Antimicrobial susceptibility testing of cystic fibrosis and non-cystic fibrosis clinical isolates of *Pseudomonas aeruginosa*: a comparison of three methods

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ABSTRACT

Pseudomonas aeruginosa is an important pathogen in humans, particularly in the context of nosocomial infection and infections of the cystic fibrosis (CF) lung. In order to provide clinicians with information about the likely effectiveness of specific antimicrobial treatment for *P. aeruginosa* infections, clinical laboratories employ *in vitro* antimicrobial susceptibility testing. Two commonly employed methods are the CLSI disc-diffusion and Etest methods. The purpose of this study is to compare the accuracy of susceptibility results generated by these two methods against agar dilution as the reference method. Susceptible or non-susceptible (resistant and intermediate) results of the Etest and CLSI disc-diffusion methods are compared with CLSI agar dilution results for a large cohort of clinical cystic fibrosis (n=71) and non-cystic fibrosis (n=83) isolates using CLSI interpretive criteria. An unacceptable number of major and very major errors were observed for various antimicrobials tested against both CF and non-CF isolates when using the Etest and CLSI disc-diffusion methods. The potential for error in standard laboratory antimicrobial susceptibility testing should be considered by clinicians when being guided by the results of such tests in the prescription of antimicrobial agents for *P. aeruginosa* infection.

KEY WORDS: Cystic fibrosis.
Microbial sensitivity tests.
Pseudomonas aeruginosa.

Introduction

Pseudomonas aeruginosa is a significant cause of nosocomial infection and is the primary infectious cause of morbidity and mortality in cystic fibrosis (CF) patients. Treatment of infections caused by *P. aeruginosa* is often hindered by the organism's intrinsic resistance to many antimicrobial agents and its capacity to develop or acquire new resistance mechanisms. To allow more specific targeting of antimicrobial therapy in patient treatment, diagnostic laboratories perform *in vitro* antimicrobial susceptibility tests on clinical isolates of *P. aeruginosa*. However, CF isolates are often phenotypically distinct from non-CF isolates, displaying slower growth rates, mucoid morphologies, phenotypic switching, and hypermutable states, all of which may compromise the accuracy of antimicrobial susceptibility testing.[1–4]

The determination of minimum inhibitory concentration (MIC) by agar dilution is an established reference method for *P. aeruginosa* antimicrobial susceptibility testing.[5] However, in most clinical laboratories, a number of simpler alternative methods of antimicrobial susceptibility testing are employed. Two such commonly used methods are the Clinical and Laboratory Standards Institute (CLSI) disc-diffusion method and the Etest method, which provide a simple and reproducible indication of the susceptibility of a given organism to specific antimicrobial agents. Discrepancies between susceptibility test results of the disc-diffusion and Etest methods and those generated by agar dilution are often observed.

This study aims to compare and determine the prevalence of inconsistencies obtained by the Etest method and the CLSI disc-diffusion method with results generated by the CLSI agar dilution as a reference method.

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Materials and methods

Isolates

A total of 153 clinical isolates of *P. aeruginosa* comprised 71 CF respiratory isolates and 82 non-CF isolates of respiratory (n=25), urine (n=24), skin and soft tissue (n=28) and otitis externa (n=5) origin. The sources, methods of collection, identification and storage of all isolates employed in this study have been published previously.[6,7]

Antimicrobial susceptibility testing

All isolates were tested using three methods for eight different antimicrobials commonly used in the treatment of

Table 1. Agar dilution antimicrobial susceptibility results of *Pseudomonas aeruginosa* isolates used in this study.

	CLSI Agar dilution (reference) method					
	CF isolates			Non-CF isolates		
Antimicrobial	Susceptible (*n*)	Not susceptible (*n*)	Not susceptible (%)	Susceptible (*n*)	Not susceptible (*n*)	Not susceptible (%)
Timentin	46	25	35	72	10	12
Aztreonam	51	20	28	67	15	18
Ceftazidime	43	28	39	65	17	21
Cefepime	45	26	37	77	5	6
Ciprofloxacin	34	37	52	74	8	10
Gentamicin	31	40	56	78	4	5
Tobramycin	61	10	14	81	1	1

P. aeruginosa infections: timentin (ticarcillin plus clavulanic acid), aztreonam, ceftazidime, cefepime, ciprofloxacin, amikacin, gentamicin and tobramycin. Agar dilution and disc diffusion were performed according to CLSI guidelines.[8] The concentration of test agent in the agar dilution plate equalled the CLSI MIC resistance breakpoint (breakpoint targeting) and any growth was interpreted as resistance. Etest (AB Biodisk, Solna, Sweden) was performed according to the manufacturer's instructions and interpreted using CLSI breakpoints. Results for all three methods were recorded as susceptible or non-susceptible, with the latter comprising both resistant and intermediate categories.[9]

With the exception of Etest, all tests were performed in duplicate. Etest was performed in duplicate only for isolates in which initial testing results did not correlate with those of either agar dilution or disc-diffusion testing. In cases where duplicate testing results were discordant, tests were performed in triplicate and the two concordant results accepted as the correct result.

Statistical analysis

Results for the Etest and disc-diffusion methods were compared with the agar dilution reference method. The percentage of very major errors (isolate categorised as susceptible to a given antimicrobial agent when non-susceptible by agar dilution) and major errors (isolate categorised as non-susceptible to the antimicrobial agent when isolate susceptible by agar dilution) were calculated for the Etest and disc-diffusion assays. Greater than 1% very major errors and greater than 5% major errors were considered unacceptable.[9]

Results

The CF isolates of *P. aeruginosa* employed in this study showed a higher percentage of non-susceptible results across all antimicrobial agents tested when compared to non-CF isolates using the agar dilution reference method. The highest percentage of non-susceptibility rates was noted in non-CF isolates for aztreonam (18%) and ceftazidime (21%). In comparison, the highest percentage of non-susceptibility in CF isolates was that for ciprofloxacin (52%) and gentamicin (56%) (Table 1).

Susceptibility testing of CF isolates by Etest yielded an unacceptable number of very major errors for all antimicrobial agents tested. An unacceptable number of major errors was observed only for cefepime testing of CF isolates. In testing non-CF isolates, an unacceptable percentage of very major errors for timentin, aztreonam, ceftazidime, cefepime and gentamicin were produced. Unacceptable levels of major error were noted only for aztreonam (Table 2).

The CLSI disc-diffusion method also performed poorly when testing CF isolates, with an unacceptable percentage of very major errors observed for all antimicrobial agents tested. Unacceptable levels of very major errors were evident for β-lactam-based agents (timentin, aztreonam, ceftazidime and cefepime), but not ciprofloxacin, gentamicin or tobramycin when using this method to test non-CF isolates. An unacceptable percentage of major errors was seen only for aztreonam in both CF and non-CF isolates (Table 3).

When Etest results were used as a hypothetical reference method for evaluation of the efficacy of CLSI disc diffusion, the latter method showed improved results compared to when the more established agar dilution reference method was employed. Unacceptable levels of very major errors were evident for timentin and ceftazidime (4% each), cefepime and gentamicin (7% each) among CF isolates. In testing non-CF isolates, an unacceptable level of very major error was seen for timentin only (5%). Indeed, the mean average percentage of very major errors observed for non-CF isolates was within acceptable limits. As with the results found in the comparison with the agar dilution reference method, unacceptable percentages of major error were seen only for aztreonam in both CF and non-CF isolates (Table 4).

Discussion

The finding of increased non-susceptibility to antimicrobials in CF *P. aeruginosa* isolates is consistent with the increased development of antibiotic resistance. This has previously been attributed to the increased number and duration of antibiotic therapy courses that most CF patients infected with *P. aeruginosa* undergo,[5] and to increased rates of hypermutability in CF isolates, leading to the rapid selection

Table 2. Comparison of the Etest antimicrobial susceptibility test with the agar dilution reference method for CF and non-CF clinical isolates of *Pseudomonas aeruginosa*.

	Etest method							
	CF isolates				Non-CF isolates			
Antimicrobial	Susceptible (*n*)	Not susceptible (*n*)	VME (%)	ME (%)	Susceptible (*n*)	Not susceptible (*n*)	VME (%)	ME (%)
Timentin	47	24	**4**	3	69	13	**2**	**6**
Aztreonam	51	20	**4**	4	71	11	**5**	0
Ceftazidime	48	23	**7**	0	78	4	**16**	0
Cefepime	43	28	**7**	**6**	78	4	**2**	1
Ciprofloxacin	39	32	**8**	1	74	8	0	0
Gentamicin	39	32	**14**	3	80	2	**4**	1
Tobramycin	65	6	**6**	0	81	1	0	0
Mean average			**7**	2			**4**	1

VME: very major errors (categorised susceptible when non-susceptible by reference method).
ME: major errors (categorised non-susceptible when susceptible by reference method).
Percentage error results outside acceptable limits are highlighted in bold.

of resistant mutants among isolates infecting this group of patients.[4]

Neither of the two antimicrobial susceptibility methods tested in this study performed well in comparison to agar dilution as a reference method for CF *P. aeruginosa* isolates. The degree of very major error returned for both methods was unacceptably high. Mean average percentage of very major error across all antimicrobial types tested was seven to eight times the acceptable limit. The results of non-CF isolate testing were not as discordant as those observed when testing CF isolates. However, the percentage of very major errors observed was still above acceptable limits for many agents tested.

A low level of major error was observed for both methods when testing CF and non-CF isolates. Etest yielded an unacceptable level of major errors only for cefepime, but this was only 1% outside the acceptable limit. The CLSI disc-diffusion method showed an unacceptable level of major errors for aztreonam only; a result seen in both CF and non-CF isolates.

Given the relatively poor results of testing CF *P. aeruginosa* isolates compared to non-CF isolates, it is worth considering that the utility of laboratory-based susceptibility testing in guiding the treatment of chronic *P. aeruginosa* infections of the CF lung has been brought into question. Various studies have found that clinical outcomes in CF patients treated with antibiotics to which the infecting organism is resistant often do not correlate.[2,10,11] Determination of antibiogram in such isolates remains an effective mechanism of monitoring the development of resistance in a given population. It may also be of limited epidemiological assistance in detecting the emergence of multidrug-resistant CF clonal complexes of *P. aeruginosa* in an otherwise sensitive population.[6,12] However, when laboratory-based antimicrobial susceptibility testing is employed to guide CF patient therapy, the high percentage error and potential lack of clinical relevance of results should be taken into account.

In many laboratories, antimicrobial gradient diffusion strips such as Etest are used as the reference method for the determination of *P. aeruginosa* isolate MIC. This study has

Table 3. Comparison of the CLSI disc-diffusion antimicrobial susceptibility test with the agar dilution reference method for CF and non-CF clinical isolates of *Pseudomonas aeruginosa*.

	CLSI disc-diffusion method							
	CF isolates				Non-CF isolates			
Antimicrobial	Susceptible (*n*)	Not susceptible (*n*)	VME (%)	ME (%)	Susceptible (*n*)	Not susceptible (*n*)	VME (%)	ME (%)
Timentin	48	23	**4**	0	73	9	**2**	1
Aztreonam	46	25	**3**	**8**	63	19	**2**	**7**
Ceftazidime	51	20	**11**	0	79	3	**16**	0
Cefepime	46	25	**6**	0	77	5	**2**	2
Ciprofloxacin	40	31	**10**	1	74	8	0	0
Gentamicin	41	29	**20**	0	78	4	0	0
Tobramycin	64	7	**4**	0	81	1	0	0
Mean average			**8**	1			**3**	2

Table 4. Comparison of the CLSI disc-diffusion antimicrobial susceptibility test with Etest as a hypothetical reference method for CF and non-CF clinical isolates of *Pseudomonas aeruginosa*.

	CLSI disc-diffusion method							
	CF isolates				Non-CF isolates			
Antimicrobial	Susceptible (*n*)	Not susceptible (*n*)	VME (%)	ME (%)	Susceptible (*n*)	Not susceptible (*n*)	VME (%)	ME (%)
Timentin	48	23	**4**	3	73	9	**5**	0
Aztreonam	46	25	1	**8**	63	19	1	**11**
Ceftazidime	51	20	**4**	0	79	3	1	0
Cefepime	46	25	**7**	3	77	5	1	2
Ciprofloxacin	40	31	1	0	74	8	0	0
Gentamicin	43	28	**7**	1	78	4	1	4
Tobramycin	64	7	0	1	81	1	0	0
Mean average			**3**	2			1	2

shown that the susceptibility results for *P. aeruginosa* obtained by Etest often do not correlate with those obtained by agar dilution testing. This raises the possibility that plastic antimicrobial gradient diffusion strips may yield more reliable results than agar dilution. This might explain why the results of two well-established methods of determining antimicrobial susceptibility performed so poorly against an established reference method. In order to investigate this possibility, the results of CLSI disc-diffusion testing were compared with Etest as the reference method. Far fewer errors were seen with this method. The degree of error observed was also lower than when agar dilution was used as a reference method; an effect seen in both CF and non-CF isolates. It should be noted, however, that disc-diffusion findings are not an effective mechanism for determining the validity of a given reference method. However, the analysis required to prove the hypothesis that antimicrobial gradient diffusion strips may represent a superior reference method to agar dilution for the susceptibility testing of *P. aeruginosa* is beyond the scope of this study.

In summary, laboratories should be mindful of the potential for unacceptably high levels of major and very major error when using the Etest or CLSI disc-diffusion methods for antimicrobial susceptibility testing of *P. aeruginosa*, particularly in the context of CF lung infection. Hypermutability and phenotypic switching will often lead to adverse results. It is further recommended that clinicians should be guided by, but be aware of the limitations of, laboratory-based antimicrobial susceptibility testing when considering appropriate treatment for *P. aeruginosa* infection. □

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