The Northern New England Cystic Fibrosis Consortium

BACTERIAL SHEDDING BY CF PATIENTS IN THE OUTPATIENT SETTING

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ABSTRACT

Increasing recognition of horizontal transmission of respiratory pathogens among patients with CF has led to scrutiny of outpatient infection control practices in this population. The objective of this study was to test the hypothesis that cystic fibrosis patients shed bacteria into the local environment during the course of an office visit. The organisms of interest in the study were *Pseudomonas aeruginosa, methicillin-resistant Staphylococcus aureus, Burkholderia cepacia, and Stenotrophomonas maltophilia*.

METHODS

The following were sampled as part of the project: air in the examination room, faucet handles, examination room inner door handle, non-disposable segment of the otoscope, physician's fingers, physical therapist's fingers, spirometer handle, spirometer tubing and the diaphragm of the examination stethoscope. All surface cultures were obtained by sampling with a culture swab moistened with Stuart's bacterial transport medium. Hand samples were obtained by taking impressions of the fingertips on blood agar and MacConkey agar plates. Air samples were obtained using a single stage impactor (Andersen 1-STG) drawing ambient air (rate of 28.3 L/min) onto a blood agar plate within 5 feet of the patient. Prior to each CF clinic session, environmental control cultures were obtained. Recovery experiments determined that the sensitivity of the surface and air sampling methods was less than 100 colony forming units (CFU) for each of the study organisms. Respiratory tract cultures were obtained from either expectorated sputum or gag specimens. Organisms that were recovered from both respiratory secretions and any of the environmental samples were subjected to pulsed-field gel electrophoresis to evaluate strain relatedness.

RESULTS

A total of 25 patients, followed in the Maine Medical Center CF Program, participated in the study. Of these, 18 were in the adult CF Program and 7 were in the pediatric CF Program. Most were studied on more than one visit, resulting in a total of 40 encounters. A total of 164 control cultures were obtained, none of which recovered any study organisms. A total of 338 environmental cultures were collected in the course of patient encounters. In one instance *Burkholderia cepacia* was recovered from patient sputum and air within the examination room. Pulsed-field gel electrophoresis confirmed that the organism recovered from the air was indistinguishable from that in the patient's sputum.
These data indicate a shedding rate of 2.5% (95% CI 0-13.2%)

CONCLUSION

The bacterial shedding rate was 2.5% during the course of CF outpatient visits at our center. However, based on the small number of encounters in this pilot study the confidence interval of the shedding rate is quite large. A larger study is planned in order to improve the confidence interval of the measurement and to examine clinical factors that may affect the rate of bacterial shedding in the outpatient setting.

HYPOTHESIS

Cystic fibrosis patients shed bacteria from the respiratory tract to the local environment during the course of outpatient visits.

BACKGROUND

Bacterial infection of the lower airways is a well-recognized complication of CF and is followed by a decline in pulmonary function. Ultimately it is the leading cause of death. The possibility of horizontal transmission of pathogenic organisms is therefore of particular interest to patients and health care providers. Organisms that are of leading concern in this patient population include *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Stenotrophomonas maltophila* and methicillin-resistant *Staphylococcus aureus*.

Patient to patient spread of *B. cepacia* has been well-described by several groups. Horizontal transmission of *P. aeruginosa*, *S. aureus* and *S. maltophila* has been reported in the inpatient setting, but few studies have addressed this issue in the outpatient setting. In addition, few studies evaluating bacterial shedding from CF patients have attempted to validate the sensitivity of the sampling methods.

METHODS

Environmental samples were obtained from the following sites:

1. Inner door handle of the patient examination room
2. Diaphragm surface of the stethoscope
3. Horizontal surfaces of the faucet handles of the examination room sinks
4. Non-disposable section of the otoscope
5. Fingers of the examining physician and physical therapist (or instructor of airway clearance)
7. Spirometry tubing distal to the disposable filter
8. Air within the examination room
9. Handle of the spirometer mouthpiece

Prior to each CF clinic session, control cultures were collected from the assigned examination rooms. After each patient visit, during which spirometry was performed and a sputum or gag specimen obtained for culture, all of the above sites were again sampled.

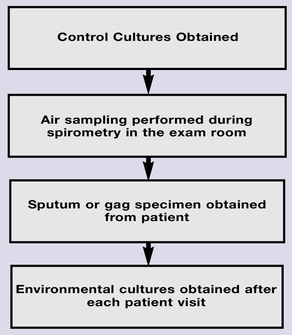
All of the physical surfaces were sampled with a Culturette swab (Becton Dickenson, Maryland) moistened with Stuart's bacterial transport medium. Air samples were collected during spirometry and were obtained using a single stage impactor (Andersen 1-STG, Georgia) drawing ambient air (rate 28.3 l/min) onto a blood agar plate within 5 feet of the patient for 15 minutes. (*Figure 1).*

Figure 1.

Anderson Single Stage Impactor with pump (1-STG) for collecting air samples. The sampler is rated to capture airborne particles down to 2μm in diameter.

Sputum samples from patients were obtained by expectoration. If the patient had a non-productive cough, a gag specimen was obtained. All bacteriological samples were sent to the microbiology laboratory within 6 hours of collection.

Figure 2.



Study procedure for environmental sampling.

If any of the four organisms under investigation recovered from the environmental samples were also present in the patient's respiratory secretions, pulsed-field gel electrophoresis was performed to evaluate strain relatedness.

VALIDATION OF METHODS

Recovery experiments using standard dilutions of stock bacteria to assess the sensitivity of the culturing techiques were performed.

Serial 10-fold dilutions (range of 10^5 cfu/ml to 10 cfu/ml of each of the four organisms under investigation were obtained. In the first experiment to test the sensitivity of the surface-sampling method, 0.5 ml of each innoculum was applied to a 10 cm by 10 cm dry area and allowed to stand for 5 minutes. That area was then sampled with a Culturette swab (Table 1).

Table 1:

RECOVERY BY SURFACE SAMPLING METHOD

Dilution (cfu/ml)	*S. aureus*	*P. aeruginosa*	*S. maltophila*	*B. cepacia*
10	No Growth	No Growth	No Growth	No Growth
10^2	1+	1+	1+	1+
10^3	2+	1+	1+	1+
10^4	3+	2+	3+	2+
10^5	4+	3+	3+	3+

Stock dilutions were applied to a dry surface (innoculum 0.5 ml) and sampled with Culturette swabs. Culturette swabs were cultured by streaking onto an agar plate.1+=rare growth (<10 colonies); 2+=light growth; 3+=moderate growth; 4+=heavy growth

In the second experiment, a similar series of stock bacterial suspensions was utilized. Each suspension was nebulized over 5 minutes from a Pari-LC nebulizer driven off a Pulmo-Aide compressor (mean nebulized volume 0.84 ml). The mouthpiece of the nebulizer was placed 12 inches from the inlet of the air sampler.

Table 2:

RECOVERY BY THE AIR SAMPLING METHOD

Dilution (cfu/ml)	*S. aureus*	*P. aeruginosa*	*S. maltophila*	*B. cepacia*
10	No Growth	No Growth	No Growth	No Growth
10^2	1 Colony	1 Colony	1 Colony	1 Colony
10^3	10 Colonies	5 Colonies	3 Colonies	3 Colonies
10^4	69 Colonies	12 Colonies	14 Colonies	13 Colonies
10^5	270 Colonies	45 Colonies	283 Colonies	50 Colonies

Stock dilutions of bacterial cultures were nebulized using a Pari-LC nebulizer driven off a Pulmo-Aide Compressor. Samples were collected using an Anderson single stage impactor (1-STG).

These experiments demonstrate that the surface and air assays are capable of detecting <100 cfu per sample.

RESULTS

A total of 25 patients participated in the study.

Table 3:

PATIENT CHARACTERISTICS

	One Visit	Two Visits	Three Visits	Four Visits
<18 years old	6	1	0	0
<18 years old	8	7	2	1

A total of 164 control cultures were obtained, none of which contained organisms found in any of the patients' respiratory secretions. A total of 338 environmental cultures were obtained during 40 encounters.

Table 4:

BACTERIAL SHEDDING

Organism	Sputum Pathogen	# of Patients	Positive for Shed
P. aeruginosa	20	18	0
B. cepacia	3	2	1
MRSA	4	4	0
S. maltophila	4	3	0
Total	31	27	1

Figure 3a **Figure 3b**

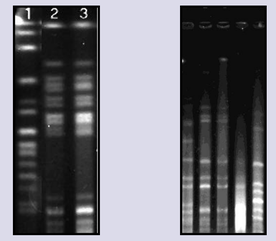


Figure 3a. Pulsed field gel electrophoresis of Spe 1 digest of Burkholderia cepacia isolates submitted to determine strain identity. Lane 1; Pseudomonas culture control, Lane 2; Air sample, Lane 3; Patient isolate. The banding pattern in lanes 2 and 3 is indistinguishable.

Figure 3b. Pulsed field gel electrophorsis of Sma 1 digest of S. aureus isolates. Lane 1, S. aureus control strain, lane 2 S. aureus MD fingers, lane 3 S. aureus MD fingers, lane 4, patient isolate of S. aureus. The isolates in lanes 2 and 3 are indistinguishable. The isolate in lane 4 is distinct from those in lanes 2 and 3.

One patient with *B. cepacia* in the sputum shed this organism into the air. The strain of both organisms was found to be indistinguishable by pulsed-field gel electrophoresis *(Figure 3a)*. Three environmental cultures were also positive for methicillin-sensitive *S. aureus* in conjunction with a sputum culture positive for the same organism. Although the antibiotic sensitivity profiles were very similar, the specimens were not sent for pulsed-field gel electrophoresis initially, as MSSA was not one of the organisms under investigation. However, a sample sent towards the end of the study was found to be different from that present in the patient's sputum *(Figure 3b)*. Therefore, confirmed shedding was detected in one out of 40 encounters, (rate 2.5%; CI 0-13.2%).

CONCLUSION

The bacterial shedding rate was 2.5% during the course of CF outpatient visits at our center.

FUTURE DIRECTIONS

We are planning a multi-center study of very similar design that will enroll up to 500 patients. The aim will be to improve the confidence interval of the measured shedding rate. In addition, this study will attempt to examine clinical factors that may affect the shedding rate in the outpatient setting.

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This project was funded by Maine Medical Center Medical Research Committee.