Measuring Bacterial Shedding in Cystic Fibrosis Clinics

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Abstract
(as submitted-data updated on poster)

OBJECTIVE: Recent studies have shown that in the cystic fibrosis (CF) population bacteria can spread from patient to patient or be acquired from the contaminated environment. However, few studies have assessed the risk in the outpatient setting. This cross sectional study, conducted at 7 CF Centers, assessed the rate of bacterial shedding by CF patients during office visits. The primary endpoint for Phase I of the study was the detection of a bacterial isolate from an environmental culture that was genotypically identical to that recovered from the subject's respiratory tract.

METHODS: During the office visit, respiratory tract specimens were collected from study subjects. The hands of the patient and members of the CF team were cultured using the glove-juice technique (1). In addition, cultures were taken from the air and environmental surfaces, including office equipment (2).Whenever possible, spirometry was performed in the examination room and air sampling occurred within 3 feet of the spirometer. *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA) [including methicillin-resistant (MRSA) strains], *Stenotrophomonas maltophilia* (SM) and *Burkholderia cepacia* complex (BC) were studied. All isolates were shipped to the core laboratory at Dartmouth-Hitchcock Medical Center for speciation and environmental isolates were compared with respiratory tract isolates by pulsed field gel electrophoresis (PFGE).

RESULTS: Samples were collected from 96 patient encounters (41 pediatric, 55 adult). Study organisms were isolated from respiratory cultures in 77% of the encounters (n= 74). The overall apparent shedding rate was 16%. Among patients harboring PA (n=45), air samples from the examination room detected organisms in 15% of encounters. Similarly, SA (n=28) was noted in 21% of air samples. PA and SA were recovered from the hands of 7% and 11% of patients, respectively. Observed rates of apparent shedding appeared to be similar between the group of patients (n=26) suffering from exacerbations (19% shedding) and patients (n=59) who were clinically stable (22% shedding), p=0.77. Contamination of surfaces such as doorknobs, stethoscopes and oximeters was detectable but infrequent (<5%). PFGE analysis is ongoing, but thus far has been useful to confirm identity between sputum and environmental isolates.

CONCLUSIONS: Bacterial shedding from CF patients was observed during office visits. Respiratory pathogens were cultured more frequently from the hands of CF patients and from airborne droplets than from environmental surfaces and equipment. Pulmonary exacerbation did not appear to increase the rate of shedding in the office setting. Understanding the pattern of bacterial shedding in the outpatient arena should provide the basis for developing rational infection control policies for outpatient settings.

Hypothesis

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

Participating Sites

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Dartmouth-Hitchcock Medical Center, NH

Eastern Maine Medical Center, ME

Fletcher-Allen Healthcare, VT

Maine Medical Center, ME

New York-Presbyterian Hospital, NY

St. Christopher's Hospital for Children, PA

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Methods

History and physical examination. A history and physical examination and review of symptoms was recorded at each visit. Elements of the history that were recorded specifically for the purposes of this study included the presence of fever, the severity of cough, and daily sputum volume. Spirometry was also performed and results recorded. Pulmonary exacerbation was identified using criteria, as previously outlined (3).

Environmental sampling. Environmental samples were obtained from the following sites :

- Inner door handle of the patient examination room
- Diaphragm surface of the stethoscope
- Horizontal surfaces of the fauct handles of the examination room sinks
- Non-disposable section of the otoscope
- The pulse oximeter finger clasp
- Spirometer handle
- Air within the examination room (sampled 3 feet from the front of the patient)
- Hands of the patient
- Hands of the examining physician, nurse and physical therapist (or hands of personnel involved with spirometry were also tested)
- Toys (if appropriate) and arm rests on chairs (if any) in the waiting area—at the end of clinic
- The counter surface in the check-out area—at the end of clinic
- Door handle and faucet handles in clinic bathroom—at the end of clinic

Culture techniques.

Environmental sampling in the examining room was performed with a Remel swab within 10 minutes of the patient leaving the room and prior to cleaning. The tip of the swab was moistened with the culture broth that is included in the kit for sampling surfaces. All specimens were transported on ice to the Dartmouth-Hitchcock Microbiology facility by overnight carrier and cultured on appropriate selective media.

Air sampling was performed with single stage impactors hooked in series to a vacuum pump (1-STG Viable Particle Sampler, Andersen Instruments Inc., Atlanta, GA) with a collection rate of 28.3 liters of air per min. The impactor containing a blood agar plate was placed 3 feet from the front of the patient (during spirometry and the patient interview) to assess for droplet shedding.

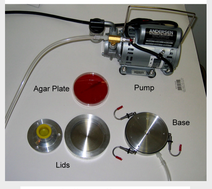
Specimens from hands were collected using the "glove juice" method (1). Briefly, a hand of the subject was placed in a clean plastic bag that contained 50 cc of solution (0.075 M phosphate buffer, pH 7.9, 0.1% polysorbate 80, 0.1% sodium thiosulfate, and 0.3% lecithin). This was done prior to hand washing or any other cleaning procedure.

Controls. Prior to each CF outpatient clinic, baseline cultures were collected from the examining rooms assigned to the CF participants for that day. Samples from the sinks, door handles, stethoscopes, otoscopes and hands (nurse, physician and physical therapist) were obtained. In addition, air sampling was performed for 30 minutes in the room prior to patient testing.

Pulsed Field Gel Electrophoresis (PFGE). A standardized innoculum of the organism under investigation was run concurrently with digested genomic DNA from a control culture and molecular mass standard. An aliquot of an overnight culture in trypticase soy broth was centrifuged for 1–2 min at 10-12,000 RPM to create a cell pellet. The pellet was then be resuspended in cold cell buffer. Lysozyme and/or lysozyme/lysostaphin was added, followed by liquid agarose solution. The resultant mixture was transferred to two plug molds and allowed to solidify. The agarose plugs were incubated with a solution of lysozyme and/or lysozyme/lysostaphin to lyse the bacterial cells and release the DNA. The plugs were then rinsed with wash buffer, and a solution of Proteinase K was added to the plugs to digest histone proteins. Following overnight incubation, the plugs were washed again prior to restriction. A cocktail consisting of buffer and restriction enzyme specific for the target organism was added and the plugs were incubated overnight. The plugs were then transferred to a comb and casting stand and an agarose gel poured. Electrophoresis was performed using a Gene Path instrument (Bio-Rad) with parameters determined by the organism in question.

Figure 1. Specimen collection techniques

A



Air Sampler Components

B

"Glove-Juice" Technique

C

Surface Sampling

Figure 2. Flow diagram for pre/post clinic samples

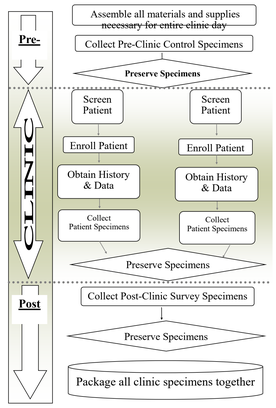
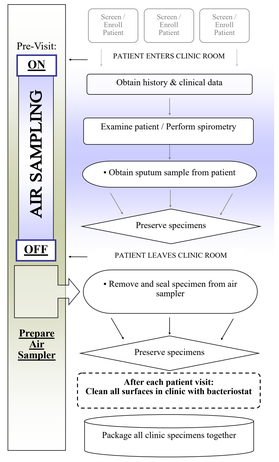


Figure 3. Flow diagram for encounter sample collection



Definitions

Shedding: Any encounter where at least one collected isolate matches (by PFGE) a respiratory tract isolate from the same encounter.

Indistiguishable isolates: Identical molecular fingerprint by PFGE.

Probably related isolates: Nearly identical PFGE pattern that can be accounted for by a single molecular event (usually 1-2 band difference).

Possibly related isolates: Similar PFGE pattern that likely requires more than a single molecular event to account for differences (usually more than 3 band difference).

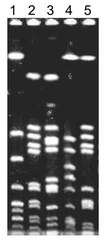
CF Exacerbation: A protocol definition for pulmonary exacerbation was utilized, as previously described (3).

Results

During the period from April, 2004– May, 2005 there were 97 study encounters at the 7 study sites. Approximately 2100 respiratory tract and environmental cultures were collected and potential matches (based on species) were subjected to PFGE. Figures 4-8 illustrate representative gels comparing sputum and environmental isolates.

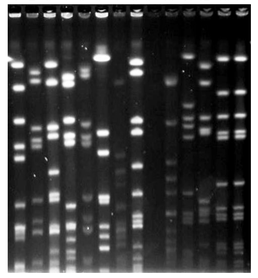
- The Table shows the distribution of sputum and environmental isolates examined by PFGE. The table also shows the pattern of environmental shedding in isolates shown to be related to sputum cultures during study encounters.
- The overall rate of bacterial shedding was 12.4% (95% CI 5.8-18.9%), Figure 9.
- There did not appear to be a significant difference in rate of shedding based on the organism isolated from sputum (though the study was not powered for this secondary endpoint), Figure 9.
- No significant difference in rate of shedding was seen between stable patients and those who were having a pulmonary exacerbation, Figure 10.

Figure 4. Isolates of Staphylococcus (SA) compared by PFGE



Lane 1 air sample pre-clinic control ; *Lane 2* faucet post-clinic sample; *Lane 3* nurse hands pre-clinic control; *Lane 4* patient sputum (005-249151, study patient identifier); *Lane 5* air sample (005-249151). **Note distinct band patterns across lanes.**

Figure 5. Isolates of Staphylococcus (SA) identified by PFGE

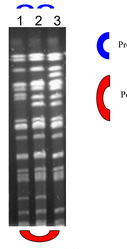


Lane 1 patient sputum (001-297096); *Lane 2* post-physician hands; *Lane 3* patient sputum (003-313428); *Lane 4* air sample; *Lane 5* post-visit counter top; *Lane 6* patient sputum (004-426087); *Lane 7* oximeter sample; *Lane 8* patient sputum (005-172263); *Lane 10* post-visit chair armrest ; *Lane 11* patient sputum (032-430527); *Lane 12* air sample; ***Lane 13*** patient sputum (035-298777); *Lane 14* post-visit bathroom door . **Note Lanes 13 and 14 are identical.**

Figure 6. Indistinguishable isolates of Pseudomonas identified by PFGE

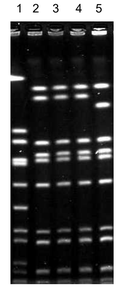
Lane 1 patient sputum (003-150724); *Lane 2* air sample (003-150724); *Lane 3* patient hands (003-150724). **Note all lanes have identical band pattern.**

Figure 7. Isolates of Pseudomonas compared by PFGE



Lane 1 patient sputum (007-536716), non-mucoid morphotype; *Lane 2* patient sputum (007-536716), mucoid morphotype; *Lane 3* air sample (007-536716). **Note similarity between Lanes 1 and 2 (two band difference) and Lanes 2 and 3 (two band difference). Lanes 1 and 3 differ by 4 bands.**

Figure 8. Isolates of Staphylococcus (MRSA) compared by PFGE



Lane 1 SA control; *Lane 2* oximeter sample (001-234255); *Lane 3* patient hand (001-234255); *Lane 4* patient sputum (001-234255); *Lane 5* clinical isolate non-CF patient. **Note all Lanes 2-4 are identical.**

Organism	MSSA	MRSA	PA	BC	SM	Total
Total Sputum Isolates	32	8	52	6	11	109
Potential Matches	13	2	15	-	-	30
Indistinguishable	2	2	5	-	-	9
Probably Related	2	-	1	-	-	3
Possibly Related	-	-	-	-	-	-
	Air Hands Other	Air Hands Other	Air Hands Other			
Indistinguishable	2	1 2 1	3 2			11
Probably Related	1 1		1			3
Possibly Related		1				1

Table. Distribution of sputum pathogens and environmental isolates. "Potential Matches" are those environmental cultures that match by species with a sputum isolate. These environmental isolates were then subjected to PFGE and categorized as "Indistinguishable", "Probably Related" or "Possibly Related" .

Indistiguishable isolates: Identical molecular fingerprint by PFGE.
Probably related isolates: Nearly identical PFGE pattern that can be accounted for by a single molecular event (usually 1-2 band difference).
Possibly related isolates: Similar PFGE pattern that likely requires more than a single molecular event to account for differences (usually more than 3 band difference).

MSSA– Methicillin sensitive Staphylococcus aureus. MRSA– Methicillin resistant Staphylococcus aureus. PA– Pseudomonas aeruginosa. BC– Burkholderia cepacia complex. SM– Stenotrophomonas maltophilia.

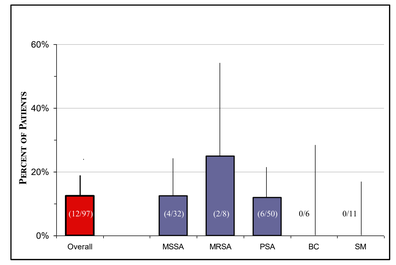


Figure 9. Overall Shedding Rate and Rate by Sputum Pathogen. Bars denote 95% confidence intervals. MSSA= Methicillin sensitive *Staphylococcus aureus;* MRSA= Methicillin resistant *Staphylococcus aureus*; PSA= *Pseudomonas aeruginosa;* BC= *Burkholderia cepacia* complex; SM= *Stenotrophomonas maltophilia*

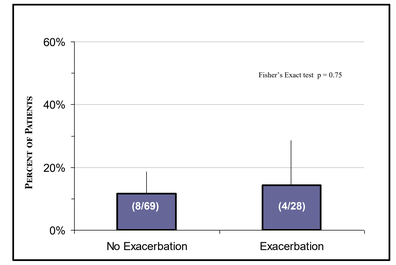


Figure 10. Shedding Rate by Clinical Status. Bars denote 95% confidence intervals. Rate of shedding is compared between clinically stable patients and those with study defined pulmonary exacerbation. Fisher's Exact Test, p = 0.75

Recovery Experiments

Surface Sampling

Dilution	*S. aureus*	*P. aeruginosa*	*S. maltophilia*	*B. cepacia*
10/ml	No growth	No growth	No growth	No growth
10^2/ml	1+	1+	1+	1+
10^3/ml	2+	1+	1+	1+
10^4/ml	3+	2+	3+	2+
10^5/ml	4+	3+	3+	3+

An aliquot (0.5 ml) of each dilution was applied to a 10 cm x 10 cm dry, flat area and allowed to stand for 10 minutes.. 1+ = rare growth (<10 colonies); 2+ = light growth; 3+ = moderate growth; 4+ = heavy growth.

Air Sampling

Dilution	*S. aureus*	*P. aeruginosa*	*S. maltophilia*	*B. cepacia*
10/ml	No growth	No growth	No growth	No growth
10^2/ml	1 colony	2 Colonies	1 Colony	1 Colony
10^3/ml	1	5	3	3
10^4/ml	69	12	14	13
10^5/ml	270	45	283	50

Serial dilutions of bacteria were nebulized over 5 minutes (0.84 cc) from a Pari-LC nebulizer driven off a PulmoAide compressor and impacted on an agar plate. The mouthpiece of the nebulizer was placed 12 inches from the inlet of the single stage impactor (Andersen, 1-STG). Numbers indicate colony counts..

References

1) Am J Infect Control 26: 513-21, 1998.

2) Pediatr Pulmonol. suppl 24: 331, 2002.

3) Microbiology and Infectious Disease in Cystic Fibrosis. Cystic Fibrosis Foundation Volume V, Section1: 1-26.

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Conclusions/Future Directions

- The overall rate of shedding during outpatient visits was 12.4% (95% CI 5.8-18.9%). The shedding rate did not appear to be affected by the clinical status of the patient (i.e., clinically stable vs. pulmonary exacerbation).
- Pulsed field gel electrophoresis (PFGE) proved to be a valuable tool to evaluate the origins of environmental isolates during this study.
- Shedding was noted on hands, environmental surfaces and in the form of airborne droplets.
- While environmental contamination is not equivalent to patient to patient transmission, these results point to opportunities to further reduce the risk of infection during outpatient visits.
- We are currently examining the effects of a decontamination protocol on hand carriage of respiratory pathogens during outpatient visits.
- PFGE may also be utilized to better understand the local epidemiology of problematic pathogens within the CF community.