Prevalence of Genital Mycoplasmas in the Vaginal Tracts of Adolescents in Nnewi, South-Eastern, Nigeria

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Abstract: This study aims to screen adolescent girls in Nnewi, South-Eastern Nigeria for the presence of potential pathogens like genital mycoplasmas and also to know the associated risk factors predisposing them to these organisms. A cross-sectional study involving the use of high vaginal swabs (HVS) from 100 adolescent girls aged between 13 and 18 years was done. Molecular method was used to analyse the swabs using the polymerase chain reaction technique. Questionnaires were also used to obtain the bio data and evaluate the risk factors to these vaginal organisms. A prevalence rate was was 20% out of which 4% represented concomitant colonization by 2 or more different species. A breakdown of the organisms showed that the Ureaplasma species- Ureaplasma urealyticum and Ureaplasma parvum had 6% and 4% colonization rates respectively while the Mycoplasma species- Mycoplasma hominis and Mycoplasma genitalium had 4% and 6% rates respectively. Poor personal hygiene and sharing of personal effects were found to be risk factors in predisposing subjects to M. hominis acquisition. The high prevalence of these organisms among asymptomatic adolescents suggests strongly that they are not always associated with symptoms thus supporting the need for screening among this population.

Key words: Genital mycoplasmas • Adolescent girls • Risk factors

INTRODUCTION

Human genital mycoplasmas are species of Mycoplasmas and Ureaplasmas isolated from the urogenital tracts of humans. Most often they exist asymptomatically in the genital tracts, however; they have been associated with bacterial vaginosis along with other pathogens like Gardnerella vaginalis, Mobiluncus species and vaginal anaerobes [1]. They have also been implicated in pelvic inflammatory disease (PID), infertility and various other genital pathologies [2].

The role of genital mycoplasma as a disease-causing agent has been reported in many parts of the world [3, 4]. Although very limited studies have been done in Nigeria [5, 6], none has been carried out on adolescents. Despite the reports on these organisms, they are still not being investigated routinely mainly due to their fastidious nature and the technically challenging culture methods needed to link the organism to clinical conditions [2]. The availability of molecular methods has substantially altered the ability to derive information about the pathogenic potentials of these groups of bacteria in affecting the reproductive health of adult females [7].

Adolescents are physiologically more vulnerable to infection than older women because changes in the reproductive tract during puberty make their vagina and...
cervix less resistant to infection [8]. It is known that it is
the many activities of genital mycoplasmas and their
prolonged asymptomatic presence in the reproductive
tract that causes infertility [9]. Since it has been shown
that interventions are not usually successful once
complications have set in, it becomes important to
generate and provide research information for policy
makers, health-care providers, parents and the general
populace, so that appropriate attention will be given to
the youth at an early age.

The adolescent years are highly influential in shaping
their adulthood, particularly with regards to hygiene and
other lifestyle habits. Intervention aimed at this
population may help reduce the risk posed by potential
genital tract pathogens. Although screening of youths for
harmful genital pathogens have been recommended by
the world health organization [10], nothing of such is
being carried out on genital mycoplasmas, probably
because little information is available here in Nigeria
among young adolescent female population. The sexual
and reproductive health indices of the adolescents are
very poor as depicted in the 2008 Nigerian demographic
and Health survey [11]. This research is therefore aimed
at bridging this gap.

In Nigeria, as well as in other developing countries,
little is known about the prevalence of genital
mycoplasmas among adolescent females, therefore the
study the study aimed to spot on the prevalence among adolescent high school female
students in Nnewi, South-Eastern, Nigeria.

**MATERIALS AND METHODS**

**Subjects:** The study population comprise 100 female high
school students aged between 13 to 18 years and
attending school in Nnewi, South-eastern, Nigeria.
Written informed consent was obtained from their
parents/guardians while oral inform consent was obtained
from the girls at collection point.

**Samples:** One high vaginal swab was taken from each of
the students by a clinician using sterile disposable cotton
swab stick. The swabs were inserted into 0.5ml of
phosphate buffered saline and stored at 4°C prior to PCR
assay. Immediately after sample collection, each subject
was given a questionnaire to fill. This contains the bio
data and various risks factors.

**Exclusion Criteria:** Menstruating girls and those that
have taken antibiotics or antifungal a month before
specimen collection were excluded.

**DNA Extraction:** The DNA was extracted from the
samples in a stepwise procedure using the Trizol reagents
as recommended by the manufacturer (Invitrogen, UK).
This procedure includes the phase separation stage, the
DNA precipitation, DNA wash and re-dissolving DNA.

**Oligonucleotide Primers:** Four (4) primer pairs based on
previously published sequences [12-14] were used. They
include;

- UMA-51-F and UMA-427 R for *Ureaplasma parvum*
  (Biovar 1),
- UMS 125-F and UMA-226R for *U. urealyticum*
  (Biovar 2),
- My-insF and Mgen-P3-AMR for *Mycoplasma*
  genitalium and
- My-ins-F and Mhom-P-10-AM-R for *Mycoplasma*
  hominis;

**Preparation of Master Mix:** The master mix used was 2X
Qiagen multiplex PCR master mix. This PCR kit provides
the multiplex PCR master mix containing hot-start Taq
DNA polymerase and a unique PCR-buffer containing
factor MP. Together with optimized salt concentrations,
the factor MP stabilizes specifically bound primers and
enables efficient extension of all primers in the reaction
without optimization.

**PCR Mix:** Each sample is tested against all four
mixes and the amplification reaction mixture for one
sample includes 7µl of primer mix, 10µl of PCR master
mix and 3µl of genomic DNA to give a final volume of
20µl.

**PCR:** The 20µl PCR mix of each subject were put in 1.5ml
micro centrifuge tubes and transferred to a thermal cycler
(2720 Applied Biosystem). The thermal profiles include
initial denaturation step at 95°C for 30seconds, annealing
at 62°C for 45 seconds and extension at 72°C for 45
seconds. This was done for 35 cycles and followed by a
final extension at 72°C for 7 minutes. This is the PCR
product and was kept at a holding temperature of 20°C
until ready to use. The PCR conditions were as previously
described [12, 7].

Two (2µl) of the PCR products of each of
the samples were analysed by electrophoresis which
was ran at 100V for 30minutes. Expected bands
for positivity were 423bp for *U. urealyticum*, 427bp
for *U. parvum;* 520bp for *M. genitalium* and 326bp for
*M. hominis.*
Statistical Analysis: Number/percentages were used to describe the data obtained and calculations between variables were explored with the chi square. P < 0.05 was considered significant.

RESULTS

An overall colonization rate of 20% was obtained from the study population out of which 4% represents concomitant colonization among the organisms while individual detection rates were as follows; *Mycoplasma genitalium* 6 (6%), *Mycoplasma hominis* 3 (3%), *Ureaplasma urealyticum* 2 (2%) and *Ureaplasma parvum* 1 (1%). *Mycoplasma genitalium* was the only species not coexisting with any of the three (3) other species. *Ureaplasma urealyticum* co-existed with both *Ureaplasma parvum* and *Mycoplasma hominis* (Table 1).

### Table 1: Concomitant colonization of Genital mycoplasmas among the positive samples

<table>
<thead>
<tr>
<th>Genital Mycoplasma species</th>
<th>U1 (No/%)</th>
<th>U2 (No/%)</th>
<th>Mh (No/%)</th>
<th>Mg (No/%)</th>
<th>Total (No/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ureaplasma urealyticum</em></td>
<td>2 (2.0%)</td>
<td>3 (3.0%)</td>
<td>1 (1.0%)</td>
<td>0 (0.0%)</td>
<td>6 (6.0%)</td>
</tr>
<tr>
<td><em>Ureaplasma parvum</em></td>
<td>3 (3.0%)</td>
<td>1 (1.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>4 (4.0%)</td>
</tr>
<tr>
<td><em>Mycoplasma hominis</em></td>
<td>1 (1.0%)</td>
<td>0 (0.0%)</td>
<td>3 (3.0%)</td>
<td>0 (0.0%)</td>
<td>4 (4.0%)</td>
</tr>
<tr>
<td><em>Mycoplasma genitalium</em></td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>6 (6.0%)</td>
<td>6 (6.0%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>6 (6.0%)</td>
<td>4 (4.0%)</td>
<td>4 (4.0%)</td>
<td>6 (6.0%)</td>
<td>20 (20.0%)</td>
</tr>
</tbody>
</table>

### Table 2: Distribution of Genital mycoplasmas among the subjects according to age

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>U. urealyticum</th>
<th>U. parvum</th>
<th>M. hominis</th>
<th>M. genitalium</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-15</td>
<td>2 (5.3%)</td>
<td>1 (2.6%)</td>
<td>2 (5.3%)</td>
<td>5 (13.2%)</td>
</tr>
<tr>
<td>16-18</td>
<td>4 (6.5%)</td>
<td>3 (4.8%)</td>
<td>2 (3.2%)</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>6 (6.0%)</td>
<td>4 (4.0%)</td>
<td>4 (4.0%)</td>
<td>6 (6.0%)</td>
</tr>
<tr>
<td>X²</td>
<td>0.059</td>
<td>0.299</td>
<td>0.255</td>
<td>5.568</td>
</tr>
<tr>
<td>p-value</td>
<td>0.808</td>
<td>0.585</td>
<td>0.614</td>
<td>0.018</td>
</tr>
</tbody>
</table>

* = significant result

### Table 3: Effect of sexual habits on isolation of genital mycoplasmas

<table>
<thead>
<tr>
<th>Genital mycoplasmas</th>
<th>Yes</th>
<th>No</th>
<th>X²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. urealyticum</em></td>
<td>Pos</td>
<td>1 (7.1%)</td>
<td>5 (5.8%)</td>
<td>0.846</td>
</tr>
<tr>
<td>Neg</td>
<td>13 (92.9%)</td>
<td>81 (94.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14 (14.0%)</td>
<td>86 (86%)</td>
<td>0.038</td>
<td>0.846</td>
</tr>
<tr>
<td><em>U. parvum</em></td>
<td>(U2) Post</td>
<td>1 (7.1%)</td>
<td>3 (3.5%)</td>
<td>0.518</td>
</tr>
<tr>
<td>Neg</td>
<td>13 (92.9%)</td>
<td>83 (96.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14 (14.0%)</td>
<td>86 (86%)</td>
<td>0.419</td>
<td>0.518</td>
</tr>
<tr>
<td><em>M. hominis</em></td>
<td>Pos</td>
<td>1 (7.1%)</td>
<td>3 (3.5%)</td>
<td>0.518</td>
</tr>
<tr>
<td>Neg</td>
<td>13 (92.9%)</td>
<td>83 (96.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14 (14.0%)</td>
<td>86 (86%)</td>
<td>0.419</td>
<td>0.518</td>
</tr>
<tr>
<td><em>M. genitalium</em></td>
<td>(Mg) Pos</td>
<td>1 (7.1%)</td>
<td>5 (5.8%)</td>
<td>0.846</td>
</tr>
<tr>
<td>Neg</td>
<td>13 (92.9%)</td>
<td>81 (94.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14 (14.0%)</td>
<td>86 (86%)</td>
<td>0.038</td>
<td>0.846</td>
</tr>
</tbody>
</table>
Table 4: Effect of other risk factors on colonization with genital mycoplasmas

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Status</th>
<th>U. urealyticum (No./%)</th>
<th>U. parvum (No./%)</th>
<th>M. hominis (No./%)</th>
<th>M. genitalium (No./%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal hygiene</td>
<td>Poor</td>
<td>1 (4.3%)</td>
<td>1 (4.3%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>Pass</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (100%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>3 (10.3%)</td>
<td>2 (6.9%)</td>
<td>1 (3.4%)</td>
<td>1 (3.4%)</td>
</tr>
<tr>
<td></td>
<td>Excellent</td>
<td>2 (4.4%)</td>
<td>1 (2.1%)</td>
<td>2 (4.3%)</td>
<td>5 (10.6%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6 (6.0%)</td>
<td>4 (4.0%)</td>
<td>4 (4.0%)</td>
<td>6 (6.0%)</td>
</tr>
<tr>
<td>X2</td>
<td>1.399</td>
<td>1.112</td>
<td>24.989</td>
<td>3.660</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.706</td>
<td>0.774</td>
<td>0.000</td>
<td>0.301</td>
<td></td>
</tr>
<tr>
<td>Sharing of personal effects</td>
<td>Yes</td>
<td>3 (25.0%)</td>
<td>2 (16.7%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3 (3.4%)</td>
<td>2 (2.3%)</td>
<td>4 (4.5%)</td>
<td>6 (6.8%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6 (6.0%)</td>
<td>4 (4.0%)</td>
<td>4 (4.0%)</td>
<td>6 (6.8%)</td>
</tr>
<tr>
<td>X2</td>
<td>8.728</td>
<td>5.698</td>
<td>0.568</td>
<td>0.870</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.003*</td>
<td>0.017*</td>
<td>0.451</td>
<td>0.351</td>
<td></td>
</tr>
<tr>
<td>Toilet type</td>
<td>Water cistern</td>
<td>5 (7.1%)</td>
<td>3 (4.3%)</td>
<td>3 (4.3%)</td>
<td>4 (5.7%)</td>
</tr>
<tr>
<td></td>
<td>Pit</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6 (6.0%)</td>
<td>4 (4.0%)</td>
<td>4 (4.0%)</td>
<td>6 (6.0%)</td>
</tr>
<tr>
<td>X2</td>
<td>0.540</td>
<td>0.050</td>
<td>0.050</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.462</td>
<td>0.824</td>
<td>0.824</td>
<td>0.854</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

An overall prevalence rate of 20% genital mycoplasmas was obtained from the adolescent girls studied. Not much report was obtained on genital mycoplasmas from adolescent girls elsewhere but Manhart et al. [15] who worked on *Mycoplasma genitalium* from young adults reported 0.8% prevalence rate. This is much lower than the 6% prevalence rate observed from this study on *M. genitalium*. However, higher prevalence rates have been reported from adult females; 35.7% from adult females in Ibadan, Nigeria [7], 32.5% from Northern Nigeria Jumbo et al. [16]; 38.0% from women attending sexually transmitted in Paris [3] and 48.0% from Turkey [17]. The lower prevalence rate obtained in this study could be as a result of the population used. They young girls probably were less exposed than the adult females.

Age distribution did not show any difference in this study except for *M. genitalium* which showed a predilection for those between 13-15 years (P<0.05). In agreement with our findings, Elias et al. [4] also found no statistical significant correlation between the age of the patient and incidence of mycoplasma. In this study, the prevalence of genital mycoplasma among the age group 13-15 years was 26.4% while among 16-18 years was 16.1%. Tibaldi et al. [18] also reported a significantly higher risk of Ureaplasma colonization among younger women than older ones. Age therefore, is an independent risk factor.

A breakdown of the result into the two major student groups studied (Day students and Boarders) showed that a higher prevalence rate of genital mycoplasmas among the Day students (18.5%) than the Boarders (11.4%). Though not statistically significant, this result probably implies that the boarders are more aware of their hygienic routine than the Day students.

From this study, genital mycoplasmas appear not to be only sexually transmitted, as only 3.0% out of the 16.0% positivity rate were detected from the sexually experienced adolescents. According to Manhart et al. [15], *M. genitalium* infection was strongly associated with having engaged in vaginal intercourse. This organism has been presumed to be sexually transmitted, a conclusion made on the basis of studies on sexually active individuals [19]. *Ureaplasma urealyticum* has also been
associated with high risk behaviour [18] although in an earlier report the association of *U. urealyticum* with sexual activity are rather contradictory [20]. In contrast with all these studies, this sample population included both sexually experienced and non-experienced adolescents and this allowed this study to show the significant association of absence of sexual activity with genital mycoplasma detection; thereby strengthening the case for other means of transmission of these organisms. Other investigators who worked on bacterial vaginosis in sexually experienced and inexperienced young women share this opinion [21].

Risk factors for genital mycoplasma colonization among the adolescents from this study showed increased risks to be significantly (P<0.05) associated with sharing of personal effects and poor personal hygiene, whereas being a day or boarding student, toilet type, sexual experience had little or no significant effect on the risk for genital mycoplasma among this category of individuals. This could imply that improving the hygiene status of adolescents may reduce the colonization rate. Manhart et al. [15], associated increased risk for *M. genitalium* with ever having lived with a sexual partner, being black and absence of condom.

Detection of genital mycoplasma in the lower genital tract has been associated with inflammation in the upper genital tract in form of histological diagnosed endometritis, clinically diagnosed pelvic inflammatory disease (PID) [22, 23] and laparoscopically diagnosed salpingitis [24]. Although the population studied are asymptomatic, such are important because asymptomatic infections represent the carrier-state which serves as a reservoir for maintaining transmission within a population.

The findings about one-quarter of early preterm infants is already infected with genital mycoplasma at birth coupled with the fact that these new-borns had a higher incidence of neonatal systemic inflammatory response syndrome (SIRS), higher incidence of broncho-pulmonary dysplasia (BPD), higher serum concentration on interleukin (IL)-6 and more evidence of placental inflammation than those with negative cultures [25,26], goes a long way in picturing the complications and consequences associated with neglected or undetected genital mycoplasma in the reproductive tract of females. The initial uncertainties of whether genital mycoplasmas can cause serious consequences are disappearing in the light of the many accumulating evidences.

In conclusion, genital mycoplasmas are not a rare cause of symptomatic and asymptomatic female genital tract infection as have been the thinking. The high prevalence rate among the asymptomatic adolescents in this study suggest strongly that these organisms are not always associated with symptoms and this needs for screening exercise among these age group.

**REFERENCES**