Characteristic Description of Multiparous Women with *Mycoplasma hominis* and *Ureaplasma urealyticum* Infection at Outpatient Clinic in Medan

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Abstract: Among the organisms that cause genital infection are *Mycoplasma hominis* and *Ureaplasma urealyticum* which are commensally in women urogenital but may become pathogens and were associated as a cause of complications of genital tract infection in pregnant women, such as ascending chorioamnionitis, premature rupture of membranes, preterm birth, miscarriage, weight and neonatal birth low and newborn deaths..The objective of this study was to describe the characteristic of multiparous women with *Mycoplasma hominis* and *Ureaplasma urealyticum* infection. Design of the study was a descriptive study with cross-sectional approach by collecting cervical swab of 50 multiparous women from outpatient clinic in Medan. Duplex PCR assay was perfomed using two primers: RNAH1 and RNAH2 that amplify the 16sRNA *M. Hominis* gene at 334bp; UMS125 and UMA226 amplifying serovar3 genes multiple banded antigen which can amplify biovar1 that appeared at 403bp and biovar2 appeared at 448bp. The result of this study showed *Mh-Uu* duplex PCR results revealed that 3(6.0%) respondents were positive Mycoplasma infection and 9(18%) respondents were positive Ureaplasma infection and 5(10.0%) from infected respondents had abortion history.

1 INTRODUCTION

Mycoplasma hominis and Ureaplasma spp. is a commensal organism that found in 30-80% women's urogenital tract, thus causing urogenital tract infection. In pregnant woman, these organisms could stay in utero and then transmission by placenta to the fetus, causing several infections and trigger premature labor (Otgonjargala, 2017). In rare cases these microorganisms may infect the central nervous system in healthy neonates and present a risk of severe complications and poor prognosis (Wildenbeest, 2016). The role of these pathogens in women with chronic urinary tract symptoms remains a problem due to difficult to detect and its intracellular nature makes conventional antibiotics ineffective (Nasution, 2007).

Polymerase Chain Protein (PCR) analysis of these bacteria should be performed if symptomatic sterile leukocytosis is present, chronic urethritis and bladder hyperactivity or interstitial cystitis/ painful bladder syndrome, recurrent infections or if microbiological culture is negative (Combaz-Söhnchen, 2017). The difficulty of detecting *M. hominis* on persistent neonatal CNS infections with unknown causes requires diagnostic protocols using a specific real-time PCR. Physicians should be aware of the pathogens as possible causes of neonatal meningoencephalitis if corrective failure is found in empirical antibiotic treatment (Wildenbeest, 2016).

A study by Manhart et al, 2003, found that from 719 young women *Mycoplasma genitalium* was detected as much as 50 (7%) in a sample previously negative for bacterial vaginosis. Nasution et al, 2007, in 40 Malaysia women, used duplex PCR Mh / Uu method (*Mycoplasma hominis/ Ureaplasma urealyticum*) and showed that Ureaplasma is the most commonly discovered pathogen (positive in 90.5% of women and 47.5% of newborns), followed by Mycoplasma (32.5% and 7.5%), and the rest are chlamydia, trichomonas and gonococcus.

The purpose of this study is to describe the characteristic of multiparous women with

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Mycoplasma hominis and *Ureaplasma urealyticum* infection detected by using duplex PCR method.

2 METHOD

2.1 Study Design

The study design is cross sectional with observational approach.

2.2 Study Area

This study was conducted at an outpatient clinic in Medan North Sumatera in 2018.

2.3 Sampling

Protocol of this study has been approved by Medical Ethics Committee Universitas Sumatera Utara (No.375/TGL/KEPK FK USU-RSUP HAM/2018)

2.2.1 Respondents Characteristics

Respondents were 50 female adolescents, multiparity, age above 18 years and signed the informed consent.

3 DATA COLLECTION

3.1 Socio-demographics Background

A questionnaire consist of social demographics, using contraception, first age of sexual intercourse and history of abortion was administered by self-reports of the participants,

3.2 Sample Collection and Laboratory Processing

3.2.1 DNA Extraction

Cervical swabs from 50 respondents on the micro tube contained 0.9% NaCl firstly centrifuged at a speed of 14,000rpm for 4 minutes. The precipitated portion is added to 1.5 ml of the PBS (phosphate buffer salin). After that tube were repeating centrifuged at a speed of 14.000 rpm for 4 minutes. Furthermore, a DNA isolation procedure is performed based on the protocol of the Invitrogen® kit. There are 200 µL sample inserted into another micro tube. Moreover, inside the tube was added 20 µL of proteinase K and 200 µL of lysis buffer, then vortexed for a few minutes. After that, the tube was incubated at 55°C for 10 minutes. Followed step is the tube were added 250 µL ethanol 96%, then vortexes for 15 seconds and move the fluid into the column spin. Then the column was centrifuged at 10.000 rpm for 2 minutes. After that, replace the collection tube, then washed with 500 µL wash buffer 1. Centrifuged at 10.000rpm for 2 minutes. Next step was replacing the collection tube then washed again with 500 µL wash buffer 2, centrifuge with maximum speed (14.000rpm) for 3 minutes. Finally, replaced the collection tube again and added 50 µL delution buffer, then tube was centrifuged at 14.000rpm for 1.5 minutes.

3.2.2 Mycoplasma hominis and Ureaplasma urealyticum Detection

M. hominis and *U.urealyticum* duplex PCR (Mh-Uu duplex PCR), using 2 primers: RNAH1 and RNAH2 that amplify the 16sRNA *M. hominis*gene at 334 bp; UMS125 and UMA226 amplifying serovar 3 genes multiple banded antigen which can amplify biovar1 that appeared at 403 bp and biovar 2 appeared at 448 bp. The amplification mixture was carried out in 12,5µl master mix PCR which consists of Taq polymerase enzyme, $MgSO_4$, and dNTP (Go Taq® PCR Core System, Promega); 7,5 µl nuclease-free water and 4µl DNA template. PCR was performed in a thermocycler (Verity 96-well Thermal Cycler, AppliedBiosystems) with an initial denaturation 94^{O}C for 1 minute 30 seconds, annealing in 55^{O}C for 2 minutes, extension for 1 minute 30 seconds and ending with a final extension step at 72^{O}C (Nasution 2007).

4 RESULTS AND DISCUSSION

4.1 Socio-demographics Background

In this study, respondents mostly women between 35-40 years old (66%), followed by women above 45 years old (22%) and the least under 35 years old (12%). Most respondents were housewife (58%) Respondents whose using contraception accounted for 26%. The first age of sexual intercourse was found mostly women between 26-30 years old (56%), following women between 20-25 years old (40%) and the least were under 20 years old (4%). The respondents whose having abortion history accounted for 23%.

Table 1: Socio-demographics characteristic

No	Socio-demographics characteristic	Number of Respondent (n=50)	%
1	Age		
	< 35 years	6	12.0
	35-40 years	33	66.0
	> 45 years	11	22.0
2	Occupation		
	Working woman	21	58.0
	Housewife	29	42.0
3	Use of contraception		
	Yes	13	26.0
	No	37	74.0
4	First age of sexual intercourse		
	< 20 years old	2	4.0
	20 – 25 years old	20	40.0
	26 – 30 years old	28	56.0
5	History of abortion		
	Yes	23	46.0
	No	27	54.0

4.2 Polymerase Chain Protein

Mh-Uu duplex PCR results revealed that 3(6.0%) respondents were *M. hominis* positive and 9(18.0%) respondents were *U. urealyticum* positive (Figure. 1)

Table 2: Distribution of detection Mycoplasma hominis (Mh) and Ureaplasma urealyticum (Uu) using Duplex PCR

	n	No (%) Detection	
		Mh	Uu
Age			
< 35 years	6	1(16.0)	2(33.0)
35-40 years	33	2(6.06)	4(12.1)
> 45 years	11	0	3(27.2)
Occupation			
Working woman	21	0	4(19.0)
Housewife	29	3(10.3)	5(17.2)
Use of contraception			
Yes	13	0	2(15.4)
No	37	3(8.1)	7(0.2)
First age of sexual intercourse			
< 20 years old	2	0	0
20 – 25 years old	20	1(5.0)	6(30.0)
26 – 30 years old	28	2(7.1)	3(10.7)
History of abortion			
Yes	23	3(13.0)	2(8.7)
No	27	0	7(25.9)

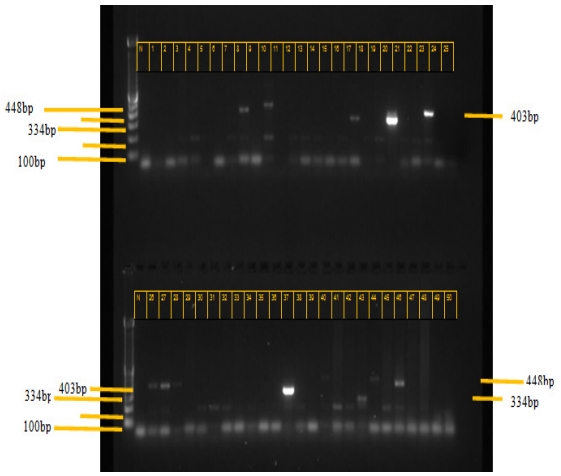

Figure 1: *Mh-Uu* duplex PCR results of 50 respondents

This study found that the respondents who had Mycoplasma infection were all housewives with the most 35-40 years old range. It is interesting that almost all respondents have had abortion; even one of the respondents had experienced abortion twice. While respondents who have Ureaplasma infection were most are housewives with the most age range 35-40 years old. Abortion history is also found in this group who was 2 respondents ever aborts 1 time.

This study was consistent with another previous study conducted in a cohort of females with bacterial vaginosi (BV) or asymptomatic, in which *U. urealyticum* infection was detected significantly more often than *M. Hominis* (Verteramo, 2013 and Padang, 2015)

The high prevalence of Ureaplasma spp. infection was also found according to previous study in Brazil with 6,810 patients with the age range of 11 to 80 years who in gynecological routine examination, found that *M. hominis* (n = 79), *Ureaplasma* spp. (n = 2,026) and the co-colonization of both (n = 199) which extract from cytological sample and detect by PCR (Milanezi, 2016). Another study in Iran using multiplex PCR to urine and genital samples from symptomatic females (20-54 years old), found that the highest incidence of *M. hominis* and *U. urealyticum* and were highly associated with habitual abortion in symptomatic females (Maleki, 2013). The inconsistent result found in case control study in Iran which conclude that no association between mycoplasma infection and spontaneous abortion (Ramazanzadeh, 2016).

5 CONCLUSION

This study has revealed that from 50 multiparous women, 3(6.0%) were positive Mycoplasma infection and 9(18.0%) were positive Ureaplasma infection and had abortion history. Further research is needed to explore whether the cause of their previous abortion was associated with this bacterial infection. Early screening is needed to detect bacterial infection of *M. hominis* and *U. urealyticum* in pregnant women with a history of preterm and premature rupture of membranes, thus decreasing the complication of this infection to newborns' morbidity and mortality

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