Genotypic and phenotypic characterization and clinical significance of 'Haemophilus quentini' isolated from the urinary tract of adult men

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‘Haemophilus quentini’ has been proposed as the name for a distinct and homogeneous Haemophilus genospecies associated with urogenital tract and neonatal-related infections. Reports of ‘H. quentini’ isolation from adult men are rare and the disease potential in this population is unknown. We report six cases where ‘H. quentini’ was isolated from the genito-urinary tract in males. The isolation of ‘H. quentini’ during routine urine and urethral culture in adult men may aid in the determination of unresolved urethritis and possible urinary tract infections.

Introduction

Descriptions of Haemophilus influenzae strains are often limited to those that inhabit the upper respiratory tract of humans, where they can be found as part of the normal flora. Infections associated with these strains include pneumonia, otitis media and sinusitis, as well as invasive diseases such as meningitis, septicaemia, cellulitis and epiglottitis (Hirschmann & Everett, 1979; Khuri-Bulos & McIntosh, 1975; Murray et al., 1983). Some H. influenzae strains express one of six distinct polysaccharide capsules, allowing division into serotypes a–f. Although encapsulated strains are associated with invasive disease, the majority of H. influenzae strains are non-encapsulated (Foxwell et al., 1998; Murray et al., 2007). Both encapsulated and non-encapsulated strains can also be characterized using phenotypic and genotypic tests. By utilizing biochemical reactions, H. influenzae strains can be classified into eight biotypes (Casin et al., 1988; Kilian, 1976; Murray et al., 2007) based on tests for indole, urease and ornithine decarboxylase. Further distinction among H. influenzae groups can be made by employing 16S rRNA gene sequence analysis; H. influenzae types a, e and f, as well as ‘Haemophilus quentini’, form unique and separate genogroups. H. influenzae types b and d are separate from these groups, but cannot be distinguished from each other.

Often overlooked, rare H. influenzae strains have been reported to be responsible for causing urinary tract infections (UTIs), Bartholinitis, endometritis, septic abortions and neonatal meningitis (Albritton et al., 1978; Casin et al., 1988). The majority of these infections have been associated with women and neonates (Foxwell et al., 1998; Kakisi et al., 2010; Khuri-Bulos & McIntosh, 1975; Wallace et al., 1983). Although there may be several genital-associated strains (Harper & Tilse, 1991), early evidence suggested that non-typable capsule-type H. influenzae strains of biotype IV were the most common (Albritton et al., 1978; Wallace et al., 1983). Further characterization of non-typable H. influenzae strains of biotype IV using multilocus enzyme electrophoresis (Quentin et al., 1990, 1993) identified a subset of strains that were distinct and homogeneous. These strains were termed H. influenzae cryptic genospecies (Quentin et al., 1996). 16S rRNA gene sequence analysis surprisingly revealed that the cryptic genospecies of biotype IV were related more closely to Haemophilus haemolyticus (Fig. 1) than to H. influenzae (Quentin et al., 1996). These studies led to the proposed naming of this distinct group of cryptic genospecies as ‘Haemophilus quentini’, a name of convenience not yet validly published in the International Journal of Systematic and Evolutionary Microbiology. Here, we report six cases of ‘H. quentini’ isolation from genito-urinary sources in males. As the clinical histories of all six cases are similar, only two representative clinical histories are presented. However, the laboratory findings and clinical significance of all cases are discussed.

Case reports

Case 1

A 28-year-old male Iraq-war veteran presented to the urgent-care clinic complaining of pain following ejaculation and a penile discharge for the past 5–6 days after unprotected intercourse 2 weeks previously. His medical history was
significant for a long history of dysuria and episodes of urethritis, complicated by possible prostatitis. Previous bacterial cultures and molecular testing for sexually transmitted infections were all negative. At this particular visit, the physical examination revealed a scant urethral discharge. The patient denied dysuria and difficulty voiding. Urine and urethral-swab specimens were collected and the patient was treated empirically with ceftriaxone and azithromycin. Urine and urethral specimen was plated onto a Thayer Martin chocolate biplate (Remel) and incubated at 35°C in air, and the latter two were incubated at 35 °C in air with 7% CO₂. After 24 h incubation, 10⁴ grey colonies were observed on the chocolate agar plate and were subsequently identified as ‘H. quentini’.

**Case 2**

A 42-year-old male presented to the alcohol-dependence treatment centre for support-group therapy, at which time a follow-up routine urine sample was obtained based upon a previous abnormal UA. The patient was asymptomatic at this time. His medical history was significant for benign prostatic hyperplasia. The patient had been monogamous with his current female partner for the past 10 years. The urine specimen was sent for UA with instructions to begin with HQ; all others with a complete name are type strains.

![Dendrogram comparing ‘H. quentini’ isolates from adult males.](Image)

Fig. 1. Dendrogram comparing ‘H. quentini’ isolates from adult males. Sequences for strains from the cases in the present report begin with HQ; all others with a complete name are type strains.

The isolates from the two aforementioned cases, together with those of four additional cases, were analysed. Gram staining of the six isolates showed tiny Gram-negative rods that were oxidase-positive and required both X and V factors for growth. Five of the six isolates were negative for β-lactamase (Table 1). Using the API Neisseria–Haemophilus (NH) system (bioMérieux), organisms were identified as H. influenzae with 99% probability. Conventional biochemical testing confirmed that all six isolates were of biotype IV (negative for indole and positive for urease and ornithine decarboxylase). Capsular serotyping performed on four of the six available isolates (HQ F3013, HQ F5096, HQ 1617, HQ 1379) showed that they were non-typable. All six isolates were subsequently identified as ‘H. quentini’ by 16S rRNA gene sequence analysis (Fig. 1) using primers and protocols described previously (Claridge, 2004; Mahlen & Claridge, 2009). All sequences were deposited in GenBank under accession numbers JF433944–JF433949.

We utilized the NH fastidious card (bioMérieux), containing biochemicals used in the identification of Neisseria spp., Haemophilus spp. and other fastidious Gram-negative and Gram-variable micro-organisms, to perform phenotypic biochemical analysis on four of the six isolates (HQ F3013, HQ F5096, HQ 1617, HQ 1379) on the Vitek 2.0 system (bioMérieux). All four isolates were identical in the following biochemical reactions based on three separate analyses: negative for γ-glutamyl transferase, arylamidases (Ala-Phe-Pro, L-proline, L-pyrrolidonyl, L-lysine and tryptophan), α-arabinosidase, β-galactopyranosidase indoxyl, glycosidase, D-mannose, maltose, L-glutamine, maltotriose, N-acetyl-D-glucosamine, phosphoryl choline, pyruvate and sucrose. Positive for arylamidases (arginine and leucine), ornithine decarboxylase, phenylphosphate, phosphate and urea. Variable reactions included D-galactose, D-glucose, D-malate, D-xylene, phenylalanine arylamidase and two proprietary biochemicals [D-ribose 2 (carbohydrate utilization) and Ellman reagent (thiosulfate utilization)].

**Discussion**

Previous reports of the isolation of ‘H. quentini’ have been from the female genito-urinary tract and neonatal-related infections (Mak et al., 2005; Quentin et al., 1989, 1990;
Wallace et al., 1983). Close examination of the literature yielded mention of the isolation of ‘H. quentini’ from two men (Quentin et al., 1989). A second report also noted the isolation of H. influenzae biotype IV from two men seen at a sexually transmitted disease clinic (Messing et al., 1989). In both reports, patient demographics and details surrounding the cases were absent or incomplete. None of these reports described the isolation of ‘H. quentini’ from urine in men. Improved recovery of all Haemophilus species from urogenital specimens in men and women has been reported by Sturm (1986) using a selective chocolate agar (Choc-VBCA) that contains vancomycin, clindamycin and amphotericin B.

Our laboratory isolated and identified six isolates of ‘H. quentini’ from genito-urinary sources in males ranging from 28 to 73 years of age (Table 1). ‘H. quentini’ was isolated from urethral exudates (n=3) and urine specimens (n=3) from adult men in association with disease (n=5) and also when the disease association was not clear (n=1). Strains associated with urethritis occurred in younger men aged 28–32 years, whilst UTIs (defined as the sole or predominant pathogen of the genito-urinary tract that can be acquired sexually (Quentin et al., 1989). Interestingly, of the three cases of ‘H. quentini’ that we isolated from urethral specimens of symptomatic adult males, two were isolated in pure culture and one was isolated concomitantly with N. gonorrhoeae. These three cases support the notion that ‘H. quentini’ may indeed be acquired sexually and can occur as a primary infection or may be isolated along with usually implicated sexually acquired organisms. Interestingly, all of the three cases in which ‘H. quentini’ was isolated from urine were associated with UTIs; to our knowledge, this is the first time that such an association has been demonstrated.

Distinguishing between ‘H. quentini’ and H. influenzae strains cannot be achieved using current automated methods and biochemical tests used routinely in the clinical microbiology laboratory. Further examination of biochemical tests on a larger number of ‘H. quentini’ isolates may yield useful biochemical properties that could be used to identify ‘H. quentini’ rapidly from genitourinary sources in the future. Two potential biochemical tests that we identified in our analysis which may prove to be useful in identifying ‘H. quentini’ are tyrosine arylamidase and phosphoryl choline. Both were negative among the four ‘H. quentini’ isolates tested (Table 1) and were positive for most H. influenzae isolates tested, as well as those in the Vitek database.

In summary, using 16S rRNA gene sequencing, we identified six strains of ‘H. quentini’ that were isolated on chocolate agar from genito-urinary sources from adult males. Reports of isolation of ‘H. quentini’ from adult men are rare and the disease potential in this population is unknown. Our isolation of ‘H. quentini’ from men, five of whom were symptomatic at the time of isolation, suggests that ‘H. quentini’ may be a cause of unrecognized urinary tract disease in certain men. The use of non-selective chocolate agar for culture, as well as identification of Haemophilus spp. from urethral and urine cultures, may aid in the treatment of unresolved urethritis and UTIs in some men. The isolation of Haemophilus spp., especially isolates that are suspected to fit the biochemical profile of H. influenzae biotype IV, from the urogenital tract should raise suspicion and warrant further workup, including 16S rRNA gene sequencing, to identify ‘H. quentini’.

### Table 1. Patient demographics and notable isolate characteristics

+ Positive; –, negative; NA, not applicable; NT, not tested.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HQ F3013</th>
<th>HQ 1394</th>
<th>HQ 1379</th>
<th>HQ F5096</th>
<th>HQ 1617</th>
<th>HQ 2522</th>
</tr>
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<tbody>
<tr>
<td>Age of patient (years)</td>
<td>28</td>
<td>30</td>
<td>32</td>
<td>42</td>
<td>69</td>
<td>73</td>
</tr>
<tr>
<td>Specimen source</td>
<td>Urethral exudate</td>
<td>Urethral exudate</td>
<td>Urethral exudate</td>
<td>Urine</td>
<td>Urine</td>
<td>Urine</td>
</tr>
<tr>
<td>Quantity of ‘H. quentini’</td>
<td>Moderate</td>
<td>NA</td>
<td>Many</td>
<td>Urine</td>
<td>Urine</td>
<td>Urine</td>
</tr>
<tr>
<td>β-Lactamase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nucleotide at position*</td>
<td>66</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>315</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>Other isolated organisms</td>
<td>None</td>
<td>None</td>
<td>Neisseria gonorrhoeae</td>
<td>None</td>
<td>None</td>
<td>Alpha streptococci</td>
</tr>
<tr>
<td>Quantity of other organisms</td>
<td>None</td>
<td>None</td>
<td>Many</td>
<td>None</td>
<td>None</td>
<td>10⁴</td>
</tr>
<tr>
<td>Tyrosine arylamidase</td>
<td>–</td>
<td>NT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NT</td>
</tr>
<tr>
<td>Phosphoryl choline</td>
<td>–</td>
<td>NT</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NT</td>
</tr>
</tbody>
</table>

*Position according to the 16S rRNA gene sequence of ‘H. quentini’ (GenBank accession no. AF224307).
Acknowledgements

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References


