Phenotypic and genetic characterisation of bacterial sexually transmitted infections in Bissau, Guinea-Bissau, West Africa: a prospective cohort study

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ABSTRACT
Background: Knowledge regarding characteristics and transmission of Neisseria gonorrhoeae, Chlamydia trachomatis and Mycoplasma genitalium and antibiotic resistance in N gonorrhoeae in Guinea-Bissau, West Africa, is entirely lacking.

Objectives: To characterise N gonorrhoeae, C trachomatis and M genitalium samples from Guinea-Bissau and to define bacterial populations, possible transmission chains and for N gonorrhoeae spread of antibiotic-resistant isolates.

Design: Prospective cohort study.

Setting: Two sexual health and family planning clinics, Bissau, Guinea-Bissau.

Participants: Positive samples from 711 women and 27 men.

Material and methods: Positive samples for N gonorrhoeae (n=31), C trachomatis (n=60) and M genitalium (n=30) were examined. The gonococcal isolates were characterised with antibiograms, serovar determination and N gonorrhoeae multiantigen sequence typing (NG-MAST). The C trachomatis ompA gene and the M genitalium mgpB gene were sequenced, and phylogenetic analyses were performed.

Results: For N gonorrhoeae, the levels of resistance (intermediate susceptibility) to ciprofloxacin, erythromycin, rifampicin, ampicillin, tetracycline, penicillin G and cefuroxime were 10% (0%), 6% (10%), 13% (10%), 68% (0%), 74% (0%), 68% (16%) and 0% (84%), respectively. All isolates were susceptible to cefixime, ceftriaxone, spectinomycin and azithromycin, and the minimum inhibitory concentrations of kanamycin (range: 8–32 mg/l) and gentamicin (range: 0.75–6 mg/l) were low (no resistance breakpoints exist for these antimicrobials). 19 NG-MAST sequence types (STs) (84% novel STs) were identified. Phylogenetic analysis of the C trachomatis ompA gene revealed genovar G as most prevalent (37%), followed by genovar D (19%). 23 mgpB STs were found among the M genitalium isolates, and 67% of isolates had unique STs.

Conclusions: The diversity among the sexually transmitted infection (STI) pathogens may be associated with suboptimal diagnostics, contact tracing, case reporting and epidemiological surveillance. In Guinea-Bissau, additional STI studies are vital to estimate the STI burden and form the basis for a national sexual health strategy for prevention, diagnosis and surveillance.

INTRODUCTION
Guinea-Bissau, West Africa, is classified as the sixth least developed country in the world.1

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The total number of inhabitants is about 1.6 million with 250,000–300,000 living in the capital city Bissau. The healthcare system is exceedingly limited and highly centralised to Bissau. Guinea-Bissau has previously presented the highest HIV-2 prevalence globally. However, after the civil war in 1998–1999, the HIV-2 prevalence has decreased and in contrast the HIV-1 prevalence has increased.2 HIV seropositive individuals have shown a higher prevalence of bacterial sexually transmitted infections (STIs), and presence of several bacterial STIs has been associated with a facilitated HIV acquisition and transmission.2 3 Present knowledge regarding the prevalence of bacterial STIs in Guinea-Bissau is highly limited. Studies from 2001 to 2002 described a prevalence of Neisseria gonorrhoeae and Chlamydia trachomatis infections of 17% and 4%, respectively, among women with vaginal discharge, and in pregnant women, the prevalence rate for Mycoplasma genitalium was 6.2%.4 5 In a recent study of women attending two sexual health clinics in Bissau, due to urogenital problems, the prevalence of N gonorrhoeae, C trachomatis and M genitalium infections was 1.3%, 12.6% and 7.7%, respectively.2

In Guinea-Bissau, in routine, the laboratory diagnosis of N gonorrhoeae is mainly based on microscopy of Gram stained urogenital smears, no appropriate diagnostics is performed for C trachomatis and M genitalium and no previous study has phenotypically and genetically typed strains of these bacterial STIs. Consequently, knowledge regarding the characteristics and transmission of circulating strains of N gonorrhoeae (including antibiotic resistance), C trachomatis and M genitalium is entirely lacking. The aims of the present study were to phenotypically and genetically characterise N gonorrhoeae and genetically characterise C trachomatis and M genitalium samples from women attending two sexual health clinics in Bissau, Guinea-Bissau and to define the bacterial populations, possible transmission chains and for N gonorrhoeae to define the presence and spread of antibiotic-resistant isolates from women and additionally a group of symptomatic men.

**MATERIALS AND METHODS**

**Study population**

The current study further examined specimens obtained in a previous prevalence study of 711 women attending two sexual health and family planning clinics (Aguibef Clinic and Centro Materno-Infantil Clinic) for urogenital problems in Bissau, Guinea-Bissau. All women visiting the clinics from February 2006 to January 2008 were offered participation. Sixty samples analysed positive for C trachomatis, 30 analysed positive for M genitalium and four culture-positive N gonorrhoeae isolates were available for further examination. In addition, 27 N gonorrhoeae isolates cultured, during February 2006 to January 2008, from men (n=27) with urogenital symptoms at the National Public Health Laboratory, Bissau, Guinea-Bissau, were included.

**Laboratory characterisation**

All 31 N gonorrhoeae isolates were initially cultured and preserved at −70°C as previously described.2 6 Briefly, endocervical (women) or urethral (men) samples were cultured within 5 h on modified Thayer–Martin medium for 48–72 h. Species confirmation was based on identification of rapid oxidase production, Gram-negative diplococci in microscopy and use of PhadeBact Monoclonal GC Test (Bactus, Stockholm, Sweden).5 Antibiotic susceptibility testing, serovar determination, DNA isolation and genetic characterisation by means of N gonorrhoeae multiantigen sequence typing (NG-MAST) were performed as previously described.7

C trachomatis and M genitalium specimens were initially diagnosed with PCR as previously described.2 Briefly, DNA was isolated from endocervical samples using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Doraville, Georgia, USA), and conventional PCRs, using previously described primers, were performed for diagnosis of M genitalium (target: 16S rRNA gene and, for confirmation, MgPa adhesion (mgpB) gene) and C trachomatis (target: ompA gene).2

For typing of C trachomatis, the ompA gene was PCR amplified, the PCR product was purified and subsequently sequenced and finally about 1100 nucleotides of the ompA sequence was used in a phylogenetic analysis for determination of the genovar, as previously described.8

For typing of M genitalium, the mgpB gene was PCR amplified and sequenced as previously described,9 with some modifications. These modifications included use of 0.4 μM of each of the primers MgPa-1 and MgPa-3 (Scandinavian Gene Synthesis AB, Köping, Sweden) and 2.0 U of AmpliTaq Gold DNA polymerase (PE Biosystems, Branchburg, New Jersey, USA). The PCR programme consisted of an AmpliTaq Gold DNA polymerase activation step at 94°C for 10 min, followed by 40 sequential cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min and finally an extension step of 72°C for 5 min. All PCR products were stored at 4°C prior to purification. The PCR products were purified using the High Pure PCR Product Purification Kit (Boehringer Mannheim, Indianapolis, Indiana, USA) according to the manufacturer’s instructions. The PCR products were sequenced with the same primers as used in the PCR, utilising the ABI PRISM BigDye Terminator Cycle Sequencing Ready...
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RESULTS

Characterisation of *N gonorrhoeae*

The results of the antibiotic susceptibility testing of all the *N gonorrhoeae* isolates (n=31) are presented in table 1.

Briefly, the levels of resistance (intermediate susceptibility) to ciprofloxacin, erythromycin, rifampicin, ampicillin, tetracycline, penicillin G and cefuroxime were 10% (0%), 6% (10%), 13% (10%), 68% (0%), 74% (0%), 68% (16%) and 0% (84%), respectively. Twenty-one (68%) of the 31 isolates were β-lactamase producing. All isolates were susceptible to cefixime, ceftriaxone, spectinomycin and azithromycin, and the minimum inhibitory concentrations (MICs) of kanamycin (8–32 mg/l) and gentamicin (0.75–6 mg/l) were low (no resistance breakpoints exist for these antimicrobials) (table 1).

All the *N gonorrhoeae* isolates were serotypeable, 20 (64.5%) were determined as serogroup WI/III (PorB1b). These isolates were assigned four different serovars: Bropt (n=16), Bropst (n=2), Bpyvust (n=1) and Bpyvust (n=1). The remaining 11 (35.5%) isolates were determined as serogroup WI (PorB1a). These isolates were assigned two different Ph serovars: Arst (n=10) and Arost (n=1).

The isolates were assigned to 19 different NG-MAST sequence types (STs), of which 16 (84%) have not been previously described (table 2). The ST3176 (n=7), ST783 (n=5) and ST3182 (n=3) were the most prevalent STs. Of these, ST783 has been described in UK, while ST3176 and ST3182 were not earlier described. The remaining 16 isolates were all of different sequence types. Of these, ST3118 has been described in isolates from Arkhangelsk, Russia, Scotland and Canada, and ST2178 in Australia (NG-MAST website: http://www.ng-mast.net).

Notable, the three ciprofloxacin-resistant isolates (all displaying MIC≥1.5 mg/l) were assigned three different STs (ST3181, ST3184 and ST3186) and two different serovars (Bropst, n=2, and Bpyvust, n=1).

Characterisation of *C trachomatis*

Phylogenetic analysis of the *ompA* gene sequences from 60 *C trachomatis* samples revealed that the most frequent genovar was G (22 isolates, 37%), followed by F (17%), I (14%), E (5%), J (5%), H (1.5%) and K (1.5%) (table 2). Accordingly, genovar E that is the most prevalent genovar followed by genovar F and genovar D in studies from other settings globally was relatively rare.10–19

Characterisation of *M genitalium*

A total of 23 *M genitalium* STs were found among the 30 *M genitalium* samples. Only three clusters, one with four isolates (‘ST1’) and two containing three isolates (‘ST2’ and ‘ST3’), were detected (figure 1, table 2). The remaining sequence types (‘ST4’–‘ST23’) were only represented by single isolates.

DISCUSSION

In the present study, bacterial STIs in Guinea-Bissau were for the first time characterised, phenotypically and genetically, demonstrating highly divergent populations of *N gonorrhoeae* and *M genitalium*, and compared with other settings worldwide an unusual genovar distribution for *C trachomatis*. However, due to the limited number of samples examined, all results need to be interpreted with caution.

The genetic characterisation of the 31 *N gonorrhoeae* isolates showed a total of 19 different NG-MAST STs, and only three (16%) of these STs have been previously
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Table 2 Distribution of different genotypes of Chlamydia trachomatis, Mycoplasma genitalium and Neisseria gonorrhoeae among infected women in Guinea-Bissau

| Chlamydia trachomatis (genovar, n=60)* | Mycoplasma genitalium (sequential numbers, n=30)† | Neisseria gonorrhoeae (NG-MAST, n=31) |
|---------------------------------------|-----------------------------------------------|--------------------------------------|
| G (37%)                               | 1 (13%)                                       | ST3176 (23%)‡                         |
| D (19%)                               | 2 (10%)                                       | ST783 (16%)‡                         |
| F (17%)                               | 3 (10%)                                       | ST3182 (10%)‡                         |
| I (14%)                               | 4–23 (single isolates 67%)                    | ST2187 (single isolate, 3%)          |
| E (5%)                                |                                               | ST1318 (single isolate, 3%)          |
| J (5%)                                |                                               | ST3177–81 (single isolates, 13%)‡     |
| H (1.5%)                              |                                               | ST3183–89 (single isolates, 26%)‡     |
| K (1.5%)                              |                                               | ST3376–77 (single isolates, 6%)‡      |

*Based on ompA genotyping.
†Based on sequencing of the M genitalium MgPa adhesion (mgpB) gene.
‡Novel NG-MAST STs.

reported, that is, ST783, ST1318 and ST2187 (NG-MAST website: http://www.ng-mast.net). Accordingly, ST783 has been described in UK; ST1318 in Russia, Scotland and Canada and ST2187 in Australia (NG-MAST website: http://www.ng-mast.net). The ST3176 (n=7), ST783 (n=5) and ST3182 (n=3), were the most prevalent STs, while the remaining 16 STs were represented by single isolates. The high number of STs represented by single isolates could be a consequence of suboptimal diagnostics, limited and biased sampling, inefficient or non-existing contact tracing, local and recent emergence of new STs and import of strains.

The serological characterisation divided the isolates into 20 (64.5%) serogroup WII/III (PorB1b) isolates and 11 (35.5%) serogroup WI (PorB1a) isolates. The proportion of serogroup WI (PorB1a) isolates was relatively high compared with, for example, Sweden with 26% WI (PorB1a) isolates reported in 2009, Italy with 4.1% WI (PorB1a) isolates in 2008 and Russia with 26% WI (PorB1a) isolates in 2007 but lower than described from India with 46.7% WI (PorB1a) isolates in 2007. The isolates from Guinea-Bissau were further divided into six different Ph serovars.

As clearly shown, the genetic characterisation (19 NG-MAST STs) had a substantially higher discriminatory power than the serovar determination (six serovars). Furthermore, no NG-MAST STs were further subdivided using serovars determination. Accordingly, genetic typing should preferably be used for epidemiological purposes, especially in geographical areas that comprise highly heterogeneous N gonorrhoeae populations.

The antibiotic susceptibility testing displayed relatively high levels of resistance and/or intermediate susceptibility to most antibiotics previously recommended as first line for treatment of gonorrhoea, for example, penicillins, tetracycline, erythromycin and cefuroxime. However, due to the low number of gonococcal isolates (n=31) examined in the present study, the resistance levels need to be interpreted with caution. The resistance level to ciprofloxacin (10%) was relatively low. Nevertheless, at the time of collection of the gonococcal isolates (2006–2008), similar low levels of resistance to ciprofloxacin were shown also in other studies from African countries, for example, from Malawi Mozambique, Central African Republic and Madagascar. These ciprofloxacin resistance levels were substantially lower than the levels described from many other countries worldwide, where it is now a fear that gonorrhoea may become untreatable in certain circumstances. However, more recent studies from African countries such as Kenya and South Africa have shown a rapidly increasing resistance to ciprofloxacin. The slower pace of emerging ciprofloxacin resistance in Guinea-Bissau and several other African countries strains may reflect the fact that fluoroquinolones for treatment of gonorrhoea were not widely used as early in Guinea-Bissau (as well as several other African countries), as in other settings worldwide. Fortunately, all isolates were susceptible to cefixime, ceftriaxone, azithromycin and spectinomycin and had low MICs of gentamicin and kanamycin. Nevertheless, due to the high and increasing resistance levels to many of these antibiotics worldwide, it is essential to enhance the gonococcal antibiotic susceptibility surveillance in African countries as well as globally. In some settings, the resistance levels to previously recommended first-line antibiotics may have remained low and these antibiotics might be valuable to continue to use for treatment of gonorrhoea in these specific settings. Nevertheless, that type of recommendation needs to be supported by sufficient local, appropriate and quality-assured antibiotic susceptibility data.

The phylogenetic analysis of ompA gene sequences in the 60 C trachomatis samples revealed that genovar G (37%) was the most frequent genovar, followed by genovar D (19%) and genovar F (17%). Studies from other settings globally, from 2000 to 2011, have shown that genovar E is the most prevalent followed by genovar F and genovar D. Furthermore, a longitudinal study over a 9-year period (1988–1996) in Seattle showed similar serovar distribution as reported from other parts of the world and also a stability in the serovar groups over time, except for the more rare serovars I and K. Genovar G, which was the most frequent genovar...
among women in Guinea-Bissau, has in other settings worldwide been the most frequently detected genovar in infections in men who have sex with men. This unusual C. trachomatis genovar distribution among women in Guinea-Bissau may reflect the limited sample size, although it can also indicate a local domestic transmission with little importation of strains from abroad.

The ompA sequences were also highly conserved within the genovars. Accordingly, insufficient epidemiological resolution is obtained by ompA sequencing, and novel molecular methods for typing of C. trachomatis, such as multiple loci variable number of tandem repeats (VNTR) analysis (MLVA) and multilocus sequence typing (MLST), which both show a substantially higher discriminatory power, are vital.

Among the 30 M. genitalium samples, 67% of the mgpB STs were only represented by single isolates. Only three small clusters, two containing three isolates and one containing four isolates, were identified. This high genetic diversity may suggest that M. genitalium is endemic in Bissau and that infection is not due to the distribution of a single strain.

Bacterial STIs are public health problems causing substantial morbidity and economical cost. Furthermore, if undetected and untreated, many bacterial STIs are associated with severe complications and sequelae such as PID, ectopic pregnancy, infertility and facilitated

Figure 1. Phylogenetic tree based on a 238 nucleotide sequence of the Mycoplasma genitalium MgPa adhesin gene (mgpB) in 30 samples from Bissau, Guinea-Bissau. The same sequence from the M. genitalium reference strain G377 (DQ248092) was used for rooting the tree.
acquisition and transmission of HIV. Ineffective or inadequate detection and/or treatment of these pathogens inevitably contribute also to further transmission of the STIs. Despite that the present study did not show any alarming tendency of \textit{N. gonorrhoeae} strains resistant to extended-spectrum cephalosporins, macrolides and spectinomycin, in Bissau, Guinea-Bissau, the rapidly increasing resistance to also these antibiotics worldwide makes it important to introduce quality-assured gonococcal antibiotic resistance surveillance also in Guinea-Bissau and in general in West Africa. Furthermore, due to the high prevalence of HIV in many of the West African countries and the fact that several of these bacterial STIs also facilitate the acquisition and transmission of HIV, more prevalence studies of bacterial STIs as well as national and regional strategies for prevention and control of bacterial STIs in this region are crucial to implement.

In conclusion, the high diversity of \textit{N. gonorrhoeae}, \textit{C. trachomatis} and \textit{M. genitalium} strains in Bissau, Guinea-Bissau, may be associated with suboptimal diagnostics, contact tracing, case reporting and epidemiological surveillance. Overall, it is exceedingly difficult to estimate the true burden, distribution and characteristics of STIs in Guinea-Bissau, as well as in many other countries in West Africa. Accordingly, additional studies in other population groups, and in other geographical areas, are crucial to perform in Guinea-Bissau and other countries in the region. The present study compiled with such future studies may form the basis for a national sexual health strategy for prevention, diagnosis and surveillance of STIs in Guinea-Bissau.

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