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Microbiology Section

Prevalence and Antibiotic Susceptibility Patterns of *Mycoplasma hominis* and *Ureaplasma urealyticum* in Females with Genital Infections from Central Kerala, India

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ABSTRACT

Introduction: Genital mycoplasmas namely *Mycoplasma Hominis* and *Ureaplasma Urealyticum* are sexually transmitted disease pathogens which are associated with genital infections, infertility, obstetric and neonatal complications. The pathogenesis, prevalence, epidemiology and antibiotic resistance of genital mycoplasmas in Indian women have been studied very minimally.

Aim: To determine the prevalence and antibiotic susceptibility pattern of *M.hominis* and *U.urealyticum* among females presenting with genital tract infections

Materials and Methods: The hospital-based descriptive cross-sectional study was carried out among 120 females of reproductive age group (15-49 years) attending Outpatient Department of Obstetrics and Gynaecology of a tertiary care centre in central Kerala, India, from January 2018 to December 2018. Endocervical/vaginal/urethral samples were collected depending on the clinical presentation. Detection, quantitation and antibiotic sensitivity pattern of *M.hominis* and *U.urealyticum* were performed using Mycoplasma IST 2 Kit. Data was analysed using Statistical Package for the Social Sciences (SPSS) version 11.5 and statistical association was analysed using Chi-square test.

Results: The prevalence of genital mycoplasmas was 25.83%. *U.urealyticum* infection was present in 20 (20.8%) and *M.hominis* infection was present in 12 (10%) of the patients. Age group of 26-35 years was commonly affected. All isolates showed high susceptibility rates to doxycycline, josamycin and pristinamycin. Intermediate to high resistance was noted to commonly prescribed macrolides and quinolones.

Conclusion: Genital mycoplasmas are prevalent in one-fourth of the study population with *U.urealyticum* infections being more common than *M.hominis* infections. The increased resistance rates to quinolones and macrolides warrants the need for routine screening and antibiotic susceptibility testing of these pathogens.

Keywords: Antibiotic resistance, Macrolides, Mycoplasma IST 2 kit, Quinolones

INTRODUCTION

Genital mycoplasmas are eubacteria belonging to the class Mollicutes which lack a rigid cell wall. Among them *Mycoplasma genitalium*, *M.hominis*, *U.urealyticum* and *Ureaplasma parvum* have emerged as common causes of Sexually Transmitted Infections (STI) both in developed and developing countries [1]. These organisms frequently colonise female urogenital tract and causes genital infections such as urethritis, cervicitis, vaginitis, Pelvic Inflammatory Disease (PID) and bacterial vaginosis. They also play a role in infertility, abortions, preterm premature rupture of membranes, low birth weight infants, postpartum endometritis with septicaemia and chorioamnionitis [2]. The relevance of screening, accurate laboratory detection and treatment of genital mycoplasma infection is also highlighted by the increasing antibiotic resistance.

There is paucity of adequate data regarding the prevalence and antimicrobial sensitivity patterns of genital mycoplasma in Indian women. Review of available literature showed prevalence of 31-44% for *U.urealyticum* infections and 14% for *M.hominis* infections [3]. Conventional culture and antibiotic susceptibility testing is rarely performed on clinical specimens due to the fastidious nature, specialised growth requirements of the organism and lack of lab infrastructure. Management of suspected infections is based on syndromic approach [4]. However, commercial broth based kits like Mycoplasma IST 2 (IST2), Mycoplasma IES and Mycofast Revolution (REV) kits enable simultaneous identification, indicative enumeration and antibiotic susceptibility testing of urogenital mycoplasma isolates. These assays are simple, rapid and accurate with high sensitivity and specificity [5].

This study was undertaken in a tertiary care centre in Central Kerala to determine the prevalence and antibiotic susceptibility pattern of *M.hominis* and *U.urealyticum* in female genital infections using a commercial broth based Mycoplasma IST 2 Kit. This study will help to understand the burden of genital mycoplasma infection in this population and local antibiotic resistance pattern which in turn facilitate the clinicians to streamline the treatment strategy.

MATERIALS AND METHODS

The hospital-based cross-sectional study was carried out in the Department of Microbiology and Department of Obstetrics and Gynaecology, Government Medical College, Thrissur, Kerala, India, from January 2018 to December 2018. The study was conducted after the approval and clearance from Institutional Research Committee and Ethical Committee (B6-8772/2016/MCTCR).

Inclusion criteria: Female patients in the age group 15-49 years presented with symptoms of genital tract infection such as abnormal vaginal discharge (increased/malodorous), dysuria, vaginal pruritus, dyspareunia and lower abdominal pain attending Gynaecology Outpatient Department were enrolled in the study.

Exclusion criteria: Pregnant women, patients with severe co-morbidities like malignancy, patients with history of antibiotic consumption in the previous four weeks were excluded from the study. Those patients diagnosed with other etiological diagnosis such as bacterial vaginosis, gonorrhoea and candidiasis were also excluded.

Sample size calculation: A sample size of 120 was obtained using the formula:

$$n=\frac{Z^2 \times pq}{d^2}$$

where 'p' is the prevalence; q=100-p; d=allowable error=20% of p, assuming the prevalence rate of genital ureaplasma as 44.5% from a previous study [3].

Written informed consent was obtained from each study participant and all patient details were kept confidential. Socio-demographic details and clinical history were recorded with the help of a proforma. Two calcium alginate swabs were used for collecting either cervical or vaginal or urethral samples from each patient depending on clinical diagnosis. This was done avoiding contamination with urine and external parts of reproductive system using a speculum. Detection, quantitation and antibiotic sensitivity pattern of *M.hominis* and *U.urealyticum* were performed using Mycoplasma IST 2 Kit (bioMerieux, Hazelwood, MO). Gram smears were also prepared to assess the presence of pus cells, evidence of candida and bacterial vaginosis.

Principle and Procedure of the Mycoplasma IST 2 Test Kit

Mycoplasma IST 2 Test Kit combines a selective Urea-Arginine culture broth with a strip containing 22 tests. The broth provides optimum growth conditions for Mycoplasma and Ureaplasma. The test was performed following manufacturer's instructions. The collected swabs were immediately placed in the Mycoplasma R1 broth incorporating the stable nutrients and selective agents and transported to the laboratory. After mixing, 3 mL of R1 solution was transferred in to the vial of R2 containing urea-arginine broth. A 55 µL of the reconstitiuted broth was dispensed into each of the 22 test cupules on the Mycoplasma IST 2 Kit strip using micropipette. Two drops of mineral oil was added to prevent drying out. The inoculated strip and the broth remaining on the R2 vial was incubated for 24 hours and 48 hours respectively at 36±2°C.

If a culture broth was positive, specific substrates (Urea for Ureaplasma and Arginine for Mycoplasma) were decomposed by enzymatic activity and phenol red indicator present in broth change colour from yellow to red due to an increase in pH. If there was red colour development at the cupules indicated for enumeration, it showed that the inoculum contains more than 10^4 colony forming unit (cfu).

The antibiotic susceptibility testing was also performed against nine antibiotics from classes of tetracyclines, fluoroquinolones, macrolides and streptogramin, each one in two concentrations based on Clinical Laboratory Standards Institute (CLSI) breakpoints [6]. The antibiotics used with their concentrations (mg/L) were as follows: tetracycline (4,8), doxycycline (4,8), ciprofloxacin (1,2), josamycin (2,8), ofloxacin (1,4), erythromycin (1,4), azithromycin (0.12,4), clarithromycin (1,4) and pristinamycin (2). Interpretation was based on the colour change of the two cupules for each antibiotic used in the strip. If both the cupules were yellow, it suggested that the organism was susceptible to the antibiotic. If cupules were red, it was suggestive of growth in the presence of antibiotic and hence a resistant result. If one cupule was yellow and the other was red, it was indicative of intermediate resistance [Table/Fig-1].

Genital mycoplasmas	Positive n (%)	Negative n (%)	Total n (%)
U.urealyticum	25 (20.8)	95 (79.2)	120 (100)
M. hominis	12 (10)	108 (90)	120 (100)
U.urealyticum alone	19 (15.8)	101 (84.2)	120 (100)
M. hominis alone	6 (5)	114 (95)	120 (100)
U.urealyticum-M. hominis coinfection	6 (5)	114 (95)	120 (100)

[Table/Fig-1]: Prevalence of genital mycoplasmas in study population.

STATISTICAL ANALYSIS

Data was analysed using Statistical Package for the Social Sciences (SPSS) version 11.5 and statistical association was analysed using Chi-square test. The p-value <0.05 was considered as statistically significant.

RESULTS

Among 120 females with genital infections enrolled in the study, *U.urealyticum* infection was present in 20.8% (25/120) and *M.hominis* infection was present in 10% (12/120) of the patients. Out of this *U.urealyticum-M.hominis* co-infection was noted in 6 (5%) patients. The overall prevalence of genital mycoplasmas was 25.83% (31/120) shown in [Table/Fig-1]. The age of the patients ranged from 19-44 years with mean age of 31.5±6.282 years. Most of the patients in which urogenital mycoplasmas isolated belonged to the reproductive age group, 26-35 years. Distribution of isolates in different age groups is shown in [Table/Fig-2]. Majority of the women, 94.2% (113/120) were married. Socio-demographic details and clinical history are depicted in [Table/Fig-3].

Age (years)	*U.urealyticum*	*M.hominis*	*M.hominis+U.urealyticum*	Total
15-20	0	0	0	0
21-25	1	1	0	2
26-30	7	3	2	12
31-35	10	1	2	13
36-40	1	1	2	4
41-49	-	-	-	-

[Table/Fig-2]: Distribution of genital mycoplasmas in different age groups.

Characteristics	Categories	Number (n)	Percentage (%)
Marital status	Married	113	94.2
	Not married	7	5.8
Parity	Nulliparous	42	35
	Uniparous	56	46.7
	Multiparous	22	18.3
Infertility		8	6.7
History of obstetric complications	Preterm labour	5	4.2
	Abortion	12	10
Symptoms	Vaginal discharge	115	95.8
	Vaginal pruritus	60	50
	Lower abdominal pain	23	19.2
	Dysuria	6	5

[Table/Fig-3]: Distribution of socio-demographic and clinical details (N=120).

Vaginal discharge was the commonest symptom (115/120, 95.8%) followed by vaginal pruritus (60/120, 50%) and lower abdominal pain (23/120, 19.2%). All patients positive for *U.urealyticum* and *M.hominis* complained of vaginal discharge. Genital infections included vaginitis, urethritis, PID, cervicitis and mixed infections. Distribution of genital mycoplasmas in genital infections along with statistical association is shown in [Table/Fig-4]. Genital mycoplasma infection showed significant association with cervicitis and PID with a p-value <0.05.

U.urealyticum isolates showed high sensitivity rates for pristinamycin (92%), josamycin (88%) and doxycycline (88%). This was followed by tetracyclines (76%), and ofloxacin (56%). High degree of resistance was observed for commonly prescribed antibiotics like ciprofloxacin, azithromycin and erythromycin. Ciprofloxacin resistance was 36% with 48% intermediate strains. A 32% isolates showed resistance to azithromycin with 20% intermediate strains. Only 40% of isolates were sensitive to erythromycin [Table/Fig-5].

Doxycycline (83.3%), pristinamycin (75%) and josamycin (75%) showed good efficacy against *M. hominis*. A 50% of *M. hominis* isolates were resistant to clarithromycin. Distribution of antibiotic resistance pattern is shown in [Table/Fig-5].

Genital infection (Total number)	*U.urealyticum* (n=25)		Statistical association using Chi-square test	*M.hominis* (n=12)		Statistical association using Chi-square test
	Present n (%)	Absent n (%)	p-value	Present n (%)	Absent n (%)	p-value
Vaginitis (55)	10 (18.2)	45 (81.8)	0.511	5 (9.1)	50 (90.9)	0.760
Urethritis (31)	5 (16.1)	26 (83.9)	0.454	3 (9.7)	28 (90.3)	1.000
PID (23)	13 (56.5)	10 (43.5)	<0.001	5 (21.7)	18 (78.3)	0.037
Cervcitis (11)	7 (63.6%)	4 (36.4%)	0.001	4 (36.4)	7 (63.6)	0.011

[Table/Fig-4]: Distribution of genital mycoplasmas in genital infections.
n: Number; PID: Pelvic inflammatory disease

Antibiotic	MIC breakpoints (mg/mL)*		*M.hominis* (n=12) Number (percentage)			*U.urealyticum* (n=25) Number (percentage)		
	S	R	S	I	R	S	I	R
Doxycycline	≤4	≥8	10 (83.3)	0	2 (16.7)	22 (88)	0	3 (12)
Tetracycline	≤4	≥8	8 (66.6)	2 (16.7)	2 (16.7)	19 (76)	4 (16)	2 (8)
Ciprofloxacin	≤1	≥2	0	6 (50)	6 (50)	4 (16)	12 (48)	9 (36)
Ofloxacin	≤1	≥4	4 (33.3)	4 (33.3)	4 (33.3)	14 (56)	8 (32)	3 (12)
Erythromycin	≤1	≥4	0	4 (33.3)	8 (66.7)	10 (40)	7 (28)	8 (32)
Azithromycin	≤0.12	≥4	3 (25)	2 (16.7)	7 (58.3)	12 (48)	5 (20)	8 (32)
Clarithromycin	≤1	≥4	2 (16.7)	4 (33.3)	6 (50)	11 (44)	9 (36)	5 (20)
Josamycin	≤2	≥8	9 (75)	2 (16.7)	1 (8.3)	22 (88)	1 (4)	2 (8)
Pristinamycin	≤2	≥2	9 (75)	0	3 (25)	23 (92)	0	2 (80)

[Table/Fig-5]: Antibiotic sensitivity pattern of *M.hominis* and *U.urealyticum*.
S: Sensitive; I: Intermediate; R: Resistant; MIC: Minimum inhibitory concentration;
*MIC values between S and R are considered intermediate

DISCUSSION

Out of the 120 women studied, the prevalence of urogenital mycoplasmas (*M. hominis, U. urealyticum* and *U.urealyticum-M. hominis* coinfection) was 26%. *U. urealyticum* infection rate (20.8%) was more than *M. hominis* infection (10%). Different studies conducted in Indian women with genital infections showed a prevalence ranging from 31-44.5% in case of *Ureaplasma species* and 14% for *M.hominis* [3]. The first study done at All India Institute of Medical Sciences, New Delhi in India by Kapur TR et al., using culture on 70 married women with leucorrhea showed a prevalence of 31.5% for *Ureaplasma sp* [7]. Bhatt R et al., reported prevalence of 14.2% for *M.hominis* and 38.6% for *Ureaplasma sp* in women with genital tract infections [8]. Study conducted using Multiplex PCR by Dhawan B et al., showed prevalence of *Ureaplasma sp* and *M.hominis* as 31% and 14.7%, respectively where as that conducted by Saigal K et al., showed 15.2% *(U.urealyticum)* and 5.4% *(M.hominis)* [9,10]. Present study was the first Indian study to utilise commercial broth based diagnostic kits to determine prevalence of genital mycoplasma infections among females to the best of our knowledge.

Prevalence reported internationally varies between 3.9-31% in Mexico, 44.8% in China and 54.9% in Turkey [11]. High prevalence was also noticed in studies conducted at Portugal and Papua New Guinea [12]. Study by Skiljevic D et al., utilising Mycoplasma IST 2 kit demonstrated a prevalence of only 14.4% in Serbia whereas a similar study conducted among Chinese females using a commercial mycoplasma strip observed a high prevalence of 62.16% [12,13]. Geographical variation in prevalence can be attributed to difference in socio-cultural factors, sexual behaviour and laboratory diagnostics utilised. Prevalence rates of genital mycoplasmas from different regions are summarised in [Table/Fig-6] [7-13].

Author name (Reference)	Region	Prevalence (Percentage)	
		Mycoplasma Hominis	*Ureaplasma urealyticum*
Kapur TR et al., [7]	New Delhi, India	Not done	31.5
Bhatt R et al., [8]	Bombay, India	14.2	38.6
Dhawan B et al., [9]	New Delhi, India	14.7	31
Saigal K et al., [10]	New Delhi, India	5.4	15.2
Díaz L et al., [11]	Cuba	4.3	68.9
Skiljevic D et al., [12]	Serbia	22.9	77.8
Zhu C et al., [13]	China	1.71	46.52
Present study	India	10	25

[Table/Fig-6]: Prevalence rates of genital mycoplasmas from different studies [7-13].

The prevalence rate was comparatively low in our study population when compared with most of the studies from India [7-9]. This could be due to the smaller sample size of the study as well as better awareness about reproductive tract infections, improved hygiene practices and treatment seeking behavior among the patients in central Kerala. As there is no pervious published data regarding the prevalence of genital mycoplasmas from the same area, further large scale studies are needed to assess the prevalence, epidemiology and risk factors. High prevalence of *U.urealyticum* when compared with *M. hominis* infection was consistent with other studies reported from India and across the world. Ureaplasma was considered to be the predominant organism by both Bhatt R et al., and Bhandari H et al., with a prevalence of 38 percent [8,14]. Our results were similar to those obtained by Elias M et al., who in the group of 222 women in a similar age range found *U. urealyticum* in 31.8% and *M. hominis* in only 3% of the cases [15].

High prevalence was noted in the age group of 26-35 years which was consistent with literature data [16,17]. In a study by Zdrodowska-Stefanow B et al., sexual Mycoplasma infections were reported among patients attending STI clinic in the age group 26-30 years [18]. Higher prevalence in this age group may be related to the sexual activity during this period. But, according to a study conducted in Korea, the prevalence of genital mycoplasmas was high in 15-19 year age groups and it was attributed to the higher sexual activity among adolescents [19].

Many studies in India and across the world have already reported the association of cervicitis and PID with mycoplasm infection as observed in the present study [20-22]. This observation emphasises the importance of screening mycoplasma in patients with cervicitis and PID in our population.

Mycoplasmas are normally susceptible to antibiotics that inhibit protein synthesis and intrinsically resistant to cell wall acting agents due to the absence of the latter. Increasing antibiotic resistance among them had been reported from several Indian and International studies [5,10,19,22,23]. Doxycycline can be considered as drug of choice for treating genital mycoplasma infection in our setting as more than 80% of the isolates were sensitive but tetracyclines showed only moderate sensitivity. Tetracycline resistance due to acquisition of tet M gene is widely reported. Skiljevic D et al., reported high tetracycline resistance of 100% and 86.5% in *M.hominis* and *U.urealyticum* respectively in Serbian population where as Tunisian study reported 22.7% and 25% among *U.urealyticum* and *M.hominis* [12,22].

High level resistance rule out the option of treating genital mycoplasma infection with fluoroquinolones in our setting. This could be attributed to the frequent prescription of quinolones for respiratory and urinary tract infections. The mechanism of resistance is probably the occurrence of a target alteration located in the DNA gyrase and topoisomerase intravenous subunits. Ofloxacin was

better choice compared to ciprofloxacin for *U.urealyticum* infections. Moderate to high fluoroquinolone resistance has been reported from many studies across the globe [5,10,19]. In a study conducted in Hangzhou, the resistance rates to ciprofloxacin and ofloxacin were 75.2% and 53.2% respectively for *U.urealyticum* [23].

M. hominis exhibit natural resistance to macrolides but sensitive to 16 membered macrolide, josamycin. Acquired résistance to macrolides is widely reported in *U.urealyticum* strains [24]. Less than half of the *U.urealyticum* strains were sensitive to erythromycin, azithromycin and clarithromycin. The less prescribed josamycin was the only best option among macrolides in the present study. In a study conducted by Saigal K et al., to determine the susceptibility of Ureaplasma, all isolates of Ureaplasma were susceptible to ofloxacin and josamycin, an intermediate level resistance towards doxycycline and azithromycin was noted in 4% and 8% of strains respectively while all *M. hominis* strains were found to be susceptible to ofloxacin and josamycin [10]. But Dhawan B et al., reported intermediate resistance to ofloxacin and azithromycin in Ureaplasma isolates [9]. High resistance rates among the study population compared to these studies point towards the importance of routine antibiotic susceptibility testing for these pathogens.

Limitation(s)

Inability to detect other important pathogens such as *Mycoplasma genitalium and Ureaplasma parvum* was the major limitation of the study. Socio-demographic factors and epidemiological risk factors were not assessed in detail. Further analytical studies involving more study subjects is the need of the hour.

CONCLUSION(S)

The prevalence of genital mycoplasma infection in almost one-fourth of study population suggests that the sexually active female population should be screened for these silent pathogens to prevent their spread and long term complications thus boosting the reproductive health programme implementation in this area. Though there is a global concern regarding the emergence of antibiotic resistance among gram positive and gram negative organisms, study of increasing resistance among these silent pathogens associated with numerous infectious diseases of public health importance is not given adequate importance. High resistance rates to quinolones and macrolides warrants the need for routine antibiotic susceptibility testing of these pathogens. Knowledge of local patterns of antimicrobial resistance facilitate clinicians to choose best treatment options for the patients.

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