Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Duplex PCR among Female Inmates in Lubuk Pakam Prison Indonesia

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Abstract: Incarcerated women are at high risk for sexually transmitted infections (STI).Chlamydia and gonorrhea are most commonly found in females less than 25 years of age, both in the general population and among incarcerated women. This is the first study using a duplex PCR assays for *C. trachomatis* and *N. gonorrhoeae* to identify both microorganisms from female inmates in Lubuk Pakam prison.The objectiove of the study is to detect multiple microbial targets simultaneously**.** From 41 subjects, endocervical swabs were taken and sent to the Laboratory for duplex CT-GC PCR identification. The result found that detection rate for *Chlamydia trachomatis* is16(39%) and *Neisseria gonorrhoeae* is 5(12%) and for both found in 3 subjects (7%) It was concluded that duplex CT-GC PCR are potentially useful for rapid from female detection *C. trachomatis* and *N.gonorrhoeae* from female genital secretions.

1 INTRODUCTION

Incarcerated women are at high risk for sexually transmitted infections (STI), which if left untreated may lead to adverse complications such as preterm delivery, infertility, pelvic inflammatory diseases, ectopic pregnancy, and even increased HIV transmission (Nijhawan, 2012) (Caviness, 2012). In some population of American women, chlamydial infection, trichomoniasis, and gonorrhea are the most common STIs, who are generally affected by STI at higher rates than men (Plitt, 2012). Two of these infections (chlamydia and gonorrhea) are most commonly found in females less than 25 years of age, both in the general population and among incarcerated women (Hardick, 2003). *Chlamydia trachomatis* infection rate in the general population in the West is 1–10% in both genders (Datta, 2012) (Cecil, 2001) (Montagne, 2004). The risk of acquiring *C. trachomatis* is associated with several socio-demographic and behavioral factors (Gollub, 2010). Particularly, incarcerated persons are at a high risk for sexually transmitted infections (STIs). Inmates were reported multiple behaviors which increased the risk of STIs such as *C. trachomatis*, including sex with multiple partners, unprotected sex and inconsistent condom use, and substance use disorders (Freudenberg, 2007) (Warner, 2006).

Genital infections caused by *C. trachomatis* closely parallel those owing to *N. gonorrhoeae* in terms of clinical manifestations. Both organisms preferentially infect columnar or transitional epithelium of the urethra, with extension to the epididymis; the endocervix, with extension to the endometrium, salpinx, and peritoneum; and the rectum. Both organisms can produce extensive subepithelial inflammation, epithelial ulceration, and scarring. In rare cases, both organisms can produce systemic manifestations. *C. trachomatis* tends to produce less exudates and a lower concentration of segmented neutrophils than *N. gonorrhoeae*; however these criteria are not always sufficient to separate the two diseases. In developing countries, traditional methods for diagnosing STIs are laborious, often not very sensitive, and have a long turnaround time with most recent commercially available diagnostic tests targeting one or, at most, two of these STIs at a time. However, studies have shown that 45.7% of persons infected with *N.*

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gonorrhoeae, are coinfected with *C. trachomatis* (Ahmad, 2015).

PCR method combines high levels of sensitivity, specificity, and accuracy, with rapid turnaround times, making for a more appealing alternative to culture-based testing methods which are often limited by long culture times and low sensitivity with certain sample types. PCR-based assays, on the other hand, are compatible with several sample types that can be used directly for DNA extraction without the need for culture (Ahmad, 2015) Multiplex PCRs are designed for the simultaneous detection of multiple microbial targets. These assays have been increasingly used for the detection of common etiologic agents in genital discharge and genital ulcers (Mahony, 1995) (Nasution, 2007) This is the first study using a duplex PCR assays for *C. trachomatis* and *N. gonorrhoeae* to identify both microorganisms from female inmates in Lubuk Pakam prison.

2 METHODS

This study was a cross-sectional study design. There were 41 subjects involved in this study. They were female inmates in Lubuk Pakam correctional facility in North Sumatera. Sampling method was total sampling which taken from all female inmates in Lubuk Pakam prison on November 2016. The inclusion criteria for this study are female inmates whom age 20-50 years old, already married or have been sexually active with partners and agree to participate in the study by signing the informed consent. The exclusion criteria were female inmates whom refused to do the examination. Samples were taken by swabbing endocervix and put inside M4RT® Microtest Tube® (http://www.remel.com) and directly send to Collaborative Laboratory in Faculty of Medicine Universitas Sumatera Utara.

2.1 DNA Extraction

Extracted DNA (4µl) were purified using Presto TM Buccal Swab gDNA Extraction Kit following the manufacturer's instructions (http://www.geneaid.com)

2.2 Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Duplex PCR

To amplify rDNA from genomic DNA, a duplex PCR was carried out. Duplex PCR was conducted by using two sets of primer which are KL1 (5'-TCCGGAGCGAGTTACGAAGA-3') and KL2 (5'-AATCAATGCCCGGGATTGGT-3') for *C. trachomatis* (Mahony, 1992); HO1 (5'-GCTACGCATACCCGCGTTGC-3') and HO3 (5'-CGAAGACCTTCGAGCAGACA-3') for *N. Gonorrhoeae* (Ho, 1992). The amplification mixture was carried out in 12,5 µl 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 4 µl DNA template (https://worldwide.promega.com) PCR was performed in a thermocycler (Verity 96-well Thermal Cycler, AppliedBiosystems) with an initial denaturation of 95°C for 5 min, followed by 35 cycles of 1 min at 95°C, 1 min at 55°C, and 2 min at 72°C. All PCR products were analyzed by electrophoresis in a 2% (wt/vol) agarose gel (Promega®) by standard procedures. The result showed DNA fragment size for *C. trachomatis* 241 bp and *N. gonorrhoeae* 390 bp

Table 1: Detection rates from duplex CT-GC PCR

No		*n*	*%*
1.	*Chlamydia trachomatis*	*61*	*39*
2.	*Neisseria gonorrhoeae*	*5*	*12*
3.	*C.trachomatis and N.gonorrheae*	*3*	*7*

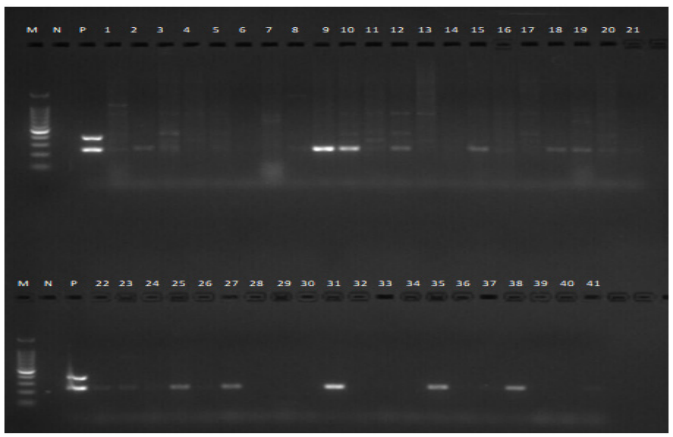

Figure 1: Duplex PCR Results.

Figure 1. M (100bp marker); N (Negative Control); P (Positive Control); Lane 2: CT (+); Lane 3: GC (+); Lane 9: CT (+); Lane 10: CT&GC (+); Lane 12: CT&GC (+); Lane 15: CT(+); Lane 17: GC(+); Lane 18: CT(+); Lane 19:CT(+); Lane 20 CT&GC (+); Lane 22: CT(+); Lane 23: CT(+); Lane 25: CT(+); Lane 27: CT(+); Lane 31: CT(+); Lane 35: CT(+); Lane 38: CT(+); Lane 41: CT(+)

3 RESULTS AND DISCUSSION

This study found that 16 female (39%) were positive for *C.trachomatis*. This study showed that chlamydial infection was the most common infection observed. This result supported the results of other studies (Parvez, 2013). The overall prevalence of chlamydial infection varied according to age, social behaviors, sexual activities, and geographic location of the patients (WHO, 2001). The high prevalence of chlamydia infection in our studied group underscored the importance of Chlamydia screening in the detained women in this area. Hence, the study also found that 5 female (12%) were positive for *N.gonorrhoeae*. The prevalence of gonorrhea has been reported to range from 0.2% to 17% among female prisoners (CDC, 2001). Furthermore, 20% of males and 40% of females with gonorrhea are co-infected with *C. trachomatis* (Holmes, 1994) The concomitant infections were the main reason for using this duplex CT-GC PCR for this study.

4 CONCLUSIONS

Duplex CT-GC PCR is potentially useful for rapid detection *C.trachomatis* and *N.gonorrhoeae* from female genital secretions.

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