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SURVIVAL OF NEISSERIA GONORRHOEAE ON SURFACES

Theodor Elmros

From the Departments of Clinical Bacteriology and Dermatology, University of Umeå, Umeå, Sweden

Abstract. The survival of *Neisseria gonorrhoeae* was investigated. It was found that gonococci could survive 24 hours in urethral secretion on a glass slide and on a towel at 22°C, and 120 hours at 4°C. A method was developed by which the survival of gonococci could be followed in vitro. With this method, gonococci grown in vitro were found to be protected by human serum, in contrast to gonococci resuspended in NaCl. The factors affecting the survival are discussed.

Key words: Neisseria gonorrhoeae; Survival

The transmission of pathogenic bacteria by indirect contact depends on their ability to survive outside their normal habitat. Various factors have been suggested as being responsible for the death of bacteria on human skin and other surfaces (13). Among these, dehydration has been considered of major importance (10, 13). During recent years, studies in this field have gained renewed interest in the discussion on the prevention of hospital infections.

Among the pathogenic gram-negative bacteria *Neisseria gonorrhoeae* is considered one of the most fragile. It has often been claimed that gonococci are killed very rapidly in urethral secretions outside human mucous membranes (2, 14). However, several case reports have suggested a non-sexual transmission of gonorrhoea (3, 8). Gonococci grown in vitro have been shown to survive about 3 hours in human serum on glass beads (1). A similar result was reported when urethral secretions from a patient with gonorrhoea were stored on a cotton stick (8). Gonococci in urethral secretions may even survive for 24 hours when kept on filter papers in special transport kits, designed for the transport of β-streptococci to the laboratory (12). In a preliminary communication the survival of gonococci in urethral secretions was described (4). The aim of the present investigation was to expand the initial data and to develop a method by which the survival of gonococci on surfaces could be studied in greater detail in vitro.

MATERIALS AND METHODS

Organisms. The gonococcal strain 82409/55 was a gift from Dr Alice Reyn, Copenhagen. The resistant strain Copenhagen V was obtained from the National Bacteriological Laboratory, Stockholm. One strain of *Staphylococcus aureus* (phage type 84) and one strain of *Escherichia coli* (06) were isolated in this laboratory from patient specimens.

Liquid cultivation. The liquid medium consisted of one part of medium 199 (Flow) layered on 3 parts of GCAB (BBL) with 3% agar. Cultivation was performed in 100 ml side-arm flasks sealed with rubber stoppers. After filling the flask with air containing 10% CO_2 about 5×10^4 colony forming units (CFU)/ml of *N. gonorrhoeae* were added and incubation was performed for 15 hours in a shaking water bath at 36°C to obtain logarithmic phase bacteria. For *Staphylococcus aureus* and *Escherichia coli* about 4×10^6 cells/ml were inoculated and grown to logarithmic phase (about 5 hours). Growth was recorded by optical density readings using a Klett-Summerson photometer with W66 filter.

Patient specimens. A drop of urethral secretion from each of 31 patients with urethral discharge and typical diplococci in methylene blue stained smears was applied to a piece of linen towel or to a glass slide. The samples were then placed in a Petri dish at room temperature. At a predetermined time 0.2 ml of a phosphate buffer with glycerol and magnesium (T2 buffer) (9) was added and, after mixing, the suspension was transferred to a hematin agar plate (GCAB, BBL) with vancomycin, colistin and nystatin and incubated for 48 hours at 36°C in air with 6% CO_2. Colonies were considered to be *N. gonorrhoeae* if they were oxidase positive, of typical colony morphology and fermenting glucose but not laevulose, maltose or sucrose. To further study their colony morphology the bacteria were transferred to plates without hematin (GCMB, Difco) and incubated for 22 hours as described above. The colony type was thereafter determined using a plate microscope with oblique transmitted light (6).

Table I. *Survival of N. gonorrhoeae in urethral discharge kept on a glass or towels*

Surface	Conditions during storage	Time of storage (hours)	Survival[a]
Glass	22 C	2–17	3/3
		24	3/4
		48	0/2
	4 C	120	4/10
Linen	22 C	3–6	2/2
		24	5/9
		48	0/2

[a] Samples with surviving gonococci/number of patient specimens tested.

In vitro survival test. Logarithmic phase cultures were centrifuged (2000 *g*) for 15 minutes at 22°C and the bacteria were resuspended in 1 ml of fresh, pooled human serum (not inactivated) or 1 ml of saline. In the serum used, no antibodies against *N. gonorrhoeae* could be detected by employing a complement fixation reaction test. 50 µl of the bacterial suspension (approximately 10^6 CFU) was applied to each well of a glass plate of a type used for blood grouping (VIGGO). The glass plate was thereafter kept at 22°C. At different times 0.2 ml of T2 buffer was added to two wells and the plate was placed on a vibrating table (Boerner shaker) for 5 minutes. Thereafter 0.1 ml of the suspension or dilutions thereof was spread on GCAB agar plates, using a glass rod. The plate was incubated as described above for 48 hours before colony counting.

RESULTS

As shown in Table I, part of a population of gonococci present in urethral secretions usually survives 24 hours on glass at 22°C and quite often for 120 hours when kept at 4°C. An equally long survival as on glass was recorded for gonococci on linen. Colony typing of the surviving bacteria was performed in specimens from a few patients. The colony morphology corresponded to type 1, while one specimen showed a predominance of type 2 colonies surviving. The number of surviving gonococci declined with increasing time of storage.

In another set of experiments an antibiotic-sensitive type 4 strain grown in vitro was used to study the survival of *N. gonorrhoeae* on glass in greater detail (Fig. 1). During the first 60 minutes in human serum there was a relatively slow decrease in the number of surviving bacteria. Between 60 and 90 minutes of storage the number of survivors decreased to approximately 0.1% of the starting number of CFU. This rapid decline of the surviving population follows the observed macroscopic drying of the bacterial suspension on the glass plate (about 60 minutes). The number of living gonococci was thereafter slowly reduced to about 0.002% at 24 hours of storage. Gonococci suspended in saline had a survival curve similar to the initial phase in serum but without a final slow phase, so that none out of 5×10^5 input CFU survived for 90 minutes. A similar survival curve as in saline was obtained with gonococci suspended in redistilled water or in phosphate buffer (0.05 M, pH 7.2).

A multiple antibiotic-resistant type 4 strain of *N. gonorrhoeae* and an antibiotic-sensitive type 1 strain showed survival curves similar to that of the type 4 strain in Fig. 1 when stored in serum. Gonococci surviving the drying experiment showed when recultured the same survival curve when the experiment was repeated.

One strain of *Staphylococcus aureus* and one strain of *Escherichia coli* were included in the glass surface survival test. During the first two hours in human serum there was a slight increase in the numbers of both staphylococci and *E. coli*. After 24 hours the number of staphylococci was unchanged. *E. coli*, however, showed a successive decline after 2 hours, leading to about 10% survival at 24 hours of storage.

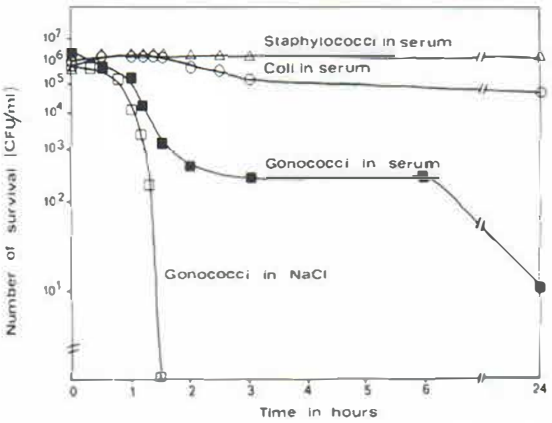

Fig. 1. Survival of *Neisseria gonorrhoeae*, *Staphylococcus aureus* and *Escherichia coli* when grown in vitro and resuspended in human serum and NaCl (*N. gonorrhoeae*).

DISCUSSION

It was early reported that *N. gonorrhoeae* may survive for 24 hours in wet secretions (16) and that the gonococci died rapidly when dried. The results of the present investigation show that gonococci in urethral secretions applied to glass or linen can survive for 24 hours at 22°C. Furthermore, in a refrigerator, gonococci were sometimes shown to survive up to 5 days in urethral secretions on glass. The number of living bacteria (CFU) in each specimen varied between none and 20 after 5 days at 4°C. In the specimens investigated with regard to colony type, only the types regarded as virulent (types 1 and 2) were found among the surviving gonococci.

The method here developed for the measurement of bacterial survival on a glass surface, gives reproducible results and is simple to perform. Most experiments were performed at a relative humidity of 35–50%. A few experiments were performed in winter (relative humidity in Umeå 15–20%). However, the difference in survival between winter and summer was insignificant. It has been noted by other authors that after drying bacteria tend to stick to glass (10). As the number of staphylococci and *E. coli* increased even after macroscopic dehydration it is most probable that the decreases in numbers of gonococci are not due to any appreciable extent to their sticking to the glass, and thus represent actual death of the gonococci.

During storage in a drop of serum the survival of gonococci seems to follow three phases: During the first phase (0–60 minutes) 70% of the gonococci die. The second phase (60–120 minutes) follows the macroscopic drying and represents the very rapid loss of most survivors of phase one. In the third phase (2 hrs to 24–48 hrs) the slow loss of the final 0.1% of the bacterial population took place.

The survival curves of different colony types (types 1 and 4) or between antibiotic sensitive and resistant strains of *N. gonorrhoeae* were similar to one another. Furthermore, when the experiment was repeated, using one of the colonies which survived for 24 hours in serum at 22°C, the same type of survival curve was obtained. Thus the survivors were not mutants resistant to drying. In saline all 10^6 input gonococci were dead by 90 minutes (after the second phase) and no third phase occurred. In all experiments, drying appeared to be the major external cause of the death of the gonococci.

The protective effect of human serum and urethral secretion might be explained in two ways. Serum and urethral secretion are probably hygroscopic—which would delay the final dehydration in deeper parts of the drop. This view is supported by the considerably prolonged survival of gonococci in a refrigerator with its high relative humidity (80%) offering poor possibilities for evaporation. It is possible that the pronounced tendency to autolyse (5, 11) shown by gonococci reduces their chances of survival markedly. The protein components of human serum and urethral secretion may also absorb molecules deleterious to gonococci. Such compounds may also be present on the glass or in the serum in rising concentrations during shrinkage of the drop. However, this latter explanation is more hypothetical. It is well known that staphylococci are relatively resistant to drying and can be transmitted airborne. As also shown here, *E. coli* is less resistant to drying than staphylococci (13) but still far more resistant than gonococci.

In order to understand the fragility of *Neisseria gonorrhoeae* in a more exact way further knowledge of the gonococcal cell is needed, its physiology, division and growth as well as the chemistry of the envelope that protects the cells. Work in these areas is in progress in this laboratory.

This investigation shows that *Neisseria gonorrhoeae* can survive over relatively long periods outside their normal habitat. It has also been demonstrated that gonorrhoea can be contracted through artificial inoculation with urethral secretions from patients with gonorrhoea (7). Furthermore, several case reports suggest the possibility of a non-sexual transmission of gonorrhoea (3, 8). Taken together, these observations suggest that a total denial of the existence of non-sexual acquisition of gonorrhoea is mistaken. Although, the number of live gonococci in urethral secretions from patients with gonorrhoea has been estimated to 10^5–10^7/ml (15) the period during which such a discharge may be infectious cannot be determined until the curves reported here can be compared with knowledge of the infectious dose in gonorrhoea.

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T. Elmros, M.D.
Department of Clinical Bacteriology
University Hospital
S-901 85 Umeå
Sweden