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Isolation of Vaginal Pathogens along with Genital Mycoplasmas from Asymptomatic Gynaecology and Antenatal Clinic Attendees

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Abstract: To examine the interaction between genital mycoplasmas and other genital pathogens from the vaginal tracts of women of reproductive age, 168 high vaginal swabs were studied microbiologically. Sixty (35.7%) genital mycoplasmas were isolated along with a total of 76 (45.2%) other microorganisms. *Candida albicans, Gardnerella vaginalis* and *Escherichia coli* had prevalence rates of 16.7%, 11.9% and 8.3% respectively. Others are *Staphylococcus aureus* (3.6%), *Trichomonas vaginalis* (3.4%) and Beta haemolytic Streptococcus (1.2%). B-haemolytic streptococci had the highest interaction rate (100%) with genital mycoplasmas and was followed by *T. vaginalis, G. vaginalis* and *C. albicans* with 55.6%, 55% and 45.5% interaction rates respectively. Others had significantly low interaction rates of 7.1% for *Escherichia coli* and 0.0% for *Staphylococcus aureus.* Apart from *Gardnerella vaginalis* the prevalence of vaginal microorganisms were more from pregnant than non-pregnant women. Though the presence of all these organisms in the urogenital tracts of these women was largely asymptomatic, they could play various adverse roles in the long run.

Key words: Vaginal pathogens · Genital mycoplasmas · Women

INTRODUCTION

The genital tracts of women consist of residents' microfloras which are made of a wide variety of species some of which play useful roles to the healthy state of the vagina[1] while others reside there as commensals but may become pathogenic if opportunity arises. The organisms associated with vaginitis are either part of the host's own microflora or exogenous microorganisms that must interact with species present as part of the host's indigenous flora [1]. Organisms in the vaginal tract may ascend to the cervical os especially during sexual intercourse and may likely cause infections there. Hence genital tract infectious agents may spread through the peritoneal cavity and toxins may produce extra-genital effects after being absorbed through the mucosa [2]. Dissemination of pathogens through the blood stream occurs with *N. gonorrhoeae* and *Treponema pallidum* thus genital tract infections can also affect the fetus and the newborn infant and some can cross the placenta and infect the developing fetus [2]. The prevalence of mycoplasmas and ureaplasmas in the genital tracts of women was earlier investigated [3]. This study therefore was carried out to identify the presence of other likely pathogens besides mycoplasmas and ureaplasmas that might be present in the vaginal tracts of the women examined in this study.

MATERIALS AND METHODS

Three high vaginal swabs were collected from each of 168 female patients (114 pregnant and 54 non-pregnant) attending both antenatal and gynaecology clinics of the University College Hospital, Ibadan, Nigeria. One of the vaginal swabs was inoculated into mycoplasma broth; the second into ureaplasma broth and the 3rd was inoculated onto blood and MacConkey agar media. Wet preparations and Gram stain were also done on the swabs

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and examined by light microscopy for the presence of clue cells, yeast cells, wbcs and Trichomonas vaginalis (wet prep). The mycoplasma/ureaplasma media used were as described by Freundt [4], though with modifications [5]. The cultural techniques used in the isolation and identification of mycoplasma and ureaplasma are as described in [5] whereby the vaginal swabs were first inoculated into broth media, incubated at 37ºC for 24 h for ureaplasma and 3 days for mycoplasma and subsequently sub-cultured onto their corresponding agar media. The agar media were also incubated at 37ºC for 24-48 h for ureaplasma and up to 10 days for mycoplasmas. The plates were examined for the characteristic "fried egg" colonies. Forty-seven (47) of the Mycoplasma strains were subjected to various biochemical and serological tests as described by Agbakoba et al. (2006) [6] while the ureaplasma strains were identified by the polymerase chain reaction as *Ureaplasma urealyticum* [7]. Other vaginal pathogens were identified using standard methods [8].

RESULTS

Other pathogens isolated from the various specimens are shown in Table 1. They include *Escherichia coli,* which were more from pregnant women 10(8.8%) than non-pregnant women 4 (7.4%) and *Candida albicans* which also has a higher isolation rate from pregnant women 20 (17.5%) than from non-pregnant women, 8 (14.8%). Others are *Trichomonas* v*aginalis* and *Gardnerella vaginalis* which were seen in 5 (4.4%) and 12 (10.5%) pregnant women clinical samples respectively; and from the non-pregnant women -1 (1.9%) and 8 (14.8%) respectively. *Staphylococcus aureus* was isolated in equal proportions from the pregnant and non-pregnant women with 4 (3.7%) and 2 (3.7%) respectively. Beta haemolytic streptococci were isolated from 2 (1.8%) of the pregnant women and none (0%) was from the non-pregnant women.

Table 1: Organisms isolated from the vaginal samples

	High vaginal swabs (No. /%)		
Pathogens	Pregnant women N=114	Non-pregnant women N=54	Total (No. /%) N=168
Escherichia coli	10(8.8)	4(7.4)	14 (8.3)
Candida albicans	20(17.5)	8(14.8)	28(16.7)
Trichomonas vaginalis	5(4.4)	1(1.9)	6(3.4)
G. vaginalis (clue cells)	12(10.5)	8(14.8)	20(11.9)
Beta haemolytic strept.	2(1.8)	0(0.0)	2(1.2)
Staphylococcus aureus	4(3.7)	2(3.7)	6(3.6)
Total	53	23	76

Table 2: Interaction of other pathogens with genital mycoplasmas

Microorganisms	No (%) seen	No (%) seen with Genital mycoplasmas
Candida albicans	28	13 (45.4)
Trichomonas. vaginalis	6	4 (55.6)
Beta haem. Strept.	2	2 (100.0)
G*ardnerella.vaginalis*	20	11 (55.0)
Escherichia. coli	14	1 (7.1)
Stapylococcus. Aureus	6	0 (0.0)
Total	76	31

Table 2 shows the isolation of other pathogens alongside genital mycoplasmas. It was observed that organisms like Beta haemolytic Streptococcus, *Trichomonas vaginalis* and *Gardnerella vaginalis* were more prevalent in the presence of genital mycoplasmas with 100% and 55.6% and 55% isolation rates respectively. *Candida.albicans* has a 45.5% isolation rate alongside genital mycoplasmas.

DISCUSSION

Microorganisms isolated from the patients include *Candida albicans*, *Trichomonas vaginalis* and *Escherichia coli* which were isolated at a higher frequency from the pregnant women than the non-pregnant women. Others were beta-haemolytic streptococci and *Staphylococcus aureus* which were also isolated more from the pregnant women. The higher prevalence rate of microorganisms in pregnant women could be attributed to their higher estrogen state, which increases their susceptibility to microorganisms [9].

The two isolates of beta-haemolytic streptococci in the pregnant women were found co-existing with genital mycoplasmas. The reason for this is not clear; though it could be that the alkaline environment of the mycoplasmas favours the streptococci species as well. Organisms of the beta-haemolytic streptococci especially Group A (S*treptococcus pyogenes*) and Group B (*Streptococcus agalactiae*) have been implicated in puerperal sepsis and neonatal infections [10]. Other investigators isolated Beta haemolytic Group B Streptococci from vagina of pregnant women [11], in intra uterine infection and spontaneous mid-gestation abortion [12] and from the milk of lactating mothers in Nigeria [13].

The higher prevalence of *Gardnerella vaginalis* from non-pregnant women in this study is supported by those of other investigators who also found the organism more from the non-pregnant women than the pregnant women [14-15]. This finding could be due to the fact that some of the non-pregnant women examined were those with

complaints of vaginal discharge similar to patients with vaginitis [14-15]. *Gardnerella vaginalis* is one of the agents of bacterial vaginosis (BV) and bacterial vaginosis has been implicated in many perinatal conditions like early pregnancy loss, premature rupture of membrane, chorioamnionitis and endometritis [16-18]. Other reported agents of BV include *Mycoplasma hominis, Ureaplasma urealyticum, Mobiluncus* spp and certain anaerobes [19]. That 55% of the *G. vaginalis* from this study was found as mixed infection with genital mycoplasmas in women with complaints of vaginal discharge further supports the possible contributory role of these organisms in bacterial vaginosis. Both groups of organisms also thrive better in near alkaline pH [20]) and this can also contribute to their isolation as mixed culture.

Three out of the five *Trichomonas vaginalis* recovered from the pregnant women and the only one from the non-pregnant women were found co-existing with genital mycoplasmas. This finding is supported by that of other investigators who reported that trichomoniasis increased colonization with *Mycoplasma hominis* [21]. On the other hand, Candidiasis occurred less frequently with genital mycoplasma, as only few of the *Candida albicans* isolated were found co-existing with the mycoplasmas thus agreeing with the finding of [21] who reported that Mycoplasmas occur less frequently with genital Candidiasis. This could also be due to the lowered pH of the vagina caused by the presence of Candida as Mycoplasmas thrive better in near alkaline pH. [20].

Escherichia coli and *U. urealyticum* have been reportedly isolated as mixed cultures in pregnant women with ruptured membranes [12]. More *E. coli* isolates were isolated from pregnant than non-pregnant women in this study and only 3 out of the 10 isolates from these women were found co-existing with genital mycoplasmas. Their role in these women (whether singly or in mixed infections) cannot be ascertained, as most of these pregnant women were normal clinic attendees, more so, as they were not followed up beyond specimen collection to know the outcome of their pregnancies.

All these isolates, both from the pregnant and non-pregnant women, may play various interactive roles in the urogenital tracts of females and though their presence was largely asymptomatic the possibility that they could lead to various adverse conditions in the long run cannot be ruled out.

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