

## Original Article

# Non-gonococcal urethritis: association of *Ureaplasma urealyticum* with non-gonococcal urethritis in men

## 非淋菌性尿道炎：男性患者中尿素分解支原體與非淋菌性尿道炎的關係

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Non-gonococcal urethritis (NGU) is the commonest sexually transmitted infection seen in male patients attending government social hygiene clinics in Hong Kong. A single-centre and prospective study had been carried out to determine the proportion of *Ureaplasma urealyticum* in patients with NGU and their association by PCR method, perform a semi-quantitative culture of *Ureaplasma urealyticum* in patients with NGU by commercial kits and study the antimicrobial susceptibility of this organism. Among the 145 patients with NGU, the proportion of *Ureaplasma urealyticum* was 22.1% which was significantly higher than that in the control group (10.1%). Ninety percent of the organism was susceptible to doxycycline.

在香港，非淋菌性尿道炎是在政府社會衛生科診所求診的男性患者中最常見的性傳播傳染病。在一項單中心的前瞻性研究中，以聚合酶鏈式反應測定非淋菌性尿道炎患者中尿素分解支原體的感染率及其聯繫；以商用檢測試劑盒半定量培養尿素分解支原體，並研究其抗菌感受性。在 145 名患者中，尿素分解支原體的感染率為 22.1%，高於對照組(10.1%)。百分之九十的尿素分解支原體對強力黴素敏感。

**Keywords:** Non-gonococcal urethritis, *Ureaplasma urealyticum*

**關鍵詞：**非淋菌性尿道炎、尿素分解支原體

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## Introduction

Urethritis, one of the commonest sexually transmitted infections (STIs), is classified as gonococcal or non-gonococcal depending on the presence or absence of *Neisseria gonorrhoeae* isolated as aetiological agent. Non-gonococcal urethritis (NGU) is the commonest STI seen in male patients attending government social hygiene

clinics. The incidence of NGU is high both in Hong Kong and the rest of the world. It represents about a quarter of the total new venereological cases including both male and female patients seen in government social hygiene clinics.<sup>1</sup>

*Chlamydia trachomatis* has been well established as a pathogen of NGU but other aetiological agents are not well defined in non-chlamydial NGU cases. *Ureaplasma urealyticum*, previously ascribed as the tiny (T)-strain mycoplasmas, was first discovered in human urogenital tract samples in 1954.<sup>2</sup> It was designated in 1974 as a new genus and species, *Ureaplasma urealyticum*<sup>3</sup> which had been suggested to be associated with NGU.<sup>4-8</sup> Previously, there was no specific test for detecting this organism in the government social hygiene clinics. However, after the availability of PCR primers and commercial culture kits for *Ureaplasma urealyticum*, we can detect this potential pathogen in clinical samples including urine and urethral swabs.

## Objectives

The main objectives are to determine the proportion of *Ureaplasma urealyticum* in patients with NGU and their association by PCR method and study the antimicrobial susceptibility of this organism.

## Method

Two groups of men (NGU and control group) were recruited from the new attendees who attended the largest male STI clinic in the Social Hygiene Service (Yaumatei Male Social Hygiene Clinic) from 1st August 2004 to 30th April 2005. NGU group is defined as patients either with symptoms of urethritis including urethral discharge, dysuria or frequency or referred as contact cases of NGU (sexual partners of confirmed NGU cases) who have positive urethral smear for gram stain showing five or more polymorphonuclear leukocytes (PML) per high-power (x 1000)

microscopic field and culture for *Neisseria gonorrhoeae* is negative. Control group is defined as patients who are asymptomatic for urethritis and not contact cases of NGU. They may complain other venereological symptoms. The study has adopted exclusion for patients who (i) are under the age of 18; (ii) are having complications of NGU. All patients gave verbal informed consent for participation of the study. First-voided urine specimens of both groups were collected for PCR- and phylogeny-based assay for detecting the presence of *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma genitalium* and *Mycoplasma hominis*. For the NGU group, urethral swab specimens were sent for culture for *Neisseria gonorrhoeae* and nuclei acid amplification test (Amplicor) for *Chlamydia trachomatis*. An additional urethral swab was taken from patients with NGU for a semi-quantitative culture of *Ureaplasma urealyticum* and *Mycoplasma hominis* with antimicrobial susceptibility testing by a commercial kit.

The multiple-banded antigen (MBA) genes of ureaplasmas contain both species- and serovar-defining regions.<sup>9</sup> Several primers sets based on the MBA sequences of ureaplasmas have been described for differentiating *Ureaplasma urealyticum* and *Ureaplasma parvum*.<sup>10-12</sup> The primer pairs used in this study were UMS-170 & UMA263 and UMS-57 & UMA222 which are specific for *Ureaplasma urealyticum* and *Ureaplasma parvum* respectively.<sup>13</sup>

Isolation of the specific causative organisms through culture remains a worthy goal as PCR technique cannot permit quantitative results and assess antibiotic sensitivity. The commercial kit used in this study is *Mycoplasma* IST 2 which is a complete kit for the culture, identification, indicative enumeration (equal to or greater than a threshold set at 10<sup>4</sup> Colony forming unit) and susceptibility testing of *Ureaplasma urealyticum* and *Mycoplasma hominis* to nine antibiotics including doxycycline, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarithromycin,

josamycin and pristinamycin. Josamycin and pristinamycin have not been marketed in Hong Kong.

Chi-square test or Fisher's Exact test was used for comparing proportions between NGU and control group. Student's t-test was used to compare means. All comparisons were performed as 2-tailed tests with significance set at  $p < 0.05$ .

## Results

There were a total of 322 patients recruited into the study. Twenty-eight patients were excluded. Six patients had GC detected by culture and were excluded from the NGU group. Eleven patients had undetermined urine PCR results because of presence of inhibitors whereas two other patients had inconclusive culture results for *Ureaplasma urealyticum* because of failure of observation of results at 48 hour. Nine patients were excluded from the control group because they were not

totally asymptomatic of urethritis on reviewing the clinical records. Hence there were remaining 294 patients with 145 NGU cases and 149 controls.

The mean age of NGU group was 38.76 years old (range: 18-80) while that of the control group was 40.17 years old (range: 18-77). The great majority of all recruited patients were heterosexual (98.3%). Only two controls and one NGU patient were homosexuals and one NGU patient was bisexual. Vaginal sex was the most common practice (81.3%) followed by vaginal and oral-genital sex (16.7%), anal sex (1%) and oral-genital sex (1%). Only 28.9% of all recruited patients always used condom in the past three months. The percentages of patients who sometimes and never used condom were 36.7% and 34.4% respectively. The mean number of sex partners over the past three months in the NGU group was 2.41 while that of the control group was 2.4. There was no significant difference between the two groups in terms of age, sex orientation,

**Table 1.** Demographic data and sexual behaviour of the NGU and the control group

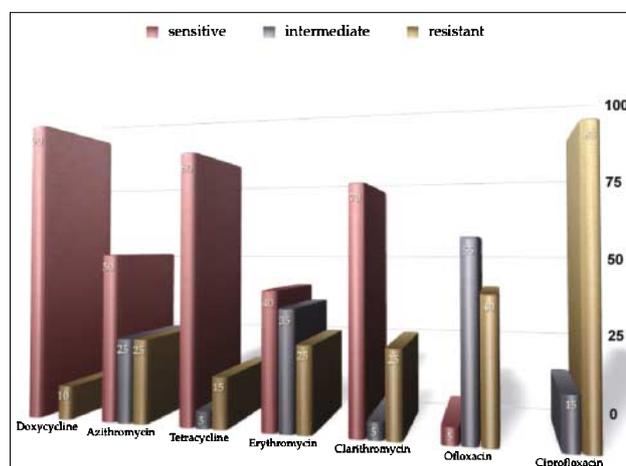
	NGU group (n=145)	Control group (n=149)	Statistical results
<b>Demographic data</b>			
Mean age (range)	38.76 (18-80)	40.17 (18-77)	$p > 0.05$ (t-test)
<b>Sexual behaviour</b>			
Sex orientation (heterosexual/ homosexual/bisexual)	143/1/1	146/3/0	$\chi^2 = 1.977$ , d.f. = 1, $p > 0.05$ (chi-square test)
Sex practice (vaginal/ vaginal + oral-genital)	116/27	123/22	$\chi^2 = 3.995$ , d.f. = 1, $p > 0.05$ (chi-square test)
Frequency of condom use (Always/sometimes/never)	36/59/50	49/49/51	$\chi^2 = 2.87$ , d.f. = 1, $p > 0.05$ (chi-square test)
Mean number of sex partners in past 3 months (range)	2.41 (0-13)	2.4 (0-20)	$p > 0.05$ (t-test)
Mean time of last casual sex contact (days)	45.23	73.05	$p = 0.028^*$ (t-test)
History of urethritis	40	14	$\chi^2 = 16.217$ , d.f. = 1, $p < 0.001^*$ (chi-square test)
Antibiotics used in past one week	18	6	$\chi^2 = 6.895$ , d.f. = 1, $p = 0.009^*$ (chi-square test)

\* $p < 0.05$  showing significant difference

sex practice, condom use pattern and number of sexual partners. The mean time of last casual sex contact was significantly longer in the control group than the NGU group. The NGU patients were more likely to have a past history of urethritis and antibiotics used in the past one week. The summary of the demographic data and sexual behaviour is shown in Table 1.

The proportion of organisms detected in the NGU group and the control group is shown in Table 2. *Chlamydia trachomatis* was detected in 43.4% of patients with NGU. For the NGU group, PCR tests for *Ureaplasma urealyticum* were positive in 32 out of 145 patients whereas for the control group, there were positive tests in 15 out of 149 patients. Proportion of *Ureaplasma urealyticum* detected in NGU patients was 22.1% while that in control patients was 10.1%. There was statistically significant difference between the NGU and the control group and hence suggested an association between NGU and detection of *Ureaplasma urealyticum* ( $p < 0.005$ ). The apparent higher isolation rate of *Ureaplasma parvum* in the control group is not statistically significant. However, when the ureaplasmas (*Ureaplasma urealyticum* and *Ureaplasma parvum*) were considered together, there was no significant difference in the detection rate of ureaplasmas between the NGU and the control group.

Of the 145 NGU patients, 20 had positive culture for *Ureaplasma urealyticum* with significant growth ( $CFU > 10^4$ ). Ninety percent of the positive cultures were susceptible to doxycycline which was used as the first line treatment for NGU patients in government social hygiene clinics. The sensitivity percentages of *Ureaplasma urealyticum* to tetracycline, clarithromycin, azithromycin and erythromycin were 80%, 70%, 50% and 40% respectively. The sensitivity to quinolones was very low with only 5% sensitive to ofloxacin and 0% to ciprofloxacin. The drug sensitivity pattern of *Ureaplasma urealyticum* detected in the present study is shown in Figure 1.



**Figure 1.** The drug sensitivity pattern of *Ureaplasma urealyticum*.

**Table 2.** Proportion of organisms detected in the NGU group and the control group by PCR method

Organisms	No. (%) of men		Statistical results
	NGU group (n=145)	Control group (n=149)	
<i>Chlamydia trachomatis</i>	63 (43.4)	N/A	N/A
<i>Ureaplasma urealyticum</i>	32 (22.1)	15 (10.1)	$\chi^2 = 7.882$ , d.f. = 1, $p < 0.005$ (chi-square test)
<i>Ureaplasma parvum</i>	23 (15.9)	30 (20.1)	$\chi^2 = 0.908$ , d.f. = 1, $p > 0.05$ (chi-square test)
<i>Ureaplasma</i> *	48 (33.1)	43 (28.9)	$\chi^2 = 0.619$ , d.f. = 1, $p > 0.05$ (chi-square test)

\**Ureaplasma* is positive when *Ureaplasma urealyticum* or *Ureaplasma parvum* is detected.

## Discussion

In this study, the proportion of NGU attributable to *Chlamydia trachomatis* was 43.4% which was comparable to the results found in the United States and United Kingdom.<sup>14-17</sup> For non-chlamydial NGU, the microbiological aetiology is not well understood. Definitive support of *Ureaplasma urealyticum* as a pathogen causing urethritis was evidenced by a volunteer experiment in 1977.<sup>18</sup> The relationship and association of NGU with *Ureaplasma urealyticum* is still in debate. Some studies have shown significant association<sup>4,7,8,19,20</sup> while some refute it.<sup>5,21-24</sup> In the present study, *Ureaplasma urealyticum* was detected in 22.1% of patients with NGU comparing to 10.1% of patients in the control group which was statistically significant. However, these studies are not directly comparable to one another. The main difference is the choice of controls. Rate of colonization of *Ureaplasma urealyticum* is affected by sexual experience. In some studies, there were controls not matched for sexual experience. Overall, there is ample evidence that *Ureaplasma urealyticum* implicated in NGU although to what extent it might be responsible is unclear.

The sensitivity pattern of *Ureaplasma urealyticum* isolated from patients with NGU reinforce our confidence of treating them with doxycycline as the first line regimen. On the other hand, only 50% of the organism were sensitive to azithromycin which is another common first line single dose regimen. This finding suggested that azithromycin may be suboptimal for treatment of *Ureaplasma urealyticum* associated NGU. Erythromycin, which is the drug of choice for patients with tetracycline allergy or pregnant women, is not recommended for treatment of *Ureaplasma urealyticum* associated NGU as only 40% of the cultured organisms were susceptible in this study. We should also avoid prescribing quinolones as only 5% of the organism was sensitive to ofloxacin and none of them to ciprofloxacin. Traditionally, minimum

inhibitory concentration (MIC) method is used for antibiotics susceptibility testing of cultured bacteria. The advantage of Mycoplasma IST 2 kit used in this study is concurrent culture and susceptibility testing of *Ureaplasma urealyticum* which provides results in a much shorter time. However, there is no universal standard for culture and susceptibility testing of *Ureaplasma urealyticum* which makes the comparison between different studies difficult.

From the epidemiological studies of NGU, there is increasing incidence of non-chlamydial NGU. If a large proportion of the aetiological agents of NGU is still unknown and there is increasing evidence of drug-resistant cases, NGU will escalate to become a major public concern. This study has provided local data on the proportion of *Ureaplasma urealyticum* in men with NGU and the drug sensitivity pattern of this organism which are useful for a good STI surveillance system. However, there are limitations in the study. For the recruitment of controls, they were asymptomatic of urethritis but we had not further categorized them into subgroups of totally asymptomatic patients for screening or with other specific symptoms of other STIs. Ideally all controls should also have urethral smear and culture done to exclude those with asymptomatic urethritis and be fully matched in demographic and epidemiological characteristics.

In conclusion, the proportion of *Ureaplasma urealyticum* in patients with NGU was found to be 22.1% from the present study and there was a significant association between *Ureaplasma urealyticum* and NGU. Ninety percent of *Ureaplasma urealyticum* cultured were susceptible to doxycycline.

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