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Prevalence of genital mycoplasmas and co-occurrence with *Gardnerella vaginalis* in high vaginal swabs from adult females in Bahrain: point-prevalence observational study

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Abstract

Purpose – Bacterial vaginosis (BV) is quite common and linked with serious public health issues such as premature delivery and spread of sexually transmitted infections. The study aims to identify different genital mycoplasmas (GM) in high vaginal swabs (HVS) from adult females in Bahrain.

Design/methodology/approach – In total, 401 HVS were collected and cultured on MYCOFAST® RevolutioN 2 test for identification and antibiotic susceptibility. Polymerase chain reaction (PCR) was performed for detection of *Mycoplasma genitalium (Mg), Mycoplasma hominis (Mh)* and *Ureaplasma* species. DNA-probe based detection for *Gardnerella, Candida* and *Trichomonas* was performed by BD Affirm Assay. Representative PCR amplicons were sequenced by Sanger sequencing.

Findings – In PCR, *Ureaplasma* sp. was the most common GM, followed by *Mg* and *Mh*; the prevalence being 21.2, 5.2 and 1.5%, respectively. On the contrary, 10.7% samples showed positivity for *Ureaplasma urealyticum (Uu)* and 1.7% for Mh in MYCOFAST® RevolutioN 2. The concordance rates between MYCOFAST® RevolutioN 2 and PCR for *Mh* and *Ureaplasma* sp. were 97.7 and 84%, respectively. Considering PCR as gold standard, sensitivity, specificity, positive predictive value, and negative predictive value of MYCOFAST® RevolutioN 2 were 33.3, 98.8, 28.6, 98.9 and 37.7, 96.5, 74.4, 85.2% for *Mh* and *Ureaplasma* sp., respectively. The *Uu* and *Mh* isolates showed antibiotic-resistance ranging from 53%–58% and 71%–86%, respectively.

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Research limitations/implications – The prevalence of *Ureaplasma* sp. was high. Significant co-occurrence of GM was noticed with BV. MYCOFAST® RevolutioN 2 had lower detection-rate than PCR, so a combination is suggested for wider diagnostic coverage.
Practical implications – The research reflects on status of prevalence of GM in adult females in Bahrain, and their co-occurrence with bacterial vaginosis. Diagnostic approach with combination of tests is suggested for wider coverage. The research has epidemiologic, diagnostic, and therapeutic implications.
Originality/value – This is the first report from the Kingdom of Bahrain reflecting on burden of GM from this geographic location. The diagnostic efficacy of MYCOFAST® RevolutionN 2 test and polymerase chain reaction was evaluated for GM detection.

Keywords *Mycoplasma, Ureaplasma*, Prevalence, Bahrain, Bacterial vaginosis, Genital mycoplasmas
Paper type Research paper

Introduction

The vaginal canal is a home to diverse microbial community that coexists with the host in a symbiotic relationship, known as the vaginal microbiota (VMB) (Andrade Pessoa Morales *et al.*, 2021). VMB is a complex microecosystem that changes with age and menstrual cycle (Andrade Pessoa Morales *et al.*, 2021). Lactobacillus, as the predominant vaginal flora, plays an important role in innate defence by maintaining vaginal pH below 4.5 (Auriemma *et al.*, 2021).

A shift in vaginal flora with diversity of bacterial species, known as bacterial vaginosis (BV), raise the vaginal pH above 4.5 making it vulnerable to infections (Vasundhara, Raju, Hemalatha, Nagpal, & Kumar, 2021). BV may increase predisposition to other sexually transmitted diseases, and may affect 23%–29% women across regions (Foessleitner *et al.*, 2021).

Mycoplasma spp. and *Ureaplasma* spp. are well-known pathogens affecting human respiratory and urogenital systems, and are responsible for a wide spectrum of inflammatory disorders in neonates, children and adults (Taylor-Robinson & Furr, 1993). Mycoplasmas are eubacteria classed with Mollicutes, and lack cell wall (Taylor-Robinson & Furr, 1993; Yiwen, Yueyue, Lianmei, Cuiming, & Xiaoxing, 2021). *M. genitalium, M. hominis*, *Ureaplasma urealyticum* and *U. parvum* are responsible for urogenital problems in both men and women and are considered as important pathogens for non-gonococcal urethritis (Yiwen *et al.*, 2021). These four species are collectively called as genital mycoplasmas (GM) (Yiwen *et al.*, 2021).

GM cause sexually transmitted infections, which are associated with critical disorders in fertile women, including cervicitis, vaginitis, and if not treated, it can cause infertility and pelvic inflammatory disease (PID) (Kelly, Garland, & Gilbert, 1987).

Ureaplasma urealyticum is a major causative agent for non-chlamydial and non-gonococcal urethritis, cervicitis, chorioamnionitis, preterm delivery and vaginitis (Zheng *et al.*, 2020). Also, in pregnant women, mycoplasma infection can lead to abortion or preterm labour (Zheng *et al.*, 2020).

For diagnosing GM infections, culture method is insufficient (Zhou, Ma, Yang, & Gu, 2018), and serological methods only allow for a retroactive diagnosis (Zhou *et al.*, 2018). Rapid methods have recently become available, including nucleic acid amplification tests (NAAT) such as polymerase chain reaction (PCR) (Coleman & Gaydos, 2018; Adebamowo *et al.*, 2017). Routine screening for reproductive tract infections (RTIs) has been implemented in countries like the USA, Denmark, UK and Sweden (Ma *et al.*, 2021), whereas, in developing countries, routine screening is not performed.

To date, the prevalence of GM, and their co-occurrence with BV, is not clearly reported from the Kingdom of Bahrain, which prompted us to perform this study. Also, we aimed at analysing the utility of MYCOFAST® RevolutioN 2 test, and PCR, in accurately diagnosing these GM.

Materials and methods

Place of study and sample collection

The study was performed in the department of Microbiology, Immunology and Infectious Diseases (DMIID), College of Medicine and Medical Sciences of the Arabian Gulf University,

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Kingdom of Bahrain. High vaginal swabs (HVS) were collected in Gynaecology Clinic of Salmaniya Medical Complex, Bahrain, from patients presenting with non-specific vaginal discharge. The samples were sent to Microbiology Department at Salmaniya Medical Complex (MDSMC) for testing on BD Affirm assay for *Gardnerella*, *Candida* and *Trichomonas* infections. Further, these HVS samples were sent to DMIID for Mycofast® RevolutioN 2 Test, and molecular testing, for GM {*Mycoplasma genitalium* (*Mg*), *Mycoplasma hominis* (*Mh*), *Ureaplasma sp.*(*Usp*)}.

Through March to August 2021, 401 random HVS samples were collected in AMIES transport media (Deltalab, Spain). The results of the BD Affirm molecular probe assay for *Gardnerella*, *Candida* and *Trichomonas* (only for the positive swabs for GM) were obtained from MDSMC for comparative analyses.

Processing of samples

A new sterile swab was inserted in the transport medium, and 500 μl of mycoplasma preservation and transport solution was added to the transport medium. The tubes were incubated at 37 °C with 5% CO_2 for 24 h.

MYCOFAST® RevolutioN 2 test

MYCOFAST® RevolutioN 2 (ELITech – France) test was used for identification and antimicrobial susceptibility of *Ureaplasma urealyticum* and *M. hominis* (Redelinghuys, Ehlers, Dreyer, Lombaard, & Kock, 2013). Then, 500 μl of the transport medium was pipetted into 3 ml UMMt-medium vial provided with MYCOFAST® RevolutioN 2 test. After mixing, 100 μl of UMMt solution each was pipetted in 24 wells of the MYCOFAST® tray, followed by adding two drops of mineral oil. Respective trays were sealed with plastic seal and incubated at 37°C for 24 h. Samples with no indication of growth after 24 h were re-incubated for further 24 h.

Growth was inferred by the change of colour of the medium from yellow to red or orange due to the metabolism of urea and arginine, respectively, by *Ureaplasma urealyticum* and *Mycoplasma hominis*. The trays had following two divisions:

Black-labelled side (for Ureaplasma urealyticum*)*

Wells 1 to 3 were for identification of *Ureaplasma urealyticum* in different concentrations: 10^3, 10^4, 10^5 CCU/ml. Wells 4 to 13 were for testing antimicrobial susceptibility of *Uu*. Following antibiotics were used: Levofloxacin (concentrations at 2 and 4 μg/ml), Moxifloxacin (2 and 4 μg/ml), Erythromycin (8 and 16 μg/ml), Tetracycline (1 and 2 μg/ml), Doxycycline (1 and 2 μg/ml).

Red-labelled side (for Mycoplasma hominis*)*

Well 14th was used for identifying *Mycoplasma hominis* in a concentration of 10^4 CCU/ml. Wells 15–24 were for antimicrobial susceptibility testing of *Mh*. Following antibiotics were used: Levofloxacin (1 and 2 μg/ml), Moxifloxacin (0.25 and 0.5 μg/ml), Clindamycin (0.25 and 0.5 μg/ml), Tetracycline (4 and 8 μg/ml), Doxycycline (4 and 8 μg/ml).

Molecular detection by conventional polymerase chain reaction

PCR was done to detect *M. genitalium*, *M. hominis* and *Ureaplasma sp.* in all samples. Swabs in the transport media were used for molecular detection. DNA was extracted from swabs by using QIAamp DNA mini kit (Qiagen, Crawley, UK). DNA controls procured from vircell MICROBIOLOGISTS were used for each GM species. A 1:10 dilution was prepared from each lyophilised DNA control. The PCR products were analysed by gel electrophoresis on 1.5% agarose.

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Polymerase chain reaction cycling and primers
The primers used in this study targeted the urease gene in *Ureaplasma*, and S16 rRNA in Mycoplasma. Following primers were used: *Mycoplasma genitalium* – F: 5′-TAC ATGCAA GTC GATCGG AAG TAG C-3′ and R: 5′-AAA CTC CAG CCA TTG CCT GCT AG-3′ (Stellrecht, Woron, Mishrik, & Venezia, 2004); *Mycoplasma hominis* – F: 5′-CAATGGCTAATGCCG GATACGC-3′ and R: 5′-GGTACCGTCAGTCTGCAAT-3′ (Waites, Katz, & Schelonka, 2005; Yoshida, Maeda, Deguchi, Miyazawa, & Ishiko, 2003); *Ureaplasma sp. (urealyticum/parvum)* – F: 5′-ACGACGTCCATAAGCAACT-3′ and R: 5′-CAATCTGCTCGTGAAGTATTAC-3′ (Timenetsky, Santos, Buzinhani, & Mettifogo, 2006). The expected amplicon sizes were 427, 344 and 429 bp, respectively. Amplification was performed using Applied Biosystems PCR system 9700 thermocycler. PCR reactions for *M. genitalium*, *M. hominis* and *Ureaplasma sp.* were run ranging between 30 and 42 cycles for different species as described by Stellrecht *et al.*, Waites *et al.*, Yoshida *et al.* and Timenetsky *et al.,* respectively.

DNA sequencing
Representative amplicons of *M. genitalium*, *M. hominis* and *Ureaplasma sp* were commercially sent to MedGenome Labs Pvt Ltd, Bangalore, India, for sanger sequencing.

Statistical analysis
Concordance rate between PCR and MYCOFAST was calculated using formula:

$$\text{Concordance rate} = a + d/a + b + c + d$$

where, a = samples positive by both PCR and MYCOFAST; b = PCR +ve and MYCOFAST –ve; c = PCR –ve and MYCOFAST +ve; d = both PCR and MYCOFAST negative.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated on MedCalc Version 20.027 statistical software package for biomedical research.

Results

Age distribution of the positive cases for genital mycoplasmas
The age of the positive cases ranged from 21–64 years. Most of the cases were between the age of 22 and 39 years; there was a predominance around the age of 34 years.

Prevalence of genital mycoplasmas based on polymerase chain reaction
Out of 401 samples, 102 (25.4%) showed positivity for GM based on molecular testing. From these 102 samples, a total of 112 GM were detected (some samples had positivity for more than one type of GM). *Ureaplasma sp*. was the most common GM, followed by *Mg* and *Mh*. Based on PCR, the prevalence of these species were 21.2% (n = 85), 5.2% (n = 21) and 1.5% (n = 6), respectively (Figure 1a). A total of ten samples were positive for more than one GM species; seven samples showed positive amplicon for *M. genitalium* + *Ureaplasma*. *sp* and three samples were positive for *M. hominis* + *Ureaplasma. sp*.

The confirmation of the PCR results was done by repeating the PCR of positive samples twice and using a positive DNA control commercially provided for each species to confirm the targeted amplicons. The representative PCR gels are shown in Figure 2(a)–(c).

MYCOFAST® RevolutioN 2 test
Detection of U. urealyticum and M. hominis. Of the 401 HVS tested, 43 (10.7%) were positive for *Ureaplasma urealyticum* and seven (1.7%) for *M. hominis*. The samples positive for

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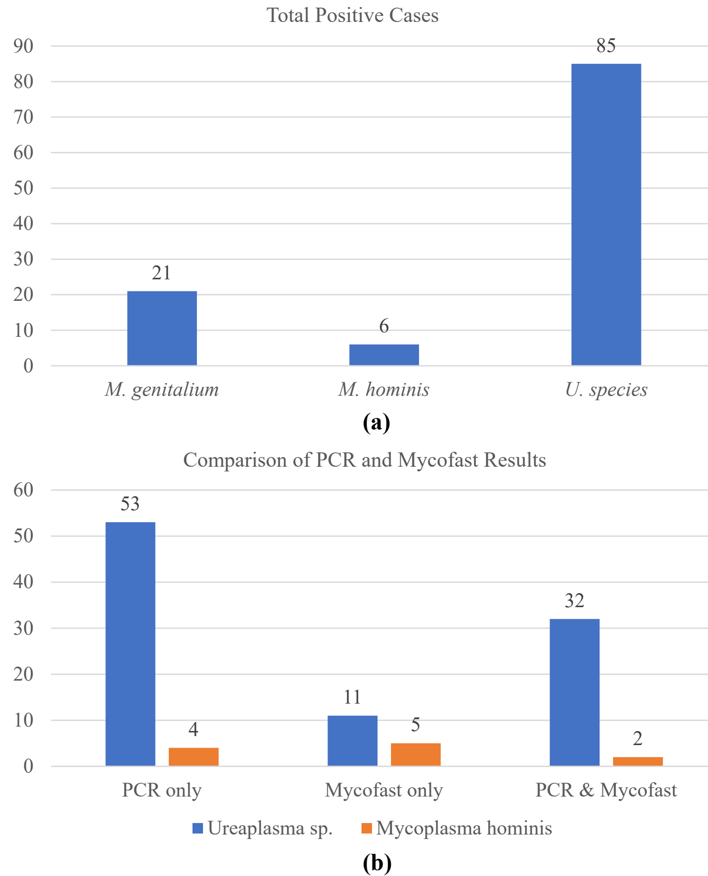

Figure 1. (a) Positivity for different types of GM by PCR test. (b) Comparison between the PCR and MYCOFAST® RevolutioN 2 test in detection of GM. **PCR primers were targeting against Ureaplasma sp. (U. urealyticum + U. parvum), whereas MYCOFAST could detect only U. urealyticum*

M. hominis were concomitantly positive for *Ureaplasma sp.* The limitation of this test is that it only detects *Ureaplasma urealyticum,* and *M. hominis* and does not detect *Mycoplasma genitalium* and *Ureaplasma parvum*.

Growth enumeration of MYCOFAST® RevolutioN 2 tests for Ureaplasma urealyticum. The tests showed 38 (88.3%) samples giving growth in 10^5 CCU/ml enumeration well, whereas only five (11.6%) samples showed growth in 10^3/10^4 CCU/ml enumeration wells.

Antibiotics susceptibility profile. The *Ureaplasma urealyticum* isolates showed resistance to the tested antibiotics ranging from 53%–58%. The *M. hominis* isolates were resistant to the tested antibiotics ranging from 71%–86%. The detailed antibiotics susceptibility profiles for *Ureaplasma urealyticum* and *M. hominis* are shown in Table 1.

Comparison between polymerase chain reaction and MYCOFAST and concordance rate

A comparative description of the detection of genital mycoplasmas by PCR alone, MYCOFAST alone and combined detection by PCR + MYCOFAST is shown in Figure 1(b). In this study, we got a total of seven positive MYCOFAST® RevolutioN 2 for *M. hominis*, and two of them were consistent with the PCR results. For *U. urealyticum,* we got a total of 43 positive samples positive in MYCOFAST® RevolutioN 2 test, and 32 of these were compatible with the PCR

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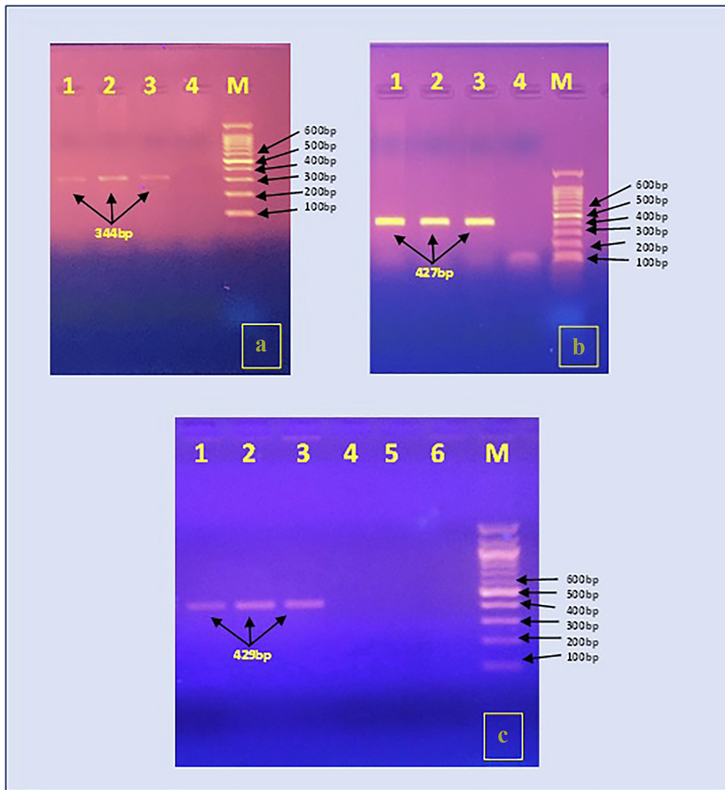

Figure 2. (a) shows the representative gel having positive amplicons for *M. hominis* (Lanes1, 2 and 3), (b) representative amplicons of *M. genitalium* (Lanes1, 2 and 3) and (c) representative amplicons of *Ureaplasma sp.* (Lanes1, 2 and 3). Lane M is 100 bp ladder

Table 1. Antibiotics resistance rates for *Ureaplasma urealyticum* ($n = 43$) and *M. hominis* ($n = 7$) tested in MYCOFAST® RevolutioN 2 test for respective antibiotics

Antibiotics tested in MYCOFAST® RevolutioN 2 panel	% resistance (n) for *Ureaplasma urealyticum*	% resistance (n) for *M. hominis*
Levofloxacin	58% (25)	86% (6)
Moxifloxacin	56% (24)	71% (5)
Erythromycin	58% (25)	NPP*
Tetracycline	53% (23)	71% (5)
Doxycycline	56% (24)	71% (5)
Clindamycin	NPP*	57% (4)

Note(s): *NPP = Not present in MYCOFAST® RevolutioN 2 panel

results (Figure 1b). The concordance rates between MYCOFAST® RevolutioN 2 test and PCR for *M. hominis* and *Ureaplasma sp.* were 97.7 and 84%, respectively.

Sensitivity, specificity, positive predictive value and negative predictive value of MYCOFAST® RevolutioN 2 test

Considering PCR as gold standard, the sensitivity, specificity, PPV and NPV of MYCOFAST® RevolutioN 2 test were 33.3, 98.8, 28.6, 98.9 and 37.7%, 96.5, 74.4, 85.2% for *M. hominis* and *Ureaplasma sp.*, respectively. The accuracy of the MYCOFAST® RevolutioN

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2 test in detecting *M. hominis* and *Ureaplasma sp*. was 95.8%–99% (95% CI) and 80%–87% (95% CI), respectively.

Results of high vaginal swabs by BD Affirm molecular assay for vaginosis/vaginitis agents
Out of 102 samples that were positive for GM in PCR, 34 were positive for *Gardnerella*, 22 were positive for *Candida* and ten cases showed concomitant infections with *Candida* and *Gardnerella*. None of these samples had positivity for *Trichomonas*. Results are described in Table 2.

Co-occurrence/association of genital mycoplasmas with main etiological agent of vaginosis (Gardnerella vaginalis) and fungal vaginitis (Candida)
On analysing the PCR results with BD Affirm assay, a significant co-occurrence of GM was noticed with *Gardnerella vaginalis*. As shown in Table 2, 23 (27%; 23/85) *Ureaplasma sp.,* three (50%; 3/6) *M. hominis* and eight (38%; 8/21) *M. genitalium* were also positive for *Gardnerella vaginalis*. Whereas, co-occurrence of *Candida* was shown in 19 (22.4%) *Ureaplasma sp.,* one (17%) *M. hominis* and one (5%) *M. genitalium* positive samples (Table 2).

DNA sequencing
Of the representative amplicons that were sent for commercial sequencing, some failed the QC check, whereas others passed. Blast search analysis of the generated sequences of *Ureaplasma sp*. confirmed them as *Ureaplasma parvum/Ureaplasma urealyticum* (majority being *Ureaplasma parvum*), and the analysis of *M. genitalium* sequencing results confirmed them as *M. genitalium*. Unfortunately, the sequencing for *M. hominis* amplicons failed due to some technical reasons, and we could not send samples again for re-sequencing due to financial constraints. Phylogenetic analyses of the sequences of *Ureaplasma sp*. and *M. genitalium* demonstrated genetic variations (Figure 3a and b).

The representative sequences were submitted and published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with Accession Number ON755316, ON755317, ON755319 for *Ureaplasma parvum* and ON755318 for *Ureaplasma urealyticum*.

Discussion

GM are often isolated from female genital tracts. Although their presence (e.g. *M. hominis*) as a normal flora makes the determination of their pathogenic roles more difficult, several studies have found that vaginal colonisation with these organisms is linked to an increased

Table 2. The results of BD Affirm molecular assay for *Gardnerella*, *Candida* and *Trichomonas* in 102 PCR positive samples for GM, and co-occurrence of GM with *Gardnerella* and *Candida*

BD Affirm molecular assay positivity for	No. of positive samples (%)
Candida	22 (21.6%)
Gardnerella	34 (33.3%)
Candida + *Gardnerella*	10 (9.8%)
Trichomonas	0 (0%)
Co-occurrence of *Gardnerella* with GM	
Gardnerella + *Ureaplasma sp.*	23 (27%)
Gardnerella + *M. hominis*	3 (50%)
Gardnerella + *M. genitalium*	8 (38%)
Co-occurrence of *Candida* with GM	
Candida + *Ureaplasma sp.*	19 (22.4%)
Candida + *M. hominis*	1 (17%)
Candida + *M. genitalium*	1 (5%)

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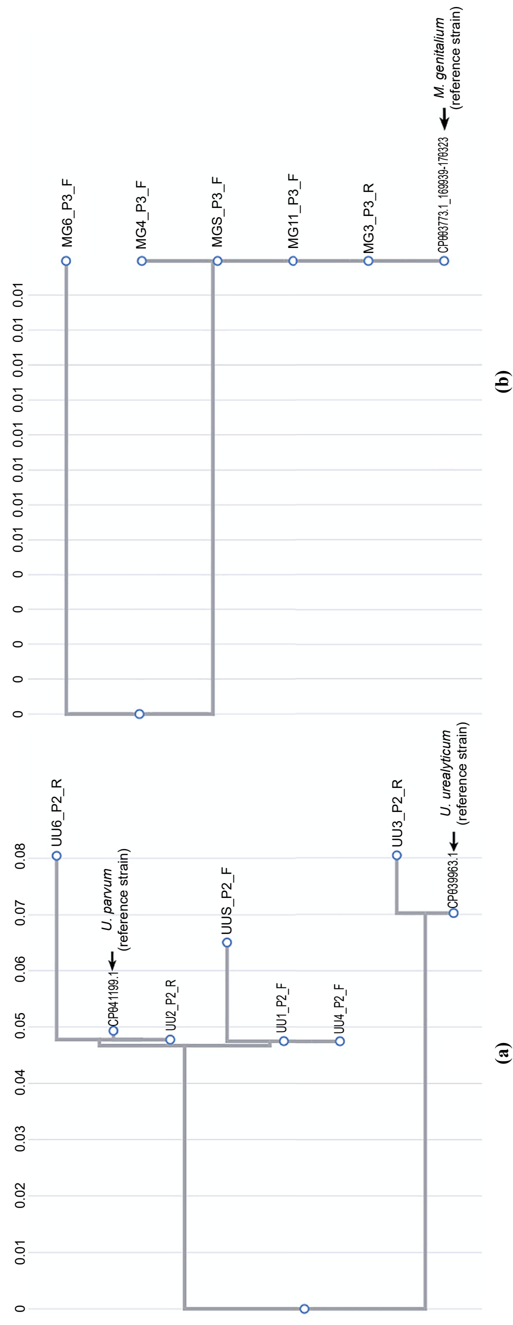

Figure 3.
(a) Dendrogram showing clonal relation/diversity in our representative sequences of *Ureaplasma sp.* analysed against *U. parvum* and *U. urealyticum* reference sequences (b) Dendrogram showing clonal relation/ diversity in our representative *Mycoplasma genitalium* analysed against *M. genitalium* ref. sequence

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risk of developing certain pathogenic conditions and linked to pregnancy abnormalities (Sarier *et al.*, 2018). With a growing body of research showing that GM can be clinically significant in some conditions, a reliable and sensitive laboratory approach for their detection is needed (Clegg, Passey, Yoannes, & Michael, 1997). Moreover, the burden of this clinical entity (infection with GM) is not reflected in published literature from our geographic region.

So, from this standpoint, we decided to screen for the prevalence of genital mycoplasmas in adult females in Kingdom of Bahrain who visited hospital with complains of vaginal discharge, firstly, to have a preliminary insight into this health burden of GM occurrence in this geographic region, and secondly, in comparing between a commercial test kit (MYCOFAST) and PCR for detection of these organisms. Moreover, we intended to analyse the co-occurrence of mycoplasmas with *Gardnerella vaginalis* and *Candida*, the organisms primarily responsible for BV and fungal vaginitis, respectively.

In our study, a total of 102 (25.4%) samples were positive either for *M. hominis*, *M. genitalium* or *Ureaplasma sp.* by PCR. For all practical purposes, considering PCR as the gold standard, we found the prevalence of *M. hominis*, *M. genitalium* and *Ureaplasma sp.* as 1.5, 5.2 and 21.2%, respectively. We could not find any published study from Bahrain for comparing our results; however, there were fragmentary studies from the nearby Middle Eastern regions. For instance, a study from Makkah reported the prevalence of 14.1% for *M. genitalium*, and 9.6% for *U. urealyticum/parvum* (Ashshi *et al.*, 2015). A study from Lebanon reported a prevalence of 35% for *Ureaplasma parvum*, 4.6% for *Ureaplasma urealyticum*, 3.6% for *Mycoplasma hominis* and 0.2% for *Mycoplasma genitalium* (Hanna *et al.*, 2020). Another study from Iran reported a prevalence of 23.2% for *M. genitalium* (Mohseni Moghadam, Kheirkhah, Mirshekari, Fasihi Harandi, & Tafsiri, 2014). The results of our study, and the published results from the nearby regions (of the Middle East), reflect a geographical variation in the prevalence of GM, which is obvious.

In MYCOFAST® RevolutioN 2 test, which is a commercial kit based on culture and colorimetric test, of all 401 samples, only 43 (10.7%) samples gave positive results. In comparison to PCR, we got comparatively lower detection rate with this test. Researchers have tried comparing between conventional culture and PCR methods for sensitive and precise identification of GM (de Barbeyrac *et al.*, 1993), and inferred that despite culture being considered gold standard, PCR enabled speedy and accurate identification of GM (Frolund, Bjornelius, Lidbrink, Ahrens, & Jensen, 2014; Sarier *et al.*, 2017). Moreover, culture requires specialised laboratories and has human subjectivity. PCR is therefore extensively employed in laboratories with better experience as opposed to culture (Kusanovic *et al.*, 2020).

It is to be emphasised here that the PCR we used in our study could detect the three types of GM, namely, *M. hominis*, *M. genitalium* and *Ureaplasma sp*. This was in contrast with MYCOFAST® RevolutioN 2, which could not detect *M. genitalium*. Redelinghuys *et al.* reported 77 and 80% sensitivity and specificity of MYCOFAST® RevolutioN 2, respectively. They assumed that the MYCOFAST® Revolution 2 assay could be a cost-effective alternative to traditional culture methods, and laboratory employees may be able to offer clinicians with the results in shorter period (Redelinghuys *et al.*, 2013). However, in our study, the sensitivity, specificity, PPV and NPV of MYCOFAST® RevolutioN 2 test were 33.3, 98.8, 28.6, 98.9 and 37.7%, 96.5, 74.4, 85.2% for *M. hominis* and *Ureaplasma sp.*, respectively. On the other hand, the accuracy of the MYCOFAST® RevolutioN 2 test in detecting *M. hominis* and *Ureaplasma sp*. was 95.8%–99% (95% CI) and 80%–87% (95% CI).

Based on MYCOFAST® RevolutioN 2 test, the *Ureaplasma* a *sp.* isolates showed resistance to the tested antibiotics ranging from 53%–58%, and *M. hominis* were resistant, ranging from 57%–86%. This was comparable with the global reports on antibiotics susceptibility of GM. The published studies have reported that the antimicrobial resistance of mycoplasmas, especially against Macrolides, Fluoroquinolones and Tetracyclines antibiotics (Bebear *et al.*, 2000; Lendamba, Mbeang Nguema, Onanga, & Mombo, 2022), has increased in previous

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years. Therefore, minocycline and josamycin are recommended as a new primary choice in anti-mycoplasmas treatment (Lee & Yang, 2020).

In our study, we also compared our PCR results of the positive cases for GM with other vaginitis and vaginosis-related microorganisms like *G. vaginalis, Candida* and *Trichomonas*, which were screened for by BD Affirm probe assay. There was a significant corrdiversity in our repr between GM and *G. vaginalis*, i.e. approximately 27% of *Ureaplasma sp.,* 50% of *M. hominis* and 38% of *M. genitalium* harbouring cases were also positive for *Gardnerella vaginalis*. Similar results have been reported in recent past where a strong association between BV and enhanced susceptibility to *M. genitalium* was suggested (Lokken *et al.*, 2017). In a recent study, Nye, Harris, Pherson and Cartwright (2020) also suggested a strong association between M. genitalium infection and BV in symptomatic females with vaginitis.

For *Candida*, 22.4% of *Ureaplasma sp.,* 17% of *M. hominis* and 5% of *M. genitalium* harbouring cases were also positive for *Candida* infection. Interestingly, we noticed that the GM were more associated with *G. vaginalis* than *Candida*.

The presence of *Ureaplasma* in the lower genitourinary tract samples equalling 10^5 CCU/ml is usually considered as the load indicating an infection that should be treated with antibiotics. Ureaplasma colonisation is thought to occur in low quantities in all lower genitourinary tract specimens (urine, vaginal swabs) and is considered as a normal flora (Kokkayil & Dhawan, 2015). Determining load of *Ureaplasmas* in upper genitourinary tract is not essential as it is always regarded as an infection (Kasprzykowska, Elias, Elias, Maczynska, & Sobieszczanska, 2014). In our study, we got about 38 (88%) *Ureaplasma* that showed growth of 10^5 CCU/ml, and only five (12%) samples gave growth only of $10^3/10^4$ CCU/ml, which indicates that most of our *Ureaplasma* species found were true infections.

In nutshell, we found a cumulative prevalence of 25.4% for GM in our study population; the prevalence of *Ureaplasma* sp. was significantly high as opposed to other GM. Sequencing and BLAST analyses demonstrated predominance of *U. parvum* in our study. The genetic variation in the derived sequences of *Ureaplasma* and *M. genitalium* is worrisome. MYCOFAST® RevolutioN 2 test showed lower sensitivity in detecting *Ureaplasma* sp. and *Mycoplasma hominis*, than PCR. Since MYCOFAST® RevolutioN 2 test has limitations in detecting *M. genitalium,* which can be detected by PCR, and that it has lower sensitivity than PCR, we suggest performing both MYCOFAST® RevolutioN 2 and PCR tests for accurate diagnosis. Moreover, the usage of MYCOFAST® RevolutioN 2 along with PCR is important because MYCOFAST® RevolutioN 2 test will reflect on antibiotics susceptibility of the isolate, which has an added therapeutic advantage. We found a significant co-occurrence of mycoplasmas with BV in our study population. To the best of our knowledge, this is the first report from the Kingdom of Bahrain reflecting on burden of GM from this geographic location.

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Ethical approval and consent to participate: All methods were carried out in accordance with relevant guidelines and regulations. The study was approved by the Research and Ethics Committee, CMMS, Arabian Gulf University (E039-PI-4/19) and by the Ministry of Health, Kingdom of Bahrain (AURS/ 403/2020). Written informed consent was obtained from all subjects and/or their legal guardian(s).

Consent for publication: Not applicable.

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