Supplemental Material

Neisseria cinerea with high ceftriaxone MIC is an origin of ceftriaxone and cefixime resistance-mediating *penA* sequences in *Neisseria gonorrhoeae*

Gene Igawa,[a] Yuka Yamagishi,[b] Ken-Ichi Lee,[a] Misato Dorin,[a] Ken Shimuta,[a] Hiroyuki Suematsu,[b] Shu-Ichi Nakayama,[a] Hiroshige Mikamo,[b] Magnus Unemo[c] and Makoto Ohnishi[a, d]*

Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan[a]; Department of Clinical Infectious Diseases, Aichi Medical University, Aichi, Japan[b]; WHO Collaborating Centre for Gonorrhoea and other STIs, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden[c]; Antimicrobial Resistance Research Center National Institute of Infectious Diseases, Tokyo, Japan[d]

*Corresponding author. Department of Bacteriology I, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku, Tokyo, 162-8640 Japan. Phone: +81 (3) 5285-1111, Fax +81 (3) 5285-1163. E-mail: ohnishi7@nih.go.jp.

Running title: Gene reservoir of ceftriaxone resistance

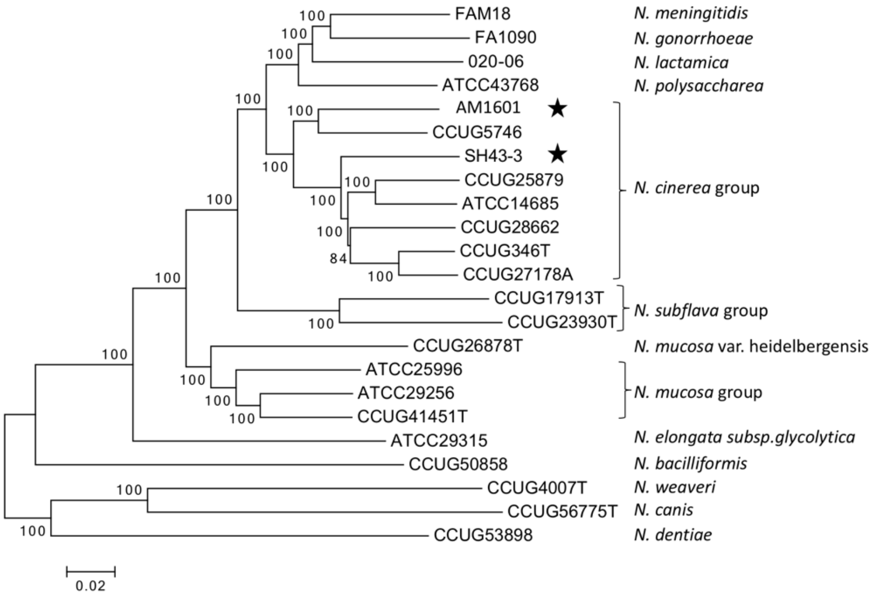

Figure. S1. Phylogenic relatedness of *Neisseria* spp. based on concatenated sequences of 53 ribosomal protein genes (*rps*). The concatenated *rps* sequences of 21 representatives of different *Neisseria* spp. (1) and the two *Neisseria cinerea* strains AM1601 and SH43-3 (indicated by asterisk), which were species verified in the present study, were analyzed using neighbor-joining method. The percentages of replicate trees in which the associated taxa were clustered together in the bootstrap test (500 replicates) are included.

Method

Ribosomal protein sequence (*rps*) comparison. To species identify the *N. cinerea* strains, 53 *rps* sequences derived from the WGS sequences, including previously described WGS sequences from *Neisseria* species (1), were used. Briefly, the SNPs of the 53 *rps* alleles were extracted and imported into MEGA version 7 software (2), and a phylogenetic tree was constructed using the neighbor joining method (2).

(1) Bennett JS, Jolley KA, Earle SG, Corton C, Bentley SD, Parkhill J, Maiden MCJ. 2012. A genomic approach to bacterial taxonomy: An examination and proposed reclassification of species within the genus Neisseria. Microbiol (United Kingdom) 158:1570–

1580.

(2) Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33:1870–1874.