Cystic fibrosis antibiotic susceptibility testing

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Cystic fibrosis (CF) is a chronic, progressive, life-limiting genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene[1]. Most CF patients suffer from acute pulmonary exacerbations resulting in progressive lung disease due to the production of thick immobile secretions, airway inflammation, chronic and recurrent infections[1, 2]. Therefore, the cornerstone of CF management is the use of mucoactive drugs and antibiotics with the goal of improving symptoms while suppressing the resident bacterial population[2]. Long term antibiotic therapy leads to infecting / colonising organisms becoming resistant to more and more antibiotics making treatment difficult[3]. Similarly, problems with antimicrobial allergy or intolerance pose challenges for appropriate antimicrobial therapy. Therefore, extended antimicrobial susceptibility testing (AST) is employed — quantitative, evidence-based *in vitro* AST results can guide prescribing of antimicrobials[4].

The Cystic Fibrosis Antibiotic Susceptibility Service (CFASS) has been funded by "NHS National Services Scotland" since 1999. It is based at the Microbiology Department in Aberdeen Royal Infirmary, Scotland and provides extended antimicrobial susceptibility testing using a minimum of six pairs of antimicrobials with results ranked in order of their *in vitro* effectiveness. The service is available for use by all Scottish CF clinicians / clinics and accepts multidrug-resistant Gram-negative microorganisms isolated from the respiratory tracts of adult individuals with CF. Microorganisms which are not multidrug-resistant are also accepted for testing where there is difficulty locally in determining appropriate antimicrobial therapy due to allergy or intolerance.

In our 20 year experience and in agreement with CF epidemiology, the most received isolate is *Pseudomonas aeruginosa* (54.31%) followed by *Burkholderia cepacia complex* (22.54%). In CF patients, *P. aeruginosa* is the most commonly isolated pathogen; more than 70% are colonised with this bacterium by the age of 25[5, 6]. This is due to its ubiquitous presence in the environment[7] and the ability to phenotypically and genotypically adapt itself to the CF lung environment. An innate adaptation of *P. aeruginosa* which enables its establishment in the airways is the ability to switch from planktonic to a biofilm mode of growth. This greatly impedes the efficacy of antibiotics due to reduced growth rate of biofilm bacterial cells and the presence of an anaerobic environment[3]. Other adaptations used by *P. aeruginosa* is the ability to exist as metabolically dormant persister cells or hypermutator strains due to increased mutation rates from defects in DNA repair / error systems[3]. The inability to eradicate these organisms from the airways and the development of resistance are the reasons combination testing is employed in CF management.

Synergy testing is an *in vitro* assessment of the interaction of two antimicrobial agents to determine if the effect of the combination is greater than the sum of their individual activities, hence classified as synergistic.[8] Data from 11,695 combination tests showed that most combinations had no interaction with only 9.8% synergy and 1.4% antagonistic combinations observed. Notably, **Table 1** shows that c.50% synergistic combinations were observed in *Stenotrophomonas maltophilia* (15.7%) compared with *P. aeruginosa* (8.4%). Furthermore, our data suggest that synergy in *S. maltophilia* primarily results from the addition of ticarcillin / clavulanate (44.94%): combination with aztreonam resulted in 50% synergy. Ciprofloxacin and ceftolozane / tazobactam was the most synergistic combination in *P. aeruginosa*. The reasons for these are unclear; research is necessary to unravel the underlying causes of species / drug synergistic interactions.

Organism ID	Total Isolates (%)	Synergy*(%)#	No interaction*(%)#	Antagonism*(%)#	Top Synergistic combination
P. aeruginosa	1089 (54.31%)	504 (8.4)	5435 (90.5)	65 (1.1)	Ciprofloxacin + Ceftolozane/ Tazobactam
Pseudomonas spp.	139 (6.93%)	51 (6.7)	708 (92.5)	6 (0.8)	Ciprofloxacin + Piperacillin/ Tazobactam
S. maltophilia	176 (8.78%)	178 (15.7)	930 (81.8)	29 (2.6)	Ticarcillin/Clavulanate + Aztreonam
B. cepacia complex	452 (22.54%)	333 (11.0)	2638 (87.4)	49 (1.6)	Tobramycin + Ceftazidime
Achromobacter spp.	117 (5.84%)	80 (10.4)	669 (87.0)	20 (2.6)	Ceftazidime + Imipenem

Table 1. Summary of isolate combination testing interpreted using FICI
* number of isolates; # percentage of isolate

Synergy testing methodology varies widely in complexity and interpretation and there is a lack of standardidation[8]. There is currently no clear consensus on the gold standard for assessing synergy; methods are time-consuming and labour-intensive with up to 25% discrepant results compared with the commercial Etest method used in most clinical laboratories[8]. The clinical relevance of synergy testing is questioned due to a lack of data.[8] Only one study used the multiple-combination bactericidal test method alone in a randomised, double-blind trial to show that CF patients who were treated with combination antibiotic regimens for pulmonary exacerbation did not exhibit significantly improved outcomes[9]. Due to insufficient evidence, the UK Cystic Fibrosis Foundation guidelines recommend that synergy testing should not be done in CF patients[10] but research has shown that it is still currently

in use in the management of pulmonary exacerbations[11,12]. Due to limited treatment options resulting from increasingly resistant bacteria, we believe there is an urgent need for further research to understand which synergy methods are predictive of clinical efficacy. This should lead to identification of an evidence-based, gold standard method for carrying out and interpreting synergy testing. Additional interpretative criteria should be explored when comparing the *in vitro* effectiveness of antimicrobial combinations. This should include the susceptibility breakpoint index[13] proposed by our laboratory, which may be more clinically relevant than the fractional inhibitory concentration index (FICI) as it is a measure of clinically relevant concentrations. **Figure 1** demonstrates that similar median susceptible breakpoint index (SBPI) values were observed for most isolates except *B. cepacia* complex. We also advocate rigorous quality control and exploration of avenues such as automation and / or the manufacture of synergy panels[14] to simplify methods for use in the clinical laboratory.

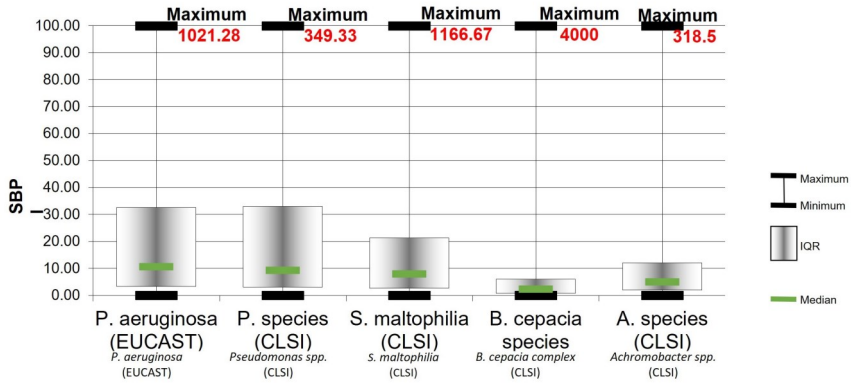

Figure 1. Susceptibility Breakpoint Indices (SBPI) for CFASS referred isolates. SBPI interquartile range denoted by the grey box, median, green line while maximum and minimum values are represented by the thick black line. P. aeruginosa was interpreted using the EUCAST guidelines for all antibiotics while CLSI was used for other species.

Despite evidence that a decrease in AST frequency is not associated with poorer outcomes[15] or lack of predictive value[16], it is still used in the management of CF. We asked service users if the AST report helped in the management or initial choice of antibiotics. 40% of respondents stated that reports helped in the initial choice of antibiotic treatment for infective exacerbations (**Figure 2**). Zemanick *et al*[4] reported that AST is rarely used to guide initial antibiotic choice and changed only when there was a lack of clinical response to current treatment: whilst we agree with this statement, we hypothesise that an AST report helps reaffirm the initial choice of antibiotics although it does not necessarily result in a change. When we explored whether there was a relationship between existing treatment and clinical progress, divergent results were seen. This suggest that assessment of clinical progress is subjective and clearer definitions should be included in CF pulmonary exacerbation management when assessing the effectiveness of treatment.

In conclusion, AST results appear not to influence treatment decisions, but our survey identified it is an important resource for clinicians: 94% of respondents proposed to use AST reports in the management of subsequent pulmonary exacerbations.

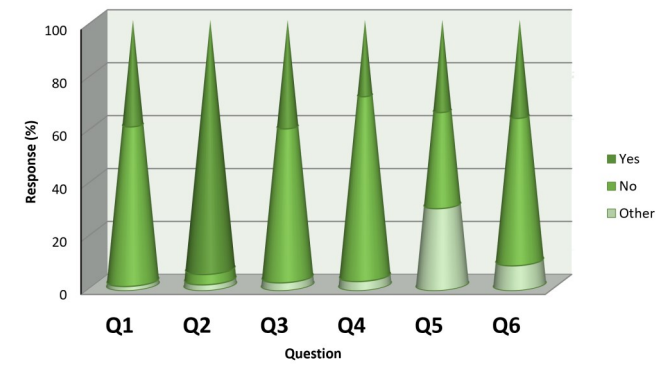

Figure 2. Clinician responses to CFASS feedback questionnaires. Combination susceptibility feedback responses (yes, no, or other) were grouped as six questions (Q1-6). A total of 817 feedback responses were received by the service.

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