An Extended-Spectrum Beta-Lactamase Production in *Neisseria gonorrhoeae*?: New Evidence Supporting Possible Evolution Pathway

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**Background:** Nowadays, treatment of gonorrhea is seemingly complicated according to multi-drug resistant strains of *Neisseria gonorrhoeae*. Our latest study revealed that the use of ceftriaxone or cefixime, a standard treatment of patients with gonorrhea, may select for an extended-spectrum beta-lactamase (ESBL) producers from strains currently circulating in Thailand that are capable of producing two restricted-spectrum TEM-1 and TEM-135 beta-lactamases with an additional point mutation, E104K or G238S.

**Materials and Methods:** One hundred and twenty-two isolates of *N. gonorrhoeae* were collected from patients in Bangkok metropolitan area from 2005-2007 for antibiotic susceptibility test by disk diffusion method and genetic analyses. Plasmid profiling and DNA sequencing were performed. Presence of specific *bla*TEM-1 or *bla*TEM-135 gene was determined by heteroduplex semi-nested polymerase chain reaction amplification. A clone with double *bla*TEM alleles has been characterized by Southern blot hybridization, gene cloning, and DNA sequencing. Multi-locus sequence typing and NG-MAST were performed for clonal analysis.

**Results:** There were 101 among 122 isolates from patients with gonorrhea that positive for penicillinase production. Antibiotic susceptibility of 101 viable isolates for penicillin, ceftriaxone, tetracycline, and ciprofloxacin were 0, 100, 0, and 9.6%, respectively. Heteroduplex semi-nested PCR for *bla*TEM-1 and *bla*TEM-135 detections showed that 11 isolates carried *bla*TEM-135 allele simultaneously with *bla*TEM-1. Southern blot results showed the *bla*TEM genes were of plasmid origin. DNA cloning and sequencing confirmed an existence of double *bla*TEM-1 and *bla*TEM-135 alleles in the same piece of 9 kb-cloned DNA segment. Plasmid profiling, MLST, and NG-MAST showed international clonal sharing, i.e. ST1588, and its variants.

**Conclusion:** Patients infected with *N. gonorrhoeae* were at risk of treatment failure due to an evolution to be multi-drug resistant of the Thai isolates. Upon using more third generation cephalosporins, penicillinase-producing *N. gonorrhoeae* or PPNGs will be selected further for an ESBL phenotype. Careful surveillance and active screening may be necessary for the identification of ESBL producing *N. gonorrhoeae* in the near future.

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