Biotypes, serotypes, and susceptibility to antibiotics of 60 *Haemophilus influenzae* strains from genitourinary tracts

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**Summary** Sixty strains of *Haemophilus influenzae* were isolated from the genitourinary tracts of adults: 19 from cervicovaginal secretions, one from a woman with bartholinitis, 37 from urethral exudates, and three from urine. Non-capsulated strains were recovered predominantly, and biotype III accounted for 28 isolates and biotype IV for 25.

Many of the *H influenzae* strains were found to be resistant to one or more of the antibiotics commonly used against sexually transmitted diseases. Resistance to tetracycline was prevalent and was found in 17 of the strains. Ten strains were ampicillin resistant and β lactamase producing. Kanamycin resistance was less common (two strains). Trospectomycin (U-6336F), a new spectinomycin analogue, was eight to 16 times more active than spectinomycin. All the quinolones tested were very active against all strains and may provide an effective alternative for the treatment of resistant *H influenzae* in genitourinary infections.

The aetiological role of *Haemophilus influenzae* has been reported in neonatal,14-15 obstetrical,4-7 and gynaecological infections;6-13 and in urethritis,11 14-16 acute epididymo-orchitis,17 and urinary tract infections.18 19 It is thought to be a cause of sexually transmitted disease (STD).20 Moreover, mixed infections with other agents of STD, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*, are common.11 14-16 In 1982–5 we isolated 60 strains of *H influenzae* from patients with genital and urinary tract infections. In the present study we classified the biotype and capsular serotype of these strains and investigated their susceptibility to eight antibiotics used for genital and urinary infections: ampicillin, chloramphenicol, tetracycline, minocycline, erythromycin, kanamycin, spectinomycin, and roxithromycin. We also assessed the usefulness of the following newer antibiotics as alternatives to the conventional drugs: trospectomycin (U-63366), a new spectinomycin analogue; roxithromycin (RU-28965), a new macrolid antibiotic; and the new quinolone derivatives, pefloxacin, norfloxacin, ofloxacin, and ciprofloxacin.

**Patients, materials, and methods**

**Bacterial strains** The *H influenzae* strains evaluated were clinical isolates from adult patients with urinary or genital symptoms. Nineteen were isolated from vaginal secretions, one from a woman with bartholinitis, 37 from male urethral exudates, and three from urine.

**Identification procedures** Requirements were assessed on tripticate soy agar (TSA, Bio-Mérieux, France) with Taxo Haemophilus differentiation strips (BBL, Microbiology Systems, Cockeysville, Maryland, USA).

The synthesis of porphyrins and porphobilinogen from 5-aminolaevulinic acid (δ-ALA) was investigated as described by Kilian et al.21 The production of urease, ornithine decarboxylase, and indole was tested with the API-10E kit (API System, La Balme les Grottes, France) inoculated heavily with the growth from a 24 hour culture on chocolate agar supplemented with 1% Isovitalex (Bio-Mérieux, France). The classification of biotypes followed the scheme of Kilian et al.21

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The capsular type of encapsulated *H. influenzae* strains was assessed by slide agglutination using a specific antiserum to type b and pooled antisera to types a, c, d, e, and f (Phadebact Haemophilus test, Pharmacia Diagnostic, Sweden).

**DETECTION AND CHARACTERISATION OF β-LACTAMASE**

Production of β-lactamase was detected by the rapid chromogenic cephalosporin test (Cefinase, Bio-Mérieux, France).

The characterisation of β-lactamase was carried out by analytical isoelectric focusing in polyacrylamide gel on an LKB 2117 Multiphor instrument (Sweden) according to a method described previously. Two *Escherichia coli* K12 strains containing the plasmids pSF 2124 (colEI::Tn3) and RP4, which code respectively for TEM1 and TEM2 type β-lactamases, were used as reference for isoelectric points.

**ANTIBIOTIC SUSCEPTIBILITY TEST**

We used Mueller-Hinton agar supplemented with 5% Fide’s extract to test for susceptibility to the following antimicrobial agents: ampicillin (Beecham), tetracycline hydrochloride (Rhône Poulenc), minocycline (Lederlé), chloramphenicol, erythromycin, roxithromycin, and ofloxacin (Roussel-Uclaf), kanamycin (Bristol), spectinomycin and trospectomycin (U-63366) (Upjohn), minocycline (Winthrop), spectinomycin, roxithromycin, pefloxacin (Roger Bellon), norfloxacin (Merck, Sharp and Dohme), and ciprofloxacin (Bayer-Pharma).

Minimum inhibitory concentrations (MICs) were measured by agar dilution. Agar plates containing serial dilutions of antibiotics were inoculated with a Steers type replicator. Colonies from overnight culture on chocolate agar plates were suspended in sterile saline to a density of 0.5 MacFarland’s scale and diluted to give a concentration of about 10⁶ colony forming units (CFU) a spot. Culture plates were incubated at 37°C in an aerobic atmosphere for 24 hours. The MIC was defined as the lowest concentration of antibiotic that completely inhibited visible growth. Reference strains *Staphylococcus aureus* ATCC 25923 and *E. coli* ATCC 25922 were included in each experiment.

### Table 1  Correlation between anatomical origin, biotype, and capsular type of 60 strains of Haemophilus influenzae

<table>
<thead>
<tr>
<th>Anatomical origin of strains</th>
<th>No</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>Capsular type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal secretions</td>
<td>19</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>b, a, c, d, e, f, Acp</td>
</tr>
<tr>
<td>Bartholin’s duct</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>Acp</td>
</tr>
<tr>
<td>Urethral exudates</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>Acp</td>
</tr>
<tr>
<td>Urine</td>
<td>37</td>
<td>1</td>
<td>2</td>
<td>19</td>
<td>15</td>
<td>0</td>
<td>Acp</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>2</td>
<td>3</td>
<td>28</td>
<td>25</td>
<td>2</td>
<td>Acp</td>
</tr>
</tbody>
</table>

b = Acp = acapsular strains.

### Table 2  Minimum inhibitory concentrations (MICs) of 14 antibiotics against 60 strains of Haemophilus influenzae

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No of strains</th>
<th>MIC (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>_MICw</td>
</tr>
<tr>
<td>Ampicillin:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>50</td>
<td>0.03-0.5</td>
</tr>
<tr>
<td>Resistant</td>
<td>10</td>
<td>16-64</td>
</tr>
<tr>
<td>Kanamycin:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>58</td>
<td>1-4</td>
</tr>
<tr>
<td>Resistant</td>
<td>2</td>
<td>≥128</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>60</td>
<td>4-8</td>
</tr>
<tr>
<td>Trospectomycin</td>
<td>60</td>
<td>0.25-1</td>
</tr>
<tr>
<td>Tetracycline:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>43</td>
<td>0.25-2</td>
</tr>
<tr>
<td>Resistant</td>
<td>17</td>
<td>8-64</td>
</tr>
<tr>
<td>Minocycline</td>
<td>60</td>
<td>0.12-8</td>
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<tr>
<td>Minocycline</td>
<td>60</td>
<td>0.05-1</td>
</tr>
<tr>
<td>Minocycline</td>
<td>60</td>
<td>0.5-5</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>60</td>
<td>4-32</td>
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<td>Erythromycin</td>
<td>60</td>
<td>0.03-0.25</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>60</td>
<td>0.016-0.25</td>
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<td>Pefloxacin</td>
<td>60</td>
<td>0.003-0.03</td>
</tr>
<tr>
<td>Ciprofl oxacin</td>
<td>60</td>
<td>0.008-0.03</td>
</tr>
</tbody>
</table>

MICw = MIC for 90% of the strains.

### Results

**BIOTYPES AND CAPSULAR TYPES**

Table 1 shows the correlation between the anatomical origin of the *H. influenzae* isolates and their biotypes and capsular types. Most of the strains belonged to biotypes III (28 strains) and IV (25 strains). All but two strains were non-capsulated.

**SUSCEPTIBILITY TESTING**

Table 2 shows the ranges of MICs and the MIC of each antibiotic required to inhibit 90% of the isolates (MIC 90). Ten out of the 60 *H. influenzae* strains tested were β-lactamase producing and had MICs of ampicillin ranging from 16 to 64 mg/l or more. Seventeen strains were resistant to tetracycline (MICs of 8 to 64 mg/l). MICs of minocycline were 8 mg/l or less for all strains. MICs of kanamycin ranged from 1 to 128 mg/l or more. Two strains were resistant to this compound. Trospectomycin (U-63366) was eight to 16 times more active than spectinomycin. All strains tested were fully susceptible to chloramphenicol (with MICs of 0.5-1
Biotypes, serotypes, and susceptibility to antibiotics of 60 H influenzae strains from genitourinary tract

mg/l). Roxithromycin was slightly less active than erythromycin.

All quinolones tested exhibited a high activity against all strains. Ciprofloxacin and ofloxacin had the lowest MICs.

**CHARACTERISATION OF β-LACTAMASE**

The same isoelectric focusing pattern was obtained from crude extracts of the 10 β-lactamase producing strains of *H influenzae* and was similar to that of the TEM1 type β-lactamase produced by pSF 2124.

**Discussion**

*H influenzae* is not usually considered to be part of the normal flora of the genital tract: it has been recovered from none to 1% of symptomless women, pregnant or not, and from none to 9% of symptomless men. *H influenzae* strains in this study were recovered from symptomatic patients. When isolated from urine, cultures showed bacteriuria of 10⁶/ml or more. All urethral and cervicovaginal specimens showed numerous polymorphonuclear leucocytes on Gram staining, and cultures yielded *H influenzae* purely or predominantly. Eight patients yielded a concomittant genital pathogen: *N gonorrhoeae* (in 1 patient), *C trachomatis* (1), *Trichomonas vaginalis* (3), and *Candida albicans* (3).

Since the first descriptions of genital and neonatal infections due to *H influenzae*, the number of cases reported has increased appreciably during the past 15 years, which suggests a possible pathogenic role of this organism in gynaecological, obstetrical, and neonatal infections. Nevertheless the aetiological role of *Haemophilus* in adult vaginitis and urethritis in man is less well established. The pathogenicity of *H influenzae* strains is closely related to the possession of capsular polysaccharide. Most strains isolated in invasive disease of infants and young children are encapsulated, and nearly all are type b. In obstetrical and neonatal systemic infections as well as in pelvic inflammation, both typeable and non-typeable *H influenzae* strains have been implicated. As noted in this and other reports, acapsulated strains predominate in the lower female genital tract and male urethra. No encapsulated strain was found by Wallace *et al* in genital infections not associated with bacteraemia or by Sturm in male urethritis. Classification into biotypes according the scheme of Kilian *et al* has shown a biotype distribution of genital *H influenzae* strains different from that of nasopharyngeal strains. Our results agree with those in other reports, which show the predominance of biotype IV *H influenzae* strains in the genitourinary tract. These findings and those of Barenkamp *et al* showing a common outer membrane protein profile of strains isolated from neonates with sepsis, led Wallace *et al* to suggest that non-typeable biotype IV *H influenzae* strains might have a particular affinity for the genital tract and special virulence for neonates, but other data do not support Wallace's opinion.

Most reports on the resistance of *Haemophilus* to antimicrobials deal with systemic or oropharyngeal infections, and few concern genitourinary isolates. In this study 18 (30%) strains were found to be resistant to one or several antibiotics. Resistance to tetracycline alone occurred in eight strains and associated with ampicillin resistance in nine strains. The two kanamycin resistant strains were also resistant to ampicillin and tetracycline. These numbers were higher than those reported in a nationwide French study in 1982 that excluded genitourinary isolates, and also than those reported by Albritton *et al* and Wallace *et al* in genital and neonatal *H influenzae* strains.

The production of TEM1 like β-lactamase was responsible for ampicillin resistance in the strains studied. We did not detect strains producing ROB-1 β-lactamase, the new type of plasmid determined β-lactamase found in two *H influenzae* strains pathogenic to man and similar to the ROB-1 produced by the porcine pathogen *H pleuropneumoniae*. Neither did we identify ampicillin resistant non-β-lactamase producing *H influenzae* strains.

Ofloxacin and ciprofloxacin may provide an effective alternative for the treatment of resistant *H influenzae* in genitourinary infections. They are also effective in vitro against genital pathogens such as *N gonorrhoeae*, *H ducyrei*, and to a lesser extent, *C trachomatis* and *U urealyticum*. Recent clinical trials with these compounds showed an excellent cure rate of gonococcal and non-gonococcal urethritis in men. Further clinical studies with these agents in the treatment of STD seem to be warranted.

Few drugs are effective against both gonococcal and non-gonococcal genital infection, and tetracyclines are the most widely used. The high rate of resistance to tetracycline alone (13%) or associated with resistance to other antibiotics as found in this study, has to be kept in mind because the common use of these antibiotics is likely to maintain a selective pressure on the genital bacteria. Studies have shown the spread of tetracycline resistance in, among other genera isolated from the genital tract, *M hominis*, *U urealyticum*, *Gardnerella vaginalis*, *S agalactiae*, *H ducyrei* (Sanson-Le Pors MJ, *et al*, unpublished observation), and *N gonorrhoeae*.

In conclusion, if we consider the possibility that the genitourinary tract environment favours plasmid transfer between bacterial species as well as genera, and as multiresistant strains of *H influenzae* appear to
be commonly recovered from patients with STD, it is important to monitor the trends in antimicrobial susceptibility of these organisms.

References