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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 baumanni*, meningococcal viability after desiccation on plastic, glass or metal surfaces decreased rapidly, but viable meningococci were present for up to 72 h. Encapsulation did not provide an advantage for meningococcal environmental survival on environmental surfaces.

Key words: Environmental management, infectious disease control, *Neisseria meningitidis*.

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 have been reported [1, 2]. Meningococcal transmission and disease have also been linked to environmental conditions including changes in humidity, smoking and 'dust' (Harmattan, a hot dry wind) [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, 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 suggested 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. 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

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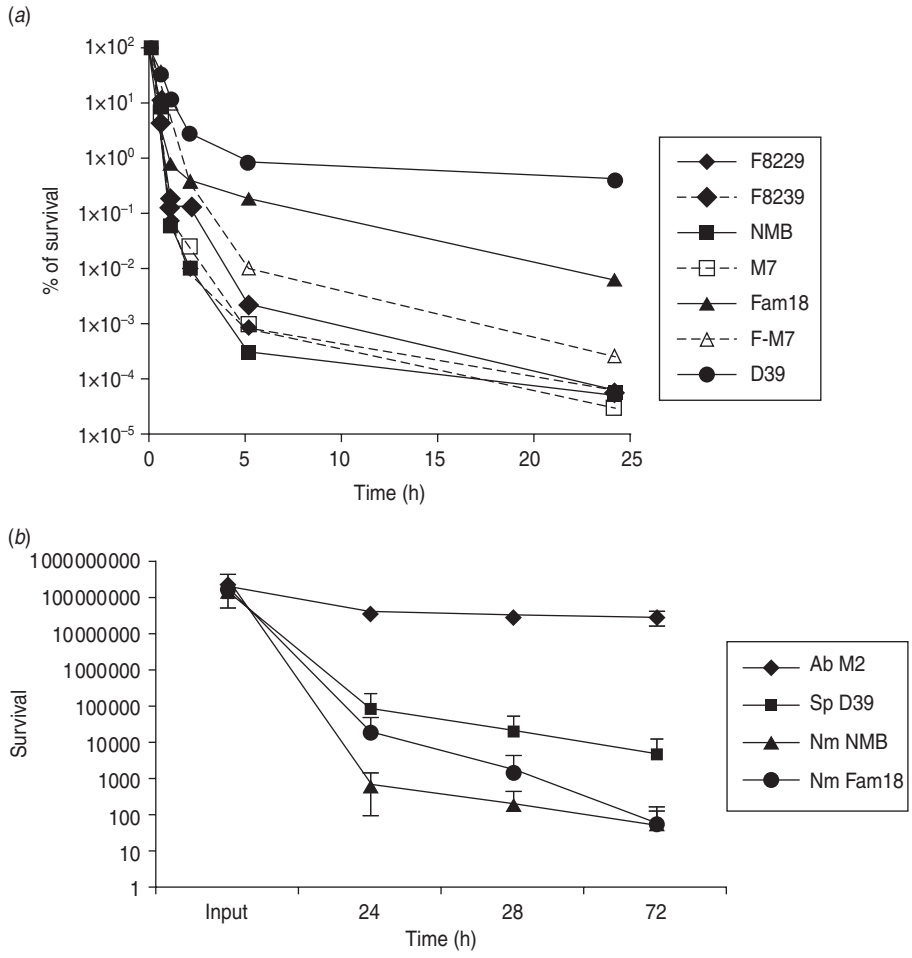

Fig. 1. (*a*) Survival of *N. meningitidis* and *S. pneumoniae* over 24 h after desiccation. Encapsulated strains F8229 (serogroup A), NMB (serogroup B), and FAM18 (serogroup C) were examined in parallel with non-encapsulated strains, serogroup A strain (F8239), NMB/*synA::tetM* (M7) and FAM18/*synA::tetM* (F18-M7), respectively. The survival of pneumococcal strain D39 was included for comparison. Viable colony-forming unit (c.f.u.) counts obtained from individual wells were normalized to the input c.f.u. 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 Student's *t* test. (*b*) Survival over 72 h 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 c.f.u. 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 Student's *t* test ($P<0{\cdot}05$).

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% CO_2 incubator. The bacterial colonies were resuspended in PBS, and the optical densities at 600 nm were adjusted to obtain $\sim 10^8$ cells/50 µl. Fifty µl aliquots of suspension were added and spread evenly within individual wells of 24-well microtitre plates. Immediately after spreading, the bacteria were recovered with 1 ml PBS, serially diluted, and plated to determine the input colony-forming units (c.f.u.). 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 1 ml PBS and appropriate dilutions were plated on appropriate agar plates to determine viable c.f.u. counts. Similar experiments were also performed with meningococci deposited onto glass and metal surfaces. Survival was calculated by dividing the viable c.f.u.s by the input c.f.u.s. Fluctuations in ambient temperature (range 23–26 °C) and relative humidity in the environmentally maintained indoor laboratory space were not further controlled.

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Under the experimental conditions, the viable counts of meningococci decreased ~3–4 logs during the first 2 h and declined ~6 logs over 24 h (Fig. 1*a*) 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, meningococcal survival under desiccation was similar between encapsulated and non-encapsulated strains for all three serogroups (Fig. 1*a*). 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 a ~2 log decline at 24 h (Fig. 1*a*).

A. baumannii and other *Acinetobacter* spp. 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 h following the same experimental procedure. *A. baumannii* strain M2 demonstrated the best survival with <1 log decline in 72 h, while *S. pneumoniae* showed an intermediate survival phenotype (Fig. 1*b*). However, viable meningococci could still be recovered after 72 h 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* spp., *Staphylococcus aureus*, or *Streptococcus pyogenes*, and Gram-negative species, such as *Acinetobacter* spp., *Escherichia coli, Klebsiella* spp., *Pseudomonas aeruginosa, Serratia marcescens*, or *Shigella* spp., 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 1940s, survival of *N. meningitidis* on either glass or fabric was reported to be ~3–30 h [9, 10], which is consistent with our observations. In a 2007 study by Swain & Martin, while rapid decline of meningococcal viability with desiccation was noted, survival was observed for up to 168 h on most surfaces [11]. *N. meningitidis* is much more susceptible to environmental desiccation compared to *S. pneumoniae* and *A. baumannii*, but meningococci survived up to 72 h in our study.

The meningococcal inoculum required for colonization or needed to cause invasive disease is not known. Moreover, 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 $\sim 10^5$ c.f.u. of gonococci, which could be further reduced to $\sim 3\times 10^2$ 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 highlight 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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DECLARATION OF INTEREST

None.

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