Evaluation of the novel culture-based FAST-T system allowing selection of optimal antibiotics for critically ill patients within 4 h (for other than bloodstream infectious).

Running title: FAST-T test system for selecting optimal antibiotics

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ABSTRACT

Rapid diagnostic tests are needed to improve patient care, particularly in immunocompromised hosts. Here, we describe the validation of a new phenotypic culture-based FAST-T method for rapid selection of antibiotics *in vitro* using specimens with mono- and polybacterial infections. FAST-T approach, which can be applied to any type of non-blood tissue, does not require isolation of pure bacterial cultures. FAST-T-selected antibiotics are those that can completely eliminate mixed bacterial infections in specimens. The method uses a novel FASM-T medium that allows more rapid bacterial growth of gram-positive and gram-negative monoisolates compared with that achieved with conventional laboratory media. The application of the FAST-T method in 122 bacterial species demonstrated overall sensitivity, specificity, positive predictive value, and negative predictive value of 99.6%, 98.1%, 98.5%, and 99.4%, respectively, already

30 after 4 h. The overall category agreement with the outcome of standard testing was 98.9% with
31 very major errors and major errors being detected in 1.2% and 0.6% of cases.

32 The use of FASM-T medium in 20 clinical polymicrobial samples allowed culturing a more
33 diverse set of bacteria, including fastidious species, compared with that achieved with the
34 standard laboratory diagnostic and enabled, already within 4 h, accurate selection of the
35 antibiotics that completely eliminated all cultivatable bacteria from clinical samples. In
36 conclusion, FAST-T system may be a valuable tool in improving phenotypic-based antibiotic
37 selection, enabling targeted empirical therapy and accurate antibiotic replacement, which is
38 especially important in high-risk patients.

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52 **Introduction**

53 Antibiotic therapy is typically started on an empirical basis, because the causative organism is
 54 not identified in an appreciable proportion of patients (1-3). However, empirical antibiotic
 55 therapy is inadequate in over 25% of cases, with 8–12% of patients receiving antibiotics that are
 56 ineffective (4). For these patients, antibiotic therapy must be adjusted following antimicrobial
 57 susceptibility testing (AST) by culture-based and/or molecular biology methods. However, even
 58 following the switch of the empirically chosen antimicrobial, in over 10% of cases, the newly
 59 selected antimicrobial remains ineffective (3). The resulting clinical failure is particularly
 60 dangerous for immunocompromised patients.

61 There are several types of diagnostic tests used currently for the selection of appropriate
 62 antibiotics, but neither of them is ideal with regard to its expedience and accuracy (5). Currently,
 63 traditional methods based on the isolation of bacterial culture, require at least 24 h to detect the
 64 growth of bacteria isolated from clinical specimens after sampling and another 6 to 24 h for
 65 detailed isolate characterization (i.e., biochemical identification) or susceptibility testing, during
 66 which time the treatment can only be empirical (6, 7). Therefore culture-based methods, owing
 67 to the slow turnaround time of results, are not suitable for tailored empirical therapy and are
 68 suboptimal in acute infection cases.

69 Another concern with culture-based tests is that media used in classical AST techniques, such as
 70 LB, Columbia and others, do not allow the growth of complex polymicrobial communities (8, 9).
 71 As a result, some clinically relevant organisms that are more difficult to culture do not get
 72 detected by standard AST of biological samples, leading to therapy failure (9). Many
 73 contemporary methods allow isolation of only the predominant species, whereas bacteria at the
 74 site of infection are rarely represented by a single species (10). The vast majority of the
 75 infections of the respiratory tract, urinary tract, skin, and soft-tissues had been previously
 76 characterized as being monomicrobial due to inaccurate results from culture-dependent isolation
 77 techniques. In contrast, the use of more advanced methods today indicates their polymicrobial

78 nature (8, 11-14). Bacteria within polymicrobial communities are significantly more resistant to
 79 antimicrobials as they share collective antibiotic resistance (15). Furthermore, they are
 80 characterized by combined virulence contributing to the increased mortality rate among critically
 81 ill and compromised patients, such as patients with HIV or those that underwent solid organ or
 82 marrow transplantation (10, 16-18).

83 Another reason for the inappropriate selection of antibiotics by the standard AST methods is that
 84 they focus only on well-known pathogenic bacteria (8, 19). However, many bacteria that are
 85 considered nonpathogenic in non-immunocompromised patients may be pathogenic in people
 86 with suppressed immune response (20). This may happen because even in the case of successful
 87 elimination of one specific pathogen with a selected antibiotic, other bacteria that remain at the
 88 site of infection continue to grow and result in disease progression. Moreover, there is no
 89 benchmark accurate enough to indicate with certainty that bacteria are definitely pathogenic (21).

90 For example, *Pandora* *apista*, *Aeromonas* *spp.*, and *Kluyvera* *spp.* are now classified as
 91 pathogenic, although several decades ago, they were believed to be opportunistic and safe
 92 microorganisms (21-24). The media used in standard AST mostly promote the growth of the
 93 predominant bacteria and do not necessarily support proliferation of minor bacterial species from
 94 biological samples that may depend on each other for growth (25). That is why, it has been
 95 estimated that less than 10% of bacteria can be cultured in laboratory conditions and assayed by
 96 conventional AST (26-28).

97 In order to overcome these obstacles, in the last few decades, several types of novel diagnostic
 98 tests for rapid identification of bacteria and AST have been developed (29, 30). Such tests are
 99 based on direct pathogen identification using nucleic acid amplification techniques (5, 31).

100 Although these methods are frequently utilized to determine the causative agents of bloodstream
 101 infections, some of them are used for the infections of other parts of the body (32-34). These
 102 methods do not depend on *in vitro* growth for bacterial detection and have become routine in
 103 many clinical microbiology laboratories. The introduction of PCR assays and sequencing

technologies shortened the turnaround time to obtain the results and enabled characterization of pomyicrobial infections (35, 36). However, these assays also have a number of limitations. In particular, although the identification of bacteria helps to determine the therapeutic strategy, it does not really inform on the extent of resistance to antibiotics (6). Moreover, the methods based on 16S RNA sequencing are not very accurate as many bacterial species share similar or identical 16S rRNA sequences (37, 38).

Some molecular biology methods are based on the detection of antimicrobial resistance genes (39, 40). However, the reliance on the presence of resistance genes may be misleading with regard to accurate antibiotic selection as not all resistant genotypes result in resistant phenotypes (6). Furthermore, these methods can only detect known antibiotic resistance genes but may omit those, which have not yet been discovered (39, 41). The available resistance markers are not sufficiently comprehensive to provide clinically actionable results and may lead to the usage of either unnecessarily broad spectrum antibiotics or those without the sufficiently strong therapeutic effect.

Consequently, culture-based AST that predicts not only the resistance but also susceptibility to different drugs is still considered the gold standard for the identification of the most appropriate antibiotic for various infections (42).

Here, we evaluate a newly developed culture-based FAST-T system that can provide rapid phenotypic-based antibiotic selection for monobacterial and polybacterial clinical isolates. The FAST-T approach is based on a novel paradigm for the selection of effective antibiotics that considers not only monobacterial infection but also polybacterial cooperative interactions at the infection site.

Materials and Methods

Study samples and laboratory settings

Study specimens were obtained from the Human Microbiology Institute (New York, NY, USA), maintained at about 4 °C (wet ice), but not frozen, and sent to TGV-Biomed within 8 h after sampling.

Bronchoalveolar lavage (BAL) or sputum samples obtained from patients with chronic obstructive pulmonary disease (COPD), community-acquired pneumonia (CAP), ventilator-associated pneumonia (VAP), hospital-acquired pneumonia (HAP), cystic fibrosis (CF) were tested by using both state-of-the-art and FAST-T system methods.

Antibiotics

AST was limited to antibiotics used for the treatment of respiratory infections, which were selected according to the American Thoracic Society/Infectious Disease Society of America guidelines (43-45). Antibiotics were added to FASM-T medium at respective maximal concentrations that could be achieved at the site of infection according to literature data: amikacin, 9 µg/mL (46); azithromycin 8 µg/mL (46, 47); aztreonam 22 µg/mL (48, 49); cefepime 24 µg/mL (46, 49, 50); gentamicin 5 µg/mL (56, 51); levofloxacin 12 µg/mL (46, 52); linezolid 25 µg/mL (53, 54); meropenem 10 µg/mL (55); piperacillin-tazobactam (25 for piperacillin and 3.5 for tazobactam) µg/mL (56); vancomycin 12 µg/mL (46, 57) (all from Sigma-Aldrich, St. Louis, MO). Probes were incubated at 37 °C, for 4, 8, and 24 h.

Bacterial strains and growth conditions

Bacterial clinical isolates included the following gram-positive and gram-negative bacteria: *Staphylococcus aureus* (n = 22), *Streptococcus pneumoniae* (n = 4), *Streptococcus epidermidis* (n = 4), *Streptococcus pyogenes* (n = 2), *Enterococcus faecalis* (n = 5), *Bacillus cereus* (n = 4), *Paenibacillus VT400* (n = 1), *Bacillus respiratorii* VT-16-64 (n = 1) *Escherichia coli* (n = 15), *Acinetobacter baumannii* (n = 2), *Stenotrophomonas maltophilia* (n = 4), *Klebsiella*

153 *pneumoniae* ($n = 10$), *Proteus vulgaris* ($n = 4$), *Proteus mirabilis* ($n = 5$), *Haemophilus*
 154 *influenzae* ($n = 1$), *Klebsiella oxytoca* ($n = 1$), *Rothia mucilaginosa* ($n = 1$), *Moraxella*
 155 *catharrhalis* ($n = 1$) *Pseudomonas aeruginosa* ($n = 16$), *Burkholderia cenocepacia* ($n = 6$),
 156 *Enterobacter cloacae* complex ($n = 6$), *Achromobacter xylosoxidans* ($n = 2$), *Serratia*
 157 *marcescens* ($n = 3$).

158 Bacteria were obtained from the Cystic Fibrosis Foundation Therapeutics Development Network
 159 Resource Center for Microbiology at the Seattle Children's Hospital (Seattle, WA, USA) and
 160 from the Human Microbiology Institute (New York, NY, USA). Individual patterns of resistance
 161 to antibiotics are summarized in Table S1.

162 All bacterial strains were subcultured from frozen stocks onto Columbia broth (Oxoid Ltd.,
 163 London, England) and incubated at 37 °C overnight. A standard bacterial inoculum of 5×10^5
 164 CFU/mL was used. All subsequent liquid subcultures were derived from colonies isolated from
 165 these plates and were further grown at different solid media: LB agar (Oxoid Ltd., London,
 166 England), Columbia agar (Oxoid Ltd., London, England), *Burkholderia cepacia*-
 167 selective agar (Hardy Diagnostics, Santa Maria, CA), chocolate agar (Oxoid UK),
 168 CHROMagar Staph aureus Medium (Becton, Dickinson and Company, Franklin Lakes, NJ),
 169 FAST-M medium (Human Microbiology Institute, New York, NY, USA). All media were
 170 supplemented with 5% lysed sheep erythrocytes (Becton Dickinson, Heidelberg, Germany).

171

172 **Biological specimen processing and bacterial isolation from them**

173 BAL or sputum biospecimens (1 mL) from the patients with ventilator-associated, community-
 174 acquired pneumonia, cystic fibrosis (CF), or chronic obstructive pulmonary disease were mixed
 175 with 1 mL of sterile H₂O until homogeneity with a plastic swab. After homogenization, 25 µL of
 176 the suspension was directly plated onto FASM-T medium in each well and incubated at 37 °C,
 177 for 4, 18, and 24 h. For control arm, probes were plated on LB agar and *Burkholderia cepacia*-

178 selective agar (Hardy Diagnostics), chocolate agar (Oxoid), CHROMagar Staph aureus Medium
179 (Becton, Dickinson and Company) and cultured according to laboratory recommendations at 37
180 °C for 24 h (8).

181

182 **Identification of bacteria in biological specimens**

183 Bacterial identification to the species level from biological specimens was done by using
184 subcultures on FAST-M or LB medium (Oxoid, UK). Isolates were examined for purity by light
185 microscopy (Leica 2500DM, Leica Microsystems, Germany). To exclude the presence of mixed
186 bacterial cultures, the isolates were assessed from at least 10 fields of view (59). After the
187 subcultivation of every mixed bacterial culture up to monocultures, subsequent biochemical
188 identification and matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass
189 spectrometry (Microflex LT, Bruker Daltonics, Germany) analysis were performed according to
190 the manufacturer's instructions after 24 h of growth.

191 The complete 16S rRNA gene sequencing of bacterial colonies was also used to identify the
192 isolates. A PCR was performed with the general bacterial primers 27f (5'-
193 AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3'). The
194 PCR mixture contained 0.2 µM of each primer and 40 ng of bacterial DNA in a total volume of
195 100 µL (HotStar Taq Master Mix Kit, Qiagen, Valencia, CA, USA) (60).

196 PCR was performed with a Mastercycler EP S gradient thermocycler (Eppendorf, Germany)
197 using the following protocol: one cycle of 15 min at 95 °C, followed by 30 cycles, of (60 s at 95
198 °C, 45 s at 50 °C, and 90 s at 72 °C), and the final extension cycle of 10 min at 72 °C. The PCR
199 products were verified by electrophoresis in 0.8% agarose gel, and the rest of the sample was
200 purified and concentrated in 30 µL of demineralized water using a Qiaquick PCR purification kit
201 (Qiagen) (61).

202 PCR amplicons were sequenced with a BigDye Terminator v1.1 cycle sequencing kit (Life
203 Technology, Foster City, CA, USA). Identification to the species level was conducted by
204 comparing the obtained sequences with those in BLAST database
205 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Identifications to the genus and species-level were done
206 on the basis of $\geq 97\%$ and 99% identity of the 16S rRNA gene sequence to the reference,
207 respectively (62).

208

209 **FAST-T system and interpretation of results**

210 FAST-T system is a 12-well plate (TGV-Biomed, USA) filled with FASM-T medium that
211 comprises pancreatic digests of casein, peptic digest of meat, heart pancreatic digest, yeast
212 extract, starch, and water (Human Microbiology Institute, New York, NY, USA).

213 Clinical specimens were directly plated onto FASM-T medium in each well with a sterile swab,
214 avoiding scratching or damaging the agar. Ten of the twelve wells contained FASM-T nutrient
215 medium with antibiotics (one antibiotic per well) at concentrations that can be practically
216 achieved at the site of infection. Two control wells contained antibiotic-free FASM-T medium.

217 Plate reading was performed following sampling and incubation at 37 °C, for 4, 8 , and 24 h.

218 The presence of microbial growth was identified with naked eye and confirmed with a
219 stereoscopic microscope (Leica S6, Leica Microsystems, Germany). The signs of early bacterial
220 growth, namely increased turbidity, hemolysis, appearance of film, and microcolonies, in the
221 wells with antibiotics were compared with bacterial growth signs in control wells.

222 Microbial growth in any well meant that in the pathological material, there were microorganisms
223 resistant to the particular antibiotic in the nutrient medium in this well. Therefore, such antibiotic
224 was categorized as “ineffective”. Absence of bacterial growth in the well meant that the
225 antibiotic present in the well killed all bacteria in the pathological material and was thus

226 categorized as “effective”. In rare cases, there was no growth in control wells after 4 h, so those
227 cultures were not analyzed further as is separately described in the Results below.

228

229 **Gold standard definition**

230 Culture-based *in vitro* AST was selected as the standard of care method. The minimal inhibitory
231 concentrations (MICs) for antimicrobials were determined by the broth microdilution method
232 according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (63, 64). The
233 isolates were categorized as susceptible or resistant according to CLSI breakpoint guidelines
234 with the only modification that “intermediate” isolates were treated as “resistant” (65). A
235 standard bacterial inoculum of 5×10^5 CFU/mL was used. Serial twofold dilutions of the
236 antimicrobials were prepared in cation-adjusted Mueller-Hinton broth. MIC was defined as the
237 lowest concentration of antibiotic that completely inhibited visible growth. Experiments were
238 conducted in triplicate.

239

240 **Data analysis**

241 The FAST-T system antibiotic selection results were compared to those obtained with culture-
242 based gold standard microdilution method as described above. For the purpose of data analysis,
243 the following definitions were used. A true-positive result occurred when both the FAST-T and
244 standard-of-care methods indicated that the microorganism was resistant to the antibiotic (i.e.,
245 bacteria gave growth on the medium with antibiotic). A true-negative result occurred when both
246 FAST-T and standard-of-care methods demonstrated sensitivity of the microorganism to the
247 antibiotic (i.e., bacteria did not grow on the medium with antibiotic). A false-positive result
248 occurred when the FAST-T system identified a strain as being sensitive to a certain antibiotic
249 (organisms did not grow in FAST-T plates), but according to the standard-of-care method, the

organism was resistant to that antibiotic, whereas a false-negative result was recorded when the FAST-T system suggested that the microorganism was resistant to the antibiotic, whereas the standard-of-care method indicated that it was sensitive. We calculated the values of accuracy, sensitivity, specificity, as well as positive predictive value (PPV) and negative predictive value (NPV) as previously described (66).

The total category agreement (CA) was determined and the results discrepant with those obtained by the state of care microdilution method were categorized (65). The very major errors were recorded in the cases when the FAST-T system indicated that an isolate was susceptible to the antibiotic, whereas according to the standard of care method, the isolate was resistant. The major errors were recorded in the cases when the FAST-T system suggested that an isolate was resistant to the antibiotic, whereas the standard of care method indicated that the isolate was susceptible. The rate of very major errors was calculated by dividing the total number of very major errors by the total number of strains determined as being resistant and multiplied by 100%. The rate of major errors was determined by dividing the total number of major errors by the number of strains determined as being susceptible and multiplied by 100%.

265

266 **Bacterial diversity analysis**

The variety of bacteria that grew on the media used was characterized by the α -diversity indices such as non-parametric abundance-based coverage estimator (ACE) and Chao 1, indices which were managed and analyzed using R version 3.4.1 software (67).

Species richness (a count of different species), that gave growth on different media were represented on a dot plot, generated by package 'ggplot2' within R version 3.4.1 (67). All statistical analyses were conducted with a significance level of $\alpha = 0.05$ ($P < 0.05$).

273

274 **Results**

275 **Comparison of bacterial growth rate on FASM-T medium to that on other** 276 **media**

277 After 4 h of cultivation, visible growth was detected in 119 of 122 monomicrobial cultures
278 (97.5%) in plates with FASM-T medium. Visible growth was not detected after 4 h only for *S.*
279 *maltophilia* ($n = 1$), *S. pneumoniae* ($n = 1$), and *A. baumannii* ($n = 1$). However, these three
280 strains became visible already after 8 h in culture (122/122; 100%).

281 Under the same conditions, visible growth on LB agar or Columbia agar after 4 h of culturing
282 was detected for fewer microorganisms. In particular, culturing on LB agar revealed growth of
283 only 69 out of 122 strains (56.5%), whereas 89 out of 122 strains (72.9%) grew on Columbia
284 agar. Moreover, some *Streptococcus* spp did not reveal clear growth even after 8 h on these
285 media and became visible only by 24 h (Fig. 1, Table S2).

286 We observed higher species richness after growth for 4 h on FASM-T medium as revealed by
287 ACE and Chao 1 indices (28.07 and 33.5, respectively), compared with the values of these
288 parameters after growth on LB agar (ACE = 25.6 and Chao 1 = 21.5) or Columbia agar
289 (ACE = 25.5 and Chao 1 = 24; Fig. 2). Therefore, FASM-T was the only medium that
290 allowed to detect visible growth of monomicrobial cultures already after 4 h with high accuracy.

291

292 **Estimation of the diversity of bacteria grown on FASM-T medium**

293 Next, we applied FASM-T medium for the studies of polymicrobial infections. We used samples
294 from patients with respiratory infections, which are usually associated with polymicrobial
295 growth. Bacterial growth on FASM-T medium was seen within 4 h in 20 out of 20 clinical
296 specimens (100%), whereas microbial growth was detected only in 8 out of 20 samples (40%)
297 grown on LB agar that was used as standard medium. Only subsequent growth for 24 h revealed
298 clear bacterial growth in all samples cultured in LB media.

299 Next, we analyzed polymicrobial growth in each sample by using FASM-T and LB media at 4 h
300 and 24 h of culturing. A detailed description of bacteria that gave growth on different media is
301 provided in Table 1. Already after 4 h, direct plating on FASM-T media allowed to cultivate
302 mixed bacterial communities in 19 of 20 clinical specimens (95%). The increase of the culturing
303 time to 24 h did not increase the diversity of cultured bacteria. Under the same conditions, no
304 mixed communities (0/100; 0%) were observed during exposure to LB media after 4 h.
305 Subsequent culturing for up to 24 h allowed to identify polymicrobial communities only in 5 out
306 of 20 samples (25%).

307 Next, we determined the overall gain of identified microbial diversity that gave growth on
308 FASM-T vs standard media, demonstrating that even after 4 h of culturing, FASM-T
309 uncovered a more diverse set of microorganisms from the biological specimens compared
310 with that revealed by the standard medium after 24 h of culturing (Fig. 3).

311 Notably, in the majority of specimens grown on FASM-T, we identified more than one well-
312 known pathogen of respiratory tract infections, whereas the standard method allowed the
313 identification of one and, only sometimes, two pathogens. The highest microbial diversity was
314 observed in sputum of the patients with CF – a disease that is known to be characterized by a
315 particularly complex, mixed lung microbiome (68). For example, from some tissue specimens,
316 we isolated *P. aeruginosa*, *S. haemolyticus*, *A. baumannii*, *A. xylosoxidans*, and *B. thailandensis*
317 with FASM-T medium. Each of these microorganisms has been shown to be associated with
318 lung infections in CF, whereas the standard method identified only the dominant *P. aeruginosa*
319 (69-71).

320 The ability of FASM-T to enable growth of fastidious species is illustrated by the isolation of *S.*
321 *milleri* from CF patient #10. This bacterium was only recently associated with pulmonary
322 complications in CF because previously, *S. milleri* could not be cultured on standard laboratory
323 media (72, 73). Another notable example was the identification with FASM-T of *K. pneumonia*,

324 *Rothia dentocariosa*, *Acinetobacter ursingii* and *Bacillus pumilus*, in sample #18 from the
325 patient with hospital-acquired pneumonia (74). Each of these species had been shown previously
326 to be associated with human infectinos. However, under the same conditions, the standard
327 method identified only *K. pneumonia*. A similar trend was noted for all other tested specimens
328 from cases of other respiratory diseases whereby FASM-T enabled identification of a wider
329 range of known respiratory pathogens compared to that revealed by the standard method.

330 Notably, by using FASM-T medium, we identified two previously unknown bacterial species
331 *Chryseobacterium mucoviscidosis* sp. nov. and *Bacillus obstructivus* sp. nov. (75, 76). These
332 bacteria have not been identified by MALDI-TOF, as they had very low similarity to known
333 species, and their further whole genome sequencing proved that they were previously unknown
334 bacterial species highly enriched in genes encoding pathogenic and antibiotic resistance factors.

335 **Antibiotic selection in monomicrobial cultures using FAST-T method**

336 The performance of the FAST-T system for direct selection of 10 different antibiotics prescribed
337 for the treatment of respiratory infections caused by gram-negative or gram-negative bacteria
338 was evaluated in a total of 122 monobacterial cultures after 4 h and 24 h of culturing. Cultures of
339 3 out of 122 strains (*S. pneumoniae* VT-SP-14, *S. maltophilia* VT-CFSM-4, and *A. baumannii*
340 ATCC 17978) were excluded from the experiment due to the lack of growth in control wells
341 after 4 h, leaving 1,990 runs in total for analysis (119 bacterial species tested against 10
342 antibiotics; 1 run = each antibiotic tested against one bacterial strain). For the tested 119 strains,
343 correct antibiotic selection, as gauged by the results of conventional AST, was achieved after 4 h
344 in 1,177 of 1,190 runs (98.9%). In rare cases, false-positive and false-negative results were
345 obtained. The overall sensitivity, specificity, PPV, and NPV were 99.6%, 98.1%, 98.5%, and
346 99.4%, respectively (Tables 2 and S3).

347 After 24 h of culturing, we preformed 1,220 runs in total, as all bacteria at this time gave growth
348 on FASM-T control antibiotic-free medium. By 18 h, all microbial strains gave growth in control

349 wells. Correct antibiotic selection was achieved at 1,209 of 1,220 runs (99.1%). The values of
350 analyzed parameters after 24 h of culturing were marginally higher than those achieved after 4 h
351 (sensitivity, 99.6%; specificity, 98.5%; PPV, 98.8%; and NPV, 99.4%; Tables 2, S4). Notably,
352 there was no difference in the overall ability to detect gram-positive and gram-negative bacteria.
353 Next, we determined CA after 4 and 24 h of culturing for each antibiotic. After 4 h, the overall
354 CA for all antibiotics used was 98.9% (1,177/1,190, 10 very major errors, 3 major errors). The
355 rate of very major errors was 1.5% (10 very major errors/666 resistance outcomes), and the rate
356 of major errors was 0.6% (3 major errors/512 susceptibility outcomes). The highest CA values
357 were observed for levofloxacin, vancomycin (absolute agreement, 100%), followed by those for
358 aztreonam, gentamicin, linezolid, piperacillin/tazobactam (for all: 118/119; 99.2%), amikacin,
359 azithromycin, cefepime (for all: 117/119; 98.3%), and meropenem (116/119; 97.4%) (Table 3).

360 CA values for the FAST-T system were even higher when the period of culturing was extended.
361 The overall CA for all antibiotics after 24 h of cultivation was 99.1% (1,209/1,220) with 8 very
362 major errors and 3 major errors. The rate of very major errors was 1.2% (8 very major errors/688
363 resistance results), and the rate of major errors was 0.6% (3 major errors/521 susceptibility
364 results). CA values became nominally higher for piperacillin/tazobactam and azithromycin.
365 Thus, after 24 h of culturing, the absolute CA was observed for piperacillin/tazobactam,
366 levofloxacin, and vancomycin with no errors detected (100%). Slightly lower, but nonetheless
367 very high CA values were noted for aztreonam, azithromycin, gentamicin, linezolid (for all:
368 121/122; 99.2%), amikacin, cefepime (for all: 120/122; 98.3%), and meropenem (119/122;
369 97.5%) (Table 3).

370 At both time points tested, the level of major errors was very low, indicating that antibiotic-
371 sensitive isolates did not grow on FASM-T medium with antibiotics added at chosen
372 concentrations. In general, there was no discernible pattern of the errors that could be explained
373 by the nature of microorganisms or antibiotics tested: AST errors occurred in various species and
374 with various drugs. CA values were not statistically different between the antibiotics used.

375

376 **Antibiotic selection in polymicrobial cultures using FAST-T method**

377 We used 10 randomly selected samples from cases of respiratory infections tested before and
 378 analyzed particularities of antibiotic selection with the FAST-T (after growth for 4 h) and
 379 standard methods (Table 4). The majority of antibiotics identified by the standard method as
 380 “ineffective” (totally N50) were also identified as “ineffective” with FAST-T (total N65). At the
 381 same time, in several cases, there was a discrepancy between the conclusions of the FAST-T and
 382 standard methods about antibiotic efficacy. Some discrepancies were detected, in particular, for
 383 narrow-spectrum antibiotics, when growth of mixed gram-positive and gram-negative species
 384 was revealed by the FAST-T method, whereas only one species gave growth on the standard
 385 medium. However, such discrepancies were rare and that narrow-spectrum antibiotics were
 386 effective against mixtures gram-positive and gram-negative pathogens grown on FASM-T
 387 medium likely indicated complicated interspecies interactions (Table 6). For example,
 388 aztreonam was effective against mixed communities formed not only by the gram-negative *P.*
 389 *aeruginosa*, but also by *S. aureus*. Furthermore, vancomycin was found to be effective against
 390 mixed infections associated with both gram-positive and gram-negative bacteria.

391 We performed follow-up subculturing of bacterial colonies from the wells containing FASM-T
 392 with those antibiotics that were identified as being effective against the identified predominant
 393 pathogen by standard AST, but, as the presence of live colonies indicated, proved to be
 394 ineffective according to FAST-T. In 22% of such cases (11 discrepancies out of 50 cases), we
 395 could isolate the remaining identified predominant pathogen. Most likely this happened because
 396 complicated polymicrobial interactions at the site of infection that resulted in the higher
 397 resistance of bacteria, including the predominant pathogen, were not adequately simulated by the
 398 standard method compared to monobacterial culture growth. However, such interactions could
 399 be modeled well with the FAST-T system.

400

401 **Discussion**

402 New diagnostic methods for the selection of antibiotics for tailored empirical therapy and for the
 403 change of antibiotic therapy that failed in immunocompromised patients are urgently needed.
 404 The availability of rapid and accurate methods of antibiotic selection for non-bloodstream
 405 infections, such as lung, urinary tract, skin, and soft tissue infections, would have a great impact
 406 on the disease outcome, length of hospital stay, possible complications, and spread of antibiotic
 407 resistance (77).

408 Our present experiments evaluated for the first time the performance of the novel FAST-T
 409 system providing culture-based antibiotic selection within a short, 4 h period. The FAST-T
 410 method can be used for initial tailored empirical therapy and for the selection of an appropriate
 411 antibiotic to those patients who have not responded to the previous therapy and require change of
 412 antimicrobial treatment strategy. Our original hypothesis that the use of the FAST-T system
 413 would provide a rapid and accurate selection of antibiotics for both monobacterial or
 414 polybacterial infections sampled directly from clinical specimens, was found to be confirmed
 415 experimentally. FAST-T system allowed faster growth of monobacterial cultures compared with
 416 that achieved by the standard method used in clinical diagnostics and demonstrated an increased
 417 richness with higher ACE and Chao 1 indices for bacteria that gave growth within 4 h on
 418 FASM-T medium than those recorded for bacteria grown on LBA or Columbia agar (Fig. 2).

419 Our study also revealed that FAST-T system provided accurate antibiotic selection compared to
 420 that achieved by the standard of care, with results being available already after 4 h of culturing.
 421 If compared with the standard method that involves the isolation of pure bacterial cultures prior
 422 to AST, the FAST-T system was faster by more than 32 h. The antibiotic selection was highly
 423 accurate already within 4 h (sensitivity, 99.6%; specificity, 98.1%; PPV, 98.5%; NPV, 99.4%,
 424 and CA, 98.9%); with no discernable bias in the error pattern toward gram-positive or gram-

negative bacteria or toward a particular antibiotic class. Furthermore, the increase of culturing time from 4 h to 24 h did not significantly change the accuracy of the FAST-T method performance, indicating that accurate results can be obtained after just several hours of culturing.

In the current study, FASM-T medium supported growth of a significantly more diverse set of bacteria from biological samples even after 4 h of culturing than the standard medium, broadly used in microbiological laboratories, after 24 h of culturing. Thus, FAST-T system allowed identification of polymicrobial cultures in 19 out of 20 samples after 4 h of culturing. This was in agreement with previous reports that emphasized the polymicrobial nature of the majority of respiratory tract infections (13). Under the same conditions, the latter approach detected polymicrobial cultures in only 5 out of 20 samples. In this study, the standard method failed to detect not only some isolated commensal bacteria, but also well-known pathogens, such as *B. cepacia*, *S. maltophilia*, *K. pneumonia*, and others.

Notably, many bacteria identified within the polybacterial samples are known as causative agents of respiratory infections. For example, we detected *S. aureus*, *R. dentocariosa*, *H. influenzae*, *E. corrodens*, *E. miricola*, *A. baumannii*, *A. xylosoxidans*, and other well-known respiratory tract pathogens in samples from CF and chronic obstructive pulmonary disease cases. These microorganisms have been shown to cause recurrent respiratory infections but are completely overlooked by standard of care diagnostics. In the current study, the FAST-T system enabled growth of different complex mixtures of bacteria without a limit to the number of species that could be isolated. This finding is of primary importance due to two considerations. First, the presence of polymicrobial infections is one of the reasons for the inappropriate antibiotic selection and mortality of high-risk patients. The elimination of the dominant strains leads to the re-growth of other bacteria at the site of the infection unaffected by the initial antibiotic treatment, which may lead to disease progression and complications, e.g., due to the lack of sufficiently strong immune response. Moreover, as it has been shown before, some bacteria that are considered nonpathogenic in the lungs of non-immunosuppressed subjects may be

451 pathogenic in immunosuppressed hosts (78, 79). This is why, for some bloodstream infections,
 452 sterilization is widely used, particularly in the patients with impaired immune response.
 453 Therefore, the fact that FAST-T system reliably detects both major and minor bacterial
 454 pathogens within the site of infection makes it promising for the use in immunocompromised
 455 patients. Second, our assessment of antibiotic sensitivity within polymicrobial communities
 456 showed that in some cases, antibiotics selected with the standard method did not eliminate even
 457 the dominant bacteria in mixed microbial communities. Such false-positive data might result in
 458 the selection of ineffective antibiotics and therapy failure (80). The reason for false-positive
 459 results generated by the gold standard method may be because this method enables growth of
 460 monobacterial cultures but does not take into consideration higher tolerance to antibiotics of
 461 bacteria within mixed communities (15). However, “real life” infections are predominantly
 462 polymicrobial by nature (10). The resulting survival of the lead pathogen leads to its re-growth
 463 and therapy failure. According to our *in vitro* data, FAST-T system is less prone to such
 464 limitations.

465 Furthermore, the use of the FAST-T system for the treatment of polybacterial infections allowed
 466 selection of not only broad spectrum but also narrow spectrum antibiotics, confirming the
 467 presence of complexed interspecies interactions in mixed bacterial communities.

468 Notably, by using FAST-T, we have isolated previously unknown bacterial species
 469 *Chryseobacterium mucoviscidosis* sp. nov. and *Bacillus obstructivus* sp. nov. that possessed
 470 several typical virulence factors, such as hemolysins, alpha-amylases, and others, which are
 471 found in other respiratory pathogens (75, 76, 81). Moreover, in these bacteria, we identified
 472 several antibiotic resistance genes that once found in endospore-forming *Bacillus* spp. are of
 473 concern because of the possible spread of antibiotic resistance among sporobiota members (82).
 474 The identification of previously unknown bacteria as well as some fastidious species, such as *S.*
 475 *milleri*, indicates that the developed FASM-T medium provides unique growth factors and
 476 cultivation environment that are not achievable with the standard media (19, 83).

477 The use of the FAST-T system enables to quickly select antibiotics that are effective for the
 478 treatment of each particular condition (i.e., those that kill *all* microorganisms present in the
 479 clinical specimen) without the need to identify precisely the causative agents of the infection or
 480 to determine MICs. Importantly, although the FAST-T method does not allow immediate
 481 identification of bacteria on the species level, bacterial cultures that gave growth on FASM-T
 482 medium can be isolated and subsequently identified by using culture-based techniques and any
 483 other standard methods for the study of bacteria.

484 FAST-T system has all benefits of phenotypic culture-based methods, but is 30–54 h faster than
 485 standard culture-based diagnostics that requires time-consuming isolation of pure bacterial
 486 cultures. Even compared with direct AST, the FAST-T method allows antibiotic selection on a
 487 much shorter timescale (84). Like direct AST, FAST-T system enables direct sampling of
 488 biological specimens without the need for culturing or time-consuming sample processing.
 489 Furthermore, the FAST-T method can indicate the suitable antibiotic in only 4 h, whereas direct
 490 AST requires 18–36 h (and that is why direct AST is not applicable for tailoring empirical
 491 antibiotic therapy) (84).

492 Despite being as fast as some of the molecular biology methods, FAST-T system lacks main
 493 disadvantages of molecular methods based on next-generation sequencing and 16S RNA
 494 sequencing, such as overestimation of antimicrobial resistance and inability to inform on
 495 antimicrobial susceptibility (37, 38).

496 In this study, we used FAST-T system with a set of antibiotics used for the treatment of
 497 respiratory infections. However, it can easily be adjusted for the diagnosis of the infections of
 498 other parts of the body, such as urinary tract, skin, or soft tissues, by including the antibiotics
 499 used for the therapy of these infections in FASM-T medium. Moreover, it can be used for the
 500 cultivation of bacteria with different oxygen requirements. The presence of bacterial growth was
 501 analyzed with naked eye and stereoscopic microscopy, but a more sophisticated device for visual
 502 monitoring can be used to increase the analysis accuracy. We believe that FAST-T system may

503 become a valuable tool in improving antibiotic selection in diagnostics, with as little as 4 h
 504 turnaround time. The use of FAST-T system in therapeutic management will allow faster
 505 development of adequate therapies, including targeted empirical therapy and accurate antibiotic
 506 replacement, especially in immunocompromised, high-risk patients. Future studies will be
 507 necessary to investigate clinical efficacy of the FAST-T system and the clinical impact of its use
 508 alone and as an auxiliary method for standard diagnostics.

509

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514

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770 **Figure captions**

771 **Figure 1:** Comparison of bacterial growth rate on FASM-T, LB, and Columbia media. Growth
772 rate is represented b

773 y a heat map with each cell indicating the percentage of bacterial strains that gave growth at 4, 8,
774 or 24 h. White color intensity represents the highest rate of growth, whereas red color indicates
775 no growth for a particular time period.

776

777 **Figure 2:** Increased diversity of bacterial species that gave growth on FASM-T medium,
778 compared to that of species that grew on LB and Columbia agar, as revealed by the values of
779 abundance-based coverage estimator (ACE), A) and Chao1 (B) indices.

780

781 **Figure 3:** Comparison of the richness of bacteria that gave growth on FASM-T medium (after 4
782 h of culturing) compared with that of bacteria that gave growth (after 24 h) on standard media
783 (LB medium, *Burkholderia cepacia*-selective agar, chocolate agar,
784 CHROMagar Staph. aureus medium. Richness values for bio samples in two types of media are
785 represented on a dot plot, displaying the distribution of numerical variables where each dot
786 represents a value. The height of the column of dots represents the frequency for that value. The
787 package ‘ggplot2’ within R (version 3.4.1) was used.

788

789 **TABLE 1.** Comparison of diversity values of bacteria grown on FAST-T and standard media for 4 and 24 h.

Number	Clinical specimen	Diagnosis	Bacteria identified with FASMT-T medium		Bacteria identified with standard media*	
			4 h	24 h	4 h	24 h
1	BAL	COPD	<i>Burkholderia gladioli</i> <i>Neisseria spp.</i> <i>Haemophilus parainfluenzae</i> <i>S. mitis</i> <i>R.mucilaginoso</i> <i>Streptomyces violaceruber</i>	<i>B. gladioli</i> <i>Neisseria spp.</i> <i>H.parainfluenzae</i> <i>S. mitis</i> <i>R.mucilaginoso</i> <i>S. violaceruber</i>	-	<i>S. mitis</i> <i>R. mucilaginoso</i>
2	BAL	COPD	<i>S.aureus</i> <i>K.pneumonia</i> <i>E. coli</i> <i>E.faecalis</i> <i>Proteus spp.</i>	<i>S.aureus</i> <i>K.pneumonia</i> <i>E. coli</i> <i>E.faecalis</i> <i>Proteus spp.</i>	<i>K.pneumonia</i>	<i>K.pneumonia</i>
3	BAL	CAP	<i>S.aureus</i> <i>S.pneumonia</i>	<i>S.aureus</i> <i>S.pneumonia</i>	-	<i>S.aureus</i>
4	BAL	CAP	<i>S.aureus</i>	<i>S.aureus</i>	<i>S.aureus</i>	<i>S.aureus</i>
5	BAL	CAP	<i>P.aeruginosa</i> <i>H. influenza</i> <i>M. catarrhalis</i>	<i>P.aeruginosa</i> <i>H. influenza</i> <i>M. catarrhalis</i>	<i>P.aeruginosa</i>	<i>P.aeruginosa</i>
6	BAL	VAP	<i>S.aureus</i> <i>E.cloacae</i> <i>P.aeruginosa</i>	<i>S.aureus</i> <i>E.cloacae</i> <i>P.aeruginosa</i>	-	<i>P.aeruginosa</i>
7	BAL	VAP	<i>S.aureus</i> <i>K.pneumonia</i> <i>E. coli</i> <i>C.mucoviscidosis</i> <i>S.aureus</i>	<i>S.aureus</i> <i>K.pneumonia</i> <i>E. coli</i> <i>C.mucoviscidosis</i> <i>S.aureus</i>	-	<i>S.aureus</i> <i>E. coli</i>

8	Sputum	CF	<i>P.aeruginosa</i> <i>S.aureus</i> <i>R.mucilaginosa</i>	<i>P.aeruginosa</i> <i>S.aureus</i> <i>R.mucilaginosa</i>	<i>P.aeruginosa</i>	<i>P.aeruginosa</i> <i>S.aureus</i>
9	Sputum	CF	<i>P.aeruginosa</i> <i>S.aureus</i> <i>S. maltophilia</i> <i>B.cepacia</i>	<i>P.aeruginosa</i> <i>S.aureus</i> <i>S. maltophilia</i> <i>B.cepacia</i>	-	<i>P.aeruginosa</i> <i>B.cepacia</i>
10	Sputum	CF	<i>P.aeruginosa</i> <i>Micrococcus luteus</i> <i>S.aureus</i> <i>S.oralis</i> <i>S.milleri</i>	<i>P.aeruginosa</i> <i>M.luteus</i> <i>S.aureus</i> <i>S.oralis</i> <i>S.milleri</i>	-	<i>P.aeruginosa</i>
11	Sputum	CF	<i>P.aeruginosa</i> <i>A.xylosoxidans</i> <i>E.coli</i>	<i>P.aeruginosa</i> <i>A.xylosoxidans</i> <i>E.coli</i>	<i>P.aeruginosa</i>	<i>P.aeruginosa</i>
12	Sputum	CF	<i>S. maltophilia</i> <i>E.coli</i> <i>Bacillaceae spp</i> <i>P.aeruginosa</i>	<i>S. maltophilia</i> <i>E.coli</i> <i>Bacillaceae spp</i> <i>P.aeruginosa</i>	<i>P.aeruginosa</i>	<i>P.aeruginosa</i>
13	Sputum	CF	<i>P. aeruginosa</i> <i>S. haemoliticus</i> <i>A. baumannii</i> <i>A. xylosoxidans</i> <i>B. thailandensis</i>	<i>P. aeruginosa</i> <i>S. haemoliticus</i> <i>A. baumannii</i> <i>A. xylosoxidans</i> <i>B. thailandensis</i>	-	<i>P. aeruginosa</i>
14	Sputum	CF	<i>B. multivorans</i> <i>Corynebacterium pseudodiphtherit</i> <i>icum</i> <i>Moraxella catharrhalis</i> <i>Paenibacillus pabuli</i>	<i>B. multivorans</i> <i>C.pseudodiphtheriticum</i> <i>M.catharrhalis</i> <i>P.pabuli</i>	-	<i>C. pseudodiphtheriticum</i>

15	Sputum	CF	<i>A. xylosoxidans</i> <i>R.mucilaginosa</i> <i>Elizabethkingia miricola</i> <i>Microbacterium paraoxydans</i>	<i>A. xylosoxidans</i> <i>R. mucilaginosa</i> <i>E.miricola</i> <i>M.paraoxydans</i>	-	<i>A. xylosoxidans</i>
16	Sputum	CF	<i>P. aeruginosa</i> <i>S. aureus</i> <i>E. coli</i> <i>S.anginosus</i> <i>B. sonorensis</i>	<i>P. aeruginosa</i> <i>S. aureus</i> <i>E. coli</i> <i>S.anginosus</i> <i>B. sonorensis</i>	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>
17	Sputum	CF	<i>P. aeruginosa</i> <i>S. aureus</i> <i>E. coli</i> <i>B. cereus</i>	<i>P. aeruginosa</i> <i>S. aureus</i> <i>E. coli</i> <i>B. cereus</i>	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> <i>S. aureus</i>
18	BAL	HAP	<i>K. pneumonia</i> <i>Rothia dentocariosa</i> <i>A.ursingii</i> <i>Bacillus pumilus</i>	<i>K. pneumonia</i> <i>R. dentocariosa</i> <i>A.ursingii</i> <i>B. pumilus</i>	-	<i>K. pneumonia</i>
19	BAL	CAP	<i>H.influenzae</i> <i>Actinobacillus suis</i> <i>Eikenella corrodens</i> <i>Actinomyces oris</i>	<i>H.influenzae</i> <i>A. suis</i> <i>E. corrodens</i> <i>A.oris</i>	-	<i>H. influenzae</i>
20	BAL	COPD	<i>K. pneumoniae</i> <i>H. influenza</i> <i>Bacillus obstructivus</i>	<i>K. pneumoniae</i> <i>H. influenza</i> <i>Bacillus obstructivus</i>	-	<i>K. pneumoniae</i>

790 Bacteria from all cystic fibrosis patient samples were plated on LB medium as well as cultivated on *Burkholderia cepacia* selective agar, chocolate agar, and
791 CHROMagar Staph aureus medium.

792 **TABLE 2.** Overall identification performance of the FAST-T system after 4 and 24 h of culturing.

Species	N true positive		N true negative		N false positive		N false negative		Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h
<i>S.aureus</i>	134	134	84	84	1	1	1	1	99,3	99,3	98,8	98,8	99,3	99,3	98,8	98,8
<i>S.pneumoniae</i>	12	16	18	24	0	0	0	0	100	100	100	100	100	100	100	100
<i>S.epidermidis</i>	16	17	23	23	1	0	0	0	100	100	95,8	100	94,1	100	100	100
<i>S.pyogenes</i>	10	10	30	30	0	0	0	0	100	100	100	100	100	100	100	100
<i>E.faecalis</i>	27	27	22	22	1	1	0	0	100	100	95,6	95,6	96,4	96,4	100	100
<i>B.cereus</i>	17	17	23	23	0	0	0	0	100	100	100	100	100	100	100	100
<i>Paenibacillus</i> VT400	5	5	4	4	1	1	0	0	100	100	80	80	83,3	83,3	100	100
<i>B.respiratorii</i> VT1664	3	3	7	7	0	0	0	0	100	100	100	100	100	100	100	100
<i>E.coli</i>	70	71	78	78	2	1	0	0	100	100	97,5	98,8	97,2	98,6	100	100
<i>A.baumannii</i>	8	16	1	4	0	0	0	0	100	100	100	100	100	100	100	100
<i>S.maltophilia</i>	19	28	10	11	1	1	0	0	100	100	90,1	91,6	95	96,6	100	100
<i>K.pneumoniae</i>	51	51	47	47	1	1	1	1	98,1	98,1	97,9	97,9	98,1	98,1	97,9	97,9
<i>P.vulgaris</i>	18	18	22	22	0	0	0	0	100	100	100	100	100	100	100	100
<i>P.mirabilis</i>	22	22	28	28	0	0	0	0	100	100	100	100	100	100	100	100
<i>H.influenzae</i>	2	2	8	8	0	0	0	0	100	100	100	100	100	100	100	100
<i>K.oxytoca</i>	6	6	4	4	0	0	0	0	100	100	100	100	100	100	100	100

<i>R.mucilaginoso</i>	4	4	6	6	0	0	0	0	100	100	100	100	100	100	100	100
<i>M.catharrhalis</i>	4	4	6	6	0	0	0	0	100	100	100	100	100	100	100	100
<i>P.aeruginosa</i>	122	122	35	35	2	2	1	1	99,2	99,2	94,6	94,6	98,4	98,4	97,2	97,2
<i>B.cenocepacia</i>	53	53	7	7	0	0	0	0	100	100	100	100	100	100	100	100
<i>E. cloacae complex</i>	31	31	29	29	0	0	0	0	100	100	100	100	100	100	100	100
<i>A.xylosoxidans</i>	16	16	4	4	0	0	0	0	100	100	100	100	100	100	100	100
<i>S. marcescens</i>	14	14	16	16	0	0	0	0	100	100	100	100	100	100	100	100
Gram-positive bacteria	403	408	259	265	6	5	2	2	99.9	99.9	96.8	97.2	97.4	97.9	99.6	99.6
Gram-negative bacteria	437	455	295	298	14	14	2	2	99.8	99.8	98.6	98,8	99.2	99.4	99.7	99.7

793

794 NPV, negative predictive value; PPV, positive predictive value.

TABLE 3. Category agreement of antibiotic sensitivity results of the FAST-T method with those obtained by the standard method after 4 and 24 h of culturing.

Antibiotic	Category agreement: correct identifications/total No of observations (%)		No of errors			
	4h	24h	Very major (false-positive)		Major (false-negative)	
	4h	24h	4h	24h	4h	24h
Piperacillin-tazobactam	118/119 (99,2)	122/122 (100)	1	0	0	0
Meropenem	116/119 (97,4)	119/122 (97,5)	2	2	1	1
Levofloxacin	119/119 (100)	122/122 (100)	0	0	0	0
Aztreonam	118/119 (99,2)	121/122 (99,2)	1	1	0	0
Gentamicin	118/119 (99,2)	121/122 (99,2)	0	0	1	1
Amikacin	117/119 (98,3)	120/122 (98,3)	2	2	0	0
Azithromycin	117/119 (98,3)	121/122 (99,2)	2	1	0	0
Vancomycin	119/119 (100)	122/122 (100)	0	0	0	0
Cefepime	117/119 (98,3)	120/122 (98,3)	2	2	0	0
Linezolid	118/119 (99,2)	121/122 (99,2)	0	0	1	1
Overall performance	1177/1190 (98,9)	1209/1220 (99,1)				

797

798 Bacterial cultures included monomicrobial cultures of *Staphylococcus aureus* ($n = 22$),
799 *Streptococcus pneumoniae* ($n = 4$), *Streptococcus epidermidis* ($n = 4$), *Streptococcus pyogenes* (n
800 $= 2$), *Enterococcus faecalis* ($n = 5$), *Bacillus cereus* ($n = 4$), *Paenibacillus VT400* ($n = 1$),
801 *Bacillus respiratorii* VT-16-64 ($n = 1$) *Escherichia coli* ($n = 15$), *Acinetobacter baumannii* ($n =$
802 2), *Stenotrophomonas maltophilia* ($n = 4$), *Klebsiella pneumoniae* ($n = 10$), *Proteus vulgaris* (n
803 $= 4$), *Proteus mirabilis* ($n = 5$), *Haemophilus influenzae* ($n = 1$), *Klebsiella oxytoca* ($n =$
804 1), *Rothia mucilaginosa* ($n = 1$), *Moraxella catharrhalis* ($n = 1$) *Pseudomonas aeruginosa* ($n =$
805 16), *Burkholderia cenocepacia* ($n = 6$), *Enterobacter cloacae complex* ($n = 6$), *Achromobacter*
806 *xylosoxidans* ($n = 2$), *Serratia marcescens* ($n = 3$).

807

808 **Table 4.** Identification of effective antibiotics selection from the biological samples with FAST-T (4 h of culturing) standard media (24 h of
809 culturing).

Number	Clinical specimen	Diagnosis	Bacteria identified with FAST-T medium		Bacteria identified with the standard media*		Follow-up sub-cultivation of bacterial communities from the wells of the FAST-T system with antibiotics that were deemed effective against identified pathogens by the standard method, but were suggested to be resistant according to the FAST-T method	
			Bacteria	Resistance pattern	Bacteria	Resistance pattern	Antibiotic	Bacteria detected despite the presence of antibiotic
1	BAL	CAP	<i>K. pneumoniae</i> <i>S.aureus</i> <i>E. coli</i> <i>E. faecalis</i> <i>Proteus spp.</i>	Amikacin Azithromycin Vancomycin Cefepime Linezolid Meropenem Aztreonam	<i>K. pneumoniae</i>	Amikacin Azithromycin Vancomycin Cefepime Linezolid	Meropenem Aztreonam	<i>K. pneumoniae</i> <i>S.aureus</i> <i>E. coli</i> <i>S.aureus</i> <i>Enterococcus faecalis</i> <i>E. coli</i>
2	BAL	Pneumonia	<i>S.aureus</i> <i>S.pneumonia</i>	Piperacillin-tazobactam Meropenem Aztreonam Cefepime Gentamicin Amikacin	<i>S.aureus</i>	Piperacillin-tazobactam Meropenem Aztreonam Cefepime	Gentamicin Amikacin	<i>S.aureus</i> <i>S.pneumonia</i> <i>S.pneumonia</i>
3	BAL	Pneumonia	<i>S.aureus</i> <i>E.cloacae</i> <i>P.aeruginosa</i>	Amikacin Vancomycin Cefepime Linezolid Piperacillin-tazobactam Levofloxacin Meropenem	<i>P.aeruginosa</i>	Amikacin Vancomycin Cefepime Linezolid Piperacillin-tazobactam Levofloxacin	Meropenem	<i>S.aureus</i> <i>E.cloacae</i>

4	Sputum	CF	<i>P.aeruginosa</i> <i>A.xylosoxidans</i> <i>E.coli</i>	Aztreonam Cefepime Linezolid Levofloxacin Meropenem Piperacillin- tazobactam Vancomycin	<i>P.aeruginosa</i>	Aztreonam Cefepime Linezolid Levofloxacin Meropenem Piperacillin- tazobactam Vancomycin	NA	NA
5	Sputum	CF	<i>S. maltophilia</i> <i>E.coli</i> <i>Bacillaceae spp</i> <i>P.aeruginosa</i>	Aztreonam Levofloxacin Vancomycin Linezolid Azithromycin Piperacillin- tazobactam Cefepime	<i>P.aeruginosa</i>	Aztreonam Levofloxacin Vancomycin Linezolid Azithromycin	Piperacillin- tazobactam Cefepime	<i>P.aeruginosa</i> <i>S. maltophilia</i> <i>E.coli</i> <i>Bacillaceae spp</i> <i>P.aeruginosa</i> <i>S. maltophilia</i> <i>E.coli</i> <i>Bacillaceae spp</i>
6	Sputum	CF	<i>P. aeruginosa</i> <i>S. haemolyticus</i> <i>A. baumannii</i> <i>A. xylosoxidans</i> <i>B. thailandensis</i>	Aztreonam Levofloxacin Vancomycin Linezolid Azithromycin Piperacillin- tazobactam Amikacin	<i>P. aeruginosa</i>	Aztreonam Levofloxacin Vancomycin Linezolid Azithromycin	Amikacin Piperacillin- tazobactam	<i>P. aeruginosa</i> <i>S. haemolyticus</i> <i>A. baumannii</i> <i>A. xylosoxidans</i> <i>B. thailandensis</i> <i>P. aeruginosa</i> <i>A. xylosoxidans</i>
7	Sputum	CF	<i>A. xylosoxidans</i> <i>R.mucilaginosa</i> <i>E.miricola</i> <i>M.paraoxydans</i>	Piperacillin- tazobactam Meropenem Aztreonam Gentamicin Amikacin Azithromycin Cefepime Vancomycin Linezolid	<i>A. xylosoxidans</i>	Azithromycin Cefepime Vancomycin Linezolid	Piperacillin- tazobactam Meropenem Aztreonam Gentamicin Amikacin	<i>E.miricola</i> <i>A. xylosoxidans</i> <i>E.miricola</i> <i>M.paraoxydans</i> <i>A. xylosoxidans</i> <i>E.miricola</i> <i>A. xylosoxidans</i> <i>E.miricola</i> <i>M.paraoxydans</i> <i>R.mucilaginosa</i> <i>E.miricola</i>

8	Sputum	CF	<i>P. aeruginosa</i> <i>S. aureus</i> <i>E. coli</i> <i>S.anginosus</i> <i>B. sonorensis</i>	Piperacillin-tazobactam Aztreonam Levofloxacin Vancomycin Linezolid Azithromycin	<i>P. aeruginosa</i>	Piperacillin-tazobactam Aztreonam Levofloxacin Vancomycin Linezolid Azithromycin	NA	NA
9	HAP	BAL?	<i>K. pneumonia</i> <i>R. dentocariosa</i> <i>A.ursingii</i> <i>B.pumilus</i>	Gentamicin Vancomycin Linezolid Aztreonam Cefepime	<i>K. pneumonia</i>	Amikacin Gentamicin Vancomycin Linezolid Piperacillin-tazobactam	Aztreonam Cefepime	<i>Bacillus pumilus</i> <i>R. dentocariosa</i> <i>K. pneumonia</i> <i>B. pumilus</i> <i>R.dentocariosa</i> <i>A.ursingii</i>
10	Exacerbation of COPD	BAL	<i>K. pneumoniae</i> <i>H. influenza</i> <i>B.obstructivus</i>	Gentamicin Vancomycin Linezolid Aztreonam Piperacillin-tazobactam	<i>K. pneumoniae</i>	Gentamicin Vancomycin Linezolid	Piperacillin-tazobactam Aztreonam	<i>K. pneumoniae</i> <i>H. influenza</i> <i>B. obstructivus</i> <i>H. influenza</i> <i>B. obstructivus</i>

810

811 Bacteria from all cystic fibrosis patient samples were plated on LB medium as well as cultivated on *Burkholderia cepacia* selective agar, chocolate agar, and
812 CHROMagar Staph aureus medium.

813 **Supplementary Table S1.** Individual patterns of resistance to antibiotics.

814 **Supplementary Table S2.** Comparison of growth speeds of monoisolates on different media.

815 **Supplementary Table S3.** Results of antimicrobial susceptibility testing by using the FAST-T
816 system (4 h culturing) and standard method (24 h culturing) for 119 gram-positive and gram-
817 negative pathogens.

818 **Supplementary Table S4.** Results of antimicrobial susceptibility testing by using the FAST-T
819 system and standard method for 122 gram-positive and gram-negative pathogens after 24 h of
820 culturing.

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FIG 1

FIG 2

FIG 3