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Effect of Drying on Neisseria Gonorrhoeae in Relation to non-Venereal Infection in Children

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Summary

Alausa, K. O. Sogbetun, A. O. and Montefiore, D. (1977). *Nigerian Journal of Paediatrics*, **4** (1), 14. **Effect of Drying on Neisseria Gonorrhoeae in Relation to non-Venereal Infection in Children.** Previous work has suggested the possibility of transmission of gonorrhoea by towels, bed-clothes and underclothes. The sensitivity of the gonococcus to the lethal effects of drying has usually been taken to imply that such articles could only remain infective for a very short time; in the case of vulvo-vaginitis of children, sexual or close personal contact with an infected adult has been considered the most likely source of infection. *In vitro* experiments reported here show that the high relative humidity of the tropics during the rainy season may allow clothes contaminated with gonococci to remain infective for at least three to four hours. It could thus transfer infection without the need for immediate or close personal contact. Adult patients with gonorrhoea should be warned that they may be a potential danger to children in the household who may share the same bed-clothes, or other articles such as underclothes.

Gonococcal vulvo-vaginitis is common among children in Nigeria (Osoba and Alausa, 1974), and usually there is no evidence of sexual contact. However, in many instances one or both parents, or other member of the close family circle, is found to have acute gonorrhoea.

The possibility of indirect, non-venereal, transmission of gonorrhoea from infected adults to children has been raised by previous workers, who have suggested that infection might be transferred by contaminated hands, or by bedclothes or under-clothes (Michalowski, 1961; Shore and Winkelstein, 1971). The gonococcus is known to be extremely sensitive to drying (Wilson and Miles, 1975), and it is implied that infection can only be transmitted by an inanimate object very shortly after it has been contaminated.

However, the rate of drying depends on the relative humidity of the atmosphere. In the rainy season in the tropics, the relative humidity is extremely high, and drying of cloth is very slow if it is shielded from the sun or other heat source. This effect can be particularly evident in over-crowded sleeping accomodation, where both children and adults may use the same bed.

It thus seemed worthwhile to study, in an *in vitro* system, the effects of drying on the survival of gonococci applied to cloth samples which were exposed to the atmosphere in a room without air-conditioning, during the rainy season.

Materials and Methods

1. Preliminary Experiment

A sample of pus from a male patient with acute gonococcal urethritis was placed on a number of microscope slides which were then left exposed to ordinary room conditions in the dark. Portions of the pus were inoculated, every half hour, onto Brain Heart Infusion blood agar (incorporating vancomycin, polymyxin and nystatin). The plates were then incubated at 36°C in candle extinction jars for 48 hours, after which they were examined for the presence of *Neisseria gonorrhoeae*. Under these conditions gonococci could be isolated for 2½ hours: after this time the pus dried, and this coincided with the disappearance of viable gonococci.

In order to get an approximate estimate of the number of viable gonococci present in urethral discharge, a second sample of pus was titrated using a standard loop dilution technique. Three replicate estimations gave a mean $\log_{10}$ count of 7.98 viable colony forming units per ml of the gonococcal pus.

2. Survival of gonococci on cloth samples

For these experiments a freshly isolated strain of *N. gonorrhoeae* was used. The organism was suspended in 50 per cent serum-glucose broth, using inactivated foetal calf serum, to simulate any protective effect of protein in inflammatory exudates.

(a) Experiment 1: Drying on white cloth at high humidity

Small discs of white cotton cloth were prepared and sterilised in the autoclave, and then dried. Each disc was then fully moistened with the suspension of gonococci, and transferred with sterile forceps into Petri dishes whose lids were kept partly closed. The experiment started (Time 0) by eluting five groups of three discs into 1.5 ml portions of glucose broth containing 1 per cent calf serum. Elution was carried out by thorough swirling and squeezing of the discs, using sterile forceps.

Serial ten-fold dilutions of the eluate were made in 1 per cent serum-glucose broth, and a surface viable count (Miles and Misral, 1938) of gonococci was then made for each dilution. The mean of five values obtained for each dilution was taken as the appropriate colony count. This procedure was repeated with further groups of discs every 30 minutes for 2½ hours.

(b) Experiment 2: Drying on white cloth in room without air-conditioning.

Small squares were marked out in pencil on a piece of the same type of white cloth that was used in Experiment 1. The cloth was sterilised, and after drying, was moistened with the suspension of gonococci. The cloth was laid out to dry on a sheet of sterile aluminium foil in a room without air-conditioning and in normal room lighting conditions, although shielded from direct sunlight from the windows. At the start of the experiment, squares were cut out with sterile scissors, and then treated in the same way as the discs had been, in Experiment 1. Titrations were carried out each hour for five hours.

(c) Experiment 3: Drying on coloured cloth in room without air-conditioning.

Since coloured cloth is frequently used for underclothing, or as a 'wrapper', the same experiment was repeated using coloured cotton cloth.

All cloth samples were first washed well with soap and water to remove any toxic 'finishes' which might have been applied by the manufacturer, and to remove excess dye.

In each of the above three experiments, the mean viable bacterial counts were calculated for each time interval and trend lines plotted using the method of least squares. Inspection showed that the rate of decline in viable bacterial count obtained in Experiment 1 clearly differed from those obtained in the other two experiments, but the trend lines for Experiments 2 and 3 were compared by calculating the standard error of the difference between the regression coefficients obtained in these experiments.

Results

Experiment 1: Drying on white cloth at high humidity.

The Table shows the log mean bacterial counts obtained at each time interval, and inspection shows that there was only a very slight fall in the numbers of viable gonococci recovered over the period of the experiment. The linear trend line calculated for these values gave a regression coefficient of -0.10, with a standard error of 0.04. Using Student's 't' test, a value of 1.25 was obtained, indicating that there was no significant fall in the numbers of bacteria during the course of the experiment ($P > 0.05$). During the course of this experiment, the discs all remained obviously wet.

Experiment 2: Drying on white cloth in room without air conditioning.

The Table shows the log mean bacterial counts obtained during this experiment. During the first hour, the cloth samples remained obviously damp, and it can be seen that there was virtually no fall in the numbers of gonococci recovered during this period. By two hours, drying was clearly apparent, and this was reflected in a fall in the numbers of viable bacteria; this fall continued during the rest of the experiment, with the viable count falling to zero by four hours.

During the first hour, when the cotton cloth was damp, the conditions apparently corresponded to those obtained during the whole of Experiment 1, but after the first hour, as drying occurred, there was a progressive decrease in the viable bacterial counts. A linear trend line calculated from Hour 1 to the end of the experiment, gave a regression coefficient of −1.62, with a standard error of 0.16.

Experiment 3: Drying on coloured cloth in room without air-conditioning.

The Table also shows the log mean bacterial counts obtained during this experiment. Here, obvious drying did not occur during the first two hours, and again the viable bacterial counts remained stable as long as the cloth remained obviously damp. As in the previous experiment, once obvious drying occurred, from 2 hours onwards, there was a progressive fall in the numbers of viable bacteria recovered. The linear trend line calculated from this point onwards gave a regression coefficient of −2.40, with a standard error of 0.38.

Effect of Drying on Viability of N. Gonorrhoeae on Cloth Samples

	Experiment 1 (High humidity)	*Experiment 2 (White Cloth)*	*Experiment 3 (Dyed cloth)*
Time (Hours)	*Log. Mean Count per ml*	*Log. Mean Count per ml*	*Log. Mean Count per ml*
0	6.1219	4.9671	7.5744
½	6.2071	–	–
1	5.6972	4.6785	7.6075
1½	6.0068	–	–
2	5.7952	3.7147	8.0585
2½	5.6355	–	–
3	–	1.5224	6.5222
4	–	0.0000	2.5185
5	–	–	1.3802

Note: Experiment 2: Dry bulb temperature 25°C Relative Humidity 83%
Experiment 3: Dry bulb temperature 25.6°C Relative Humidity 81%

The difference in values between the two regression coefficients obtained in Experiments 2 and 3, was 0.78; the standard error of the difference between the two regression coefficients was 0.41. Thus there is no significant difference ($P > 0.05$) between the rate of loss of viable gonococci observed in the two experiments, and it can be concluded that the dyes used on the cloth samples in Experiment 3 did not exert any detectable toxic effect on the gonococci.

Figures 1, 2 and 3 illustrate in graphic form the values given in the Table, together with the calculated linear regression lines described in the text.

Discussion

The problem of gonococcal vulvovaginitis in children was reviewed by Lang (1955), who noted that while the infection results from direct or indirect contact with infected adults, it may also spread from child to child in institutions. He mentioned the belief that infection may be spread by linen, towels, bath tubs and thermometers, but inclined towards the opinion expressed by Cohn, Stear and Adler, (1940) that some form of sexual contact is the usual source; this view was repeated by Holmes (1974) and by Branch and Paxton (1965).

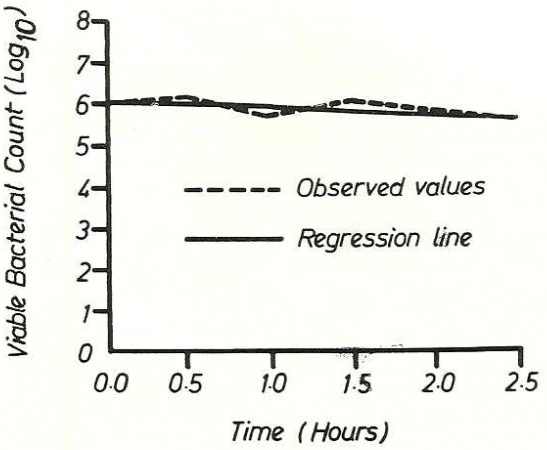


Fig. 1. Regression curves showing the survival of N. gonorrhoeae on moist white cotton cloth discs in partially covered Petri dishes. Note that there is no difference between the observed and expected values.

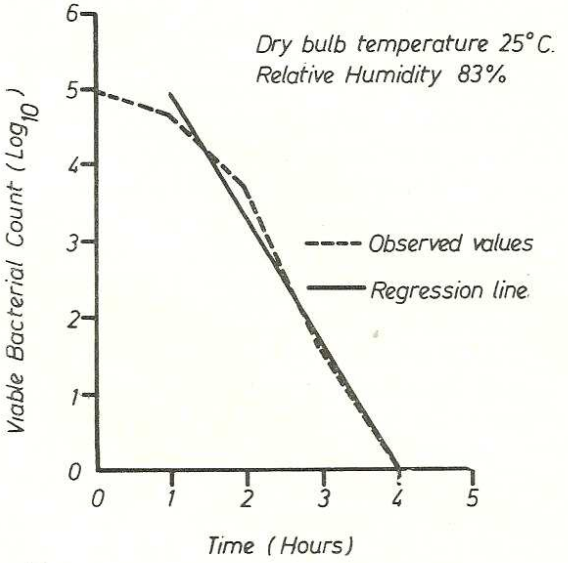
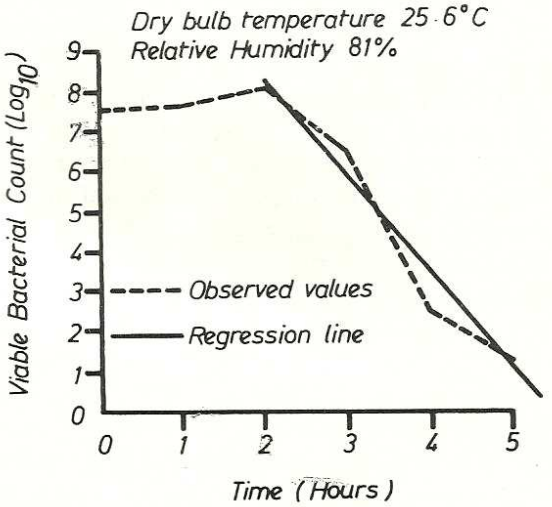


Fig. 2. Regression curves showing the effect of drying on the survival of N. gonorrhoeae applied to white cloth under room conditions. Note that after 4 hours there are no viable gonococci on the cloth.



Ftg. 3. Regression curves showing the effect of drying on the survival of N. gonorrhoeae applied to dyed cloth under room conditions. After 4 hours there are still some viable gonococci.

However, Osoba and Alausa (1974) in describing 17 cases of vulvovaginitis in Nigerian children, could detect evidence of close personal contact with infected adults in only four cases, and sexual assault in a further two cases. In three cases they found evidence that the infected child had shared towels or underclothes with a similarly infected sister, while in eight cases they were unable to determine the route of transmission.

As already mentioned, the possibility of transmission of infection via inanimate objects has been considered in the past, and evidence supporting this view has been put forward from time to time (Shore and Winkelstein 1971). It has, however, usually been emphasised that the gonococcus is a very delicate organism which is extremely sensitive to drying, with the implication that survival outside the human body must be quite short.

That this may not be so was evidenced by Elmross and Larsson (1972) who showed that gonococci could still be isolated from urethral pus kept on microscope slides in a Petri dish at room temperature for up to a maximum of 24 hours.

Osoba and Alausa (1974) made the suggestion that in most conditions such as obtain in the humid tropics, contaminated towels, underclothes and bedding might remain sufficiently damp to allow transmission of infection for relatively long periods of time. In this connection it may be noted that most urban family units in Nigeria live in crowded one or two-room dwellings, and the practice of adults and children using the same bed and 'wrapper' is still very common. It is also common practice for a child to share the use of a towel with an adult in the family unit.

The present experiments have shown that it would be possible for gonococci to survive long enough for infection to be transmitted by contaminated cloth materials. With the relative humidity of approximately 80 per cent during the periods of our experiments, obvious drying did not occur for between two and three hours, and this was followed by a reduction of viable gonococci at a rate of up to 2.4 $\log_{10}$ units per hour. In titrating the gonococcal pus, we found approximately 8.0 $\log_{10}$ units per ml of pus for the viable *N. gonorrhoeae* content, and this would suggest that an area of cloth contaminated by exudate sufficiently to moisten it, or which was already moist, could remain infective for three or four hours. However, at certain times of the year the relative humidity can rise much higher than 80 per cent, especially at night, and this could further delay drying, and hence increase the survival time of the gonococci.

Our findings thus lend support to the belief that, at least in the tropics, gonorrhoea can be transmitted by articles such as clothes, bedding and towels. They also suggest that such articles can remain infective for substantial periods of time, so that close or immediate contact with an infected person is not required. It would therefore seem justifiable to emphasise to adults who present for treatment of acute gonorrhoea, that they may not only spread infection by sexual contact, but may also be a source of danger to young children who may not even come into immediate contact with them.

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