

## Comparison of Three Methods for Culturing Throat Swabs from Cystic Fibrosis Patients

JÖRG E. HOPPE\*, UTE THEURER-MAINKA, AND MARTIN STERN

University Children's Hospital, Eberhard Karl University, D-72070 Tübingen, Germany

Received 24 January 1995/Returned for modification 31 March 1995/Accepted 26 April 1995

**In patients with cystic fibrosis who do not produce sputum, deep throat swabs are cultured for potential respiratory pathogens. Usually these swabs are directly streaked onto selective agar media. In a study of 50 pediatric cystic fibrosis patients, we compared this traditional method using rayon swabs with two methods having quantitative modifications: calcium alginate swabs eluted in Ringer's lactate and rayon swabs eluted in normal saline. The eluates were then processed quantitatively (three-step dilution series). The yield of potential pathogens was significantly higher with the two quantitative methods. Overall, the combination of alginate with Ringer's lactate was superior to the combination of rayon with saline, although only some of these differences achieved statistical significance.**

Young cystic fibrosis (CF) patients usually do not produce sputum. Consequently, alternative strategies have to be used for obtaining material from the respiratory tract for bacteriological culture. The most commonly used technique for these patients has been the deep throat swab. Placing a swab in the pharynx induces coughing, resulting in lung secretions being brought up into the pharynx. The flora cultured by this technique is likely to consist of both throat flora and lower respiratory tract flora (3).

Usually throat swabs are taken with standard rayon swabs and are processed semiquantitatively (i.e., they are streaked directly onto various nonselective and selective agar media). In the present study, we compared this direct plating (DP) method which uses rayon swabs with two modified methods: rayon swabs eluted in saline (RS) and alginate swabs eluted in Ringer's lactate (AR). These eluates were then processed quantitatively.

### MATERIALS AND METHODS

Patients attending the CF clinic at the University Children's Hospital, Tübingen, Germany, were subjected to three throat swabs after coughing. The swabs were always taken by one of three physicians in a randomized order and by a standardized swabbing technique. Two swab tips consisted of rayon (Cultureswab Twin Transport System; Difco, Augsburg, Germany), and one consisted of calcium alginate (em-te; Hamburg, Germany). All swab tips were roughly equal in size and took up ca. 0.01 ml of secretions as determined in pretest experiments. The swabs were sent in Amies transport medium at room temperature to the bacteriology laboratory in the same building, where they were processed within 6 h.

One rayon swab was streaked on half an agar plate of each of five media (see below). These inocula were fractionated twice with a sterile platinum loop. Growth after incubation at 36°C for 48 h was recorded semiquantitatively (+ to +++).

The second rayon swab and the alginate swab were vortexed in 0.9 ml of saline and Ringer's lactate, respectively, and left for 30 min at room temperature. They were then vortexed again, expressed, and discarded. A total of 0.1 ml of this eluate was spread with a sterile glass rod on the surface of each of five different agar plates. Two further 100-fold dilutions were prepared in saline. A total of 0.1 ml of each dilution was spread on one plate of each of the five agar media. After incubation for 48 h at 36°C, colonies were counted at the dilution that showed countable numbers of CFU and the total number of CFU per milliliter of secretion was calculated.

Commercially prepared plates (BioMérieux, Nürtingen, Germany) of the following media were used: Columbia agar with 5% sheep blood (CB) as universal

medium; Columbia sheep blood agar supplemented with colistin (10 mg/liter) and nalidixic acid (15 mg/liter) (CNA) as selective medium for *Staphylococcus aureus*, pneumococci, and group A streptococci; chocolate agar with bacitracin (50,000 IU/liter) as selective medium for *Haemophilus influenzae* (HS); MacConkey agar (MAC) for pseudomonads and members of the family *Enterobacteriaceae*; and Sabouraud agar (SAB) supplemented with gentamicin (100 mg/liter) and chloramphenicol (50 mg/liter) for yeasts and molds.

Bacteria, yeasts, and molds were identified by standard methods (1). Components of the normal throat flora (alpha-hemolytic streptococci, *Neisseria* spp., *Corynebacterium* spp., and nonencapsulated *Haemophilus influenzae*) were not identified to the species level and were not counted. Growth of normal throat flora was recorded but excluded from further analysis. Isolates of *Pseudomonas aeruginosa* of different morphotypes and different antibiograms were counted as separate isolates.

For statistical analysis of both the qualitative and quantitative data, the maximum likelihood procedure followed by the chi-square test was used (4).

### RESULTS

Fifty CF patients 6 months to 34 years of age (mean age, 11.8 years; median, 10.0 years) were studied. Forty-six of them received antibiotics per os (most frequently trimethoprim-sulfamethoxazole [ $n = 16$ ], cefaclor [ $n = 10$ ], and doxycycline [ $n = 6$ ]) and/or by aerosol (tobramycin [ $n = 17$ ] and colistin [ $n = 6$ ]). Components of normal throat flora were found in all 50 patients. Forty-eight patients (96.0%) harbored organisms that do not belong to the normal throat flora. The 124 strains of 17 species isolated from these 48 patients are shown in Table 1. Pneumococci, group A streptococci, and *Pseudomonas cepacia* were not found in this study.

Patients harbored up to six different species aside from the normal throat flora. Most frequently, patients harbored one ( $n = 11$ ), two ( $n = 12$ ), or three ( $n = 15$ ) species.

**Qualitative comparison of the three methods.** None of the three methods evaluated in this study was able to detect all of the 124 isolates. The yield of the quantitative AR method was higher (114 isolates [91.9%]) than that of the quantitative RS method (102 isolates [82.3%]), while the semiquantitative DP method detected only 74 isolates (59.7%; AR method versus DP,  $P < 0.0001$ ; RS method versus DP,  $P = 0.002$ ). The difference between the AR and RS methods was statistically not significant.

Thirty-six strains of *P. aeruginosa* were isolated from 24 patients (colonization rate, 48.0%) (Table 2). Approximately 40% of the strains were mucoid. Eight patients harbored more than one strain of *P. aeruginosa*. Two of these patients har-

\* Corresponding author. Mailing address: University Children's Hospital, Rümelinstrasse 23, D-72070 Tübingen, Germany. Phone: 49/7071/293781. Fax: 49/7071/294713.

TABLE 1. Organisms not belonging to the normal throat flora (n = 124) isolated from throat swabs of CF patients (n = 50)

Organism(s)	No. of isolates
<i>Candida albicans</i> .....	39
<i>Pseudomonas aeruginosa</i> .....	36
<i>Staphylococcus aureus</i> .....	22
Members of the family <i>Enterobacteriaceae</i> <sup>a</sup> .....	12
<i>Pseudomonads</i> <sup>b</sup> .....	5
<i>Aspergillus fumigatus</i> .....	5
<i>Haemophilus influenzae</i> type b.....	3
<i>Candida parapsilosis</i> .....	2

<sup>a</sup> Isolates included *Enterobacter cloacae* (n = 4), *Escherichia coli* (n = 2), *Citrobacter amalonaticus* (n = 1), *Escherichia vulneris* (n = 1), *Enterobacter amnigenus* (n = 1), *Klebsiella pneumoniae* (n = 1), *Morganella morganii* (n = 1), and *Serratia* spp. (n = 1).

<sup>b</sup> Isolates included *Pseudomonas pickettii* (n = 2), *Xanthomonas maltophilia* (n = 2), and *Pseudomonas putida* (n = 1).

bored three strains, and one had four strains. These third and fourth strains were not detected by the DP method.

The analysis of the results with regard to *Staphylococcus aureus*, members of the family *Enterobacteriaceae*, and *Candida albicans* shows that the yield was highest with the AR method for all of these organisms (Table 2). The RS method detected more isolates than DP, with the exception of members of the family *Enterobacteriaceae*. The two isolates of *Candida parapsilosis* were exclusively detected by the AR method.

**Qualitative comparison of culture media.** The qualitative performance of the various agar media is shown in Table 3, which combines the results obtained with all three methods. The highest numbers of *C. albicans* isolates were detected with SAB. MAC yielded the highest number of *P. aeruginosa* isolates, but this organism also showed good growth on HS and CB. *S. aureus* was isolated significantly more frequently and in higher numbers on the universal CB than on CNA. MAC proved useful for members of the family *Enterobacteriaceae*, but many isolates of this group were also detected on CB and HS. The three isolates of *H. influenzae* type b found in this study were detected exclusively on HS.

**Quantitative comparison of the AR and RS methods.** Colonies of *C. albicans*, *P. aeruginosa*, and *S. aureus* were counted on the plates of the best-performing media (SAB, MAC, and CB, respectively). The mean values are shown in Table 4. When the AR method was employed, mean numbers of CFU of *C. albicans* and *P. aeruginosa* were significantly higher.

DISCUSSION

This study compared two quantitative methods of processing throat swabs of CF patients with the traditional semiquantita-

TABLE 2. Detection of organisms isolated from throat swabs of CF patients by three methods

Organism(s)	Total no. of isolates	No. of isolates detected by:		
		AR	RS	DP
<i>C. albicans</i>	39	38 <sup>a</sup>	35 <sup>b</sup>	21 <sup>a,b</sup>
<i>P. aeruginosa</i>	36	34 <sup>b</sup>	30	22 <sup>b</sup>
<i>S. aureus</i>	22	20 <sup>c</sup>	17	13 <sup>c</sup>
Members of the family <i>Enterobacteriaceae</i>	12	11	8	9

<sup>a</sup> P < 0.0001.  
<sup>b</sup> P = 0.002.  
<sup>c</sup> P = 0.01.

TABLE 3. Performance of five agar media with respect to the most frequently isolated organisms

Organism(s)	No. of plates with growth					
	Total	CB	CNA	HS	MAC	SAB
<i>C. albicans</i>	186	25	44 <sup>a</sup>	37		80 <sup>a</sup>
<i>P. aeruginosa</i>	176	39		59	78	
<i>S. aureus</i>	69	44 <sup>b</sup>	25 <sup>b</sup>			
Members of the family <i>Enterobacteriaceae</i>	51	16		13	22	

<sup>a</sup> P < 0.0001.  
<sup>b</sup> P = 0.006.

tive streaking technique. Both quantitative methods significantly increased the number of isolates. With regard to the qualitative overall performance of the quantitative methods, use of the AR method was superior to use of the RS method, although the difference was statistically not significant. In addition, significantly higher numbers of *C. albicans* and *P. aeruginosa* CFU were found with the AR method.

Several reasons may account for the observed differences. In a study of the recovery of group A streptococci from cotton swabs, Ross showed that only a small percentage of the initial inoculum could be recovered on solid media. This was due to insufficient release of the organisms from the swabs (7). When swabs are eluted for 30 min in a liquid like saline or Ringer's lactate, release of organisms is very likely much better. This effect may partly explain the superiority of the quantitative methods.

When throat swabs of CF patients are directly streaked onto agar plates, very heavy growth is often observed, since both the normal flora and potential pathogens are usually present in the specimens. In spite of the use of selective media, this heavy growth may obscure the presence of species which grow in small colonies or may be present only in low numbers. The dilution steps inherent in quantitative processing may facilitate detection of organisms that would be missed by semiquantitative plating.

The superiority of calcium alginate swabs over rayon swabs has been demonstrated in studies with *Bordetella pertussis* (5). Alginate acid (polymannuronic acid) is an extract from seaweed. Calcium alginate is soluble in alkaline solutions such as Ringer's lactate (6). This solubility of calcium alginate fibers can be observed macroscopically. Alginate swab tips eluted for some time in Ringer's lactate (AR method) look frayed out, while rayon swab tips eluted in saline (RS method) do not. Therefore, the release of organisms from alginate swab tips is likely to be better than that from rayon-tipped swabs. Doern and Brogden-Torres recommend elution of throat swabs of CF patients in Trypticase soy broth, but they do not state if this method was compared with others (2).

The evaluation of the agar media employed in our study confirms the usefulness of SAB for *C. albicans* and MAC for *P.*

TABLE 4. Quantitative comparison of AR and RS methods with regard to most frequently isolated organisms

Organism	Mean (SD) CFU isolated		P
	AR	RS	
<i>C. albicans</i>	7.2 × 10 <sup>4</sup> (2.1 × 10 <sup>5</sup> )	2.2 × 10 <sup>4</sup> (5.7 × 10 <sup>4</sup> )	0.002
<i>P. aeruginosa</i>	3.2 × 10 <sup>7</sup> (1.5 × 10 <sup>8</sup> )	4.9 × 10 <sup>6</sup> (1.1 × 10 <sup>7</sup> )	0.04
<i>S. aureus</i>	3.5 × 10 <sup>6</sup> (1.4 × 10 <sup>7</sup> )	3.7 × 10 <sup>6</sup> (8.9 × 10 <sup>6</sup> )	0.9

*aeruginosa* and members of the family *Enterobacteriaceae* (2, 3). A considerable number of isolates of *P. aeruginosa*, other pseudomonads, *C. albicans*, and members of the family *Enterobacteriaceae* were also found on HS. The degree of selectivity of this medium is quite low. On the other hand, the only three isolates of *H. influenzae* type b in this study were detected exclusively on HS. This agar may therefore be indispensable. The relatively poor recovery of *P. aeruginosa* on CB is surprising. Since less than half of the isolates were mucoid, it is possible that some of the nonmucoid strains were overlooked on the nonselective CB, which usually shows very heavy growth of both flora constituents and potential pathogens. Our results also confirm the limited usefulness of CNA (2, 3).

In conclusion, our study demonstrates a significant increase in the yield of potential pathogens from throat swabs of CF patients if the swabs are processed quantitatively. We recommend the use of calcium alginate swabs and elution of these swabs in Ringer's lactate (AR method) because of the better overall performance of this method.

#### ACKNOWLEDGMENTS

We thank E. Mayer and M. Reuter for taking some of the throat swabs and K. Dietz and J. Sand for help with the statistical evaluation.

#### REFERENCES

1. Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.). 1991. Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
2. Doern, G. V., and B. Brogden-Torres. 1992. Optimum use of selective plated media in primary processing of respiratory tract specimens from patients with cystic fibrosis. *J. Clin. Microbiol.* **30**:2740-2742.
3. Gilligan, P. H. 1991. Microbiology of airway disease in patients with cystic fibrosis. *Clin. Microbiol. Rev.* **4**:35-51.
4. Hartung, J., B. Elpelt, and K. H. Klösener. 1985. Statistik, p. 435. R. Oldenbourg, Munich.
5. Hoppe, J. E., and A. Weiss. 1987. Recovery of *Bordetella pertussis* from four kinds of swabs. *Eur. J. Clin. Microbiol.* **6**:203-205.
6. Merck and Co., Inc. 1989. The Merck index: an encyclopedia of chemicals, drugs, and biologicals, p. 41-42. Merck and Co., Inc., Rahway, N.J.
7. Ross, P. W. 1977. The isolation of *Streptococcus pyogenes* from throat swabs. *J. Med. Microbiol.* **10**:69-76.