Plasmid-mediated resistance to tetracyclines among *Neisseria gonorrhoeae* strains isolated in Poland between 2012 and 2013

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**Abstract**

**Introduction:** One of two main mechanisms of resistance in tetracycline-resistant *Neisseria gonorrhoeae* (TRNG) is associated with the presence of TetM protein responsible for actively blocking of the tetracycline target site in the 30S ribosomal subunit. This mechanism is encoded by conjugative plasmids. The second mechanism is chromosomal in nature and due to mutations in specific genes.

**Aim:** To determine the incidence and type of *tetM* determinants in TRNG strains isolated from patients presenting with gonorrhea infection to the Dermatology and Venereology Clinic in Warsaw in 2012–2013.

**Material and methods:** Tetracycline and doxycycline susceptibility was determined by E-Tests. The presence and type of the *tetM* gene were determined by polymerase chain reaction.

**Results:** Tetracycline resistance was detected in 50.8% of the evaluated strains. The TRNG strains containing the *tetM* plasmid constituted 13.8% of all the evaluated strains. Dutch type *tetM* constituted 12.3% and American type *tetM* 1.5% of all the evaluated strains. In the remaining TRNG strains, resistance to tetracyclines was presumably chromosome-encoded. The minimal inhibitory concentration (MIC) of tetracycline ranged from 0.25 to 32.0 mg/l, MIC₅₀ = 2.0 mg/l, MIC₉₀ = 32.0 mg/l. The MIC of doxycycline ranged from 0.25 to 32.0 mg/l, MIC₅₀ = 4.0 mg/l, MIC₉₀ = 16.0 mg/l.

**Conclusions:** Unlike most of European countries, in 2012–2013 in Poland, the Dutch type *tetM* was found to be much more common than the American type. Minimal inhibitory concentration values of tetracycline and doxycycline were similar, with doxycycline exhibiting a somewhat lower effectiveness *in vitro* than tetracycline towards chromosome-mediated tetracycline resistant strains of *N. gonorrhoeae*.

**Key words:** *Neisseria gonorrhoeae*, *tetM* Dutch type, *tetM* American type, tetracycline resistance.
Tetracycline resistance in epidemic *N. gonorrhoeae* strains (e.g. G1407 genogroup) is often a result of mutations in *penB* (encoding porin B) [6–9], *penC* (encoding PilQ secretin) [6, 8, 10] and overproduction of the MtrCDE efflux pump, associated with mutations in the *mtrR* gene [9, 11, 12]. Chromosomal mutations cause an increase in the minimal inhibitory concentration (MIC) of tetracycline, usually up to 2–4 mg/L. A comparable level of resistance is also provided by much more rare mutations in the *psl* gene encoding the S10 ribosomal protein [13]. As a result of increasing resistance of *N. gonorrhoeae* to tetracyclines these antibiotics are not used in monotherapy to treat *N. gonorrhoeae* infections in Europe. However, either tetracycline or doxycycline may be used instead of azithromycin in combination with third-generation cephalosporin, especially in mixed infections, e.g. gonorrhea and chlamydia [14–17].

**Results**

Thirty-three (50.8%) out of the 65 evaluated *N. gonorrhoeae* strains were found to be tetracycline resistant according to both EUCAST and CLSI criteria. Table 1 shows MIC value distribution and result interpretation according to EUCAST and CLSI. Table 2 presents the MIC$_{90}$ and MIC$_{50}$ values of tetracycline and doxycycline. In light of no existing criteria for interpretation, susceptibility testing results for doxycycline are not presented here. Tetracycline-resistant strains were mainly (72.8%) those with tetracycline MIC of 2–4 mg/L consistent with chromosome-mediated resistance, whereas strains with tetracycline MIC of 16–32 mg/L consistent with the plasmid tetM determinant constituted 21.2% of TRNG strains. We also assessed the presence and type of the plasmid tetM determinant. The results are shown in Table 3. The plasmid tetM determinant was found in 9 out of 33 TRNG strains (27.3%), which constituted 13.8% of all evaluated *N. gonorrhoeae* strains. Out of the 9 strains with the tetM determinant, 8 (88.9%) were of the Dutch type, and only 1 (11.1%) exhibited the American-type determinant. None of the evaluated strains exhibited either different
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The prevalence of tetracycline resistance in N. gonorrhoeae depends on the time period and the country of strain isolation. In 2013, in India there were 12% of tetracycline resistant strains [22]; in 2010–2012, in Sri Lanka, 16.3% [23]; in 2010–2011/2012/2013, in Belarus it was 36/35/40%, respectively [24]; in 2010–2011, in Germany the percentage was 41.3% [25]; similarly in 2010/2011/2012, in Poland resistant strains constituted 42.9/38/49%, respectively [18]; in 2010–2012, in Indonesia 100% strains were resistant to tetracycline [23]. Studies on the worldwide prevalence of Dutch and American type conjugative plasmids encoding the TetM protein in N. gonorrhoeae strains showed the Dutch type plasmids to be more prevalent in Asian countries such as Indonesia (100%), Philippines (100%), and Thailand (100%) in 1988–1995 [21], in China in 1999–2006 (99.2%) [26] and in 2011–2012 (96.2%) [27], in Bangladesh (98.7%) [28], in Brazil (76.5%), in Guyana (61.1%), in Trinidad (95.5%), in Saint Vincent (93.3%) [29, 30], whereas the American type plasmids were more prevalent in Europe in 1988–1995 (80.5%) [21], in the UK in 1988–1995 (81.8%) [21], in Italy in 2003–2005 (77.8%) [31], in several African countries in 1988–1995 (98.3%) [21], and in Jamaica in 1988–1995 (63%), with a total Caribbean prevalence of 64.3% [21]. Our study showed that TetM-synthesizing N. gonorrhoeae strains isolated in Poland in 2013 are more often of the tetM Dutch type (88.9%). Meanwhile, we observe an increase in the prevalence of the tetM determinant in different countries. For example, the prevalence of tetM determinant in N. gonorrhoeae strains in China increased 18-fold in 1999–2005 (from 1.8% in 1999 to 32.8% in 2006) [26]. In Poland the prevalence of tetM in N. gonorrhoeae strains was 17.9/17.4/6.1% in 2010/2011/2012, respectively.

| Antibiotic | MIC range [mg/l] | MIC<sub>50</sub> [mg/l] | MIC<sub>90</sub> [mg/l] | Criteria | S (%) of strains | I (%) of strains | R (%) of strains |
|------------|------------------|------------------|------------------|----------|------------------|------------------|------------------|
| Tetracycline | 0.25–32.0 | 2.0 | 32.0 | EUCAST | 6 (9.2) | 26 (40.0) | 33 (50.8) |
| | | | | CLSI | 1 (1.5) | 31 (47.7) | 33 (50.8) |
| Doxycycline | 0.25–32.0 | 4.0 | 16.0 | | | |

Table 3. The tetM determinant type in N. gonorrhoeae strains

| Strain | Tetracycline MIC | Doxycycline MIC | tetM type |
|--------|-----------------|-----------------|----------|
| 1 | 16.0 | 8.0 | Dutch |
| 2 | 32.0 | 16.0 | Dutch |
| 3 | 32.0 | 16.0 | Dutch |
| 4 | 32.0 | 16.0 | Dutch |
| 5 | 16.0 | 16.0 | American |
| 6 | 32.0 | 16.0 | Dutch |
| 7 | 32.0 | 32.0 | Dutch |
| 8 | 32.0 | 16.0 | Dutch |
| 9 | 32.0 | 16.0 | Dutch |
In recent years, tetracycline-resistance of \textit{N. gonorrhoeae} isolates in Poland has remained at the same high level, with approximately 1/3 of isolates exhibiting plasmid-mediated resistance. Unlike in many other European countries, in Poland the prevalence of the Dutch type of \textit{tetM} was much higher than that of the American type in 2012–2013. The reason of the observed difference is unknown. It does not seem to come from neighboring eastern countries either, because although the percentage of Dutch and American type of \textit{tetM} in Russia was not investigated, \textit{tetM} accounts only for 3% of \textit{N. gonorrhoeae} tetracycline resistance in this country [32]. We have no data about \textit{tetM} prevalence in Belarus and Ukraine, however the percentage of resistant strains in Belarus was about 40 [24]. The observed pattern of tetracycline resistance seems to be regional to Poland.

The comparison with NG-MAST types of the previously isolated strains (data published before) [18] revealed that the \textit{tetM} determinant was most often found in NG-MAST sequence type (ST) 1405. The type of the \textit{tetM} gene was not determined at that time. The ST 1405 was the most prevalent type in Poland in 2010, 2011 and at the beginning of 2012 it was still relatively common, although it was partially replaced by epidemic ST 1407. Unlike 1407 that prevails in many European countries, ST 1405 hardly ever occurs outside Poland.

The MIC values for tetracycline and doxycycline were comparable, with doxycycline showing a slightly lower activity in vitro against TRNG not possessing \textit{tetM} determinant, with tetracycline-resistance most likely to be chromosome mediated.

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Conflict of interest

The authors declare no conflict of interest.

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Plasmid-mediated resistance to tetracyclines among \textit{Neisseria gonorrhoeae} strains isolated in Poland between 2012 and 2013

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