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Snow angels – The microbiology of freshly fallen snow: implications for immunocompromised patients

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ABSTRACT

The frequency of seasonal snowfall results in the transient covering of gardens/amenity sites/open public spaces, which encourages recreational interaction mainly with children. No data is available demonstrating the microbiological composition of such fallen snow and therefore a study was undertaken to examine the microbiology of snow from 37 sites, estimating (i) total viable count (TVC), (ii) identification of bacteria, and (iii) the presence of *Pseudomonas aeruginosa.* Mean TVC count of 8.3 colony-forming units (cfu)/ml snow melt water, 51.7 cfu/ml, 865 cfu/ml and 2,197 cfu/ml, was obtained for public amenity sites, domestic gardens, public open spaces and melting snow from public footpaths, respectively. No bacterial organisms (<10 cfu/ml) were detected in 5/14 (35.7%) open public spaces, 2/5 (40%) amenity sites and in 1/10 (10%) domestic gardens. *Pseudomonas aeruginosa* was not detected from any snow sample examined. Bacterial diversity consisted of 15 bacterial species (11 Gram-positive/four Gram-negative). The six Gram-positive genera identified from snow were *Actinomyces, Bacillus, Brevibacillus, Micrococcus, Staphylococcus* and *Streptococcus.* The four Gram-negative genera identified were *Enterobacter, Pantoea, Pseudomonas* and *Xanthomonas*. *Bacillus licheniformis* was the most commonly isolated organism from snow; it was isolated from every snow type. Snow may contain a diverse range of bacteria, many of which are capable of causing human infections.

Key words | bacteria, cystic fibrosis, microbiology, *Pseudomonas aeruginosa*, snow, water

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INTRODUCTION

The recreational exposure of individuals to contaminated environmental water sources has been well documented in the literature (Ayi 2015; Fewtrell & Kay 2015). Such water-related infections can occur mainly through swimming or bathing in contaminated waters and can result in inhalation, ingestion or direct contact with water. However, to date, there has been a paucity of evidence describing infection due to recreational association with snow. Equally, there is a gap in the evidence base relating to a description of the microbiology of freshly fallen snow and a subsequent risk assessment to help decide on the infection risks (if any) associated with the seasonal recreational involvement of UK families enjoying playing in the snow, making snow angels, building snowmen and having snowball fights, while transient snowfalls last and before they melt away.

While the microbiology of polar and high altitude snow is well described (Liu *et al.* 2009; Yang *et al.* 2016), no data currently exists describing the microbiology of transient snow, while it lies for short periods in populated, temperate zones such as the British Isles. Knowledge of the bacteriological status of snow could help guide behavioural decisions as to how immunocompromised children, e.g.

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children with cystic fibrosis (CF), should/should not play in such snow environments. It was therefore the aim of this study to examine the microbiology of freshly fallen snow in Northern Ireland during the snow storms of January 2018 and to examine the potential of such snow sources harbouring potential bacteriological aetiological agents of infection for immunocompromised children, who enjoy playing seasonally in the snow, while it lasts.

MATERIALS AND METHODS

Location of snow sampling

Snow samples (30 ml) were collected aseptically in sterile plastic containers (Sterilin Ltd, UK) from freshly fallen snow (within 12 h after falling) at 37 sites, from the following four categories: (i) domestic gardens ($n = 10$), (ii) public amenity sites ($n = 5$; including public playing fields/grass football pitches/tennis courts), (iii) public places ($n = 14$; including public parks and open spaces), and (iv) public footpaths during the melting process ($n = 8$). The location of sites sampled included the surface and immediate sub-surface snow layers, specifically avoiding the interface zone between the snow and the ground (soil). All samples were transported to the laboratory and were processed within 24 h following collection. Sampling was performed at three rural locations in County Antrim, Northern Ireland, in January 2018.

Microbiological examination of snow

Total viable count (TVC)

The total number of culturable bacteria was enumerated quantitatively in each sample as follows: Snow samples were allowed to melt at ambient temperature to produce water from the melted snow. Each snow melt water sample (100 ul) and serial 1,000-fold (10^{-3}) dilutions was spread onto the surface of Standard Plate Count (SPC) agar (Oxoid CM0463; Oxoid Ltd, Basingstoke, UK) and incubated aerobically at 37 °C for 24 h in duplicate. After this period, all colonies on the plate were counted and the count expressed as colony forming units (cfu) per ml rainwater (cfu/ml).

Identification of organisms from snow melt water

SPC plates from (i) above were examined for different colonial types. Phenotypically different organisms were purified and subcultured on SPC agar, as detailed above prior to identification using the MALDI-TOF system (Biomerieux, France), in accordance with the manufacturer's instructions. All identifications were performed in triplicate.

Isolation of *Pseudomonas aeruginosa* from snow melt water

Snow melt water (1 ml) was selectively enriched by adding it to *Pseudomonas* Selective Broth (9 ml) and incubating at 37 °C for 48 h, following which 20 μl was inoculated on *Pseudomonas* CFC Selective Agar (Oxoid PO0291) and incubated as above. Following incubation, plates were examined for the typical presence of colonies displaying characteristic appearance of *Pseudomonas aeruginosa*. *Pseudomonas* Selective Broth was prepared by employing Todd-Hewitt broth (CM0189) as the basal broth, to which *Pseudomonas* CFC selective supplement (SR0103), containing the following active constituents was added (cetrimide (10 mg/L), fucidin (10 mg/L) and cephalosporin (50 mg/L)).

RESULTS AND DISCUSSION

Microbiological results for each of the four snow locations examined are shown in Table 1. Public amenity sites had the lowest mean total viable count (TVC) [8.3 cfu/ml] followed by domestic gardens, public open spaces and melting snow from public footpaths (2,197 cfu/ml). Melting snow from public footpaths was markedly more heavily contaminated by at least 1–2 logs, than snow which remained untouched. No bacterial organisms (<10 cfu/ml) were detected in 5/14 (35.7%) open public spaces, 2/5 (40%) amenity sites and in 1/10 (10%) domestic gardens. *Pseudomonas aeruginosa* was not isolated in any snow sample examined. A total of 15 bacterial species were identified from the snow samples, comprising 11 Gram-positive

Table 1 | Description of microbiological characteristics of snow

Snow location	Number of samples examined	Mean total viable count (TVC) (cfu/ml)	Bacteria identified	Presence of *Pseudomonas aeruginosa*
Domestic gardens	10	51.7	*Bacillus clausii* *Bacillus licheniformis* *Bacillus subtilis* *Brevibacillus* sp. *Enterobacter aerosaccus* *Micrococcus luteus* *Pseudomonas fluorescens* *Streptococcus vestibularis* *Xanthomonas translucens*	−ve
Public amenity sites	5	8.3	*Bacillus cereus* *Bacillus licheniformis* *Micrococcus luteus* *Staphylococcus warnerii*	−ve
Public open spaces	14	865	*Bacillus licheniformis* *Brevibacillus* sp. *Pantoea agglomerans* *Streptococcus mitis/oralis*	−ve
Melting snow from public footpaths	8	2,197	*Bacillus altitudinus* *Bacillus licheniformis* *Actinomyces viscosus*	−ve
Total	37	780	15 organisms identified 11 Gram +ve & 4 Gram –ve)	−ve

organisms and four Gram-negative. The six Gram-positive genera identified from snow were *Actinomyces, Bacillus, Brevibacillus, Micrococcus, Staphylococcus* and *Streptococcus*. The four Gram-negative genera identified were *Enterobacter, Pantoea, Pseudomonas* and *Xanthomonas*. *Bacillus licheniformis* was the most commonly isolated organism from snow, which was isolated from every snow type, as detailed in Table 1.

The aim of this study was to culturally isolate and identify bacterial species in freshly fallen snow, which may be potentially pathogenic and an infection risk to immunocompromised individuals, who could have a recreational association with snow, for example making snow angels in domestic gardens or public amenity areas, as well as in open public space, or using snow for sledging. For this reason, all incubations were performed aerobically and at 37 °C, so as to attempt to maximise the isolation of potential pathogens. We appreciate that with the methods employed in this study, we excluded being able to identify most of the environmental organisms, which were unable to tolerate this incubation temperature, but equally were probably not an infection risk. Additionally, we employed the relatively new phenotypic bacterial identification technique, matrix-assisted laser desorption/ionization – time-of-flight (MALDI-TOF), to identify the bacterial isolates from snow. This technique has recently been used to differentiate environmental aquatic bacterial isolates (Popović *et al.* 2017).

Although there have been no reports in the literature describing an immunocompromised child acquiring a water-related infection from recreational exposure to snow, the perceived risk to concerned parents remains real. For instance, parents of children with CF know the risks from environmental water sources and *Pseudomonas aeruginosa*. Therefore, any fun activity involving water, such as jumping in muddy puddles (Furukawa *et al.* 2018) or making snow angels generates the anxiety of heightening the risk of potentially acquiring *Pseudomonas aeruginosa*. With the exception of four bacteria, namely *Bacillus clausii, Enterobacter aerosaccus, Xanthomonas translucens* and *Bacillus altitudinus*, all the other isolated bacteria have been shown previously in the literature to have been pathogenic to humans, through the description of several case reports and case series. The question as to whether or not to allow immunocompromised children to play in the snow and not risk the acquisition of these opportunistic pathogens, is difficult to answer because of a number of variables, as discussed recently by Furukawa *et al.* (2018). For these reasons, it remains impossible to give an evidence-based response to such questions. Recently, Furukawa *et al.* (2018) set out a rationale that influences whether a susceptible patient could acquire such an infection from an environmental source. In particular, our concern is for patients with CF, who may acquire a *Pseudomonas aeruginosa* isolate potentially from contaminated snow. However, we were unable to isolate any *Pseudomonas aeruginosa* from any snow source in this study.

This study showed that untouched snow has a low bacterial TVC, ranging from 8.3 to 865 cfu/ml melt water from snow. This therefore prompts the question as to where such fresh snow acquires this bacterial load. Various potential sources may have led to the contamination of fresh snow, including: (i) wind – blowing aerial contaminants from soil onto fresh snow, (ii) wildlife (including aerial contamination via birds), (iii) aerosols containing bacteria taken up during the evaporation of water with the water cycle and deposited back down to earth via a meteorological snow storm. One interesting finding from this study was the isolation of *Bacillus altitudinus* from meltwater on public footpaths. This species has been associated with high altitudes (approximately 24 km above the earth) and was originally isolated from cryogenic tubes used for collecting air samples from high altitudes (Shivaji *et al.* 2006). The isolation of such an organism raises the possibility that this organism could have been aerosolised remotely to the location where snow precipitation occurred and carried at high altitudes before returning to earth through precipitation/snowing.

This study demonstrated that freshly fallen snow is not sterile and may be a source of a wide diversity of bacterial taxa (10 bacterial genera and 15 species), albeit in low numbers, ranging from <10 (non-detectible) to 2,197 colony-forming units (cfu)/ml snow melt water. Many of these identified organisms from snow are capable of causing human disease. The presence of potentially disease-causing bacteria in snow identifies a new potential environmental reservoir of infection-causing bacteria, which may be of importance to immunocompromised individuals.

In conclusion, snow contains a diverse range of bacteria, many of which are capable of causing infections, particularly in immunocompromised hosts. This study adds value to the current evidence base, by detailing the numbers and taxa of bacteria found in freshly fallen snow. Additionally, further work is required to help elucidate the findings of the current study, including an examination of the survival dynamics of potential pathogens in snow, as well as the potential transmission of such pathogens from the environment to humans.

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