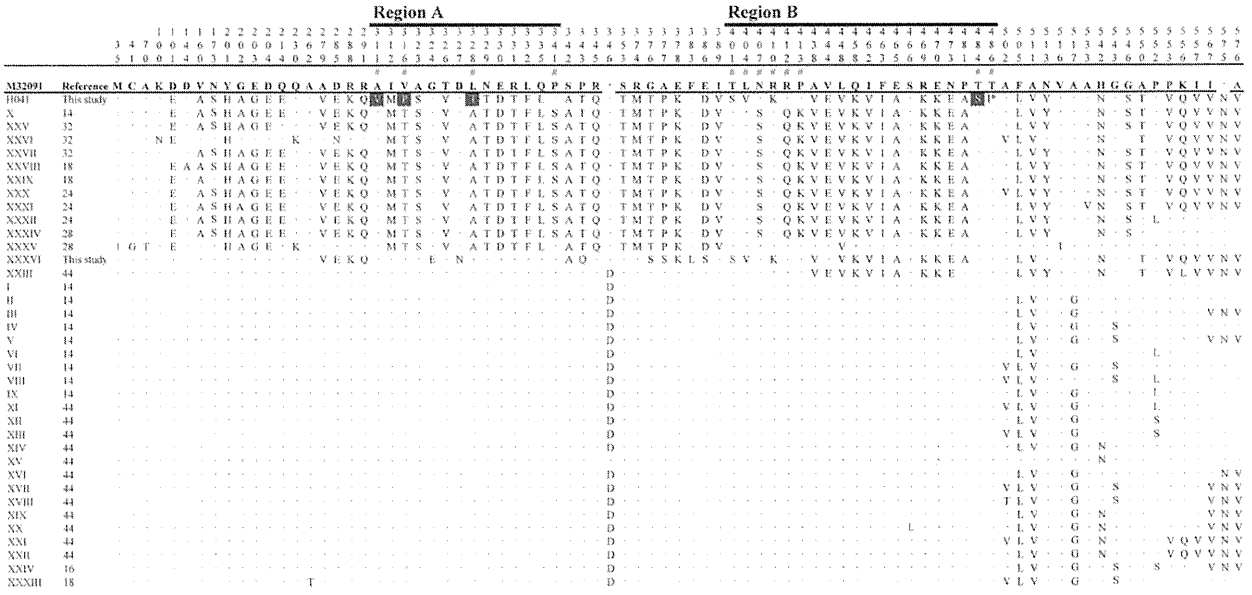

FIG. 2. A schematic figure describing all reported penicillin-binding protein 2 (PBP2) amino acid sequences in *Neisseria gonorrhoeae*, which are aligned to the wild-type PBP2 sequence M32091. All amino acid alterations in the different PBP2 sequences are illustrated with a single capital letter. The amino acids in PBP2 of H041 differing from PBP2 mosaic X (n = 12) are indicated (#). The four amino acid residues in the highly ceftriaxone-resistant *N. gonorrhoeae* strain H041 not previously observed in any *Neisseria* species and which explained the ceftriaxone resistance are shown by white letters on a blue ground. The amino acid residue marked with an asterisk has previously been found in *N. meningitidis* (unpublished) and *N. flavescens* (GenBank accession number M26645).

coccal strains in cocultivation experiments (data not shown) performed as previously described (24), which shows that this ceftriaxone resistance can rapidly spread within the *N. gonorrhoeae* population.

H041 seems to represent a subclone of the previously described MLST ST7363 cefixime-resistant *N. gonorrhoeae* circulating in Japan (23, 24). This clone has caused treatment failures using oral ESCs, has successfully spread worldwide, and now seems to have evolved further and developed resistance to ceftriaxone as well. The fear is that this ceftriaxone-resistant subclone will now spread in Japan, to WHO WPR countries, to Pacific Rim countries, and globally, which has been the scenario for emergence and worldwide spread of most gonococcal AMR. Based on previous experience (e.g., for fluoroquinolones), AMR can be widely disseminated internationally only 1 or 2 decades after the first emergence of AMR in WHO WPR (34, 36). The finding of this single high-level ceftriaxone-resistant gonococcal strain is important, especially because it was identified in a female commercial sex worker belonging to a high-risk, frequently transmitting population and because no national gonococcal antimicrobial resistance surveillance programs (including no sentinel sites for identification of gonorrhea treatment failures) are active in Japan. Accordingly, the strain should have excellent opportunities for a rapid spread. An enhanced but still limited gonococcal antimicrobial resistance surveillance in Kyoto, Japan, was initiated after the finding of H041; however, any secondary spread of H041 (or additional treatment failures) has yet not been identified. Despite the suboptimal Japanese surveillance systems, this fact may indicate that H041 has a lower biological fitness that results in limited further spread. Accordingly, the biological fitness of H041, compared to that of its wild type lacking $penA_{H041}$ that causes the ceftriaxone resistance, would be valuable to examine in a well-designed study, i.e., investigating quantitatively the fitness *in vitro* (different culture media, solid and liquid based) and also in an appropriate animal model, i.e., *in vivo*.

Nevertheless, *N. gonorrhoeae* has now shown its ability to develop resistance to ceftriaxone also, in which case gonorrhea may become untreatable in certain circumstances; although the biological fitness of H041 remains unknown, a serious public health problem seems to be approaching. To at least limit the spread of ESC (cefixime and ceftriaxone) resistance, timely and decisive multidisciplinary and multicomponent public health actions are essential not only in Japan but also globally. A recent expert review described WHO initiatives and approaches to AMR containment and how to meet public health challenges of untreatable gonorrhea (36). Nevertheless, to succeed with any AMR containment, enhanced gonorrhea control activities are needed to reduce the burden of infection (36). Furthermore, it is crucial to explore options, in industrialized settings as well as in less-resourced settings, for future treatment of ESC-resistant gonorrhea. This includes exploration of optimized dose regimens of presently used antimicrobials, new antimicrobials (or rediscovery of old drugs, such as gentamicin, ertapenem, and perhaps, piperacillin-tazobactam in emergent situations of ESC-resistant *N. gonorrhoeae*) or other substances, and combination therapy (6, 7, 19, 20, 22, 31, 36, 37; M. Unemo and J. Tapsall, unpublished data).

In conclusion, the first high-level ceftriaxone-resistant *N. gonorrhoeae* strain has now been characterized in detail, including an elucidation of its resistance mechanisms. Accordingly, *N. gonorrhoeae* has now shown its ability to develop

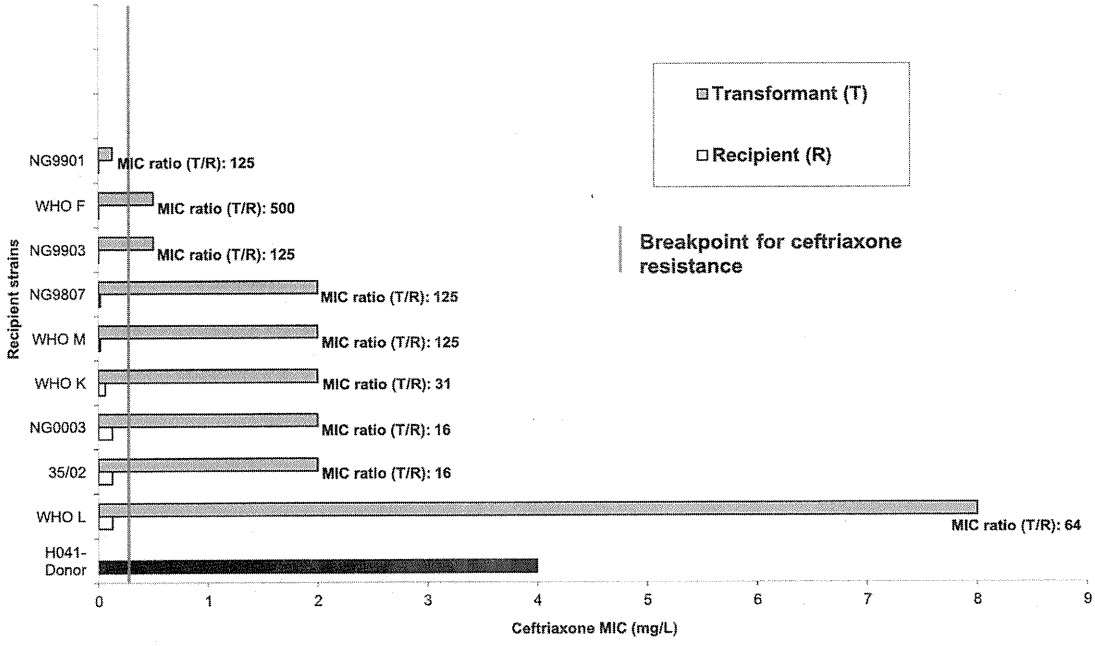

FIG. 3. Transformation of the full-length *penA* allele ($penA_{H041}$) from the high-level ceftriaxone-resistant *Neisseria gonorrhoeae* strain H041 (Donor) into *N. gonorrhoeae* strains (Recipients) with different ceftriaxone MICs and genetic resistance determinants affecting the susceptibility to ceftriaxone. The ceftriaxone MICs using the Etest method (shown as mean results of three repeated experiments) of the donor strain, recipient strains (R), and transformants (T) and the MIC ratio (T/R) are given. The breakpoint for ceftriaxone resistance is according to reference 8.

ceftriaxone resistance also and, although the biological fitness of ceftriaxone resistance in *N. gonorrhoeae* remains unknown, the gonococcus may soon become a true superbug that initiates a future era of untreatable gonorrhea. To at least slow the spread of ESC (cefixime and ceftriaxone) resistance, a reduction in global gonorrhea burden by enhanced disease prevention and control activities is crucial. As well, the implementation of much wider strategies for general AMR control, better understanding of the mechanisms and global monitoring of the emergence and spread of AMR, and global and national public health response plans (including sustainable clinical, microbiological, and epidemiological components) are needed. Any such plan alone will most probably not be able to prevent the emergence, establishment, and spread of ceftriaxone resistance; nevertheless, these plans will be valuable to delay and limit a global spread of ESC resistance (cefixime and ceftriaxone). Ultimately, a major focus important for public health globally is the timely development of effective new drugs (for single or combined use) for the treatment of gonorrhea.

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The work was performed at the Department of Bacteriology I, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku, Tokyo 162-8640, Japan, and the Swedish Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, SE-701 85 Örebro, Sweden.

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研究代表者　小野寺 昭一

連絡先　東京慈恵会医科大学医学部
〒105-8461　東京都港区西新橋3-25-8
TEL.03-3433-1111　FAX.03-5400-1249