

## Original Article

# A study on in-vitro survival of *Neisseria gonorrhoeae* in Hong Kong

## 香港奈瑟氏淋球菌體外存活之研究

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A prospective study was carried out in Lek Yuen Social Hygiene Clinic in Hong Kong during 1996-1997 to study the longest survival time of *Neisseria gonorrhoeae* outside the human body. Fifteen patients with confirmed gonorrhoea were recruited. The patients' urethral discharges were taken for interval cultural analysis in standardised Thayer Martin medium with and without physiological normal saline. The result showed that *Neisseria gonorrhoeae* could survive up to 20 hours outside human body. The twenty hours in-vitro survival of *Neisseria gonorrhoeae* provide important information especially when a genitourinary physician is facing a suspected child abuse case in which the clients claimed that the infection was acquired through fomites.

香港瀝源社會衛生科診所於 1996 至 1997 年進行一項前瞻性研究，以確定奈瑟氏淋球菌於人體外的最長存活時間。研究選定十五名經確診的淋病患者。將患者的尿道分泌物以 Thayer Martin 培養基作間段性培養分析，部份標本加有生理鹽水。研究結果顯示奈瑟氏淋球菌可於人體外存活長達二十小時。此項研究結果提供重要臨床資訊，特別是當泌尿生殖科醫生面對一宗懷疑虐兒個案，而當事人堅稱感染是由污染物引起。

**Keywords:** Hong Kong, in-vitro survival, *Neisseria gonorrhoeae*

關鍵詞：香港，體外存活，奈瑟氏淋球菌

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## Introduction

It is the experience of some genitourinary physicians in his practice to encounter clients diagnosed gonorrhoea and who attributed acquisition of the infection to contact with inanimate objects rather than through a sexual route. Rarely, there are cases of suspected child abuse in which the victim acquired gonorrhoea but detailed case investigation failed to find the abuser. At present, gonorrhoea is still considered

as an exclusively sexually transmitted disease. There is no firm evidence reported in the literature that gonorrhoea could be acquired through contaminated fomites. However, such chance cannot be totally denied if the causal agent, *Neisseria gonorrhoeae* could survive outside human body for a prolonged period of time. A study on the in-vitro survival time of *Neisseria gonorrhoeae* was therefore carried out in a group of gonorrhoea patients attending Social Hygiene Service, Department of Health, Hong Kong.

## Objectives

1. To find out the longest survival time of *Neisseria gonorrhoeae* outside the human body.
2. To evaluate if factors of moisture, ambient temperature and humidity that would affect the in-vitro survival of *Neisseria gonorrhoeae*.

## Patients and method

A prospective study was carried out in Lek Yuen Social Hygiene Clinic from May 1996 to December 1997. A total of 15 patients with proven gonorrhoea infection (initially diagnosed by microscopic detection of gram-negative intracellular diplococci and later re-confirmed by culture) were recruited and their urethral discharges collected. Fresh urethral discharge from each of these patients was taken for interval cultural analysis at recorded room temperature and humidity. To standardise collection, the urethral discharge was collected by a 0.02 ml pipette to give a volume of 0.05 ml in a clock glass. Another 0.05 ml of urethral discharge was also collected by the pipette in a second clock glass and mixed with an equal volume of 0.05 ml normal saline.

At the designated time intervals of one hour, two hours, four hours, six hours, eight hours and up to 20 hours from the time when urethral discharge

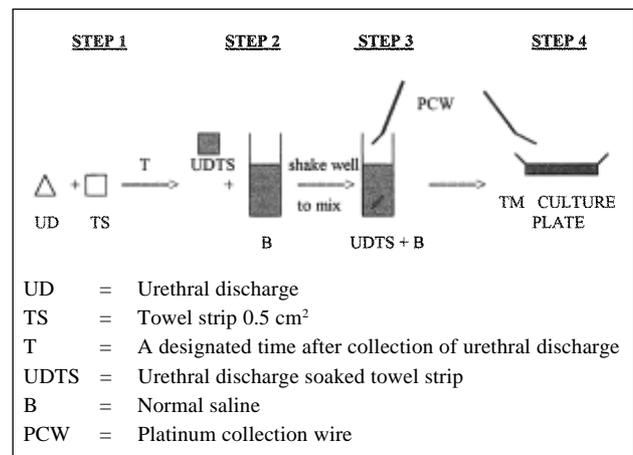
was collected, a specimen from each of these two clock glasses was obtained using the standard platinum collecting wire and inoculated directly onto a modified Thayer Martin (TM) culture plate. All culture plates were incubated at 35°C in a 5% carbon dioxide atmosphere. Their subsequent isolation, identification and sensitivity tests were carried out by microbiologists and recorded. The collection of urethral discharge and the culture procedure were all done by one single investigator to avoid technical variation (Figure 1).

In this study, the time from onset of symptoms and any preceding systemic medications of each patient were also noted. At commencement, the specimen at zero hour was also sent for:

1. Microscopic examination for the number of white blood cells.
2. Chlamydiazyme for concomitant *Chlamydia trachomatis* infection.

## Results

A total of 15 patients with culture proven gonorrhoea were recruited. The results showed that their symptoms onset from three to 14 days (mean = 8.1 days) after their last venereal exposure. All patients except patient 8 have not taken any



**Figure 1.** Steps involved in the experiment in performing cultures of *Neisseria gonorrhoeae* from urethral discharge with and without normal saline.

medications prior to examination. Their urethral smears showed two or more white blood cells per high power field on microscopic examinations (>20-29 white blood cells per high power field) but all their Chlamydiazyme tests were negative.

The results of tests for the maximal survival time for a positive culture in all these patients were shown in Table 1.

## Discussion

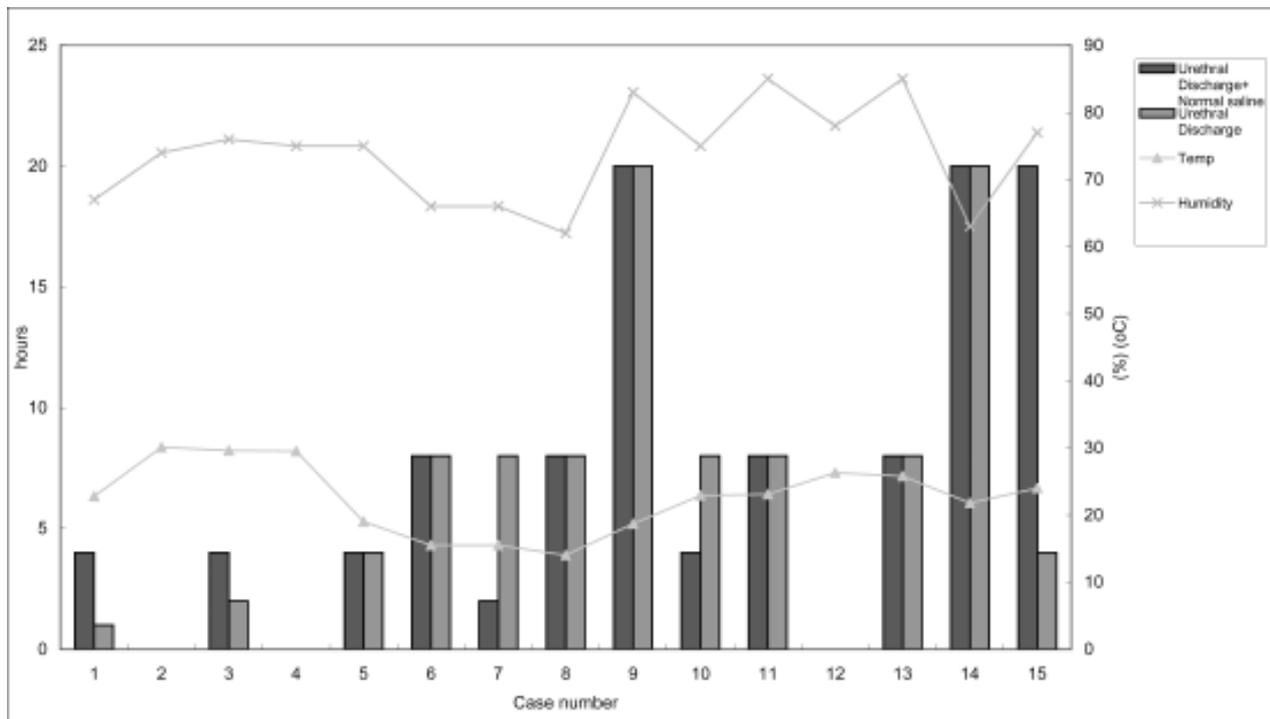
Our results showed that *Neisseria gonorrhoeae* could survive up to 20 hours outside human body in both pure urethral discharge and discharge mixed with physiological saline at a temperature of 35°C. The mean duration of the survival of the gonococcus in the pure urethral discharge is 6.6 hours while the mean survival time of the organism in the urethral discharge with normal saline is 7.3 hours. There is no significant difference in the

mean survival time between the two groups ( $t=0.31$ ,  $p<0.62$ ). Besides, the study results also showed that the maximum survival time of the gonococcus in the pure urethral discharge and urethral discharge in normal saline has no relationship with the number of white blood cells microscopically examined per urethral smears, environmental temperature and humidity recorded (Figure 2).

The result is in agreement with those documented in the literature. Tulloch in 1929 found that gonococcus could survive in fluid pus for 24 hours at room temperature.<sup>1</sup> Elmros showed that the organism could be cultured positive after an interval of 24 hours on a towel and 17 hours on a clear glass slide contaminated with gonococcus.<sup>2</sup> Perez and colleagues recovered the gonococcus after 24 hours on plastic and linen bed sheets soaked with gonococci containing fluids.<sup>3</sup> Srivastava confirmed the presence of gonococcus on most of his studied materials after 24-48 hours.<sup>4</sup>

**Table 1.** The maximum survival time for a positive culture outside human body in hours of the pure urethral and urethral discharge in normal saline (N=15)

Patient number	Temp (°C)	Humidity (%)	Maximal survival time for a positive culture outside human body (hours)		
			Urethral discharge	Urethral discharge + Normal saline	Difference
1	22.8	67	1	4	-3
2	30.1	74	0	0	0
3	29.6	76	2	4	-2
4	29.5	75	0	0	0
5	19.0	75	4	4	0
6	15.5	66	8	8	0
7	15.5	66	8	2	6
8	14.0	62	8	8	0
9	18.7	83	20	20	0
10	22.9	75	8	4	4
11	23.1	85	8	8	0
12	26.3	78	0	0	0
13	25.8	85	8	8	0
14	21.8	63	20	20	0
15	24.0	77	4	20	-16
<b>Mean</b>	<b>22.6</b>	<b>73.8</b>	<b>6.6</b>	<b>7.3</b>	<b>0.7</b>



**Figure 2.** The maximum survival time for a positive culture of *Neisseria gonorrhoeae* outside the human body in hours under different environmental conditions.

Although different authors used different study methods, results of all these studies including ours, consistently showed that *Neisseria gonorrhoeae* could survive for a long time outside human body in the environment. The literature reported a range from 6.6 hours to 48 hours.

We also observed that the incubation period of gonorrhoea of our studied subjects had also increased. All our patients had onset of their symptoms at three to 14 days (mean 8.13 days) from their last date of sexual exposure. This is longer than the generally documented incubation period of two to five days.<sup>5</sup> The presenting symptoms of gonorrhoea have changed from a usual purulent profuse discharge to a less severe, serous urethral secretions resembling non-gonococcal urethritis. This less symptomatic presentation of gonorrhoea may delay the patients in seeking medical treatment and hence more opportunities for further transmission.

Although our study did not provide all the data needed to substantiate and delineate the mechanism of nonsexual transmission of gonorrhoea in human subjects, it provides evidence that *Neisseria gonorrhoeae* could survive in vitro both under physiological and non-physiological conditions. This is an important step before confirming objectively that *Neisseria gonorrhoeae* might be acquired through contacts with contaminated fomites especially in the unwearied, prepubescent child.

Our study may have the following limitations. The study did not provide information on the mode of transmission of the *Neisseria gonorrhoeae* from the environment to the patients. It did not examine the importance of the vector and the environmental factors that enabled the transfer of the organisms. It has been verified in various experimental studies that survival of *Neisseria gonorrhoeae* need a very precise environmental temperature and humidity.

Cooling and desiccation destroy the gonococcus. Polymorphs found in the purulent discharge protect the gonococcus from external insults.<sup>6</sup> Any postulated vector like fingertips of the hands of the patients or their parents should provide the pre-requisite conditions for the survival. In order to show that transmission of *Neisseria gonorrhoeae* did occur nonsexually, the exact mode of transmission should be delineated and explained in a plausible manner taking into account of the environmental factors and vectors described.

The study did not offer us information on the "sufficient" dose of *Neisseria gonorrhoeae* needed to allow such nonsexual acquaintance of the organisms. The size of the inoculum and the nature of fomite are crucial in the process of transmission and as the host defense. *Neisseria gonorrhoeae* may not survive very long in the harsh environment and inevitably destroyed by the host immune mechanism. The number or concentration of the organisms that is minimally required for the successful transmission should be determined by experimental study. The number of subjects participated in the study is small and the results obtained are less generalisable due to selection biases. Nevertheless, up to 80% of all the collected urethral discharges showed in-vitro survival of gonococci occurs up to 20 hours give

credibility to the validity of the appropriateness of the sample size.

## Conclusion

Our study showed that gonococcus can survive for hours outside the human body and the longest recorded time being 20 hours. Although there is no concrete evidence that gonorrhoea can be transmitted through fomites. Our study result supports the need for future large scale studies on evaluating a possible role of fomite transmission of gonorrhoea.

## References

1. Tulloch WJ. The gonococcus. In A system of bacteriology in relation to medicine, edited by the Bacteriological Committee of the Medical Research Council. London: HMSO, 1929;2:239-69.
2. Elmros T, Larsson PA. Survival of gonococci outside the body. *Br Med J* 1972;2:403-4.
3. Perez JL, Gomez E, Sauca G. Survival of gonococci from urethral discharge on fomites. *Eur J Clin Microbiol Infect Dis* 1990;9:54-5.
4. Srivastava AC. Survival of gonococci in urethral secretions with reference to the nonsexual transmission of gonococcal infection. *J Med Microbiol* 1980;13:593-6.
5. Edward W. Gonococcal infections in the adult. In: K K Holmes, editors. Sexually transmitted diseases. 3rd ed. New York: McGraw-Hill, 1999;451-66.
6. Gilbaugh JH Jr, Fuchs PC. The gonococcus and the toilet seat. *N Engl J Med* 1979;301:91-3.