A closely related transposon, here designated Tn*2008*B, that appears to be derived from Tn*2006* is prevalent in China and has also been found in three independent locations.[4] Though movement has not been observed experimentally, these findings support the conclusion that Tn*2008* and Tn*2008*B are discrete transposons, i.e. the same unit can move repeatedly. This is in contrast to the case of ISEcp1 that can mobilize adjacent DNA segments but the length of the segment is not discrete.[13]

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Transparency declarations

None to declare.

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Detailed characterization of the first high-level azithromycin-resistant *Neisseria gonorrhoeae* cases in Ireland

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Sir,

Neisseria gonorrhoeae has developed resistance to all antibiotics used as first-line empirical monotherapy for gonococcal infections, including the last option, ceftriaxone.[1,2] Consequently, in Europe, the USA and several other countries, dual antimicrobial therapy consisting of ceftriaxone (250 or 500 mg intramuscularly) combined with azithromycin (1 or 2 g orally) has been introduced recently.[3,4] However, this combination is compromised by the emergence of resistance to ceftriaxone and azithromycin. More worryingly, gonococcal strains with high-level resistance to azithromycin (MIC $\geq$256 mg/L) have been described in the UK, Italy, Sweden, the USA, Argentina and Australia.[2,5] Here we report the detailed characterization of the first two high-level azithromycin-resistant *N. gonorrhoeae* cases in Ireland.

Azithromycin susceptibility by Etest methodology (bioMérieux, Marcy-l'Étolie, France) was retrospectively determined for all available urethral isolates of *N. gonorrhoeae* (n=300) collected at St James's Hospital, Dublin, from 2008 to 2014. Fourteen (4.7%) isolates were resistant to azithromycin according to breakpoints of EUCAST (Version 5.0)[6] and two of the isolates showed high-level resistance to azithromycin (MIC>256 mg/L).

The first *N. gonorrhoeae* isolate (NGSJH7) was cultured in April 2008 from a urethral swab of a 22-year-old heterosexual male. He presented to his general practitioner with mild urethritis. He was *Chlamydia trachomatis* and HIV negative, and had not been diagnosed previously with any sexually transmitted infection. The patient was treated empirically with 500 mg of ciprofloxacin orally (recommended in 2008) and was negative in test of cure. One sexual contact was traced and samples collected, but these tested negative for *N. gonorrhoeae* both by molecular and culture methods.

Table 1. Detailed characteristics of the first *N. gonorrhoeae* isolates with high-level resistance to azithromycin (MIC ≥256 mg/L) identified in Ireland

Isolate	Year	Sex/age (years)	MIC in mg/L (susceptibility category[a])				23S rRNA gene mutation (no. of mutated alleles)	NG-MAST	MLST
			azithromycin	ceftriaxone	ciprofloxacin	spectinomycin			
NGSJH7	2008	male/22	>256 (R)	0.016 (S)	≤0.03 (S)	8 (S)	A2059G (4/4)	ST3311	ST1580
NGSJH11	2014	male/20	>256 (R)	0.016 (S)	≤0.03 (S)	8 (S)	A2059G (3/4)	ST649	ST1580

S, susceptible; R, resistant.
[a]According to EUCAST.

The second *N. gonorrhoeae* isolate (NGSJH11) was cultured from a 20-year-old heterosexual male who presented to his general practitioner with urethritis in May 2014. The patient was negative for *C. trachomatis*. He was referred to a genitourinary medicine specialist who treated him empirically with 500 mg of ceftriaxone intramuscularly and 1 g of azithromycin orally. The patient did not return for test of cure and no sexual contacts were traced.

Isolate NGSJH7 was confirmed as *N. gonorrhoeae* based on Gram's stain, oxidase testing and carbohydrate utilization tests while isolate NGSJH11 was species verified using MS (VITEK MS®, bioMérieux). Both isolates showed high-level resistance to azithromycin (MIC > 256 mg/L), but were susceptible to ceftriaxone (MIC = 0.016 mg/L), ciprofloxacin (MIC ≤0.03 mg/L) and spectinomycin (MIC = 8 mg/L) (Table 1).

Sequencing libraries of genomic DNA from the two *N. gonorrhoeae* were generated using NexteraXT library preparation reagents (Illumina, Eindhoven, The Netherlands) and sequenced on an Illumina MiSeq platform. An A2059G mutation (*Escherichia coli* numbering), in the peptidyltransferase loop in domain V of 23S rRNA, was found in three of the four alleles of the 23S rRNA gene in NGSJH7 and in all four alleles in NGSJH11. The A2059G SNP confers high-level resistance to azithromycin if present in at least three of the four 23S rRNA gene alleles.[2,7] No resistance mutations were identified in the promoter region or the coding sequence of the *mtrR* gene, which indicates a normal expression of the MtrCDE efflux pump. Acquired macrolide resistance genes including *ermA*, *ermB*, *ermC*, *ermF*, *ereA*, *ereB*, *mefA*, *mefE* and *mphA* were not identified in any isolate.

MLST, analysing seven slowly evolving housekeeping genes,[8] assigned both isolates as ST1580. *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST), analysing the more variable *porB* and *tbpB* genes,[9] assigned NGSJH7 and NGSJH11 as NG-MAST ST3311 (*porB* allele, 2008; *tbpB* allele, 29) and ST649 (*porB* allele, 442; *tbpB* allele, 29), respectively. Accordingly, the two isolates belonged to two different NG-MAST STs. However, each had an identical *tbpB* allele and the *porB* alleles (490 bp) only differed by two SNPs, which likely reflect the ongoing evolution of the same strain. This observation is further strengthened by the identical antibiogram and MLST, as well as the similar genome sequences (data not shown) of the isolates. NG-MAST ST3311 and ST649 have also been previously identified in *N. gonorrhoeae* isolates with high-level azithromycin resistance in the UK (both ST3311 and ST649)[7] and the USA (ST649).[10] Therefore, it is likely that this high-level azithromycin-resistant *N. gonorrhoeae* strain might have been spreading for several years internationally.

The identification of an internationally transmitted *N. gonorrhoeae* strain with high-level azithromycin resistance stresses the need for antimicrobial surveillance nationally (including in Ireland) and internationally. The efficacy of dual antimicrobial therapy internationally can be compromised by these strains. While nucleic acid amplification tests allow a rapid diagnosis of gonorrhoea, culture of *N. gonorrhoeae* remains essential for antimicrobial resistance surveillance. Testing for ceftriaxone and azithromycin susceptibility must be included in all routine antimicrobial susceptibility testing.

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None to declare.

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Non-susceptibility to ceftaroline in healthcare-associated multiresistant MRSA in Eastern Australia

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Sir,

Ceftaroline is the active metabolite of the cephalosporin prodrug ceftaroline fosamil. With *in vitro* activity and clinical efficacy against MRSA due to its high affinity for PBP2a,[1] it is approved for the treatment of complicated acute bacterial skin and skin-structure infections and community-acquired bacterial pneumonia. Reduced ceftaroline susceptibility has been associated with PBP2a alterations conferring reduced drug affinity.[2]

We assessed the *in vitro* activity of ceftaroline against MRSA strains isolated in our teaching hospital laboratory, prior to the introduction of the drug to the hospital formulary or any substantial use in our geographical region. Consecutive MRSA isolates from clinical and infection control screening specimens on unique patients were prospectively collected between August and December 2013. Susceptibility testing was performed by both EUCAST[3] and CLSI[4] disc diffusion testing methods. CLSI and EUCAST ceftaroline disc diffusion testing were performed on Mueller–Hinton agar (MHA) and ceftaroline MICs were determined by gradient diffusion (Etest, Oxoid, UK) on MHA. Broth microdilution (BMD) testing was performed in accordance with CLSI guidelines using pure ceftaroline powder, obtained from AstraZeneca Australia, in triplicate.

MRSA isolates were classified as non-multiply-resistant (NMR-MRSA) if they were susceptible to two or more of erythromycin, tetracycline, ciprofloxacin and trimethoprim/sulfamethoxazole, or as multiply-resistant (MR-MRSA) if susceptible to one or none of these agents. Many laboratories in our region without access to real-time strain typing make this distinction. Our historically dominant hospital-acquired (HA)-MRSA clone ST239-III (Aus2/3 EMRSA) is the main MR-MRSA type seen, although ST22-IV (EMRSA-15) may also have this pattern.[5] In addition, the MLST and SCC*mec* type were predicted using the binary type pattern, using a previously published technique.[6]

Of the 100 isolates, 73 were NMR-MRSA and 27 MR-MRSA. By Etest, ceftaroline MICs ranged from 0.25 to 2.0 mg/L (MIC_{50} 1.0 mg/L, MIC_{90} 2.0 mg/L). All 15 ceftaroline-non-susceptible isolates by Etest had MIC values of 2 mg/L and had an MR-MRSA pattern. By BMD, ceftaroline MICs ranged from 0.5 to 2.0 mg/L (MIC_{50} 0.5 mg/L, MIC_{90} 2.0 mg/L). No isolate had an MIC ≥4 mg/L (resistant by CLSI breakpoint). Of 15 isolates with ceftaroline non-susceptibility by BMD, 14 (93%) had an MR-MRSA pattern, compared with 13/85 (15.3%) of ceftaroline-susceptible isolates. Of the 27 isolates with an MR-MRSA susceptibility pattern, 14 (52%) were ceftaroline non-susceptible by BMD. The relationship between CLSI and EUCAST ceftaroline zone diameters according to BMD results is shown in Figure 1.

Binary typing of the 27 MR-MRSA isolates revealed that 21 (77.8%) and 2 (7.4%) isolates belonged to the predicted ST239-III and ST22-IV clones, respectively. Clone prediction was unable to be performed for the remaining 4 (15%) as they showed rare binary types for which MLST relationships have not been determined. All 14 MR-MRSA isolates that demonstrated ceftaroline non-susceptibility by BMD had binary types consistent with the ST239-III clone. Nine of these belonged to a single binary type (280841) and three to a second type (281865).

The majority of MR-MRSA isolates in our study were non-susceptible to ceftaroline (MIC ≥ 2 mg/L), significantly higher than recent Australian studies in which 15% (3/20)[7] and 3.04% (13/428)[8] HA-MRSA isolates were non-susceptible. All ceftaroline-non-susceptible MR-MRSA isolates in our study belonged to the

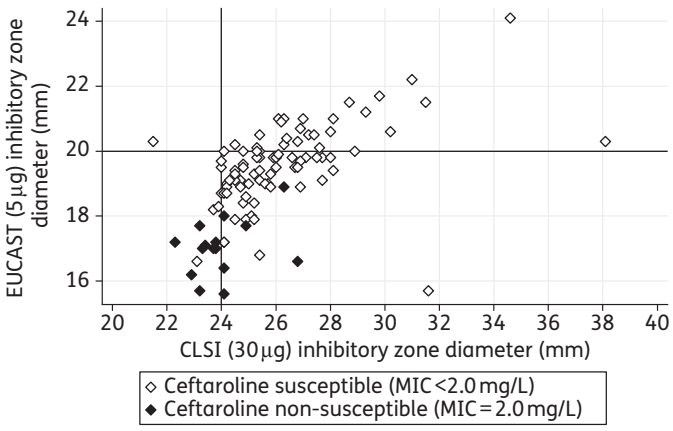

Figure 1. EUCAST versus CLSI disc diffusion zone diameters.