Centers for Disease Control (TW-CDC) is a reference laboratory for *N. gonorrhoeae* in Taiwan. Since 2006, we have launched the G-NICE surveillance programme, with 40 hospitals and clinics voluntarily contributing isolates and demographic data (age, gender and date of gonococci isolation). Specimens of *N. gonorrhoeae* were cultured on selected media and identified according to the characteristics of biochemical tests (Gram staining and catalase, oxidase and superoxol tests). Anti-HIV antibody was tested for using ELISA (Genscreen HIV 1/2 version 2, Bio-Rad, France) and particle agglutination (Serodia HIV 1/2 Mix, Japan) and HIV infection was confirmed using western blotting (New LAV Blot I, Bio-Rad, France). Assays for rapid plasma reagin (Pulse Scientific Inc., USA) and Treponema pallidum particle agglutination (FTI-Serodia, Taiwan) were simultaneously performed for diagnosis of syphilis.

During April 2006 to June 2012, the G-NICE programme collected 2357 gonococcal isolates. Upon receipt at the TW-CDC, the isolates were subcultured onto chocolate agar, phenotypically and genotypically characterized and stored at −80°C for further experiments, as described previously.2 All isolates were typed by NG-MAST and screened for mosaic penA alleles using our duplex PCR method.8 In G-NICE, only 47 ST4378 isolates have been identified. The 47 isolates were collected from nine hospitals (Taipei H1 (n = 33), Taipei H2 (n = 5), Taipei H3 (n = 1), Taipei H4 (n = 1), Taipei H5 (n = 2), Taipei H6 (n = 2), Chungli H7 (n = 1), Hsinchu H8 (n = 1) and Taichung H9 (n = 1)); 44 of the 47 isolates were collected in Taipei City.

**Molecular typing of isolates**

Genomic DNA of *N. gonorrhoeae* clinical isolates was extracted using the QIAGEN DNA Mini Kit (Qiagen, Hilden, Germany). PCR amplification was performed using a T3000 Thermocycler (Biometra, Gottingen, Germany). Sequencing was carried out bidirectionally with the appropriate sequencing primers using an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence data were aligned and analysed by using BioNumerics 6.5 software (Applied Maths). All isolates were typed by MLST (defined by seven housekeeping alleles: abcZ, adk, aroE, fumC, gdh, pdhC and pgm)9 and NG-MAST (porB and tspB).10 The sequences of the MLST alleles were compared in the PubMLST database (http://pubmlst.org) to obtain the allele numbers of the STs. The NG-MAST STs of the isolates were assigned by querying the NG-MAST database (http://www.ng-mast.net).

**Antibiotic susceptibility**

Gonococcal isolates were inoculated on chocolate agar and incubated at 37°C for 16–18 h. GC Agar Base supplemented with IsoViteX Enrichment was used for antimicrobial susceptibility testing. The susceptibilities to six antimicrobials (penicillin G, spectinomycin, cefoxitin, ciprofloxacin, cefixime and ceftriaxone) were measured by using the disc diffusion test. The MICs of cefixime and ceftriaxone were determined by using Etest (AB bioMérieux, Solna, Sweden) following the CLSI-2010 guidelines for *N. gonorrhoeae* (M100-S20).11

**Molecular characterization of the resistance genes**

PCR amplification and sequencing of the penA alleles were conducted as described previously.12 We assigned penA-encoded penicillin-binding protein (PBP) 2 into types I–XL, based on alterations in the amino acid sequences.13,14 The genes related to β-lactam resistance (penA),15 quinolone resistance (gyrA and parC),16 and multidrug resistance (mtrR,15 porB1b15 and pilQ19) were amplified by PCR and sequenced by published methods.

**Ethics**

The study was approved by the local Ethics Committee (TCHIRB-970601-E). Informed consent was waived.

**Results**

In G-NICE, only 47 ST4378 isolates were identified. In this ST4378 strain cluster, all patients were male. Among the 33 patients with medical records, 25 (76%) were men who have sex with men (MSM), 3 (9%) were bisexual and 5 (15%) were heterosexual. In addition, 14 (42%) of the 33 patients had HIV, 8 (24%) had syphilis and 7 (21%) had both infections. Clinically, patients in this strain cluster were treated with 500 mg of injectable ceftriaxone (Rocephin) combined with 100 mg of doxycycline (oral, twice per day). Within 1–2 weeks, their symptoms were resolved. A test of cure was performed by bacterial culture 1 week after treatment.

All isolates of the cluster belonged to MLST ST1901. MLST ST1901 is an ST that has successfully spread worldwide over the past decade. Two PBP2 types were identified in the strain cluster; mosaic PBP2-XXIV (n = 46) and a new type found in this study, designated as non-mosaic PBP2-XX (n = 1, GenBank accession number JQ782218). The mosaic penA allele XXXIV has been reported in other countries,18,19 but has not been identified in Taiwan previously. The PBP2-XXL allele is different from all of the previously published penA types. Figure 1 shows the comparison of nucleotide and translated amino acid sequences of five penA alleles: wild-type (GenBank accession number M32091), PBP2-II (NZ_EQ973053), PBP2-XXL (JQ782218), PBP2-XXIV (GU723422) and PBP2-X (CP002440). The novel PBP2-XXL allele was identical to PBP2-XXIV at amino acid positions 1–291, but its region between positions 292 and 582 was evidently similar to that of non-mosaic PBP2s, such as PBP2-II, suggesting that the penA gene encoding PBP2-XXL was a recombinant allele. Similarly, based on the identical region of positions 1–545 (Figure 1), PBP2-XXIV may also be a derivative of PBP2-X, the earliest and predominant mosaic PBP2 type, and has undergone several independent recombination events. Apart from PBP2 alteration, the mutations in other resistance genes were also determined. All of the 47 isolates had the same polymorphism: penA (T375A and L421P), mtrR (deletion of A, except for two isolates), parC (wild-type), gyrA (S91F and D95G), porB1b (G120K and A121N) and pilQ (wild-type). The susceptibility profiles determined by the disc diffusion test indicated that the rates of resistance of the 47 isolates to penicillin G, ciprofloxacin, cefoxitin, cefixime and ceftriaxone were 83% (39/47), 100%, 26% (12/47), 13% (6/47) and 4% (2/47), respectively; all isolates were susceptible to spectinomycin. The cefixime MICs of the 47 isolates ranged from 0.016 to 0.19 mg/L and the ceftriaxone MICs ranged from 0.012 to 0.094 mg/L. According to the CLSI-2010 guidelines, the lower...
cut-offs for decreased susceptibility to cefixime and ceftriaxone were both 0.125 mg/L. In this cluster, 12 isolates had cefixime MICs ≥ 0.125 mg/L, but none had a ceftriaxone MIC ≥ 0.125 mg/L. In light of the same ST, identical polymorphism in the resistance genes and geographical and temporal proximity, the 47 isolates were considered to belong to the same clonal population, disseminated in a distinct high-risk MSM/bisexual network.

**Discussion**

The emergence and worldwide spread of the NG-MAST ST1407 clonal complex, associated with treatment failure and decreased susceptibility to the third-generation cephalosporins, has threatened to limit the last treatment options for gonorrhoea. This study identified the first cluster of ST1407-related strains transmitted during April 2010 to May 2012 in an MSM/bisexual network in Taiwan. Isolates of the ST4378 cluster are of the same MLST molecular type 1901 and exhibited elevated MICs of cefixime and ceftriaxone.

The majority of the ST4378 isolates harboured PBP2-XXXIV and one contained a novel recombinant PBP2-XL. PBP2-XXXIV was first reported in strains of ST1407 in 2008 in California, USA and in strains of ST225 and ST51 in Ontario, Canada. Among the 2357 isolates of G-NICE collected during 2006–12, apart from the 46 isolates in this study and the two ST1407 isolates in 2008 and 2011, no other gonococcal isolate with PBP2-XXXIV was identified in Taiwan. According to our G-NICE surveillance, the ST1407 isolates were both sporadic events and not associated with this persistence.
ST4378 cluster. A possibility is that ST1407 and its derivative ST4378 containing penA-XXXIV were introduced from other countries and subsequently circulated into the sexual network in Taiwan. Previous studies have reported that the mosaic PBP2-XXXIV (in ST1407 strains) was a major factor contributing to decreased susceptibility to penicillin and expanded-spectrum cephalosporins (ESCs) in N. gonorrhoeae. The MICs in this study were similar to those reported by Golparian et al., the median MICs of cefixime and ceftriaxone for the isolates carrying mosaic penA-XXXIV were 0.094 and 0.047 mg/L, respectively, which were higher than for those isolates harbouring non-mosaic penA. Also, the ST1407 isolate with penA-XXXIV collected in 2008 showed elevated MICs of 0.19 and 0.094 mg/L for cefixime and ceftriaxone, respectively. A recent study of a high-level ESC-resistant strain (F89) isolated in France indicated that an additional A501P mutation may additionally confer high-level resistance to both cefixime and ceftriaxone.

The emergence of the gonococcal strain harbouring the recombinant penA allele suggests that there might be bridging patients, who provided transmission links between MSM and heterosexual networks. These patients may serve as a hub for transmission of specific clones from MSM networks into heterosexual networks. The treatment failure and high resistance to cefixime and ceftriaxone of ST1407 strains has been increasingly reported in many countries. Hence, it is pivotal to closely monitor the dissemination of ST1407 and its related strains, such as ST4378, across networks as well as their propensity to become untreatable.

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References

Transparency declarations
None to declare.
A cluster of an ST1407-related clone with mosaic penA


