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Abstract (poster session)

Optimisation of Phoenix automated identification and susceptibility testing of mucoid Pseudomonas species

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Objectives: Pseudomonas aeruginosa (PSA) is a clinically important pathogen causing serious infections such as pneumonia in immunosuppressed patients. They are a major cause of morbidity and mortality in cystic fibrosis (CF) patients. Often in these patients, organisms adopt a mucoid phenotype, making species identification and susceptibility testing difficult. Species identification and in particular susceptibilities are of the upmost importance in CF patients and many labs now use automated instruments to perform this task. Automated instruments fail to identify/issue susceptibility results for mucoid isolates, possibly due to the additional glycocalyx present. This study investigated various techniques to optimise the performance of an automated instrument to identify (ID) and susceptibility test (ST) these isolates. Methods: 2 mucoid PSA, which previously failed tests (ID + ST) on Phoenix (PHX) were used. Both isolates were pre-treated with 12 methods prior to re-testing on PHX and compared with the standard method (addition of 25ul of McFarland (McF) 0.5 inoculum to AST broth). Pre-treatments include; 1- wash bacteria in sterile water, 2- 30min incubation in saline + 20u/ml alginate lyase (AL), 3- 4hrs growth in tryptone soya broth (TSB) + 20u/ml AL, 4- growth on agar + 20u/ml AL, 5- heat to 60°C for 30mins, 6- add 50ul of McF 0.5 to AST broth, 7- growth on DNAse plate, 8- addition of 20u/ml AL to AST broth, 9- add 100ul on McF 0.5 to AST broth, 10- add 50ul of McF 1 to AST broth, 11- add 50ul of McF 2 to AST broth, 12- add 50ul to McF 4 to AST broth. The ability to produce an ID + ST was compiled on 5 occasions. Growth curves for the best pre-treatments were performed in AST broth. Results: Results are shown in Table 1. Positive ID results were seen in all pre-treatments except 5 where ID results failed for both PSA. Positive ST results were seen in PSA (A) & (B) in pre-treatments 10 & 12 and 2, 6, 7, 9, 10, 11 & 12 respectively. Growth of PSA (A) / (B) for standard, pre-treatment 9, 10, 11 &12 methods after 4 hours were: 5.2x106/3x106, 7.9x106/1.5x107, 6.9x106/1.2x107, 1.3x107/1.3x107 and 1.7x107/3.4x107 respectively. Conclusions: The most effective pre-treatment for optimising ID and ST from the PHX was addition of higher density inoculums (pre-treatments 9 to 12). Susceptibility testing in PHX is reliant on growth of isolate in AST broths, increasing starting inoculums increases density of growth in AST broths and therefore ability to produce ST results.

	PHX ID present		PHX AST present	
Pre-treatment	PSA (A)	PSA (B)	PSA (A)	PSA (B)
1	5 / 5	5 / 5	0 / 5	0 / 5
2	5 / 5	5 / 5	0 / 5	**2 / 5**
3	5 / 5	5 / 5	0 / 5	0 / 5
4	5 / 5	4 / 5	0 / 5	0 / 5
5	2 / 5	1 / 5	0 / 5	0 / 5
6	5 / 5	3 / 5	0 / 5	**3 / 5**
7	5 / 5	4 / 5	0 / 5	**2 / 5**
8	5 / 5	5 / 5	0 / 5	0 / 5
9	5 / 5	4 / 5	0 / 5	5 / 5
10	5 / 5	5 / 5	**1 / 5**	**5 / 5**
11	5 / 5	5 / 5	0 / 5	5 / 5
12	5 / 5	5 / 5	**1 / 5**	**5 / 5**