Case Report

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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.*, 2007). 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-capsulated (Foxwell *et al.*, 1998; Murray *et al.*, 2007). Both encapsulated and non-capsulated 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

Abbreviations: UA, urine analysis; UTI, urinary tract infection.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences determined in this study are JF433944–JF433949.

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.

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 analysis (UA) was remarkable for trace amounts of leukocyte esterase and 4–8 red blood cells per high-power field. Gram staining of the urethral discharge indicated many white blood cells, no epithelial cells and no organisms seen. On molecular testing, the urine was negative for *Chlamydia* spp. and *Neisseria gonorrhoeae* by the Aptima test (Gen-Probe). The urethral specimen was plated onto a Thayer Martin chocolate bipate (Remel) and incubated at 35 °C in air with 7 % CO₂. After 24 h incubation, the culture was negative for *N. gonorrhoeae*; however, moderate numbers of a pure culture of '*H. quentini*' were isolated on chocolate agar only.

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

culture if indicated, and the patient was treated empirically with amoxicillin. The UA showed 3+ leukocyte esterase and elevated white blood cells, which warranted culture. The urine specimen was plated onto Columbia agar with 5 % sheep blood, MacConkey agar, Columbia agar with the addition of colistin and nalidixic acid and chocolate agar (all media from Remel). The first two plates were incubated at 35 °C in ambient 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*'.

Laboratory analysis

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 (Clarridge, 2004; Mahlen & Clarridge, 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 tyrosine), α -arabinosidase, β -galactopyranosidase indoxyl, glycogen, D-mannose, maltose, L-glutamine, maltotriose, N-acetyl-D-glucosamine, phosphoryl choline, pyruvate and sucrose. Positive for arylamidases (arginine and leucine), ornithine decarboxylase, phenylphosphate, phosphatase and urea. Variable reactions included D-galactose, D-glucose, D-malate, D-xylose, 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;

Table 1. Patient demographics and notable isolate characteristics

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

Characteristic	Isolate					
	HQ F3013	HQ 1394	HQ 1379	HQ F5096	HQ 1617	HQ 2522
Age of patient (years)	28	30	32	42	69	73
Specimen source	Urethral exudate	Urethral exudate	Urethral exudate	Urine	Urine	Urine
Quantity of ' <i>H. quentini</i> '	Moderate	NA	Many	10 ⁴	10 ⁴	10 ⁵
β -Lactamase	–	–	+	–	–	+
Nucleotide at position*:						
66	A	A	G	G	G	A
315	C	G	C	C	C	G
Other isolated organisms	None	None	<i>Neisseria gonorrhoeae</i>	None	Alpha streptococci	None
Quantity of other organisms	None	None	Many	None	10 ⁴	None
Tyrosine arylamidase	–	NT	–	–	–	NT
Phosphoryl choline	–	NT	–	–	–	NT

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

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.*, 1983). 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 isolate from urine at $>10^4$ c.f.u. in the presence of polymorphonuclear leukocytes) occurred in older men (aged 42–73 years). Two genogroups were defined, which differed at two base-pair positions. These genogroups may correlate with severity of disease, but did not correlate with specimen type or β -lactamase production (Table 1).

'*H. quentini*' has been described as an opportunistic 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 genito-urinary 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*'.

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References

- Albritton, W. L., Hammond, G. W. & Ronald, A. R. (1978). Bacteremic *Haemophilus influenzae* genitourinary tract infections in adults. *Arch Intern Med* **138**, 1819–1821.
- Casin, I., Sanson-Le Pors, M. J., Felten, A. & Perol, Y. (1988). Biotypes, serotypes, and susceptibility to antibiotics of 60 *Haemophilus influenzae* strains from genitourinary tracts. *Genitourin Med* **64**, 185–188.
- Clarridge, J. E., III (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev* **17**, 840–862.
- Foxwell, A. R., Kyd, J. M. & Cripps, A. W. (1998). Nontypeable *Haemophilus influenzae*: pathogenesis and prevention. *Microbiol Mol Biol Rev* **62**, 294–308.
- Harper, J. J. & Tilse, M. H. (1991). Biotypes of *Haemophilus influenzae* that are associated with noninvasive infections. *J Clin Microbiol* **29**, 2539–2542.
- Hirschmann, J. V. & Everett, E. D. (1979). *Haemophilus influenzae* infections in adults: report of nine cases and a review of the literature. *Medicine (Baltimore)* **58**, 80–94.
- Kakisi, O. K., Kalkakos, P., Spyropoulos, V., Lazaraki, T. P., Moraitou, H., Avdeliodi, K., Kada, H. & Diakakis, I. (2010). Non-typeable *Haemophilus influenzae* and tubo-ovarian abscesses: case report and brief review. *Eur J Obstet Gynecol Reprod Biol* **152**, 226–227.
- Khuri-Bulos, N. & McIntosh, K. (1975). Neonatal *Haemophilus influenzae* infection. Report of eight cases and review of the literature. *Am J Dis Child* **129**, 57–62.
- Kilian, M. (1976). A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. *J Gen Microbiol* **93**, 9–62.
- Mahlen, S. D. & Clarridge, J. E., III (2009). Oral abscess caused by *Campylobacter rectus*: case report and literature review. *J Clin Microbiol* **47**, 848–851.
- Mak, G. C., Ho, P. L., Tse, C. W., Lau, S. K. & Wong, S. S. (2005). Reduced levofloxacin susceptibility and tetracycline resistance in a clinical isolate of *Haemophilus quentini* identified by 16S rRNA sequencing. *J Clin Microbiol* **43**, 5391–5392.
- Messing, M., Sottnek, F. O., Biddle, J. W., Schlater, L. K., Kramer, M. A. & Kraus, S. J. (1983). Isolation of *Haemophilus* species from the genital tract. *Sex Transm Dis* **10**, 56–61.
- Murray, P. R., Baron, E. J., Jorgensen, J., Tenover, P. C. & Tenover, M. C. (2007). *Manual of Clinical Microbiology*, 9th edn. Washington, DC: American Society for Microbiology.
- Quentin, R., Musser, J. M., Mellouet, M., Sizaret, P. Y., Selander, R. K. & Goudeau, A. (1989). Typing of urogenital, maternal, and neonatal isolates of *Haemophilus influenzae* and *Haemophilus parainfluenzae* in correlation with clinical source of isolation and evidence for a genital specificity of *H. influenzae* biotype IV. *J Clin Microbiol* **27**, 2286–2294.
- Quentin, R., Goudeau, A., Wallace, R. J., Jr, Smith, A. L., Selander, R. K. & Musser, J. M. (1990). Urogenital, maternal and neonatal isolates of *Haemophilus influenzae*: identification of unusually virulent serologically non-typable clone families and evidence for a new *Haemophilus* species. *J Gen Microbiol* **136**, 1203–1209.
- Quentin, R., Martin, C., Musser, J. M., Pasquier-Picard, N. & Goudeau, A. (1993). Genetic characterization of a cryptic genospecies of *Haemophilus* causing urogenital and neonatal infections. *J Clin Microbiol* **31**, 1111–1116.
- Quentin, R., Ruimy, R., Rosenau, A., Musser, J. M. & Christen, R. (1996). Genetic identification of cryptic genospecies of *Haemophilus* causing urogenital and neonatal infections by PCR using specific primers targeting genes coding for 16S rRNA. *J Clin Microbiol* **34**, 1380–1385.
- Sturm, A. W. (1986). Isolation of *Haemophilus influenzae* and *Haemophilus parainfluenzae* from genital-tract specimens with a selective culture medium. *J Med Microbiol* **21**, 349–352.
- Wallace, R. J., Jr, Baker, C. J., Quinones, F. J., Hollis, D. G., Weaver, R. E. & Wiss, K. (1983). Nontypable *Haemophilus influenzae* (biotype 4) as a neonatal, maternal, and genital pathogen. *Rev Infect Dis* **5**, 123–136.