Original article

Prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* infection in female sex workers and its association with douching: A study in East Jakarta, Indonesia using *Mycoplasma System Plus*

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Abstract

**Background:** *Mycoplasma hominis* and *Ureaplasma urealyticum* are commonly involved in pathogenesis of bacterial vaginosis and non-specific genital infection, while doing vaginal douching also already known as predisposing factor.

**Methods:** To evaluate the prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* infection in female sex workers and its association with vaginal douching, we conducted a study through 180 female sex workers in East Jakarta, Indonesia. Vaginal fluid samples were collected from these women. It was revealed that most of the women used betel soap, baby soap, tooth paste, or herbal medicine as vaginal douching materials. The association of vaginal douching and prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* was analyzed using cross sectional statistical methods.

**Results:** There is a high prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* infection (72%), but there are no association between vaginal douching practice and infection of *Mycoplasma hominis* and *Ureaplasma urealyticum*.

**Conclusions:** The high prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* infection in female sex workers in East Jakarta warrants a routine screening of these infections. Unusual materials used for vaginal douching in this study might cause the negative association between high prevalence of these bacteria with vaginal douching.

**Keywords:** *Mycoplasma hominis*, *Ureaplasma urealyticum*, female sex workers, vaginal douching.

Introduction

*Mycoplasma hominis* (*M. hominis*) and *Ureaplasma urealyticum* (*U. urealyticum*) are often related with bacterial vaginosis (BV) and non-specific genital infection (NSGI) in women.\(^1\)\(^2\) Those bacteria also cause pelvic inflammatory disease, premature birth, and infertility.\(^3\)\(^4\) These bacteria could be found in sexually active women\(^5\) and the amount rises along with the number of sexual partners. The prevalence of *M. hominis* and *U. urealyticum* infection is higher in female sex workers (FSW) compared to normal population.\(^6\) Infection occurs when colonization of those bacteria found in culture is more than 10\(^4\) colony forming unit (CFU)/ml.\(^5\)\(^6\)\(^7\)\(^8\)

Casari et al.\(^7\) found that the presence of *U. urealyticum* in vaginal fluid more than 10\(^4\) CFU/ml is correlated with lower amount of Lactobacilli, causing imbalance of normal vaginal flora. There are doubts regarding the ability of *M. hominis* alone causing genital infection. *M. hominis* is a
co-bacteria in BV pathogenesis.\textsuperscript{6,9} While infection by \textit{U. urealyticum} alone can cause discharge in 43.3\% patients, itch in 18.9\% patients, and dysuria in 10.8\% patients.\textsuperscript{3}

The risk factors for \textit{M. hominis} and \textit{U. urealyticum} infection are multiple sex partners, a new sex partner in the last 6 months, commencement of sexual activity at an early age and unprotected sexual intercourse.\textsuperscript{10} Other risk factors are age less than 30 years old, low education level, poverty, vaginal douching, using IUD, and smoking.\textsuperscript{11-15} The risk of \textit{M. hominis} infection increase 1.3 times in women who practice vaginal douching. Other study showed vaginal douching practice in the past 1 week, would increase the risk of \textit{M. hominis} infection into 1.7 times.\textsuperscript{14} The prevalence of \textit{U. urealyticum}'s infection is higher (33\%) in women who practice vaginal douching.\textsuperscript{15} The frequency of douching might cause imbalance in vaginal flora, increasing vagina's pH level and thus increasing the risk of having \textit{M. hominis} and \textit{U. urealyticum} infection.\textsuperscript{16}

This study aimed to know the prevalence of \textit{M. hominis} and \textit{U. urealyticum} infection in female sex workers (FSW) and its association with vaginal douching. This study only analyzes vaginal douching as the risk factor. Other risk factors such as young age, poverty, and low education level were not analyzed in this study. IUD users in FSW are low or even none and not all FSW are smoker.

Both standard culture (pleuropneumonia-like organism/PPLO) and polymerase chain reaction (PCR) are currently used to detect \textit{M. hominis} and \textit{U. urealyticum}.\textsuperscript{17} PCR, method is more accurate than culture in detecting these bacteria, but the cost is more expensive.\textsuperscript{17,18} This study used Mycoplasma System Plus (MSP) (Liofilchem\textregistered), a culture based examination method. The advantage of MSP is easy to use, had lower cost as compared to PCR method and the result can be read in 1-2 days (quicker than standard culture). To obtain the result, the MSP materials need to be incubated for 18-24 hours, therefore it is not intended for mass screening.

This study was conducted as part of the Ministry of Health of Republic Indonesia survey program in East Jakarta. The survey program was focusing in antibiotic resistance of \textit{Neisseria gonorrhoeae}a in FSW.

**Material and Method**

A total of 180 FSWs were enrolled in this study from October until November 2012. Participants were recruited from 3 different areas in East Jakarta. All participants were low-paid undercover FSW. Exclusions were made to women who bled per vaginam, took metronidazole 1000 mg daily for 1 week or azithromycin 1 gr single dose or doxycycline 200 mg daily for 1 week. History were taken including epidemiological data, vaginal douching practices, symptoms, and treatments. Informed consent was taken prior to data collection.

**Sample collection and analyzes**

Vaginal samples were collected using speculum. Upon collection, the samples were stored in mycoplasma system broth for transportation and sent to Clinical Pathology Laboratory in Dr. Cipto Mangunkusumo Hospital within 24 hours to be processed. Samples were then seeded into MSP (Liofilchem\textregistered) and incubated at 37°C. Results were read after 24-48 hours. Infection of \textit{M. hominis} and \textit{U. urealyticum} were considered when the result showed amount of the bacteria more than 10\(^4\) CFU/ml. Data was then analyzed statistically with cross sectional design to find the association between infection prevalence and vaginal douching.

**Results**

**Sociodemographic data**

Sociodemographic characteristic of subjects in this study can be seen in table 1. The youngest FSW was 13 years old and the oldest was 55 years old. Mean age is 30 ± 8.7 years old.

Most FSW (22.4\%) aged between 25-29 years old. Based on marital status, most of FSW were married (67.3\%). About 46.1\% FSW had low education level (from no education until primary school). The results are similar to the Integrated Biological and Behavioral Surveillance (IBBS) 2011 on FSW in Indonesia.\textsuperscript{19,20}

**Characteristic of symptoms**

Symptoms experienced by subjects can be seen in table 2. Most often is vaginal discharge (experienced by 116 [64.4\%] FSW). About 35.6\% did not complain about vaginal discharge. From those 116 subjects, 42 subjects (37.1\%) reported fishy odor vaginal discharge.
Table 1. Sociodemographic data of female sex workers in East Jakarta (N=180)

| No. | Characteristic       | N* | Percentage (%) |
|-----|----------------------|----|----------------|
| 1   | Age group (years)    |    |                |
|     | <20                  | 17 | 9.4            |
|     | 20-24                | 32 | 17.7           |
|     | 25-29                | 40 | 22.2           |
|     | 30-34                | 34 | 18.9           |
|     | 35-39                | 31 | 17.3           |
|     | 40-50                | 24 | 13.4           |
|     | >50                  | 2  | 1.1            |
| 2   | Marital status       |    |                |
|     | Single               | 59 | 32.7           |
|     | Married              | 121| 67.3           |
| 3   | Education level      |    |                |
|     | No education         | 10 | 5.5            |
|     | Primary school       | 83 | 46.1           |
|     | Junior high school   | 61 | 33.9           |
|     | Senior high school   | 23 | 12.8           |
|     | Academy or university| 3  | 1.7            |

*N=Number of subjects

Table 2. Symptoms reported by subjects (N=180)

| Symptoms                                | N  | Percentage (%) |
|-----------------------------------------|----|----------------|
| Vaginal discharge                       | 57 | 31.7           |
| Asymptomatic                            | 54 | 30             |
| Fishy odor vaginal discharge            | 24 | 13.3           |
| Vaginal discharge + itch                | 12 | 6.8            |
| Fishy odor vaginal discharge + itch + dysuria | 8  | 4.5            |
| Itch                                    | 7  | 3.9            |
| Fishy odor vaginal discharge + itch     | 5  | 2.7            |
| Fishy odor vaginal discharge + dysuria  | 5  | 2.7            |
| Vaginal discharge + dysuria             | 5  | 2.7            |
| Dysuria                                 | 3  | 1.7            |
| Total                                   | 180| 100            |

*N=Number of subjects

Clinical findings
Clinical findings obtained in this study can be seen in table 3. Physical examination revealed that vulva inflammation were experienced by a small percentage of subjects which were erythema 3.3%, edema 0.6%, and erosion 2.8%. Vaginal erythema was observed in 18.3% subjects, erosion and edema found in 6.1% and 8.3% subjects respectively. Vaginal discharge is the most common symptom (86%). Consistency of vaginal discharge were mucopurulent (53%), seropurulent (43%), and serous (4%). The clinical features of the cervix on speculum examination were edema (69%), erythema (68%), erosion (36%). Cervical discharge were found in 79.4% subjects devided in mucopurulent (41.1%), seropurulent (33, 3%), and serous (5.6%) discharge.

Table 3. Clinical findings found on physical examination of female sex workers in East Jakarta (N=180)

| Clinical findings       | N  | Percentage (%) |
|-------------------------|----|----------------|
| Vulva:                  |    |                |
| - Erythema              | 6  | 3.3            |
| - Edema                 | 1  | 0.6            |
| - Erosi                 | 5  | 2.8            |
| Vagina:                 |    |                |
| - Erythema              | 33 | 18.3           |
| - Erosion               | 11 | 6.1            |
| - Edema                 | 15 | 8.3            |
| - Discharge             | 155| 86             |
| - Consistency           |    |                |
| - Mucopurulent          | 95 | 53             |
| - Seropurulent          | 77 | 43             |
| - Other (seroid)        | 8  | 4              |
| Cervix:                 |    |                |
| - Edema                 | 124| 69             |
| - Erythema              | 123| 68             |
| - Erosion               | 65 | 36             |
| - Discharge             | 143| 79.4           |
| - Consistency           |    |                |
| - Mucopurulent          | 74 | 41.1           |
| - Seropurulent          | 60 | 33.3           |
| - Other (seroid)        | 10 | 5.6            |

*N=Number of subjects

Prevalence of M. hominis and U. urealyticum infection
Table 4 showed the result of material culture of vaginal discharge embedded in Mycoplasma System Plus. Infection is established when the number of bacterial culture results were found more than $10^4$ CFU/ml. Out of 180 subjects, there were 130 subjects (72%) who were infected with M. hominis and U. urealyticum, while the remaining 50 subjects declared uninfected.

As additional information, the prevalence of M. hominis and U. urealyticum infection by age group can be seen in table 5. The table showed that prevalence of M. hominis and U. urealyticum infection were found mostly in the age group 25-29 years.
Table 4. Prevalence of *M. hominis* and *U. urealyticum* infection in female sex workers in East Jakarta

| Culture          | N (%) | Bacterial count in CFU/ML (%) |
|------------------|-------|-------------------------------|
|                  |       | Negative | <10<sup>4</sup> | 10<sup>4</sup>-10<sup>5</sup> | >10<sup>5</sup> |
| *M. hominis*     | 10 (5.6) | - | 5 (50) | 4 (40) | 1 (10) |
| *U. urealyticum* | 60 (44.4) | - | 15 (25) | 63 (78.6) | 2 (2.5) |
| *Mixed*          | 63 (35) | - | 18 (28.6) | 45 (71.4) | 15 (23.8) |
| *Negative*       | 27 (15) | 27 (15) | 3 (4.8) | - | - |
| Total            | 180 (100) | 27 (15) | 23 (12.8) | 112 (62.2) | 18 (10) |

*N=Number of subjects

In detail, in the age group less than 20 years as many as 15 subjects (11.5%) were infected by *M. hominis* and *U. urealyticum*, in the age group 20-24 years 24 subjects (18.5%), 25-29 years 28 (21.5%), 30-34 years 22 (17%), 35-39 years 25 (19.2%), 40-50 years 15 (11.5%) and more than 50 years 1 (0.8%).

Table 5. Prevalence of *M. hominis* and *U. urealyticum* infection or both (N=130) in female sex workers in East Jakarta, based on age group (years).

| Age group (years) | N | Percentage (%) |
|-------------------|---|----------------|
| <20               | 15 | 11.5           |
| 20-24             | 24 | 18.5           |
| 25-29             | 28 | 21.5           |
| 30-34             | 22 | 17             |
| 35-39             | 25 | 19.2           |
| 40-50             | 15 | 11.5           |
| >50               | 1  | 0.8            |

*N=the amount of subjects infected with *M. hominis* and or *U. urealyticum*

Relevance of vaginal douching with prevalence of *M. hominis* and *U. urealyticum* infection

Vaginal douching was performed by the majority of subjects (134 subjects (74.4%)). The frequency of vaginal douching varied between 1 to 31 times per week. Most of the subjects (106 subjects or 58.9%) douch 1 to 14 times per week. A total of 26 subjects (14.4%) douched 15 to 28 times per week, 2 subjects (1.1%) douched more than 28 times per week and 46 subjects (25.6%) did not douche (Figure 1). The materials used in douching were baby soap, toothpaste, betel soap, povidone iodine, albothyl® (policresulen), potassium permanganate solution and herbs. Many subjects used more than one type of material for vaginal douching.

Association between vaginal douching and prevalence of *M. hominis* and *U. urealyticum* infection can be seen in table 6. From 134 subjects who performed vaginal douching, there were as many as 93 subjects (69.4%) were infected with *M. hominis* and *U. urealyticum*. While in 46 people who did not perform vaginal douching, there were 37 subjects (80.4%) infected. Statistical analysis revealed no difference between vaginal douching habits with the prevalence of infection (*p* = 0.149; OR 0.552; CI 0.244 to 1.248).

Table 6. Correlation between vaginal douching and prevalence of *M. hominis* and *U. urealyticum* infection in female sex workers in East Jakarta (N=180)

| Vaginal douching   | N infected (%) | N not infected (%) | Total |
|--------------------|----------------|--------------------|-------|
| Vaginal douching   | 93 (69.4)      | 41 (30.6)          | 134 (74.4) |
| No vaginal douching| 37 (80.4)      | 9 (19.6)           | 46 (25.6) |
| Total              |                |                    | 180 (100) |

*N= Number of subjects

*p* = 0.149; OR = 0.552; CI 0.244 to 1.248
Table 7. Prevalence of *M. hominis* and *U. urealyticum* infection based on culture results and vaginal douching (N=180)

| Douche | Culture results (N) | Total |
|---|---|---|
|   | MH infection | UU infection | Mixed infection | Not infected |
| Yes | 4 | 47 | 42 | 41 | 134 |
| No  | 1 | 18 | 18 | 9  | 46  |
| Total | 5 | 65 | 60 | 50 | 180 |

N= Number of subjects; MH= *M. hominis*; UU= *U. urealyticum*

Table 8. The prevalence of *M. hominis* and *U. urealyticum* infection by douching frequency (N=180)

| Douching frequency per week | Culture Result (N) | Value of p |
|---|---|---|
|   | MH infection (%) | UU infection (%) | Mixed infection (%) | Not infected (%) |   |
| Never | 1 (20) | 18 (27.7) | 18 (30) | 9 (18) |   |
| 1-14 | 4 (80) | 35 (53.8) | 35 (58.3) | 32 (64) |   |
| 15-28 | 0 (0) | 11 (16.9) | 6 (10) | 9 (18) |   |
| >28 | 0 (0) | 1 (1.6) | 1 (1.7) | 0 (0) | 0.7615 |

N= Number of subjects; MH= *M. hominis*; UU= *U. urealyticum*

In detail, the prevalence of *M. hominis* and *U. urealyticum* infection based on culture results and vaginal douching can be seen in Table 7. Of 93 subjects who were infected and doused, the prevalence of *M. hominis* infection was found in 4 subjects (4.2%), *U. urealyticum* 47 subjects (50%) and mixed infections 42 (45.8%). While in 37 subjects who were infected and did not douch, the prevalence of *M. hominis* infection was found in 1 (2.7%), *U. urealyticum* 18 (50%) and mixed infections 18 (47.3%) subjects respectively.

The prevalence of *M. hominis* and *U. urealyticum* infection in association with dousing frequency can be seen in table 8. The frequency of dousing did not affect the prevalence of *M. hominis* and *U. urealyticum* infection (p = 0.7615). The 35 different types of douching material used by subjects is showed in table 9. Betel soap is the most frequently used (34.33%).

**Discussions**

This study showed a high prevalence of *M. hominis* and *U. urealyticum* infection in FSW in East Jakarta, Indonesia. These results agree with other study conducted in China (Pingmin, *et al.*, 2005) who found a total prevalence of infection of *M. hominis* and *U. urealyticum* approximately 80% in FSW. This might related to the effects of seminal fluid that potentially increase vaginal pH. The growth of *M. hominis* and *U. urealyticum* are is optimum at high pH level. Seminal fluid has a normal pH ranging from 7.2 to 8. The increasing number of sex partners tend to increase the vagina pH level.21

The results also indicated that infection of *U. urealyticum* was more frequent than *M. hominis* for unknown reason. *M. hominis* is known to be found in small quantities in asymptomatic female and generally increase in BV infection.6

Research conducted of FSW in Asia such as China and Japan reported infections of *M. hominis* as much as 19.6% -35% and infection of *U. urealyticum* between 10.2% -72%.2,22 A similar study conducted in Poland in high-risk sexual-behavior-women found infection of *M. hominis* was 9.1% and *U. urealyticum* 59.1%.3 The difference in prevalence found in various studies possibly because of geographical differences, as well as research methods.

Research in China, Japan, and Poland were using PCR detection methods and did not report
the percentage of mixed infections. In Papua New Guinea, the prevalence of *M. hominis* infection was 7%, *U. urealyticum* 20% and mixed infection 65% in women with high risk behavior, using Mycoplasma IST kit, similar to our study.\(^8\)

Table 9. Vaginal douche combinations used by subjects (N=134)

| Douching material | N using douching material | % |
|-------------------|---------------------------|---|
| 1. Betel soap      | 46                        | 34.33 |
| 2. Other soaps     | 21                        | 15.7 |
| 3. Baby soap       | 15                        | 11.2 |
| 4. Povidone iodine | 6                         | 4.5  |
| 5. Toothpaste      | 6                         | 4.5  |
| 6. Betel leaf water| 4                         | 2.8  |
| 7. Absolut®        | 3                         | 2.22 |
| 8. Herbs           | 2                         | 1.5  |
| 9. Albothyl®       | 1                         | 0.75 |
| 10. Lactacyd®      | 1                         | 0.75 |
| 11. Dettol® soap   | 1                         | 0.75 |
| 12. Other antiseptic soap | 2 | 1.5 |
| 13. Absolut®, PP solution | 1 | 0.75 |
| 14. Salt water, betel leaf water | 1 | 0.75 |
| 15. Betel leaf water, herbs | 1 | 0.75 |
| 16. Albothyl®, povidon iodine | 1 | 0.75 |
| 17. Povidone iodine, betel leaf water | 2 | 1.5 |
| 18. Herbs, toothpaste | 1 | 0.75 |
| 19. Herbs, soap    | 1                         | 0.75 |
| 20. Lactacyd®, povidon iodine | 1 | 0.75 |
| 21. Lactacyd®, baby soap | 1 | 0.75 |
| 22. Toothpaste, absolut® | 1 | 0.75 |
| 23. Toothpaste, other soaps | 2 | 1.5 |
| 24. Toothpaste, betel soap | 2 | 1.5 |
| 25. Toothpaste, betel leaf water | 1 | 0.75 |
| 26. Toothpaste, baby soap | 1 | 0.75 |
| 27. Baby soap, herbs | 1 | 0.75 |
| 28. Baby soap, betel soap | 1 | 0.75 |
| 29. Povidone iodine, other soaps | 1 | 0.75 |
| 30. Other soaps, betel soap | 1 | 0.75 |
| 31. Other soaps, shampoo | 1 | 0.75 |
| 32. Betel soap, other soap, absolut® | 1 | 0.75 |
| 33. Betel soap, albothyl® | 1 | 0.75 |
| 34. Other soaps, povidon iodine | 1 | 0.75 |
| 35. Other soaps, shampoo | 1 | 0.75 |

\(^*N=\) Number of subjects
\(^{**}\) PP = Potassium permannaganas

African American women are at risk of being infected with *M. hominis* and *U. urealyticum* higher than other race. Reason was likely due to higher normal vaginal pH of African American women than other races.\(^23\)

The discovery of high prevalence rate of *M. hominis* and or *U. urealyticum* in this study should be of particular interest, given the role of both bacteria in NSGI, preterm delivery, pelvic inflammation and infertility. Routine examination should be considered for both bacteria, especially in high-risk sexual-behavior women.

This study found no association between prevalence of infection and vaginal douching habit. Result of this study differs from what was found by Ness et al.\(^14\) and Djigma et al.\(^15\) who found douching increases the rate of *M. hominis* and *U. urealyticum* infection. According to these studies, douching change vaginal pH, the balance of vaginal flora of thus increasing the risk of infection of *M. hominis* and *U. urealyticum*. Result difference found in this study may be due to differences in vaginal douching materials used by each subjects. The effect of vaginal douching depends on its type, for example Onderdonk et al.\(^24\) found that women who douche with 4% acetic acid have a temporary decrease of vaginal bacteria, while povidone iodine usage could result a drastic reduction of vaginal bacteria including Lactobacillus. It is said that it was antiseptic effect of douching. Further research by Monif et al.\(^25\) found the decrease of bacteria due to povidone iodine usage only lasted for 120 minutes.

Both of these studies did not examine the effects of routine vaginal douching and using different materials in douching. A number of subjects in this study used more than one material for vaginal douching per week. Furthermore, the douching materials used in this study are not typically used by subjects in other studies in other countries, such as toothpaste, betel soap, and herbs. To the author’s knowledge, there has been no study on the effect of using these douching materials routinely. It is assumed the materials for vaginal douching in this study did not affect vaginal pH, balance of normal vaginal flora or bacterial colonization of *M. hominis* and *U. urealyticum* as no statistical association was found between vaginal douching practice and infection prevalence of *M. hominis* and *U. urealyticum*.

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