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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 meningitidis*, *Haemophilus influenzae*, 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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Funding for this study was provided by the Institut de Recherche Robert-Sauvé en Santé et en Sécurité du Travail (Quebec Occupational Health and Safety Research Institute) (grant No. 0099-8990).

Conflicts of Interest: None to report.

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 m³ 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 m³. 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/m³ 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).

Previously published primers and probes were purchased from Integrated DNA Technologies (Coralville, IA). The detection systems used were specific for influenza A and B viruses and for bacteria of the genus *Mycobacterium*.^{32–34} The polymerase chain reaction mixture contained 2 µL DNA/RNA template, 0.4 µmol/L (each) primer, 0.08 µmol/L probe, and 10 µL 2 × QuantiTect Probe PCR master mix (Qiagen, Mississauga, Ontario, Canada) in a 20-µL reaction mixture. The results were analyzed using Bio-Rad CFX Manager software, release 3.0.1224.1015 (Bio-Rad Laboratories).

Statistical tests

An analysis of variance statistical test followed by a Tukey–Kramer multiple-comparison test was performed on log-transformed concentrations measured in the 2 rooms to determine whether a difference could be observed between the periods. Four periods were compared: the background measured in the morning, the waiting and preparation of the patient, the bronchoscopy procedure, and the return to background at the end of the day.

RESULTS

The average concentrations (colony forming units/meters³ of air) and the standard deviations of the culturable bacteria measured in the 2 bronchoscopy rooms are presented in Table 1.

The bacterial identifications obtained are shown in Table 2. Because of the high bacterial diversity found, pure culture isolation was done on the most frequently observed colony phenotypes to perform the strain identifications.

All the *Staphylococcus* spp strains were confirmed not to be *Staphylococcus aureus*. The average concentrations measured in room A varied from 43–100 CFU/m³ air. In room B, the average concentrations were higher, ranging from 40–370 CFU/m³. The highest concentration (580 CFU/m³ air) was measured during the second bronchoscopy procedure of the day in room B. Neither *Mycobacterium* spp nor influenza A and B viruses were detected in our investigations. The results of the Tukey–Kramer test done on the log-transformed concentrations are presented in Figure 1.

The test confirmed that the concentrations, measured during the bronchoscopies and the preparation of the patient, were significantly higher than the ones measured during the return to background at the end of the day. The morning background concentrations, on the other hand, were not significantly different, although they appeared to be lower than those found during the medical procedures.

Table 1

Average concentrations of culturable bacterial flora measured in the air of 2 bronchoscopy procedure rooms

Patients and periods	Room A			Room B		
	n	Mean CFU/m ³	Standard deviation	n	Mean CFU/m ³	Standard deviation
Background	4	58	64	2	50	14
1	2	60	0	4	208	105
2	2	100	42	3	370	193
3	1	60		4	105	37
4	8	76	24	3	136	46
5	3	53	25	3	183	64
6				2	50	28
7				2	165	106
8				1	310	
9				1	60	
10				1	40	
Return to background	4	43	47	8	68	44

Table 2

Bacterial flora identified during morning background, during patient bronchoscopies, and at the end of the day, in rooms A and B

Period	Room A	Room B
Morning background	<i>Corynebacterium</i> spp, <i>Dermaococcus nishinomiyaensis</i> , <i>Micrococcus</i> spp, <i>Staphylococcus epidermidis</i> , <i>Staphylococcus hominis</i> , <i>Staphylococcus warneri</i> , <i>Staphylococcus xylosum</i> , <i>Staphylococcus</i> spp	<i>Bacillus pumilus</i> , <i>Bacillus</i> spp, <i>Corynebacterium</i> spp, <i>Micrococcus luteus</i> , <i>Micrococcus</i> spp, <i>Staphylococcus epidermidis</i> , <i>Staphylococcus pasteurii</i> , <i>Staphylococcus</i> spp
Patients not identified during morning background	<i>Acinetobacter radioresistens</i> , <i>Bacillus circulans</i> , <i>Brevundimonas</i> sp, <i>Corynebacterium aurissanis</i> , <i>Paenibacillus pabuli</i> , <i>Staphylococcus equorum</i> , <i>Staphylococcus haemolyticus</i> , <i>Staphylococcus intermidis</i> , <i>Staphylococcus saprophyticus</i> , <i>Streptococcus</i> spp	<i>Acinetobacter lwoffii</i> , <i>Actinomycetes</i> , <i>Bacillus subtilis</i> , <i>Dermabacter hominis</i> , <i>Escherichia</i> sp, <i>Micrococcus lylae</i> , <i>Corynebacterium pseudodiphtheriticum</i> , <i>Neisseria</i> sp, <i>Rothia</i> sp, <i>Staphylococcus capitis</i> , <i>Staphylococcus haemolyticus</i> , <i>Streptococcus pneumoniae</i> , <i>Streptococcus mitis</i> , <i>Streptococcus</i> spp
Return to background	<i>Brevibacterium</i> spp, <i>Brevundimonas diminuta</i> , <i>Corynebacterium</i> spp, <i>Micrococcus luteus</i> , <i>Staphylococcus cohnii</i> , <i>Streptococcus</i> spp	<i>Actinomycetes</i> , <i>Moraxella</i> sp, <i>Rhodococcus</i> sp, <i>Staphylococcus capitis</i> , <i>Streptococcus</i> spp

DISCUSSION

To our knowledge, this is the first study to measure and identify the actual bacterial flora present in the ambient air of a room while bronchoscopies are being performed on patients. Although bronchoscopy has been identified as a high-risk procedure for HCWs,^{10,15,35,36} this recognition had until now been based on risk guesstimate and epidemiologic studies, but actual exposure had never been documented as it is here. Sampling of the ambient air of the 2 bronchoscopy rooms yielded a wide diversity of bacteria. Many sources of bioaerosols were identified, including outdoor air, medical staff, patients, and resuspension from surfaces. During the background and medical procedure periods, the concentrations and diversity of bacteria in the 2 rooms were noticeably different. According to Brandl and Mandal,³⁷ the main factors affecting the levels and diversity of airborne microorganisms in the indoor air of hospitals are the lack of cleanliness of the hospital, human activities, organic materials brought in from outdoors, and the efficiency of the hospital ventilation system. In general, the predominant genera of airborne bacteria reported in hospitals by other researchers are *Staphylococcus*, *Bacillus*, *Micrococcus*, and *Corynebacterium*.^{37–39} This was confirmed by our study, because at least 1 species of *Staphylococcus* spp was identified in every sample. Microorganisms from environmental and human sources were anticipated, and their presence was confirmed by our investigations.

Some of the bacteria identified are known to originate from the human buccal cavity and respiratory tract. Patients undergoing bronchoscopy cough numerous times, especially when the bronchoscope is being inserted. Because it is well known that coughing produces a high number of aerosol particles,^{17,19–21,40} it would seem logical to attribute a proportion of the microorganisms found in the ambient air to the patient's respiratory system. Short-term variation in, or sporadic appearance of, specific bacteria cannot be attributed to the

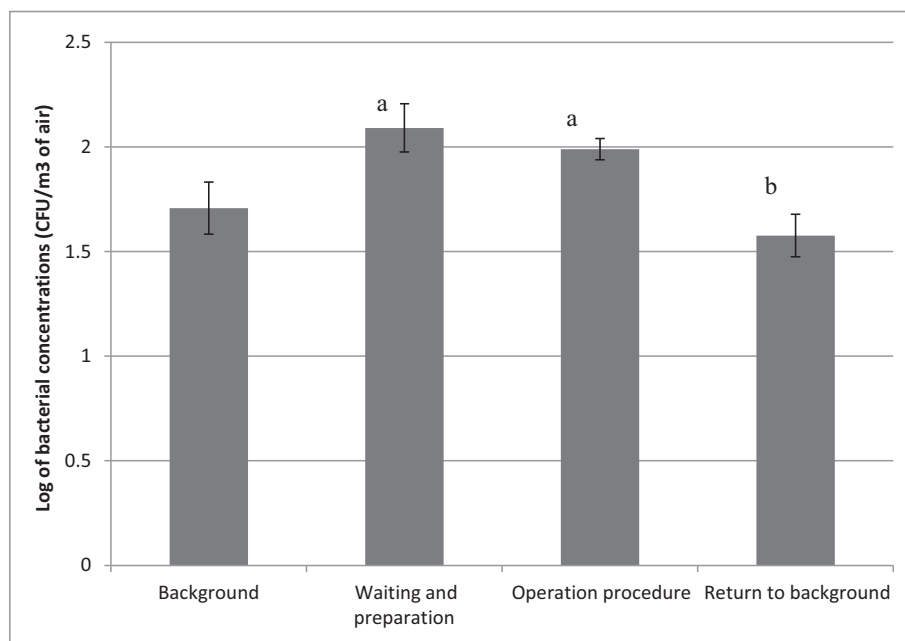


Fig 1. Bar chart showing results of Tukey-Kramer multiple-comparison analysis of the combined concentrations measured in rooms A and B for culturable bacteria using the impactor (5 minutes at 28.3 Lpm). Bars represent average concentrations for the period studied. A and B indicate statistical differences between the sampling periods ($P < .05$).

HCWs because they are present during all bronchoscopies. Certain bacteria most definitely originate from the patients, such as the *Streptococcus pneumoniae* found in room B during the second bronchoscopy. The fact that culturable bacteria from patients were found in the air 1.5 m from their mouths indicates that HCWs in closer proximity can be exposed to some pathogenic microorganisms.

The survival capacity of microorganisms is often overlooked, but it can greatly affect the bioaerosol content of the air in a room. Nosocomial pathogens can persist on inanimate surfaces.⁴¹ Like dust, microorganisms deposited on the floor or other surfaces can be re-suspended in the air as a result of people moving around, rolling stretchers in and out of the room, or any other air disturbance that might occur.^{42,43} Pathogens present in the air can therefore originate from previous patients, which underscores the importance of thorough room cleaning. The higher concentrations measured during the waiting/preparation period may well be explained by bacteria from the floor being re-suspended in the air by the rolling of stretchers and by nurses walking around the room during that period.

In this study, whereas most of the culturable bacteria identified (*Brevundimonas diminuta*, *Corynebacterium pseudodiphthericum*, *Gordonia terrae*, *Moraxella* sp, *Psychrobacter phenylpyruviens*, and *Actinomyces*) are nonpathogenic to humans in good health, some opportunistic pathogens (*Streptococcus pneumoniae*, *Acinetobacter radiorisistens*, *Acinetobacter lwoffii*, and *Escherichia* sp) were found. *Mycobacterium* spp and influenza A and B viruses were not detected. Pathogens present in the air are undoubtedly dependent on patient pathology, and the absence of any specific pathogens from the samples collected in this study does not mean they will always be absent. The identification of *Streptococcus pneumoniae*, *Neisseria* sp, and *Corynebacterium* sp shows that culturable bacteria from oral, nasal, and pulmonary flora were present in the air of the rooms during bronchoscopy procedures. HCW exposure to bioaerosols needs to be managed by implementation of prevention protocols and by providing them with appropriate personal respiratory protection if necessary. As previously pointed out, the main problem is not the treatment of known tuberculosis patients, but that of patients admitted for other problems with unsuspected or undiagnosed

tuberculosis.^{15,44} Because HCWs never know what is hiding in the lungs of their patients, emphasis must be placed on prevention and protection.

Bioaerosol concentrations in the working zone of HCWs are certainly higher than those reported here. In fact, given the distance between the sampling zone and the HCWs, the concentrations measured may well have been reduced as a result of dilution or sedimentation of the particles. The implication is that a sampling zone located closer to a patient's mouth would have revealed higher concentrations and perhaps the presence of other strains of microorganisms. Sample size was also a limitation of the study. Our presence in the room needed to be accepted by the medical staff. Only 1 sampling day was allowed in each hospital, and the number of bronchoscopy procedures was limited on those days. Still, in this study, the number of bronchoscopies necessary to be statistically representative ($P \leq .05$) with an acceptable error of 20% was calculated to be 14.⁴⁵ Nevertheless, this study still raises concerns about the occupational hazards to which HCWs are exposed. It provides clear evidence of the presence of culturable opportunistic bacteria originating from the respiratory tract of patients in the air of bronchoscopy rooms. The presence of pathogenic microorganisms in the air of these rooms is to be expected from time to time, depending on patient pathology.

Acknowledgments

The authors thank Yves Beudet, Carole Pépin, and Nancy Lacombe for their technical assistance.

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