



Major Article

Bacteria emitted in ambient air during bronchoscopy—a risk to health care workers?

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Key Words:

Occupational exposure
Bioaerosols
Indoor air**Background:** Health care workers are at risk of occupational infections, and some procedures are known to increase this risk. The aim of this study was to qualify and quantify bioaerosol concentrations during bronchoscopy to estimate the occupational risk.**Methods:** Full-day sampling was conducted in 2 rooms while bronchoscopies were performed on patients. Two microbial air samplers were used, a wet wall cyclonic sampler and an impactor, on culture media. Identification of the culturable bacterial flora was performed with chromatographic analysis of cellular fatty acid of the isolated strain and additional biochemical tests if needed. Specific polymerase chain reaction analysis was completed on wet wall cyclonic samples for the detection of influenza A and B and *Mycobacterium* spp.**Results:** A wide variety of bacteria were collected from the ambient air. All samples yielded at least 1 *Staphylococcus* species. Although most of the culturable bacteria identified were normal nonpathogenic flora, such as *Streptococcus* spp, *Neisseria* spp, and *Corynebacterium* spp, some opportunistic pathogens, such as *Streptococcus pneumoniae*, were found. Neither *Mycobacterium* spp nor influenza virus was detected with the polymerase chain reaction method during this study.**Conclusions:** Culturable bacteria from oral, nasal, and pulmonary flora are aerosolized during bronchoscopy and could be inhaled by medical staff. The potential presence of pathogens in those aerosols could represent an occupational infection risk.

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Health care workers (HCWs) are at risk of occupational infections due to the nature of their work.¹ Even when safety protocols are implemented, HCWs are still considered to be at continued occupational risk of many infectious diseases transmitted from ill patients.² Although transmission of highly infectious diseases from patients to HCWs is uncommon, a number of cases have been reported.³ Several cases of transmission of *Streptococcus pyogenes* to HCWs have been described.^{3–6} *Neisseria meningitis*, *Haemophilus influenza*, and *Acinetobacter baumannii* are other well-documented occupational pathogen infections acquired by hospital personnel.^{3,7–9} The risk of influenza pandemics, emerging infections, and

antimicrobial resistance of bacteria has raised concerns about the health of HCWs¹⁰ and therefore about prevention practices that should be followed during particular procedures. Worldwide, HCWs are reported to account for 20% of all cases of acquired severe acute respiratory syndrome.¹¹

Some medical procedures increase the risk of occupational infections because of exposure to airborne pathogenic microorganisms. Staff can be infected during routine endoscopy procedures.¹² Accordingly, endoscopists show a higher seropositivity to *Helicobacter pylori*.^{13,14} Transmission of tuberculosis from infected patients undergoing bronchoscopy is another recognized occupational risk.^{15,16} Catanzaro¹⁵ calculated that during intubation and bronchoscopy, more than 200 units per hour of infectious mycobacteria are aerosolized from a patient.

Intubation with a bronchoscope stimulates a patient's coughing reflex.^{17,18} Coughing produces droplets of various sizes,¹⁸ many of which are inhalable and can be drawn deep down into the lungs.^{19–21} Particles of saliva, mucus, and pathogenic microbes can be emitted when a patient coughs. Because particles of this kind

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originate from a deeper area of the respiratory system, the droplets released by coughing may be more infectious than those from sneezing.^{22–25} Many of these particles are small enough to remain suspended in the air for a long time, and through evaporation, larger particles can become small enough to remain suspended in the air for an extended period also.²⁶

From the perspective of infectious disease spread by the airborne route, inhalable particles include particles in the size range from 0.1–10 μm in diameter. If particles carrying pathogens are inhaled, they may be deposited in parts of the respiratory tract where they are likely to cause infection and disease.^{20,26}

Although some researchers have reported on the infectious risk to HCWs performing high-risk procedures, to our knowledge, no study has ever documented, during bronchoscopy procedure, the real bioaerosol exposure of HCWs. Davies et al²⁷ reported in their 2009 review that “no quantitative study has yet been carried out on aerosol generating procedures,” and that “uncertainty surrounding aerosol generating procedures make it difficult to construct effective infection control policy.”

The aim of this study was to qualify and quantify bioaerosol concentrations during bronchoscopy to evaluate the occupational risk to HCWs. Knowing the real exposure is essential to encouraging HCWs to implement better prevention protocols and wear personal protective equipment if needed.

METHODS

This study has been approved by the ethics boards of the 2 involved hospitals and by the University of Quebec in Montreal Ethics Board.

Sampled rooms

Two bronchoscopy rooms in 2 different hospitals were investigated. The first room had a volume of 79 m^3 and was located in a recently constructed building. It had negative pressure in relation to its anteroom, with 12 air changes per hour. Room B was smaller, with a volume of 59.8 m^3 . Three air outlets equipped with high-efficiency particulate air filters expelled the air directly outdoors. The room is located in an older hospital built in the 1930s. Extensive renovations have been done over the years, and the current configuration dates from 2010.

Bioaerosol sampling strategy

Bacteria collected were analyzed by culture, whereas *Mycobacterium* spp and influenza A and B viruses were analyzed by molecular biology methods. One full day of sampling was carried out in each bronchoscopy room. Depending on the bronchoscopy procedure time, up to 8 samples were collected per patient. In room A, 5 bronchoscopies were performed during the sampling day, for a total of 24 culturable samples. In room B, 10 bronchoscopies were performed, for a total of 37 culturable samples. The measurements were taken consecutively for all steps in the bronchoscopy procedure, from the arrival of the patient through his or her departure. At the beginning of the day, before the first bronchoscopy, samples were taken to establish the background concentrations in the room. At both hospitals, 1 member of the research team was permitted by the medical staff to remain in the bronchoscopy room.

All samples were collected at a fixed station located within a radius of 1.5 m from the patient's mouth and the workers' breathing zone. Sampling continued for 20 minutes at the end of the day to determine whether bioaerosol concentrations would return to their morning background levels during this time. Twenty minutes was chosen because that is the recommended waiting time before

re-entering a room after a procedure has been performed on a patient with tuberculosis. On average, 4 people (research team member, doctor, nurse, and patient) were present in the room.

Air sampling

The sampling devices used to assess the bioaerosols were the Andersen N6 impactor (Andersen Instruments, Atlanta, GA) for the culturable bacteria and the Coriolis μ biological air sampler (Bertin Technologies, Montigny-le-Bretonneux, France) for the molecular biology analysis. The Andersen impactor is known as a standard for culturable bioaerosol analysis.²⁸ Five-minute sampling periods were used. The pump flow rate was adjusted in the bronchoscopy room to 28.3 Lpm using a TSI Mass Flowmeter 4199 (TSI Inc, Shoreview, MN) and checked between patients. The total volume of air sampled was used to calculate the culturable bacterial concentrations. The Andersen samplers were loaded with 90-mm petri dishes containing trypticase soy agar media to which 5% defibrinated sheep blood was added (Oxoid, Ontario, Canada). All dishes were incubated at 37°C for 48 hours. All colonies were enumerated according to the total count method.²⁹ The limit of detection was 7 CFU/ m^3 air for the Andersen impactor. The cyclonic Coriolis μ sampler was used at a flow rate of 150 Lpm. Fifteen milliliters of sterile 1× phosphate-buffered saline solution, pH 7.4 (Life Technology, Ontario, Canada), were placed in the conical vials. A sampling time of 10 minutes was used to obtain an adequate detection limit of 1,200 genomes. As with the Andersen impactor, > 1 sample was taken for some patients.

Bacteria identification

Identification was performed with the Sherlock Microbial Identification System (MIDI, Newark, DE) using fatty acid extraction analysis by Instant FAME (Fatty Acid Methyl Ester) on pure culture of each isolated strain.³⁰ The Clinical Aerobes (IBA) method following the manufacturer's protocol was used. Each strain was grown on trypticase soy agar blood and incubated at 35°C for 24 ± 2 hours. Some slow grower strains needed an extra 24 hours. Two to 3 mg bacteria cells were harvested for the fatty acid extraction. Identification to the species level was completed if the similarity index was > 0.6; the Gram stain and the phenotypic characteristics needed to match. When identification was not possible with the Sherlock Microbial Identification System, the GEN-III microplate (Biolog, Hayward, CA) or the Microscan Neg ID Type 2 panel or Pos ID Type 3 panel (Beckman Coulter, Mississauga, Ontario, Canada) was used to complete the identification.³¹ Even with the 3 systems, some strains could not be identified to the species level.

DNA and RNA extractions

Aliquots of Coriolis μ air samples (1.5 mL) were centrifuged (10 minutes at 14,000 × g) and the pellets were stored at –20°C until extraction. Total genomic DNA was extracted using the PowerLyzer UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturer's instructions. The DNA extraction homogenization was performed with a Mixer Mill MM301 (Retsch, Düsseldorf, Germany) at 20 movements per minute for 10 minutes. Total DNA was eluted in 50 μL elution buffer. The RNA was extracted with the MagMAX Viral RNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA).

Detection of mycobacteria and influenza by quantitative polymerase chain reaction

Amplifications were performed using the Bio-Rad CFX384 thermocycler (Bio-Rad Laboratories, Mississauga, Ontario, Canada).

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