J Infect Chemother xxx (2017) 1–4

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Note

'*Haemophilus quentini*' in the urethra of men complaining of urethritis symptoms

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ARTICLE INFO

Article history:
Received 30 May 2017
Received in revised form
24 July 2017
Accepted 10 August 2017
Available online xxx

Keywords:
Haemophilus quentini
Haemophilus influenzae
Urogenital infection

ABSTRACT

We isolated a cryptic genospecies of *Haemophilus influenzae* referred to as '*Haemophilus quentini*' in the urethra of 3 men complaining of urethritis symptoms. *H. influenzae* strains, which had been isolated from the urethra in 77 of 1518 men complaining of urethritis symptoms, identified by the conventional test, and stored, were re-cultured for this study. Sixty-seven strains surviving storage were screened by a PCR-based assay specific for the cryptic genital *Haemophilus* genospecies. Three strains (HI09003, HI11006, and HI14016) were screened by PCR and identified as '*H. quentini*' by 16S rRNA sequencing. The men positive for HI09003 and HI11006 were diagnosed as having non-chlamydial non-gonococcal urethritis (NGU), and their demographic and clinical features were similar to those of NGU caused by other pathogens. The man positive for HI14016 was ultimately diagnosed as having condyloma acuminatum on the glans. The 3 strains of '*H. quentini*' produced no β-lactamase and were susceptible to ampicillin and other antimicrobial agents, including cephalosporins, fluoroquinolones, tetracyclines, and macrolides, recommended for treatment for urethritis. '*H. quentini*' would be an uncommon pathogen in men with urogenital infections. Based on the clinical features of the two patients with '*H. quentini*'-positive NGU, it would be difficult to predict the presence of '*H. quentini*' in the urethra. The 3 strains of '*H. quentini*' were susceptible to a variety of antimicrobial agents. Further accumulation of data regarding '*H. quentini*' infections is needed to characterize the pathogenic roles of this genospecies in urogenital infections and to establish appropriate management of '*H. quentini*' infections.

Haemophilus influenzae strains have been isolated from urogenital, maternal, and neonatal infections and are reported to predominate over non-encapsulated and biotype IV strains [1]. Among these strains of *H. influenzae*, Quentin et al. found a distinct and homogeneous subset of biotype IV strains that had unusual phenotypic features [2,3]. Furthermore, they demonstrated that this group constituted a cryptic genospecies, which was referred to as '*Haemophilus quentini*', by genetic analyses [4–6]. However, '*H. quentini*' is yet to be validly published in the International Journal of Systematic and Evolutionary Microbiology.

Some studies have reported that non-encapsulated and type IV strains of *H. influenzae* have been isolated from the urethra of men with urethritis since the early 1980s [1,7]. However, '*H. quentini*' that cannot be distinguished from *H. influenzae* by the conventional tests would be overlooked in clinical settings. Recently, clinical stains have been identified as '*H. quentini*' by 16S rRNA sequencing [8–10], but '*H. quentini*' infections are still poorly understood. In our previous studies, we conventionally identified clinical strains, which had been isolated from the urethra of men complaining of urethral symptoms, as *H. influnezae* [11,12]. We wondered if some strains of '*H. quentini*' might be contained in our strains of *H. influnezae*. In this study, therefore, we screened our *H. influenzae*

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http://dx.doi.org/10.1016/j.jiac.2017.08.007
1341-321X/

strains for the cryptic genital *Haemophilus* genospecies by a PCR-based assay [6]. We determined the 16S rRNA genes of the screened strains to identify them as '*H. quentini*'. We retrieved the medical records of the patients with urogenital '*H. quentini*' infections to demonstrate their demographic and clinical features. We also examined the strains for β-lactamase (BL) production and antimicrobial susceptibility.

This retrospective study was approved by the Institutional Review Board of the Graduate School of Medicine, Gifu University, Gifu, Japan (reference number 20–94). *H. influenzae* strains were isolated from the urethra of 77 (5.1%) of 1518 men with urethritis symptoms who were reported in our previous studies [11,12]. The 77 patients comprised 69 with acute urethritis, 6 with acute epididymitis, 1 with acute prostatitis, and 1 with condyloma acuminatum. The 77 strains of *H. influenzae* identified by the conventional test and stored frozen at −70 °C were re-cultured for this study.

The strains were screened for the cryptic genital *Haemophilus* genospecies by a PCR-based assay with the primers derived from the 16S rRNA gene of genital *Haemophilus* strain 16 N as previously described [6]. The PCR could amplify a 282-bp fragment from stains belonging to the cryptic genital *Haemophilus* genospecies.

The strains screened by the PCR were then subjected to 16S rRNA sequencing. Their 16S rRNA genes were amplified by PCR with the primers of 27f and 1492r as previously described [13]. The sequences of the PCR products were compared to the 16S rRNA genes in the GenBank database by BLAST searches and aligned with the 16S rRNA genes of other *Haemophilus* spp. listed in Supplemental Table 1 by means of the multi-alignment software in the MEGA6 program package. A dendrogram was drawn to visualize the phylogenic distances among *Haemophilus* spp. with the neighbor-joining method.

The demographic and clinical information of the patients from whom '*H. quentini*' was isolated were retrieved from their medical records. The informed consent of the patients was not obtained when this study was conducted because they could not be traced. Therefore, their information was sufficiently anonymized.

BL activities were tested by nitrocefin disks (Nippon Becton Dickinson Company, Ltd., Tokyo, Japan). For the strains identified as '*H. quentini*' by the 16S rRNA sequencing, minimum inhibitory concentrations (MICs) of 41 antimicrobial agents, ampicillin (ABPC), amoxicillin (AMPC), clavulanic acid/AMPC, piperacillin (PIPC), tazobactam/PIPC, cefaclor, cefixime, cefdinir, cefpodoxime, cefditoren, cefcapene, cefazolin, cefotiam, cefotaxime, ceftriaxone, ceftazidime, cefpirome, flomoxef, aztreonam, faropenem, imipenem, panipenem, meropenem, doripenem, tebipenem, ciprofloxacin, levofloxacin (LVFX), tosufloxacin (TFLX), sitafloxacin (STFX), garenoxacin, clarithromycin (CAM), azithromycin (AZM), tetracycline (TC), doxycycline (DOXY), minocycline (MINO), gentamicin, tobramycin, amikacin, spectinomycin (SPCM), sulfamethoxazole-trimethoprim, and chloramphenicol, were determined by the broth dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) [14].

Of the 77 stored strains of *H. influenzae* isolated from the 77 men, 67 survived storage and were re-cultured in this study. They comprised 59, 6, 1, and 1 strain derived from men with acute urethritis, acute epididymitis, acute prostatitis, and condyloma acuminatum, respectively.

A 282-bp fragment was amplified by the PCR specific for the cryptic genital *Haemophilus* genospecies from 3 (4.5%) of the 67 strains. The remaining 64 strains were negative for PCR amplification. The sequences of the 16S ribosomal RNA gene amplified with the primers of 27f and 1492r from the 3 strains were identical to that of the '*H. quentini*' 16S ribosomal RNA gene (GenBank accession no. AF224307). They were named HI09003, HI11006, and HI14016, respectively. These strains formed a cluster with '*H.quentini*' that was described as a genospecies of *H. influenzae*. However, these strains and the described '*H. quentini*' (AF224307) were not related to *H. influenzae* and located in a same clade of *H. haemolyticus* (Fig. 1).

Demographic and clinical characteristics of the patients with '*H. quentini*' infections are summarized in Table 1. Two patients, from whom HI09003 and HI11006 were isolated, had mild symptoms and signs of acute urethritis and were diagnosed as having non-chlamydial non-gonococcal urethritis (NGU). Another patient, from whom HI14016 was isolated, was ultimately diagnosed as having condyloma acuminatum because he did not have signs of acute urethritis, including a significant number of leukocytes in his first-voided urine, but had condyloma acuminatum observed on the glans on genital examination. All 3 of the patients underwent antimicrobial chemotherapies, and '*H. quentini*' was eradicated.

No BL activities were detected in HI09003, HI11006, and HI14016. For these 3 strains, MICs of the 41 antimicrobial agents are summarized in Table 2. MICs of ABPC for the 3 strains were 0.06 μg/ml. The strains were assigned to BL non-producing ABPC-sensitive (BLNAS) strains and were susceptible to all of the antimicrobial agents for which the MIC interpretive criteria were recommended for *H. influenzae* strains by the CLSI [15]. MICs of other agents, which were not listed by the CLSI, for the 3 strains were as low as those of the respective analogous agents. Therefore, these strains were susceptible to all of the examined agents, including CTRX, LVFX, TFLX, STFX, CAM, AZM, DOXY, MINO, and SPCM that are recommended for the treatment of acute urethritis in Japan.

Three (4.5%) of the 67 strains of *H. influenzae* identified by the conventional test were identified as '*H. quentini*' by the 16S rRNA sequencing. '*H. quentini*' was isolated in at least 3 (3.9%) of 77 men whose urethral swabs were positive for *H. influenzae* and in at least 3 (0.2%) of 1518 men who complained of urethritis symptoms. '*H. quentini*' would not commonly be isolated from the urethra of men with urogenital infections.

The demographic and clinical characteristics of the 2 men with '*H. quentini*'-positive NGU would not stand out compared to those with NGU caused by other pathogens such as *Chlamydia trachomatis* [11]. By contrast, the man with condyloma acuminatum had no inflammatory responses on the urethra although '*H. quentini*' was isolated from the urethra. Therefore, this study could not verify that '*H. quentini*' is a definite pathogen causing NGU.

HI09003, HI11006, and HI14016 were BLNAS and susceptible to antimicrobial agents, including several agents recommended for the treatment for acute urethritis. Recently, a clinical strain of '*H. quentini*' was reported to harbor amino acid changes in penicillin binding protein 3 associated with β-lactam resistance, which were found in BL non-producing ABPC-resistant (BLNAR) strains of *H. influenzae* [10]. For this BLNAR strain of '*H. quentini*', however, the CTRX MIC was low. The co-infection of '*H. quentini*' in gonococcal infections could be cured microbiologically with the CTRX regimen recommended for the treatment of gonococcal infections.

Another study reported that *H. influenzae* biotype IV, which probably included some '*H. quentini*' strains, would be more susceptible to fluoroquinolones than the other biotypes [7]. In the current study, HI09003, HI11006, and HI14016 were susceptible to LVFX, which was recommended as an alternative for the treatment of chlamydial NGU. The MICs of other fluoroquinolones for the strains were as low as those of LVFX. Although a study reported that a clinical fluoroquinolone-resistant strain of '*H. quentini*', harboring amino acid changes in GyrA and ParC, emerged in 2005 [8], fluoroquinolones could be effective against '*H. quentini*' infections.

HI09003, HI11006, and HI14016, which were assigned to the AZM-susceptible strains, exhibited an AZM MIC of 1 μg/ml [15]. In our previous study, AZM MICs for 3 of 4 strains of *H. influenzae*

Please cite this article in press as: Horie K, et al., '*Haemophilus quentini*' in the urethra of men complaining of urethritis symptoms, J Infect Chemother (2017), http://dx.doi.org/10.1016/j.jiac.2017.08.007