Environmental Survival of *Neisseria meningitidis*

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Summary

*Neisseria meningitidis* is transmitted through the inhalation of large human respiratory droplets, but the risk from contaminated environmental surfaces is controversial. Compared to *Streptococcus pneumoniae* and *Acinetobacter baumannii*, meningococcal viability after desiccation on plastic, glass or metal surfaces decreased rapidly; but viable meningococci were present for up to 72 hours. Encapsulation did not provide an advantage for meningococcal environmental survival on environmental surfaces.

*Neisseria meningitidis*, an obligate pathogen of humans often carried asymptomatically in the human nasopharynx, may cause epidemic as well as endemic disease [1]. Meningococcal transmission can be the result of the inhalation of large airborne droplets (produced by coughing or sneezing) from colonized individuals, but disease after direct contact with respiratory secretions, saliva and laboratory acquired cases are reported [1, 2].

Meningococcal transmission and disease have also been linked to environmental conditions including changes in humidity, smoking and “dust” (Harmattan) [3–5].

Capsular polysaccharide is a major meningococcal virulence factor. Serological typing and the biochemical composition of capsular polysaccharides have classified *N. meningitidis* into a total of 12 serogroups [1]. However, six capsular serogroups (A, B, C, W-135, X, and Y) cause almost all meningococcal disease. Serogroups B and C capsular polysaccharides are sialic acid homopolymers of (α2→8) and (α2→9) linkages, respectively; while serogroups Y and W-135 are alternating units of D-glucose or D-galactose and sialic acid, respectively. Serogroup A *N. meningitidis* expresses a N-acetyl mannosamine-1-phosphate capsule, while serogroup X expresses (α1→4) linked N-acetyl-D-glucosamine 1-phosphate. Capsular polysaccharides have been proposed to prevent desiccation and provide anti-adherent properties, thereby promoting meningococcal loss from mucosal surfaces and survival outside the human host.

We investigated the environmental survival of *N. meningitidis* and determined whether encapsulation of *N. meningitidis* indeed enhanced survival upon environmental desiccation.

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Declaration of Interest
None.
Representative strains from serogroups A (F8229), B (NMB) and C (FAM18) and their non-encapsulated isogenic derivatives, which include a non-encapsulated serogroup A strain (F8239), NMB/synA∷tetM (M7) and FAM18/synA∷tetM (F-M7), were examined. *Streptococcus pneumoniae*, which has been found to be desiccation tolerant [6], and *Acinetobacter baumannii*, known for its ability to survive in the environments [7], were also examined under identical conditions. *N. meningitidis*, *S. pneumoniae* (strain D39) and *A. baumannii* (strain M2) were streaked onto appropriate agar plates and grown at 37°C overnight in a 5% CO₂ incubator. The bacterial colonies were resuspended in PBS, and the optical densities at 600 nm were adjusted to obtain ~10^8 cells/50 μl. Fifty μl aliquots of suspensions were added and spread evenly within individual wells of 24-well microtiter plates. Immediately after spreading, the bacteria were recovered with one millilitre of PBS, serially diluted, and plated to determine the input colony forming units (CFU). The uncovered plates were dried at 37°C for 30 min and then placed in the dark at room temperature. At specific times bacteria were recovered by resuspending in one millilitre of PBS and appropriate dilutions were plated on appropriate agar plates to determine viable CFU counts. Similar experiments were also performed with meningococci deposited onto glass and metal surfaces. Survival was calculated by dividing the viable CFUs by the input CFUs. Fluctuations in ambient temperature (ranged between 23 to 26°C) and relative humidity in the environmentally maintained indoor laboratory space were not further controlled.

Under the experimental conditions, the viable counts of meningococci decreased approximately three to four logs during the first 2 hours and declined ~6 logs over 24 hours (Figure 1A) for all three serogroups. Although a trend of increased survival of the serogroup C strain FAM18 was seen, no statistically significant difference was found between the serogroups. In addition, the meningococcal survival under desiccation was similar between encapsulated and non-encapsulated strains for all three serogroups (Figure 1A). No differences in meningococcal survival were seen when environmental survival was assessed on plastic, glass or metal surfaces (data not shown). In comparison, *S. pneumoniae* showed significantly better survival with only an approximately two log decline at 24 hr (Figure 1A).

*A. baumannii* and other *Acinetobacter* species have previously been shown to exhibit remarkable ability to resist environmental conditions and desiccation [7]. To compare with *N. meningitidis*, desiccation was extended to 72 hours following the same experimental procedure. *A. baumannii* strain M2 demonstrated the best survival with less than 1 log decline in 72 hours; while *S. pneumoniae* showed an intermediate survival phenotype (Figure 1B). However, viable meningococci could still be recovered after 72 hours of desiccation.

Several reviews of the persistence of pathogens on various surfaces have been published [8, 9]. It is widely recognized that many common nosocomial pathogens have prolonged survival on environmental surfaces, thereby providing a source of repeated transmission. Both gram-positive pathogens, such as *Enterococcus* sp., *Staphylococcus aureus*, or *Streptococcus pyogenes*, and gram-negative species, such as *Acinetobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Serratia marcescens*, or *Shigella* sp., have been shown to be capable of survival for months on dry surfaces; while others, such as
Bordetella pertussis, Haemophilus influenzae, Proteus vulgaris, or Vibrio cholerae, persist for days [8].

Meningococcal environmental survival has importance for estimating the risk of transmission from exposure to contaminated surfaces. There is a widespread belief that meningococci do not survive for more than a few minutes on environmental surfaces. However, in literature dating to the 1940’s, survival of *N. meningitidis* on either glass or fabric was reported to be ~3–30 hours [9, 10] consistent with our observations. In a 2008 study by Swain and Martin, while rapid decline of meningococcal viability with desiccation was noted, survival was observed for up to 168 hours on most surfaces [11]. *N. meningitidis* is much more susceptible to environmental desiccation compared to *S. pneumoniae* and *A. baumannii*, but meningococcal survived up to 72 hours in our study.

The meningococcal inoculum required for colonization or needed to cause invasive disease is not known. Also, whether meningococci after prolonged environmental desiccation remain infectious has not been determined. However, data from experimental *N. gonorrhoeae* urethral infections in male subjects observed an ID$_{50}$ of ~10$^5$ CFU of gonococci, which could be further reduced to ~3 × 10$^2$ if gonococci obtained from the initial passage after infection were used [12]. If the infectious inoculum is similar for *N. meningitidis*, environmental surfaces could be a source of transmission for at least several hours. Although a limited number of strains were examined in this study, they are well characterized clinical isolates that represent the three worldwide major invasive serogroups. The data support a role of environmental surfaces or objects contaminated by saliva or nasal secretions in meningococcal transmission, emphasize the avoidance of sharing potentially contaminated items, promote the disinfection of surfaces contaminated by meningococci in both community, laboratory and hospital settings, and point out the need for preventive vaccination where there is increased risk. In addition, our study found that contrary to another common opinion, encapsulation did not provide a survival advantage to meningococci against desiccation. Our data and those of others suggest that contamination of environmental objects can be a risk for meningococcal transmission.

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**References**


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Figure 1.
(A) Survival of *N. meningitidis* and *S. pneumoniae* over 24 hours after desiccation. Encapsulated strains F8229 (serogroup A), NMB (serogroup B), and FAM18 (serogroup C) were examined in parallel with non-encapsulated strains, serogroup A strain (F8239), NMB/synA::tetM (M7) and FAM18/synA::tetM (F18-M7), respectively. The survival of pneumococcal strain D39 was included for comparison. Viable CFU counts obtained from individual wells were normalized to the input CFU counts, which are set as 100%. The mean values from at least five independent experiments are shown. No significant differences were observed between encapsulated and non-encapsulated meningococcal strains by Students’ t test. (B) Survival over 72 hours after desiccation. Encapsulated meningococcal strains NMB and FAM18 were examined in parallel with *S. pneumoniae* strain D39 and *A. baumannii* strain M2. The mean values and standard deviations of viable CFU counts at each time point from five independent experiments are plotted. *A. baumannii* survived significantly better than *S. pneumoniae* and *N. meningitidis* as calculated by Students’ t test (p < 0.05).