

Assessment of Microbiological Diagnostic Procedures for Respiratory Specimens from Cystic Fibrosis Patients in German Laboratories by Use of a Questionnaire

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Respiratory specimens from cystic fibrosis (CF) patients challenge microbiological laboratories with their complexity of pathogens and atypical variants. We evaluated the diagnostic procedures in German laboratories by use of a questionnaire. Although most laboratories followed guidelines, some of them served only a small number of patients, while others did not use the recommended selective agars to culture the particular CF-relevant species.

Cystic fibrosis (CF) patients suffer from chronic recurrent airway infections, which cause phenotypical changes (small-colony variants [SCVs]; mucoid phenotypes) of typical CF pathogens and the selection of multiresistant and rare bacteria such as *Burkholderia cepacia* complex (BCC) or nontuberculous mycobacteria due to repeated antimicrobial therapies and adaptation of the bacteria to this particular niche. To provide optimal microbiological diagnostic special culture conditions, knowledge and experience about the typical CF pathogens and their phenotypical changes are required.

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The status of the microbiological diagnostic procedures of laboratories serving CF centers, which participate in the German “Quality assurance cystic fibrosis project” (1), was evaluated by use of a questionnaire. First, contributing CF centers were asked to provide the address of their microbiological laboratories. Second, a questionnaire based on the German microbiological infectious quality standards for airway infections in CF (Mikrobiologisch-infektiologische Qualitätsstandards [MiQ]) (2) which were published by the German Society for Hygiene and Microbiology (3) and on the protocols provided by the consultant laboratories for CF microbiology (Institute of Microbiology, Medical School Hannover, Hannover, Germany; Max von Pettenkofer-Institute for Hygiene and Medical Microbiology, Munich, Germany) was sent to the laboratories. A translation of the main recommendations by the MiQ, which are comparable to the guidelines of the UK Cystic Fibrosis Trust (4) is provided in the supplemental material (see Table S1 in the supplemental material).

Seventy-eight of 83 (94%) CF centers provided the address of the microbiological laboratory, and all these laboratories were contacted. Forty-five of 61 (74%) laboratories responsible for the microbiological testing of 4,664 CF patients (56% of CF patients in Germany in 2010) responded to the questionnaire. Eight laboratories (18%) provide microbiological service for less than 25 CF patients. Eighty-nine percent of the laboratories reported that they used guidelines, and most of them used the MiQ (88%).

All laboratories used media to culture the most prevalent CF pathogens such as *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, supportively and/or selectively (Table 1) (2). Some reported the use of more than one medium.

Thirteen laboratories used mannitol salt agar as a selective agar for *S. aureus*, and eight laboratories employed chromogenic agar (Table 1). Although thymidine auxotroph *S. aureus* has been reported for the first time to be cultured on mannitol salt agar (5), the study of Kipp et al. (6) showed that the newer chromogenic agar media facilitate a selective and more sensitive growth of *S. aureus* without further confirmatory testing or subculture for susceptibility testing as well as the identification of *S. aureus* SCVs (2, 6–8). However, in this study (6), the authors did use only mannitol salt agar provided by one company. Therefore, it is not clear whether the use of mannitol salt agar of other companies may yield better results for SCVs (6). It is important to use special culture conditions for the isolation of SCVs, because these phenotypes are easily overgrown on standard agar by cocolonizing bacteria due to their slow growth and their small colony size and are therefore difficult to isolate. Furthermore, it is of special interest to improve culture conditions for these phenotypes, because they are associated with chronic or persistent infections and with decreased pulmonary function and are more resistant to antibiotics than the normal phenotype (9–14). Whereas all laboratories reported that they informed the clinic about pathogens with mucoid phenotypes, the culture of SCVs was reported by only 70% of investigators.

Only 91% (41/45) of laboratories indicated the application of a selective agar to culture BCC (Table 1) (2). In contrast, Zhou et al. reported the use of such an agar in the United States in 99% (136/138) of laboratories (15). Since four of the German laboratories did not use a selective agar, which is recommended by the MiQ as well as by the United Kingdom guidelines as one of the basic culture media, it might be the case that these laboratories will miss BCC isolates (2, 4). As shown in the study of Gilligan et al., in

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TABLE 1 Culture media used for the detection of CF-related pathogens

Culture medium	No. of laboratories reporting use of the indicated culture medium (%)
Columbia blood agar	41 (91)
Chocolate agar ^a	45 (100)
MacConkey agar	32 (71)
Selective media for:	
<i>S. aureus</i> ^b	31 (69)
<i>P. aeruginosa</i> ^c	19 (42)
BCC ^d	41 (91)

^a Chocolate agar alone ($n = 18$) and chocolate agar with bacitracin ($n = 18$), oleandomycin ($n = 8$), or vancomycin ($n = 1$).

^b Columbia blood agar with nalidixic acid ($n = 1$) or aztreonam ($n = 1$), colistin-nalidixic acid agar ($n = 7$), mannitol salt agar ($n = 13$), CHROMagar Staph. aureus ($n = 5$), *S. aureus* ID agar ($n = 3$), and brain heart infusion NaCl agar ($n = 1$).

^c *Pseudomonas* isolation agar ($n = 3$), cefrimid agar ($n = 15$), and cefrimid broth ($n = 1$).

^d *Burkholderia cepacia* selective agar ($n = 27$) (29), *B. cepacia* agar ($n = 1$) (16), cepacia medium ($n = 5$) (30), Oxoid *B. cepacia* agar ($n = 1$) (31), and oxidative-fermentative polymyxin B-bactriacin lactose agar ($n = 7$) (32). One laboratory did not specify the medium used for the culture of BCC.

cultures of sputum samples from 169 CF patients, BCC isolates were cultured on BCC selective agar from 35 patients, but when they were cultured on MacConkey agar, BCC isolates were found for only 21 patients, thereby missing 40% of BCC isolates (16). Because of the slow growth of some BCC strains, they can easily be overgrown by other microorganisms, thereby making their isolation very difficult (17). Shreve et al. showed that the prevalence of typical CF pathogens and their epidemiology were influenced by the frequency of the microbiological testing, by the employment of selective media for *S. aureus*, *P. aeruginosa*, and BCC as well as adequate culture conditions (18). The correct isolation of pathogens is important not only to treat CF patients appropriately but also to allow conclusions about the impact of special bacteria on disease progression (18).

In the case of culture of BCC isolates, only 60% of laboratories contacted their CF center directly to initiate special hygiene measures. Considering that patient-to-patient transmission is reported for BCC isolates (19), it becomes clear how important it is to detect this pathogen correctly and to provide rapid information of positive cultures (20). Moreover, infection with BCC strains is associated with higher mortality and high resistance to antibiotics (21–23).

All laboratories reported the identification of Gram-negative nonfermenting bacteria, including *P. aeruginosa* and BCC by using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry ($n = 29$) and/or API 20 NE ($n = 26$). Some laboratories indicated the use of multiple systems. In general, the laboratories used MALDI-TOF mass spectrometry ($n = 28$), an API system ($n = 28$), and/or Vitek 2 ($n = 24$) for bacterial identification.

Although 93% of the laboratories indicated that they performed confirmatory tests for special pathogens, only 56% used such tests to confirm BCC (Table 2). Seven laboratories specified that they used sequencing or PCR of the *recA* gene as the method of choice (2, 4). It has been shown that phylogenetic analysis of *recA* can be used to confirm BCC and that PCR-restriction fragment length polymorphism (RFLP) analysis of *recA* is superior to that of 16S rRNA gene with regard to discrimination of BCC species (20, 24).

TABLE 2 Confirmatory tests for special pathogens

Pathogen	No. of laboratories using the following confirmatory test ^a :			
	PCR	16S sequencing	Probe	Others
MRSA ^b ($n = 36$)	24	0	1	17
BCC ($n = 25$)	9	18	0	10

^a Some laboratories reported the use of more than one confirmatory test, 7 for MRSA and 8 for BCC.

^b MRSA, methicillin-resistant *S. aureus*.

For susceptibility testing, 89% of laboratories reported that they utilized more than one method. Agar diffusion ($n = 34$) and/or Etest ($n = 31$) were employed most frequently, followed by Vitek 2 ($n = 27$). In the EuroCareCF quality assessment (25), most laboratories used disc diffusion and/or Etest without any major errors. Earlier studies showed that automated systems do not always produce reliable results for susceptibility testing of *P. aeruginosa* and other nonfermenting Gram-negative bacteria, especially if adapted phenotypes, such as mucoid isolates or SCVs, were cultured and are therefore not recommended (26, 27). There were efforts made by Otto-Karg et al. to improve the automated susceptibility testing of Gram-negative nonfermenters with Vitek 2, providing good results for bacteria, which were clearly categorized resistant or susceptible (28). However, the results were poorer for strains with MICs near the breakpoints (28).

To conclude, most, but not all, consulted laboratories in Germany are using quality standards specifically elaborated for the microbiological diagnostic of CF respiratory specimens (2). In our questionnaire, we identified a lack of use of selective media especially for such important pathogens as BCC and special phenotypes such as *S. aureus* SCVs. To overcome uncertainties concerning culture media, including selective and new media and their specific culture conditions, further instructions for microbiological laboratories serving CF centers are necessary. Moreover, the culture of CF specimens should be performed by laboratories serving a certain number of patients to be able to provide special culture methods and experience in identifying unusual phenotypes and species.

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