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Full Length Research Paper

Patients with Cystic Fibrosis have Higher Rates of Fungal Detection in Their Respiratory Tracts when They Use Selective Fungal Culture Media

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Fungi have become more common in the respiratory tracts of people with cystic fibrosis (CF). However, the majority of clinical facilities in the United States do not regularly conduct fungal surveillance, which could result in an overestimate of the problem's actual prevalence. We used standard bacterial and selective fungal culture media, such as Sabouraud dextrose agar with gentamicin (SDA), inhibitory mold agar (IMA), and brain heart infusion (BHI) agar with chloramphenicol and gentamicin, to compare the rates of detection for clinically important fungi (CIF), which are defined as Aspergillus, Scedosporium, and Trichosporon species and Exophiala dermatitidis, in CF sputa. We reported how common these fungi were in a population of adults with cystic fibrosis. From 211 distinct subjects, 487 CF respiratory samples were gathered. CIF was found in 184 samples, or 37.8%. In bacterial culture medium, only 26.1% of CIF-positive samples were recognized; in contrast, higher rates of fungal identification were seen in IMA (65.8%; P < 0.001), SDA (64.7%; P = 0.005), and BHI agar (63.0%; P = 0.001). Aspergillus and Scedosporium species prevalences were 40.8% and 5.2%, respectively, higher than the 20.4% and 1.9% nationally reported prevalences. Longer incubation times and selective fungal culture media produced greater rates of CIF detection in CF sputum samples than those found in bacterial culture medium, which led to an underdetection of fungi by bacterial culture alone. Using selected fungal culture media may improve the estimation of fungal prevalence in CF, which could lead to significant clinical decisions.

Key words: Fungi, Cystic fibrosis, Fungal culture, Inhibitory mold agar, Brain heart infusion agar,

Sabouraud dextrose agar, Aspergillus, Scedosporium, Trichosporon, Exophiala.

INTRODUCTION

About 70,000 people worldwide suffer from cystic fibrosis (CF), the most prevalent fatal autosomal recessive illness in Caucasians. In the epithelial cells of the sinopulmonary, pancreatic, hepatobiliary, intestinal, cutaneous, and reproductive systems, the cystic fibrosis transmembrane conductance regulator gene malfunctions, causing CF, a multiorgan disease (1). The primary cause of death in CF is respiratory failure, which is brought on by recurrent infections and lower respiratory tract bronchiectasis. However, life expectancies for people with cystic fibrosis have increased to over 40 years of age due to improved knowledge of chronic infections and antimicrobial therapies. The vast majority of antimicrobial treatments for cystic fibrosis target bacteria, such as Burk-holderia cepacia complex, Staphylococcus aureus, and Pseudomonas aeruginosa.

Fungi isolated from CF respiratory secretions are now much more common. Over the previous 20 years, the prevalence of Aspergillus species, the most prevalent filamentous fungi in the CF airway, has risen from 6% to 12% (2,3). Furthermore, there has been a growing detection of Exophiala, Trichosporon, and Scedosporium species in CF

sputa (4–11). Additionally, there is mounting evidence that pulmonary morbidity, such as respiratory symptoms and pulmonary exacerbations, and even death in the post-lung transplant population are linked to the chronic presence of Aspergillus, Scedosporium, Trichosporon, or Exophiala species in CF patients (5–9, 11, 12). For Aspergillus and Scedosporium, the reported prevalences in CF centers have varied from 0 to 36% and 0 to 9%, respectively (3, 13, 14). A lack of standardized laboratory procedures for identifying fungus in CF respiratory specimens and variations in the clinical use of fungal surveillance could be the cause of the observed variability (15, 16). The separation of potentially clinically relevant fungi in normal culture conditions may be hampered by the presence of quickly growing Gram-negative organisms, such as Pseudomonas aeruginosa (5). However, according to data from a pilot survey, 80% of microbiology labs do not regularly do mycological analyses using selective fungal media in CF respiratory samples unless clinicians request it (Hong, unpublished data). Therefore, the only organisms reported are molds and yeasts that were isolated in bacterial growth medium. Although it's unclear how frequently this happens, some clinicians order targeted fungal respiratory cultures when there is a high suspicion of an unusual illness. Additionally, our survey results showed that roughly 57% (95% CI 18 to 90) of US practitioners report routinely monitoring fungus in CF patients. However, this is probably an overestimation of fungal surveillance in U.S. CF facilities due to the small sample size and populations restricted to large academic centers.

More selective fungal culture media are linked to a higher identification of fungi in CF sputa, and prior research indicates that nonselective standard culture media are inadequate for correctly diagnosing fungal infections (17–21). However, a direct comparison of the rates of fungal detection by selective fungal media and by standard bacterial culture medium (based on the Cystic Fibrosis Foundation [CFF] infection control guidelines) has not yet been conducted in a US CF cohort (22). In the CF population, accurate fungal infection diagnosis may have major clinical ramifications, especially for ill patients and lung transplant candidates.

Our study's goal was to assess how well bacterial and selective fungal culture media performed in identifying clinically important fungi (CIF) that were present in the CF respiratory tract. We postulated that the prevalences of Aspergillus, Scedosporium, Trichosporon, and Exophiala species in an adult CF population would be better described by selective fungal culture media, which would show higher rates of fungal detection.

RESULTS

487 of the 501 independent respiratory samples that were gathered were examined. Due to protocol violations, ten samples that were inoculated in just two of the three culture media in the selective fungal research procedure were not included in the analysis. Four samples were not included in the analysis since they were injected on culture medium that had expired. Over the course of the 12-month trial, there were 211 distinct subjects, with an average of 2.28 samples per participant. Yeast or filamentous fungi were found in 223 out of 487 samples (45.8%). Sixty specimens (12.3%) had two or more fungus, making them polymi-crobial. The most prevalent Aspergillus species was found in the 184 (37.8%) samples from which CIF were isolated.

Traits of the cohort. The baseline clinical and demographic characteristics of patients from whom CIF was isolated during the study period were compared to those of those from whom they were not (Table 1). Both groups' demographic characteristics were comparable. The F508 genotype, pancreatic insufficiency, and pulmonary function, which is measured as the expected percentage of forced expiratory volume in 1 second (FEV1), were among the clinical features that indicate the severity of the illness and were comparable in both groups. However, the CIF-positive group had higher exposures to chronic nonmacrolide oral antibiotics and inhaled anti-pseudomonal antibiotics than the CIF-negative group (oral antibiotic, 27.7% versus 13.6%, respectively, P = 0.01; inhaled antibiotic, 81.2% versus 69.1%, respectively, P = 0.05). prevalence of fungus with clinical significance. During the study period, 101 (47.9%) samples had CIF isolated from them. Figure 1 summarizes the prevalences of each organism. Aspergillus fumigatus was the most common species, with prevalences of Aspergillus and persistent Aspergillus being 40.8% and 17.1%, respectively. Trichosporon, Exophiala, and Scedosporium species had lower prevalences—5.2%, 4.7%, and 3.3%, respectively. Notably, the investigation found more filamentous fungus species. In samples from 2 (<1%) of the group, Rasamsonia species, an emerging pathogen seen in the CF airway (23), were detected. Other rare filamentous fungi such as Rhizopus, Libertella, Rhinocladiella, Cercospora, and Basidiomycete species were also observed, along with Paecilomyces variotii (4.3%) and Penicillium (11.2%).

Exophiala, Trichosporon, Scedosporium, and Aspergillus can all be detected using selective fungal media.

It was discovered that 184 samples had CIF isolates. Table 2 provides a summary of each culture media's performances. Of these, 48 (26.1%) samples had bacterial cultures that contained CIF. At 37°C, CIF were found in 103 (56.0%) samples using the selective fungal culture medium Sabouraud dextrose agar with gentamicin (SDA). This was statistically substantially higher than the rate of detection in bacterial culture medium (26.1%, P < 0.001).

We found that each of the fungal media had significantly higher rates of detection when comparing their

performances to the rates of detection of CIF using bacterial medium (26.1%). For example, CIF was detected in 119 samples (64.7%, P = 0.005) in SDA at 30°C, 121 samples (65.8%, P < 0.001) in inhibitory mold agar (IMA), and 116 samples (63.0%, P = 0.001) in brain heart infusion (BHI) agar.

According to Table S1 in the supplemental article, we also examined the effectiveness of selective fungal media for identifying each of the CIF: Aspergillus, Scedosporium, Trichosporon, and Exophiala. Of the 144 samples that tested positive for Aspergillus species, 134 (93%), contained Aspergillus fumigatus. Additionally noted were A. terreus, A. niger, A. flavus, and A. nidulans. The bacterial culture medium has a 30.6% Aspergillus detection rate. Comparatively, the detection rates were higher using the following selective fungal culture media: BHI agar (47.9%, P = 0.003), IMA (50.0%, P = 0.001), SDA at 37°C (54.9%, P < 0.001), and SDA at 30°C (50.0%, P < 0.001). Only the bacterial culture medium was used to identify 25 out of the 144 Aspergillus-positive samples. Three out of the 20 samples that tested positive for S. prolificans (n = 3) and Scedosporium apiospermum (n = 17) were found by bacterial culture medium (15%), but the rates for detecting Scedosporium using selective fungal media were higher. Only one specimen (5.9%) out of the 17 Trichosporon-positive samples was identified by bacterial culture. IMA, BHI agar, and SDA at 30°C and 37°C all outperformed bacterial medium. Finally, following three days of bacterial culture incubation, we were unable to find any Exophiala-positive specimens. In contrast, IMA had a high detection rate (95.7%), while SDA at 30°C and 37°C and BHI agar had somewhat lower detection rates.

Average time to identify Exophiala, Trichosporon, Scedosporium, and Aspergillus.

For a maximum incubation period of three days, the mean times-to-isolation of CIF on bacterial growth medium and SDA at 37°C were 2.35 days and 2.21 days, respectively (Table 3). The mean times-to-isolation of CIF for SDA at 30°C, IMA, and BHI agar incubated for 14 days were 3.49, 3.85, and 3.43 days, respectively, and did not surpass 4 days for each of the media. Aspergillus grew on IMA in 3.48 days, Scedosporium in 3.61 days, Trichosporon in 2.75 days, and Exophiala in 5.87 days, according to our analysis of each CIF (see Table S2). At 30°C, the mean times-to-isolation for each CIF were comparable to those on BHI agar and SDA.

DISCUSSION

We are the first to use different culture media to analyze and evaluate the frequencies of fungal detection in the CF airways of people in a North American CF cohort. Our study's findings imply that when bacterial culture medium is used only for fungal surveillance, CIF in CF patients susceptible to fungal isolation in the respiratory tract is underreported. Moreover, the prevalence of CIF is significantly higher than the known prevalence recorded in the CFF registry when combinations of selective fungal media are employed (3).

In our cohort, CIF was more than 40% prevalent. In typical bacterial culture medium, only 25% of the clinically relevant fungal species were effectively identified. Compared to bacterial culture, CIF was detected at statistically considerably higher rates using selective fungal culture conditions and longer incubation times. SDA was most effective in detecting Aspergillus species at 37°C for incubation. A bacterial culture was used to isolate Aspergillus from one-third of the positive samples. The selective fungal media outperformed the bacterial culture for additional clinically relevant fungi. On bacterial culture media, 15% of the Scedosporium isolates were found. Likewise, 5% and 0% of Trichosporon and Exophiala were found in bacterial culture media, respectively. The combination of IMA, SDA, and BHI agar yielded the highest detection rates among all the CIF, which could be in favor of a multimodal strategy for fungal culturing. With the exception of Exophiala dermatitidis, the rates of detection of each of the selective fungal media for Aspergillus, Scedosporium, and Trichosporon species seemed comparable, despite the fact that statistical comparisons between SDA, IMA, and BHI agar were not carried out. IMA had a greater detection rate than SDA or BHI agar for the Exophiala-positive samples. These findings imply that undetected occurrences of fungal respiratory infection or colonization in CF patients, namely for Scedosporium, Trichosporon, and Exophiala species, may occur in CF centers lacking regular mycological tests utilizing selective fungal media.

Our study's Aspergillus (40.8%) and Scedosporium (5.2%) prevalences were higher than those found in the national registry data of US facilities with CFF accreditation (43). Adults with cystic fibrosis have been found to have prevalences of Aspergillus and Scedosporium species of 20.4% and 1.9%, respectively (24). Our study's prevalence figures are similar to those of studies using selective fungal media, like SDA, but lower than those of studies using Scedosporium-selective media (SceSel+) containing dichloran and benomyl, which have been reported to reach up to 10–16% (15, 17, 18, 20, 25). The CFF registry does not provide a detailed description of the prevalences of Trichosporon and Exophiala. According to epidemiologic statistics, the estimated prevalence of Trichosporon in an American cohort is 2.1% (4, 11). There is no description of Exophiala's prevalence in the US.

New culturing techniques and selective media for fungal species in the CF population have been the subject of several earlier investigations. SDA plus antibiotics (such as co-trimethoxazole, chloramphenicol, ceftazidime, and colistin) and self-formulated Medium B+ demonstrated high sensitivity (96%) and specificity (82%) for the detection of fungi in CF sputa, according to experiments conducted by Nagano et al. to develop novel selective media for isolating yeasts and filamentous fungi (26). For isolating Scedosporium species, a number of groups have shown that SceSel+ agar containing benomyl performs better (18, 25, 27). Yeast potato dextrose agar, SceSel+ agar, and B+ medium were found to be among the most sensitive for detecting fungi, including Aspergillus, Scedosporium, and

Exophiala species, in a multicenter study conducted in Europe and Australia that compared the efficacy of semiselective fungal media in 469 samples of CF sputum (28). One drawback of these investigations is the lack of commercial availability of those selective medium, which can hinder clinical labs from establishing and further standardizing fungal isolation laboratory procedures. There is no defined method for mycological investigation in Europe or the US, despite these data and the known heterogeneity of fungal prevalence estimates due to nonuniform laboratory techniques (15, 16). Unlike earlier research, we directly compared the detection rates of bacterial culture medium and commercially available selective fungal media. We also showed that bacterium-specific culture medium performed less well than ideal in identifying clinically significant molds and yeasts in CF sputum samples. Our results imply that doctors should not rely exclusively on bacterial culture to make accurate fungal diagnosis in the adult CF population that is susceptible to respiratory fungal infection and colonization.

To find the ideal incubation period to maximize fungal detection, the amount of time between inoculation and the detection of a fungal isolate was examined. Using selected fungal culture media at 30°C, the mean time-to-isolation of CIF was roughly 3.5 days, which is similar to findings from other investigations (18). The observed timeframes varied significantly amongst fungal species when the mean time-to-detection for each fungal species group during the 2-week research protocol was examined. While Exophiala dermatitidis was recovered at a mean time approaching 6 days on IMA, Trichosporon was identified after a brief period of incubation (see Table S2). According to these findings, the majority of fungus in CF sputa may be detectable after a 7-day incubation period.

Conventional culture techniques are currently the standard of care protocol for CF respiratory specimen analysis in clinical microbiology labs. Even while there is proof that culture-independent procedures, including molecular sequencing, increase the sensitivity for diagnosis, the great majority of clinical laboratories do not currently have widespread access to these methods (29, 30). Furthermore, it has not yet been established if the presence of organism DNA has any clinical significance.

Research indicates that a subgroup of CF patients may be more susceptible to fungal infections and clinically significant colonizations. The isolation of filamentous fungi, primarily Aspergillus, has been linked to the administration of inhaled corticosteroids, macrolide treatment, anti-staphylococcal oral medicines, and inhaled antibiotics (4, 5, 31–36). Chronic anti-methicillin-resistant Staphylococcus aureus (MRSA) oral antibiotics and chronic inhalation anti-pseudomonal antibiotics were used more frequently at baseline by those in our cohort from whom CIF was isolated during the study period than by those from whom CIF was not isolated. By using more precise selective fungal culture in a subgroup of patients who may be more susceptible to contracting certain infections, these connections may also help clinicians determine whether fungi, such as Aspergillus, Scedosporium, Trichosporon, or Exophiala species, are present.

It is important to recognize that our study has limitations. First, the study's generalizability to the full CF community was impacted by the fact that it only included individuals who could expectorate sputum. But according to a number of studies, older individuals with CF are more likely than youngsters to have fungus (4, 5, 37, 38). According to CFF registry data, the average age at which Aspergillus was persistently isolated was 17.3 years (Hong, unpublished data). Second, the bacterial culture medium incubation period could not be prolonged past three days. Therefore, it is important to use caution when interpreting comparison data between bacterial culture medium and selective fungal media. However, compared to a selective fungal medium technique that involves a longer incubation period, the general conventional lab practice of bacterial culture for a 3-day incubation period has a lower rate of detection for CIF. Furthermore, since there isn't a gold standard test for a true fungal diagnosis, we couldn't ascertain true test properties like sensitivity and specificity. Even though a number of diagnostic techniques, including molecular testing and sputum galactomannan, have demonstrated superior efficacy in identifying specific fungus species in cystic fibrosis, they are not frequently carried out in the clinical laboratory environment (30, 39). Lastly, unmeasured variables, such as acute oral or intravenous antibacterial medication for pulmonary exacerbation at the time of specimen collection, may influence the probability of fungal isolation on bacterial culture. Nonetheless, there has been debate regarding the relationship between Aspergillus isolation and intravenous (i.v.) antibiotic use (35, 40).

In conclusion, despite mounting evidence of respiratory morbidity, the clinical significance of certain filamentous fungus and yeast in CF is still up for debate. To fully comprehend the clinical effects linked to fungal disease, however, precise diagnoses are required. Our research indicates that if selective fungal culture techniques are not used, the present procedures of certain CF doctors and clinical microbiology labs may be restricting the fungal diagnoses. According to these findings, a CF population would isolate more CIF if a combination of selective fungal culture medium, such as IMA, SDA, and BHI agar, were cultured for roughly seven days. To direct the creation of a standardized method for identifying fungus in the CF airway, more research is required.

MATERIALS AND METHODS

Study design and participants. In this 12-month prospective study, expectorated sputa from adult CF patients at Johns Hopkins Hospital in Baltimore, Maryland, USA, was collected and analyzed. The Johns Hopkins clinical microbiology laboratory analyzed consecutive expectorated sputum specimens obtained from patients in the outpatient clinic, irrespective of their clinical state. Subjects who could

expectorate sputum and had a baseline age of 18 years or older met the eligibility requirements. Sputum samples were taken, with a minimum volume of 0.5 ml. No liquefaction, sonication, or other methods of specimen alteration were used. Sputum specimens in equal quantities were used to inoculate each plate. Each specimen was subjected to two simultaneous procedures: (i) a standard laboratory procedure that involved inoculating on the bacterial culture medium suggested by the CFF (MacConkey, Columbia CNA, sheep blood, chocolate with and without bacitracin, Burkholderia cepacia selective, or Staphylococcus aureus CHROMagar, [Remel, Lenexa, KS]) (22, 41) plus SDA (Remel, Lenexa, KS) and incubating for three days at 37°C. Additionally, each specimen was inoculated on IMA (Hardy Diagnostics, Santa Maria, California), SDA, and BHI agar with gentamicin and chloramphenicol (Becton Dickinson, Sparks, Maryland) and incubating for fourteen days at 30°C (Fig. 2). The presence of fungal isolates was assessed daily in the cultures. Upon identifying a fungal isolate, we used microscopy to characterize the colony and examine its morphology. If required, we also used DNA sequencing techniques (using the Applied Biosystems 3500 genetic analyzer to analyze the 26S to 28S rDNA and internal transcribed regions) (42). We gathered the participants' demographic and clinical data. The rate of detection of CIF, which is defined as Aspergillus, Scedosporium, Trichosporon, and Exophiala species, was the main result. These organisms were chosen based on published research linked to heightened respiratory symptoms, pulmonary exacerbations, and problems following lung transplantation (5–7, 10–13, 31). Every filamentous fungal isolate was documented. The mean time-to-detection, or the number of days between the specimen's inoculation and the fungal isolation, was the secondary outcome.

Statistical analysis.

The percentage of positive culture diagnoses for each culture medium over the total number of positive culture diagnoses was used to calculate the detection rate of each medium. The isolation of CIF in one or more of the culture media (bacterial culture media, SDA, IMA, or BHI agar) was considered a positive culture. The percentage of fungus found in each participant in the cohort was used to compute prevalence. Two or more instances of the same fungus during the study period were considered persistent isolates. Calculations of means and standard deviations for continuous variables and proportions for categorical variables were used to conduct a descriptive analysis of the baseline cohort. Age, sex, F508del genotype, pancreatic insufficiency, FEV1 percent predicted, Pseudomonas aeruginosa colonization, use of inhaled anti-pseudomonal antibiotics (tobramycin powder/solution, aztreomycin, colistin, and ceftazidime), nonmacrolide oral antibiotics (defined as anti-staphylococcal oral antibiotics, including sulfamethoxazole/trimethoprim, minocycline, doxycycline, and linezolid), azithromycin, and antifungals (itraconazole, voriconazole, and posaconazole) were among the demographic and clinical variables used in the analysis. Using chi-square and Fisher's exact tests, the rates of fungal detection were compared between conventional bacterial culture medium and selective fungal media (IMA, SDA, and BHI agar). To compare the mean time-to-detection in the bacterial and selective fungal media, we employed Wilcoxon rank-sum tests and two sample t tests.

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