Gonorrhoea: auxotypes, serovars, and clinical manifestations among female sex workers from Kinshasa, Zaire

L Mukenge-Tshibaka, M Alary, E Van Dyck, M Laga, N Nzila

The main question in this paper was to look at the distribution of auxotypes and serovars of *Neisseria gonorrhoeae* and check whether they correlate with clinical symptoms/signs among female sex workers (FSW) from Kinshasa, Zaire. The subjects were 1233 FSW enrolled in a cross sectional study on STDs and HIV infection in 1988; 771 of them were followed prospectively for a median duration of 23 months. At each visit, clinical symptoms and signs of cervicitis were recorded and the subjects were screened for gonococcal and chlamydial infection. The predominant auxotypes were prototrophic (35.2%), proline requiring (29.6%), and proline requiring phenylalanine inhibition (19%). Serovars 1A–6 (42.5%) and 1B–1 (16.7%) were the commonest. Infection with auxotype prototrophic and phenylalanine inhibition (Prot/Phen) was significantly associated with both mucopurulent cervicitis and pelvic inflammatory disease; (OR = 8.9; p = 0.002 and OR = 19.9; p = 0.002; respectively). Despite the few associations found in this study, there was no clear pattern linking clinical manifestations to auxotype/serovar profiles.

Keywords: *Neisseria gonorrhoeae*; auxotypes; serovars; symptoms/signs

Introduction
It is well known that the epidemic of *Neisseria gonorrhoeae* infection is influenced by auxotypes and serovars that are structurally and antigenically different.1,2 Geographical, temporal, and ethnic variations of auxotypes and serovars have been documented.3 In industrialised countries, the availability of serotyping has allowed detailed epidemiological and clinical studies of gonococcal infection over the past 20 years. However, only a few studies have been conducted in developing countries of tropical Africa on gonococcal resistance according to the serological classification of isolates.4 To our knowledge, no survey has been published from this part of the world on the expression of gonococcal disease according to the phenotype of isolates. This study was undertaken to look at the distribution of auxotypes and serovars of *N gonorrhoeae* and to check whether they correlate with clinical symptoms/signs among female sex workers from a developing country, Zaire.

Methods
In 1988, 1233 female sex workers were enrolled in a cross sectional study, screened for STDS and HIV antibodies in Matonge, Kinshasa by the Projet/SIDA Zaïre. This population has been described extensively elsewhere.6 Briefly, 771 of these women were followed prospectively, once every month for a median duration of 23 months (range 3–36 months). Demographic characteristics, medical and sexual history were recorded by a nurse in a face to face interview. The nurse also recorded information about urogenital symptoms (vaginal discharge, lower abdominal pain, and vulvar itching). A physician performed a gynaecological examination to record clinical signs (vaginal discharge, cervical motion tenderness, cervix erosion, and mucopurulent cervicitis).

Samples from gynaecological examination were tested for *N gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and yeasts. Gonococci were isolated on modified Thayer-Martin medium incubated in a candle extinction jar at 35°C for 24–48 hours. Isolates were identified on the basis of typical colony morphology, oxidase reaction, and sugar utilisation patterns. Strains were tested for their requirements for proline, arginine, hypoxanthine, uracil, methionine, amino acids, vitamins, and for inhibition by phenylalanine according to the auxotyping method described by Hendy and Stewart.2 For serotyping, strains were tested by coagglutination with monoclonal antibodies. The Knapp’s serovar nomenclature was used.1 Data analysis concerned 1094 infective episodes with *N gonorrhoeae*; 286 of them were diagnosed at enrolment whereas 808 were incident episodes (defined as a patient presenting a positive test at a follow up visit with a negative test at the previous visit or with a suitable treatment in the event of a positive test at the previous visit). Statistical analysis to compare the prevalence of recorded symptoms/signs measured according to different auxotypes, serovars, and auxotype/serovar classes was performed using χ². When the proportions were significantly different, we calculated the
<table>
<thead>
<tr>
<th>Auxotype (n = 622)</th>
<th>No of strains (%)</th>
<th>Serovar (n = 659)</th>
<th>No of strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proto</td>
<td>219 (35 2)</td>
<td>Serogroup IA</td>
<td>348 (52 8)</td>
</tr>
<tr>
<td>Proto/Phenalin</td>
<td>11 (1 6)</td>
<td>IA0</td>
<td>2 (0 3)</td>
</tr>
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<td>Proto/Phenalin</td>
<td>184 (29 6)</td>
<td>IA1</td>
<td>12 (1 8)</td>
</tr>
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<td>118 (19 0)</td>
<td>IA2</td>
<td>12 (1 8)</td>
</tr>
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<td>76 (12 2)</td>
<td>IA3</td>
<td>2 (0 3)</td>
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<td>Proto/Phenalin</td>
<td>1 (0 2)</td>
<td>IA4</td>
<td>21 (3 2)</td>
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<td>1 (0 2)</td>
<td>IA5</td>
<td>8 (1 2)</td>
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<td>2 (0 3)</td>
<td>IA6</td>
<td>280 (45 2)</td>
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<td>5 (0 5)</td>
<td>IA8</td>
<td>9 (1 40)</td>
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<td>IA17</td>
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<td>Serogroup IB</td>
<td>273 (41 4)</td>
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<td>1 (0 2)</td>
<td>IB0</td>
<td>7 (1 0)</td>
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<tr>
<td>Proto/Phenalin</td>
<td>1 (0 2)</td>
<td>IB1</td>
<td>110 (16 7)</td>
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<td>Proto/Phenalin</td>
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<td>IB2</td>
<td>5 (0 8)</td>
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<td>1 (0 2)</td>
<td>IB6</td>
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<td>1 (0 2)</td>
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<td>IB24</td>
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</tr>
<tr>
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</table>

Proto = prototrophic; Phenalin = phenylalanine inhibition; Pro = proline requiring; AA = amino acids requiring; Arg = arginine requiring; Hypx = hypoxanthine requiring; Ura = uracil requiring; Vit = vitamins requiring; Met = methionine requiring.

Results

Clinical symptoms/signs of cervicitis were recorded respectively in 542 (87 1%) of the 622 episodes with gonococcal auxotype data, and in 577 (87 6%) of the 659 episodes with gonococcal serovar data.

As shown in table 1, the predominant auxotypes identified were prototrophic (Proto), proline requiring (Pro), proline requiring/phenylalanine inhibition (Pro/Phenalin) and proline/arginine requiring (Pro/Arg). These four commonest strains accounted for 96% of infections. The three most frequent serovars which encompassed 69 4% of infections were IA-6, 1B-1, and 1B-7. Of the 659 strains serotyped, 348 (52 8%) were serogroup IA, 273 (41 4%) belonged to serogroup IB, and 38 (5 8%) strains were ungroupable.

Combining the two classification systems, a total of 63 distinct auxotype/serovar classes were identified. The prevalence of the majority of these classes remained stable over the study period with the exception of Proto/1B-7 and Pro/1A-6 classes that had changed dramatically in the second 6 months. The former was increased by a factor of 5 (from 4-6% to 23-6%; p < 0 01) and the second decreased very significantly (from 26 1% to 10 6%; p < 0 01).

Table 2 summarises the prevalence of recorded clinical symptoms/signs according to different auxotypes. Mucopurulent cervicitis (MPC) was more common in women infected with auxotype Proto/Phenalin than in women with all other strains; (5/10 (50%) women infected with auxotype Proto/Phenalin had MPC v 54/532 (10 2%) women infected with all other auxotypes (OR = 8 9; p = 0 002)). When we restricted analysis to women without chlamydial infection, MPC and PID remained significantly associated with auxotype Proto/Phenalin. None of the symptoms/signs was significantly associated with any particular serovar. However, serovar 1B-7 was significantly associated with the absence of signs (44/64 (68 8%) women infected with serovar 1B-7 did not present any sign v 229/513 (44 6%) for all other serovars (OR = 2 2; p = 0 003)).

Discussion

Our data show that the most frequent auxotypes were prototrophic and proline requiring. We noticed that the predominance of these two auxotypes was also reported elsewhere in Africa (Gabon) and in several studies performed in America and Europe.5,8 However, auxotypes Arg Hyp x Ura x and Pro Cit x Hyp x which have been isolated in Europe and America were not isolated in our study population. We also found auxotype Pro/Phenalin very frequent (19%). This auxotype was not isolated in other studies of which or...
we are aware since strains were not tested for
the inhibitory activity of phenylalanine.

In industrialised countries, the serovars of
serogroup B seem to predominate whereas in
our study, as in Gabon, more than half of the
strains were serogroup A. Indeed, in Spain,
serovars of serogroup B accounted for 93.5%
all strains. This serogroup also predominated
in Canada (66.8%) and in Sweden (70%).

We observed dramatic changes in the gonococcal
population infecting the cohort over the
study period with the introduction of new
strains and loss of other strains. This has been
previously reported. The significant loss of
certain strains supports the notion of gonococcal
strain specific acquired (partial) immunity,
as proposed by Plummer and colleagues who
reported that women experiencing an infection
with a specific gonococcal serovar were at 2- to
10-fold reduced risk of reinfection with the
same strain.

Despite the fact we made multiple compar-
isons, we found that only a few auxotypes/
serovars of \textit{N. gonorrhoeae} were significantly
associated with clinical manifestations of
gonococcal infection and the strength of some
associations may suggest that they did not
occur by chance only. Indeed, we found that
auxotype Proto/Phenal was strongly associ-
ated with both MPC and PID. However, this
apparently increased virulence of auxotype
Proto/Phenal should be interpreted with cau-
tion because of the small number of women
infected with this strain (only 11 of the 622
auxotypes) and the impossibility of comparing
our results with those reported by other
authors since this auxotype was not isolated in
their studies.

In summary, we did not identify many asso-
ciations between auxotypes/serovars of \textit{N. gon-
rhoeae} and symptoms/signs. To compare the
associations we observed and to obtain a bet-
ter understanding of the epidemiology of
gonococcal infection in developing countries,
there is a need for additional studies using
similar methods distinguishing between strains
of \textit{N. gonorrhoeae} (including testing for phenyl-
alanine inhibition) in areas where the same
isolates can be found.

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