Use of respiratory tract bacteria to assess aerosol and droplet dispersal: impact of respiratory activity

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PROTECT
A COVID-19 National Core Study

INTRODUCTION

Transmission of respiratory pathogens including SARS-CoV-2 can occur when droplets and/or aerosols containing infectious organisms are dispersed from the nasal and oral cavity of infected individuals[1]. Respiratory droplets (typically larger particles >5 µm in diameter) tend to settle rapidly, get inhaled into the upper respiratory tract or get deposited onto the mucosal surfaces of another person[2]. Respiratory aerosols are smaller (usually <5 µm in diameter), allowing their suspension in the air, and inhalation into the lower respiratory tract[3].

The risk of respiratory droplet and aerosol transmission depends on the complex interplay between environmental and physicochemical characteristics (e.g evaporation, chemical composition, relative humidity), expiratory activity, social behaviours, and mitigation strategy. Non-microbiology based studies have demonstrated that respiratory activities such as increased vocalisation and sneezing can generate large numbers of respiratory particles[4]. However, these studies provide limited information about potential infectivity (i.e. presence of target organism).

Carrying out controlled microbiology-based simulation studies is challenging particularly if the target organism is highly pathogenic. To overcome this, we have been investigating the use of normal oral and respiratory tract bacteria as index organisms for respiratory pathogens and applying this method to study how droplet and aerosol dispersal differs with activity, individual and mitigation strategy. To facilitate these investigations, we have designed IMADGENN (an **I**solator to **M**easure **A**erosol and **D**roplet **GEN**eratio**N**, Figure 1).

METHODS

- **Activities:** participants (n=16) were asked to count from one to 100 in their normal conversational voice. They were then asked to repeat the activity whilst shouting. A decibel meter was used to monitor sound level. No face covering was worn. Participants (n=5) were asked to self-induce a sneeze using a nasopharyngeal swab. Each participant sneezed three times – when wearing a disposable IIR medical face mask correctly (over the mouth and nose), incorrectly (only over the mouth) or not at all.

- **Droplets** were collected using Columbia blood agar settle plates placed at 10 cm intervals (up to 1 m) to the left, right and centre) of the isolator.

- **Aerosols** were collected using an R2S slit-to-agar sampler (17 litres min^{-1}) and a six stage Andersen sampler (28.3 min^{-1}) positioned 1.2 meter from source.

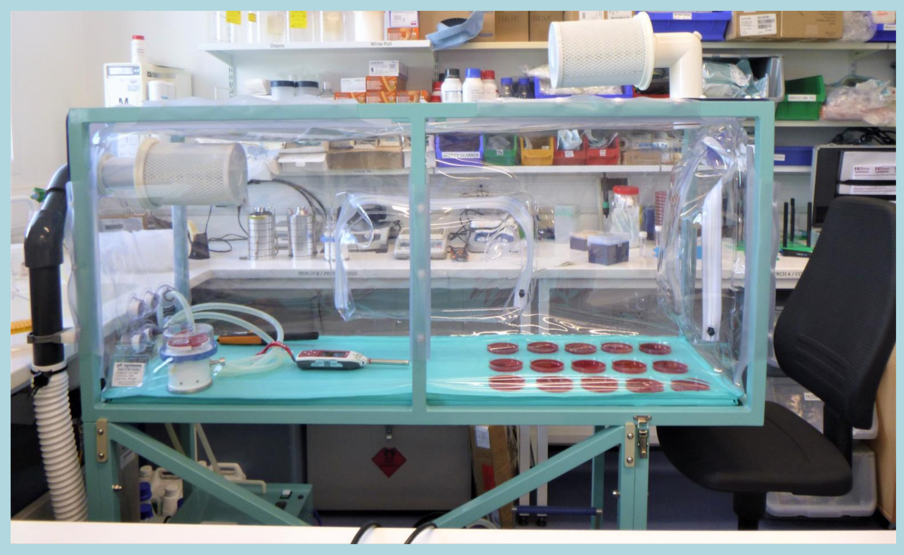

Figure 1 IMADGENN set up

- **Culturing and identifying oral/respiratory bacteria**: agar plates were incubated at 37 ºC for 48 hours. Colonies were identified using MALDI-TOF.

RESULTS

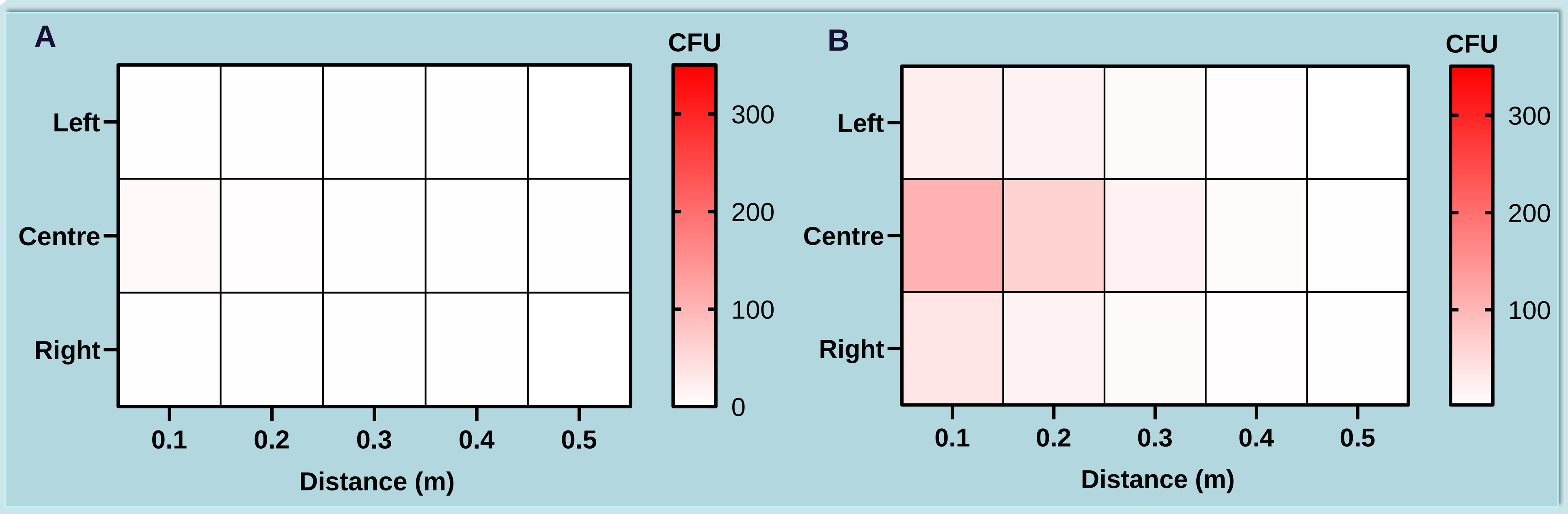

Figure 2 Heatmaps illustrating the dispersal of respiratory droplets when counting from one to 100 in normal conversational voice **(A)** and when shouting **(B).** Each rectangle represent the mean number of respiratory bacteria (CFU) recovered from the corresponding settle plate (n = 16).

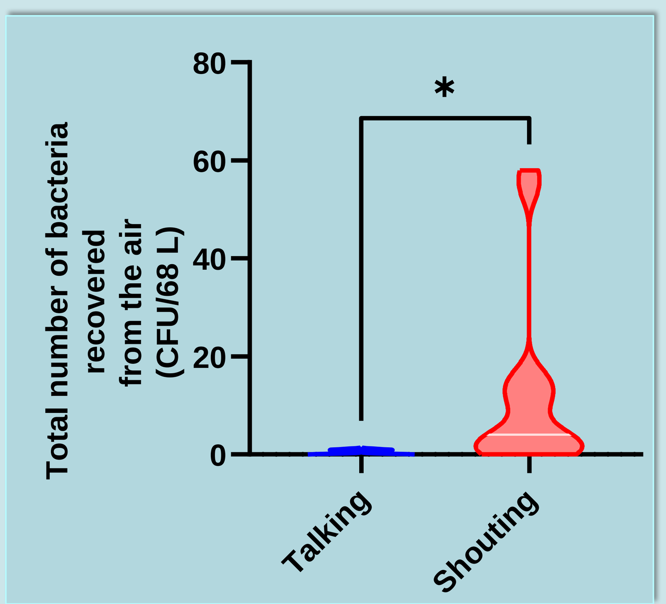

Figure 3 Violin plot illustrating the number of respiratory bacteria recovered from the air (slit-to-agar sampler) when talking and shouting.

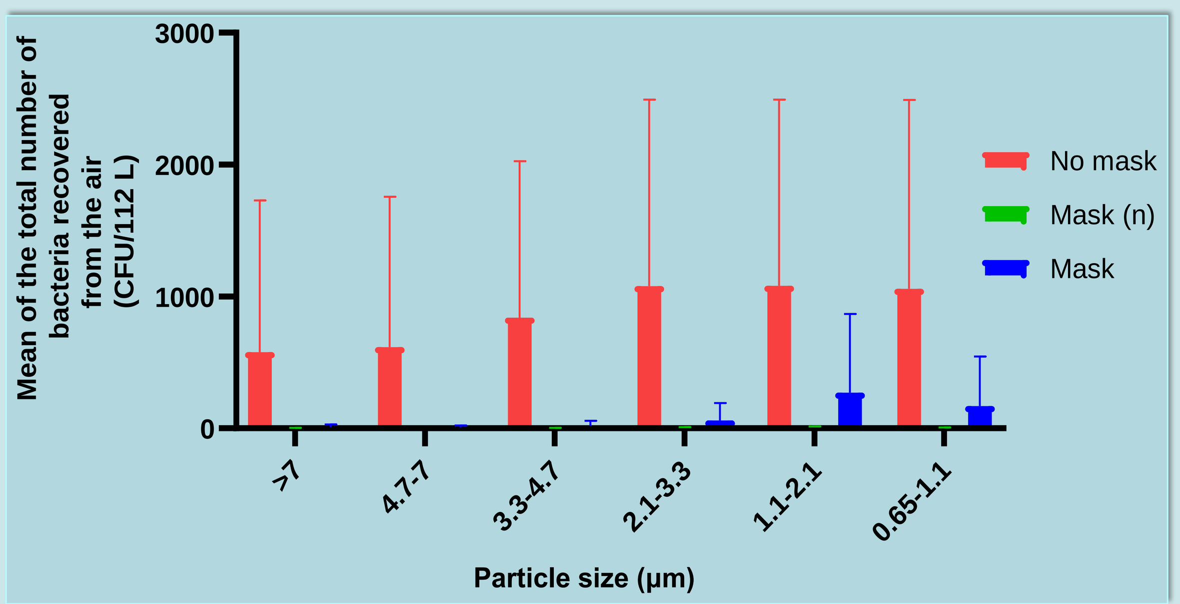

Figure 4 The mean number (± standard deviation) of respiratory bacteria recovered from the air (Andersen sampler) when a participant self-induced a sneeze when (IIR) face mask was worn correctly, only covering the nose (mask (n)) or not worn at all.

- When participants were asked to count from 1 to 100 in a normal conversational voice, the mean dB level recorded was 75 dB and the number of respiratory bacteria within 0.5 m from source ranged from 1 to 48 CFU (mean = 15 CFU; Figure 2A). An increase in vocal effort (to a mean voice level of 92 dB) generated significantly more respiratory droplets (p= 0.002) with the number of bacteria recovered ranging from 36 to 1251 CFU (mean = 324 CFU; Figure 2B). Regardless of activity, the number of bacteria recovered decreased significantly (p < 0.001) with increasing distance from source (Figure 2A,B).

- Similarly, shouting led to a significantly larger number of bacteria being recovered from the air (p= 0.016; Figure 3). After adjusting for other variables, the likelihood of more airborne bacteria being recovered increased by 34% with each dB increase in (mean) voice level (95% CI: 1.06, 1.70).

- When participants were asked to self-induce a sneeze when not wearing a IIR face mask, very high numbers of bacteria were recovered from settle plates up to 1 m from source (Figure 5A) and from the air. The highest proportion of airborne bacteria were impacted on stages 4, 5, 6 of the Andersen sampler (with quantitative limit for positive hole correction exceeding 2628 particles) implying that the majority of airborne particles were < 3 µm (Figure 4).

- Regardless of how it was worn (i. e. covering the nose and/or mouth), wearing the IIR mask while sneezing reduced the number of respiratory bacteria recovered from the air (Figure 4) and settle plates (Figure 5) by 1.5 and 2.35 log_{10} values respectively.

- However, whilst high numbers of respiratory particles can be produced during sneezing, 2 of the 5 participants dispersed little to no respiratory bacteria, highlighting large inter-individual variability, particularly within a small study cohort.

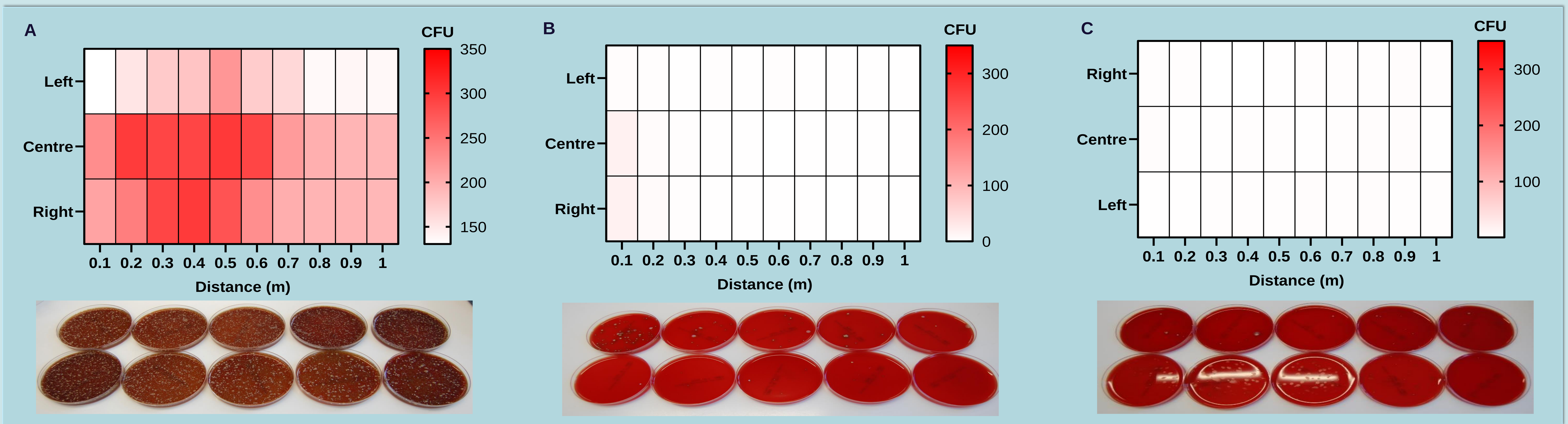

Figure 5 Heatmaps (top) illustrating the dispersal of respiratory droplets when sneezing-without the IIR mask **(A)** with mask only covering mouth **(B)** and with mask covering both nose and mouth **(C)** (n=5). Images (bottom) show the settle plates positioned at the center from 0.1 to 1 m from source when participant 5 was asked to self-induce sneeze.

CONCLUSIONS

- Compared to normal conversational speech, increased vocalisation generates significantly more bacteria-laden respiratory particles. Droplet deposition decreases with increasing distance from source, suggesting that during speech, if no face covering is worn, 1 meter social distancing can reduce the risk of droplet transmission.
- Preliminary results show the sneeze, as a violent expiratory event, can generate a high concentration of aerosols and high numbers of droplets over a distance of at least 1 meter reinforcing the need for respiratory hygiene.
- The results also suggest that IIR face masks can reduce or even prevent the dispersal of respiratory droplets and aerosols, even when the nose is exposed, confirming the effectiveness of face coverings as a mitigation strategy.
- Large inter-individual variability was observed during both respiratory activities, reinforcing the idea of "super-spreaders".
- The cohort participating in the sneezing experiment was small, partly due to the challenges sneezing on demand posed. Alternative methods for induction of the sneeze will be revisited and more data collected.

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