ORIGINAL ARTICLE

Genital Mycoplasma Infections Among Women In An Urban Community Of Northern Nigeria: Do We Need To Search For Them?

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Abstract

Methods: *To determine the incidence of genital Mycoplasma infection among females in Jos. High vaginal swab (HVS) and or Endocervical swab (ECS) samples were obtained from 476 females undergoing vaginal examinations along with other females who volunteered to enroll in the study. Samples were processed using standard laboratory procedures for the isolation of Mycoplasma species while information such as age, marital status, occupation and other clinical data were obtained using a questionnaire. The results obtained were analysed using SPSS 11.0 statistical methods and P values = or < 0.05 were considered significant.*

Results: *The overall incidence of genital Mycoplasma infection was found to be 29.6% (n=141); M. hominis, 12.1% (n=57); U. urealyticum 9.4% (n=45); mixed infection, 6.7% (n=32), and other Mycoplasmas, 1.4% (n=7). Majority of the isolates were from those aged 20-35 years old (most sexually active group); 83% (n=52) of those who presented with vaginal discharge were infected with Mycoplasma spp. (P< 0.05); also, the incidence of infection among the separated/divorce/widowed group was significantly higher than the married group (P<0.05).*

Conclusion: *Mycoplasmas are common genital organisms, hence should be sought out for from ECS probably on routine basis for suspected genital tract infections.*

Key Words: *Infection, Mycoplasma, Genital, ECS, Women, Urban*

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Introduction

Mycoplasmas are the smallest free living bacteria capable of independent metabolism and replication[1,2]. They however appear to possess less complex cellular machinery compared to other bacteria such as *H. influenzae* and *E. coli*[3]. The number of genes involved in cellular processes, such as the *fts* genes associated with cell division, heat shock proteins, and genes for chaperones functioning in protein secretion are much smaller than that of other bacteria[4,5]. In addition to the absent cell wall, the channel-forming proteins, SecG, SecF, SecE, and SecD and the cytosolic receptor proteins SecB are equally deficient in *Mycoplasmas*[6,7]. They are however richer in cell surface lipoproteins with high propensity towards antigenic variation[8].

Mycoplasmas are well established as one of the causes of sexually transmitted diseases (STDs) world over[9,10], and are recognized as one of the commonest agents of nongonococcal urethritis/cervicitis (NGU), and postgonococcal urethritis/cervicitis (PGU)[11,12]. In a study carried out in Papua New Guinea on 100 women in the Eastern Highlands; over 70% of the women and over 78% of them were colonized by *Mycoplasma hominis* and *Ureaplasma urealyticum* respectively, while over 60% of them were colonized by both *M. hominis* and *U. urealyticum*[13]. Findings from Boston, Massachusetts[14] showed that *M. hominis* and *U. urealyticum* were common causes of nongonococcal urethritis, pelvic inflammatory disease, and a wide range of complications such as: infertility, abortion, still birth, low birth weight and puerperal fever. Other species, example, *M. pneumoniae* can cause upper respiratory tract infection in about 30% of the new born infants. Findings from England similarly showed that, the first species of *Mycoplasmas* to colonize the neonate and hence cause disease were *M. hominis* and *U. urealyticum*[15]. In India, *U. urealyticum* was isolated from 9% of the cerebrospinal fluid samples, and 14% of the tracheal aspirates of the 100 low birth weight infants who had suspected meningitis and/or respiratory distress respectively[16].

Reports on the isolation of genital *Mycoplasmas* and the laboratory procedures meant to isolate these agents is lacking from most referral laboratories in Nigeria, several African countries and other tropical and

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sub-tropical regions of the world[17-21]. This gives room for probably underreporting of the disease with its attendant social and public health implications[22,23]. Lack of adequate information about such a disease would make its control at the hospital level difficult; and at the community level, a faulty or absent control programme[24,25].

Recent findings have shown that, HIV thrives better at the background infection with genital *Mycoplasmas*, while background infection with HIV has also led to the recruitment of a newer species of genital *Mycoplasmas* (*Mycoplasma penetrans*) previously unknown[26-28].

This study was therefore set up to establish the incidence of genital *Mycoplasmas* among women in a Nigerian city, with or without obvious features of genital tract infection. The findings would give an insight about the depth of the disease in the community and hence stir up for the desired control measures.

Materials and Methods

Settings: The study was conducted in Jos between February and April 2006; Jos is the Plateau state capital. Based on 1991 population census, her population is estimated at 1.1 million people. The weather is temperate in nature suitable for habitation and is endowed with interesting tourist sites; it is predominantly a Christian city but has a sizable number of Moslems, while believers of other faiths are much fewer; the majority of the occupants are traders, farmers, civil servants, students and applicants. There are three major hospitals in the city: Jos University Teaching Hospital (JUTH), Plateau State Specialist Hospital (PSSH), and Evangel Hospital.

Selection of Subjects: An active hospital based survey was carried out at PSSH, Evangel Hospital and Nassarawa Medical Centre, Nassarawa Gwom all in Jos. All patients who were undergoing vaginal examination who volunteered to enroll in the study and other willing females attending the designated health centres for various ailments within the four months study period were consecutively recruited for the study including those who presented with symptoms suggestive of a sexually transmitted infection (STI) especially vaginal discharge. Subjects with symptoms and signs of vaginal discharge served as test (T) subjects, while those without these symptoms served as control (C).

Procedure: A well structured questionnaire was administered either self or through an interviewer and items such as: age, marital status, occupation, clinical symptoms such as vaginal discharge, itching and lower abdominal pain were highlighted. All the samples were processed at the National Veterinary Research Institute (NVRI) Vom, about 35 kilometres south of Jos city.

Collection and Transport of Endocervical Swab (ECS) Samples: These were carefully taken avoiding contact with antiseptic solutions, creams, or jellies, and the cotton wool portion was dropped immediately into *Mycoplasma* broth medium. The swab was agitated in the medium, squeezed against the side of the container and then discarded to avoid the effect of the inhibitory substances that might be in the cotton wool itself. The samples were transported to the laboratory within 2 hours after collection. In the laboratory, the specimens were inoculated as rapidly as possible or were stored at 4^0C for not more than 48 hours before processing. Microscopy of the wet preparation was carried out.

Culture: Tryptose molten agar and broth, the preferred culture media for the study were prepared with several supplements to support the growth of the organisms such as[29]: Di-sodium hydrogen orthophosphate, 25% yeast extract solution, 35% sulphamethazine, penicillin G (40,000iu/ml), inactivated bovine/equine serum (70.0 mls), mycostatin (24,000 iu/ml solution), 10% urea solution, 2% L-cystein hydrochloride solution and glycerol, and the final preparations adjusted to the final PH of 7.6 and 6 for *M hominis* and *U. urealyticum* respectively. The specimens in the transport medium were subcultured on Tryptose agar plates and incubated in the presence of 95% CO_2 (Gaspak disposable gas generator envelopes in anaerobic cabin). The plates were incubated 37^0C for 2 to 7 days with daily examination for the presence of growth[29]. Control organisms were obtained from Central Public Health Laboratory, 61 Colindale Avenue, London, United Kingdom. Specimens were also inoculated unto MacConkey, blood agar, chocolate and sabouraud dextrose agar and incubated at 37^0C.

At the end of appropriate incubation period, the colonies of *M. hominis* appeared pale and tiny in nature with the typical 'fried egg' appearance while those *U. urealyticum* appeared similar but darker in colour.

Biochemical Tests: The specimen in transport medium was subcultured into fluid medium for biochemical tests, (*U. urealyticum* and *M. homonis* appropriate biochemical medium). A wire loopful of a standard control test species of *Mycoplasmas* (*U. urealyticum* and *M. hominis*) was aseptically inoculated appropriately into each type of biochemical fluid media. A tube each for negative control tests (un-inoculated

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media tubes) from the two types of the biochemical fluid media were also prepared. The test proper (specimens), the control tests (positive and negative tests) were all incubated anaerobically at 37^{0}C for 1 to 7 days[30]. *U. urealyticum* produces colour change rapidly; the speed depending upon the number of organisms in the original specimen, but it occurs usually within 24 to 72 hours and infrequently thereafter. *M. hominis* produces a colour change usually well within a week. The PH (colour change) was determined by visual observation, by comparing with the negative and positive control tubes.

Serologic Growth Inhibition Test: *Mycoplasma* growth is inhibited by its specific antiserum[30]. Venous blood 3-5 mls was collected from each female from whom HVS was obtained into sterile bijou bottle. The blood was allowed to clot and the serum was pipetted into clean dry tubes and stored at -20^{0}C. Tryptose agar plates were dried at 37^{0}C for 20 minutes. *Mycoplasma* broth cultures were standardized to 10^5 McFarland's standard, and one drop (about 0.1 ml) of each *mycoplasma* species culture (*U. urealyticum* and *M. hominis*) was deposited on each pre-dried agar plates surface and spread evenly using a sterile swab and allowed to adsorb. Each plate was labeled accordingly. Wells of about 0.4 mm were bored on each inoculated agar plate (at least 2cm apart) and agar removed with capillary pipettes and discarded into disinfectant jar. A well was labeled 'positive control' (used for specific antisera for the cultured species of *Mycoplasma* on agar plate). The remaining wells were labeled with appropriate particulars of each patient on the agar plate. Positive control antisera was added to its labeled well and each patient's antisera to their corresponding wells using different pipettes at each time or one pipette and rinsed at least three times in clean sterile normal saline after each use. The plates were packed into Gaspak anaerobic cabin. Nitrogen and CO_2 were introduced into the anaerobic cabin with the aid of Gaspak gas generating disposable envelops and incubated at 37^{0}C. The plates were examined after 2 to 3 days of incubation for *U. urealyticum* zone of inhibition by the antisera (positive control and the patient's antisera) and within 3 to 7 days for *M. hominis*. The plates were examined using X40 light microscope objective. Patients with present or recent history of antibiotics consumption were excluded from the test except those with evidence of being on penicillins and similar drugs. Zone of inhibition around test wells and control wells of antisera were measured and compared for distinct zone of inhibition.

Interpretation of Results: The isolation of *M. hominis* and *U. urealyticum* was established if the colonial appearance was characteristic along with positive biochemical characteristics, with or with out a positive growth inhibition test and where growth inhibition test alone was highly characteristic. Where the biochemical and growth inhibition tests were inconclusive, the isolates were considered as other *Mycoplasmas* such as *M. genitalium,* and *M. fermentans*. This procedure, if properly carried out and quality control maintained is able to detect over 98% of *Mycoplasmas* in a sample[29,30]. Reagents for specie identification of *M. genitalium* were however lacking for the study.

Analysis of Results: The results were analysed using simple descriptive methods and SPSS 11.0 statistical software where applicable while p values = or < 0.05 were considered significant.

Ethical Considerations: Ethical approval for the study was obtained from the Ethical committee of the Plateau state ministry of Health.

Results

The incidence of genital *Mycoplasma* infections among the 476 female subjects studied in Jos was found to be 29.6% (n=141), (fig. 1).

Among the isolates, *M. hominis* was recovered from 12.1% (n=57), *U. urealyticum,* 9.4% (n=45), mixed *M. hominis* and *U. urealyticum*, 6.7% (n=32), and other Mycoplasmas, 1.4% (n=7), (Table 1). Age distribution pattern of genital *Mycoplasma* infection showed a gradual increase from 14 years (2.1%, n= 10), and peaked at 25-29 years old (6.2%; n=29), and declined steadily thereafter to 0.2% (n=1) among those 55 years and above, (Table 1).

An analysis of the incidence of genital *Mycoplasma* infection among females in Jos based on occupation showed that, civil servants, Petty traders, applicants, and students recorded 2.3% (n=11), 7.1% (n=34), 9% (n=43), and 3.4% (n=16) infection rates respectively, while that among farmers was 5.9% (n=28), and businesswomen, 1.9% (n=9), (Table I).

Based on marital status, the incidence of genital *Mycoplasma* infection among females in Jos showed that, 18.4% (n=56) married among married, 48.1% (n=64) among singles and 67.9% (n=19) among the separated/divorced/widowed group, (P<0.05), (Fig. II).
Among those who presented with a positive history of vaginal discharge, the rate of infection with genital *Mycoplasma* was found to be 83% (52 out of 63) while 17% (11 out of 413) infection rate was recorded among

those with out symptoms and signs of vaginal discharge, (P< 0.05), (Fig. 3).

Concerning the microorganisms recovered along with genital *Mycoplasmas,* all the isolates of *Proteus mirabilis,* 11 (100%), and *Enterococcus specie*s, 3 (100%) were recovered from patients with genital *Mycoplasma* infections. Also, 25% (3 out of 12) of *T. vaginalis;* 88.9% (8 out of 9) *Escherichia coli*; 80% (4 out of 5) *Klebsiella spp.*, and 21.1% (4 out of 19) of *Candida spp.* were recovered from those with genital *Mycoplasma* infections. There was significant association of the bacteria recovered with genital *Mycoplasma* infection, (p< 0.05), (Fig. 4). Also *Mycoplasma* alone was recovered from 17 (27.0%) subjects with symptoms of vaginal discharge.

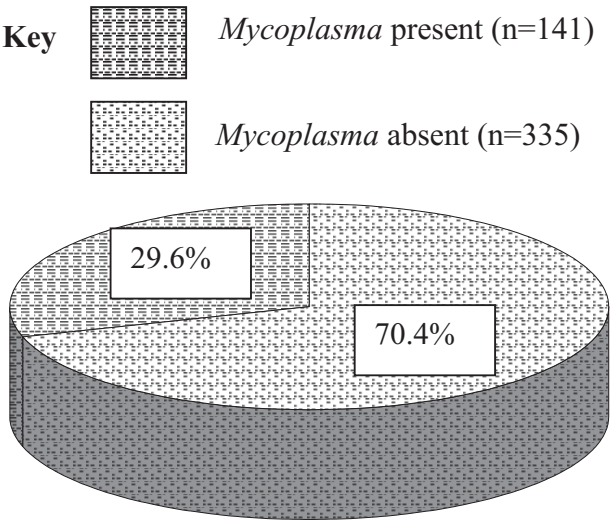


Figure 1 Incidence of genital *Mycoplasma* infection among women in Jos.

Table I Isolation of genital *Mycoplasmas* from women in Jos based on species and age distribution patterns

Age (Years)	*M. hominis(%)*	*U. urealyticum(%)*	Mixed(%)	Others(%)	Nil(%)	Total(%)
10-14	7(1.5)	2(0.4)	1(0.2)	0(0.0)	27(5.7)	37(7.8)
15-19	9(1.9)	6(1.3)	3(0.6)	0(0.0)	38(8.0)	56(11.8)
20-24	9(1.9)	9(1.9)	4(0.8)	2(0.4)	56(11.8)	80(16.8)
25-29	16(3.4)	7(1.5)	5(1.1)	1(0.2)	47(9.9)	76(16.0)
30-34	5(1.1)	13(2.7)	6(1.3)	3(0.6)	49(10.3)	76(16.0)
35-39	6(1.3)	4(0.8)	8(1.7)	1(0.2)	42(8.8)	61(12.8)
40-44	3(0.6)	2(0.4)	4(0.8)	0(0.0)	37(7.8)	46(9.6)
45-49	1(0.2)	1(0.2)	0(0.0)	0(0.0)	14(2.9)	16(3.4)
50-54	1(0.2)	0(0.0)	1(0.2)	0(0.0)	11(2.3)	13(2.7)
=55	0(0.0)	1(0.2)	0(0.0)	0(0.0)	14(1.7)	15(3.1)
Total	57(12.1)	45(9.4)	32(6.7)	7(1.4)	335(70.4)	476(100)

Table II. Rate of genital *Mycoplasma* infection among women in Jos based on occupation

Occupation	*Mycoplasma* present (%)	*Mycoplasma* absent (%)	Total (%)
Civil servant	11 (2.3)	36 (7.6)	47 (9.9)
Petty trading	34 (7.1)	53 (11.1)	87 (18.2)
Applicant	43 (9.0)	68 (14.3)	111 (23.3)
Student	16 (3.4)	45 (9.5)	61 (12.9)
Farming	28 (5.9)	94 (19.7)	122 (25.6)
Business	9 (1.9)	39 (8.2)	48 (10.1)
Total	141 (29.6)	335 (70.4)	476 (100)

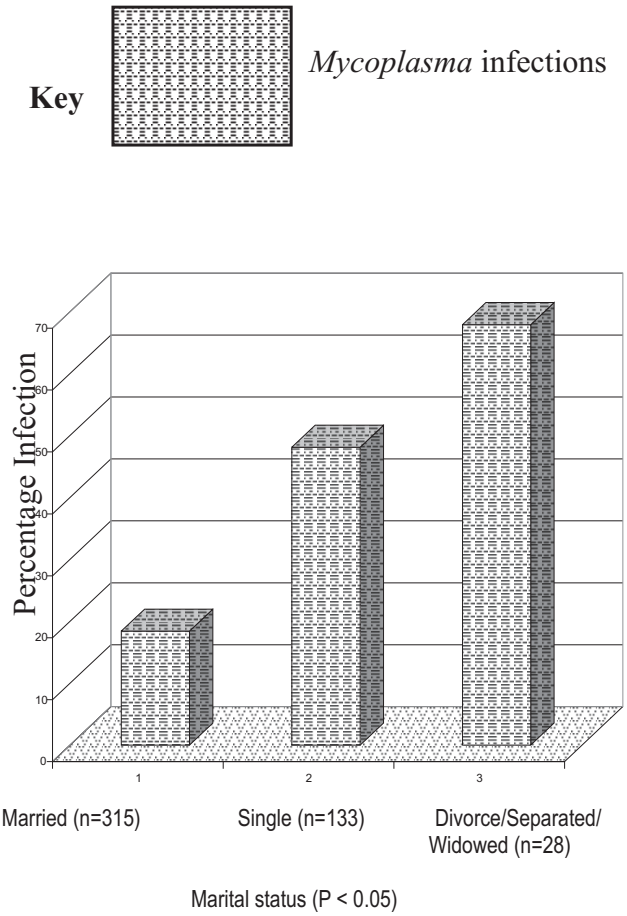


Figure 2 Rate of genital *Mycoplasma* infection among women in Jos based on marital status.

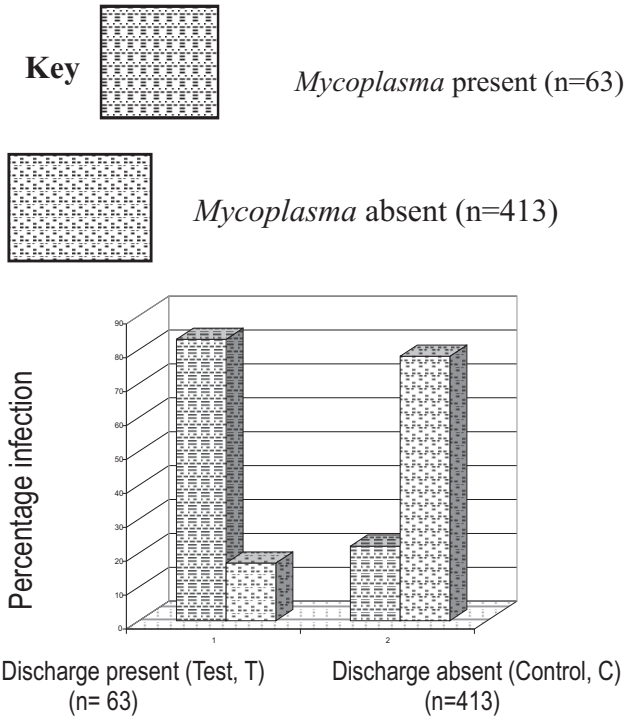
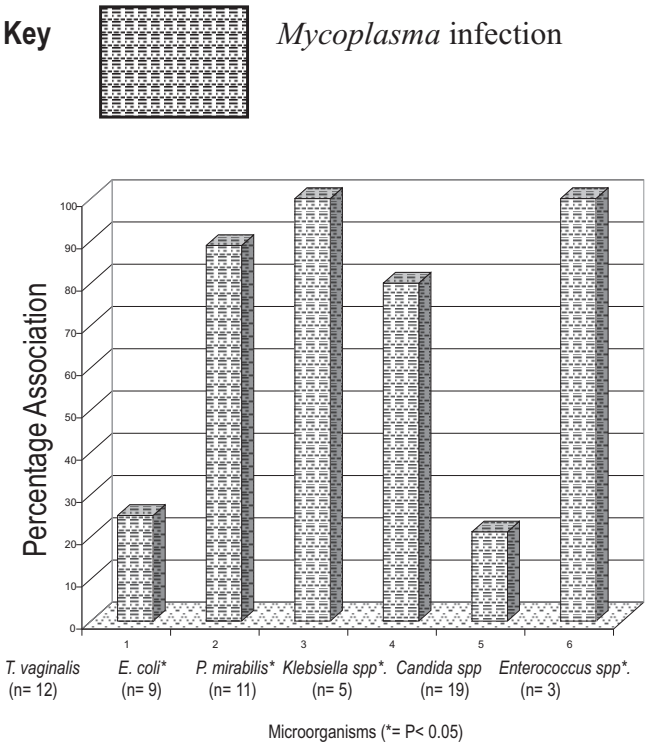


Figure 3 Rate of genital Mycoplasma infection among women in Jos based on a positive history of vaginal discharge.



NB: *Mycoplasma* only was recovered from 17 (27.0%) subjects with symptoms of vaginal discharge.

Figure 4 Other microrganisms recovered from women with vaginal discharge in Jos and their association with genital *Mycoplasma* infection.

DISCUSSION

The incidence of genital *Mycoplasma* infection among females in Jos was found to be 29.6%, while *M. hominis, U. urealyticum*, and mixed infections were 12.1%, 9.4%, and 6.7% respectively, and other *Mycoplasmas,* 1.4%. This shows how prevalent the organisms are among female genital samples and most importantly the need to look out for them from genital samples[31].

Findings from the present study compare fairly with that of: Kovacs *et al*[32] in Australia who obtained higher figures of 48.8% and 16.4% *U. urealyticum* and *M. hominis* respectively among 1,000 sexually active women undergoing vaginal examination; Simms *et al*[33] in London, who recovered *M. genitalium* from 13% of the cases of pelvic inflammatory disease; Casin *et al*[34] in Paris, France, who similarly recovered genital *Mycoplasmas* from 38% of women attending sexually transmitted diseases (STD) clinic; and Morency, *et al*[35] in Bangui, Central African Republic, who recovered genital *Mycoplasmas* from over 42% of the male subjects that presented with urethritis. Similar findings were also reported by: Steen, *et al*[36] in South Africa, Papin, *et al*[37] from a survey across West Africa, and, Mukange-Tshibaka, *et al*[38] in Cotonou, Benin Republic.

Genital *Mycoplasmas* were recovered from a significant 83% of the patients that presented with vaginal discharge as against the 17% from those who had no such symptoms, ($p<0.05$); also majority of the nonspecific bacteria (*E. coli, Proteus mirabilis*., *Klebsiella spp.,* and *Enterococcus spp*) isolates were recovered from those with genital *Mycoplasma* infections ($p<0.05$). The association of these bacteria with background *Mycoplasma* and or *Chlamydia* infection has been well reported by Sagey, *et al*, in Nigeria[39], Abdulrazak, *et al*, in Egypt[40], and Hocking, *et al*, in Australia[41]. Routine laboratory procedures on endocervical swab specimens should incorporate those procedures meant for the isolation of genital *Mycoplasmas* as well, especially in instances where nonspecific bacteria are the common isolates. This would help guard against the array of clinical features associated with the organism[11-14].

The isolation of majority of the *Mycoplasmas* from the most sexually active age range (20-35 years) underscores the role sexual transmission contributes to the spread of the infection[39,40]. Arya *et al*[41], and, Taylor-Robinson and McCormack[42] in their separate studies did not view genital *Mycoplasmas* as pathogenic organisms but mere commensals in the female genital tract. Findings from the present study however prove contrary, as well as that of some other researchers[43-45].

The incidence of genital *Mycoplasma* was found to be significantly higher among the separated/divorced/widowed group ($P<0.05$), and the singles ($P< 0.05$), compared to the married; due to possible vulnerability of these groups and likelihood of multiple sexual contacts.

The findings from present study are however different from that of : Holst *et al*[46] in Sweden where *Mycoplasmas* were not recovered from vaginal cultures of the 49 women studied probably due to variation in the degree of exposure to this organism on the two sides of the divide, or an incidental finding; Ekwempu *et al*[47] in Zaria, Nigeria, where also *Mycoplasma* was not recovered from the 187 women in labour studied since the laboratory procedures were not designed for its isolation; and Ndoye *et al*[48] in Dakar, Senegal who also did not recover *Mycoplasma* for similar reasons.

In conclusion, this study has shown that, *Mycoplasmas* are common organisms in female genital tract and constitute a significant proportion of the infectious agents there; hence, laboratory procedures for the investigation of female genital tract infections should be

constituted bearing in mind that *Mycoplasma* could be a probable isolate.

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