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Relative resistance index (RRI) – a scoring system for antibiotic resistance in *Pseudomonas aeruginosa*

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ABSTRACT

Background: There is a need to measure antibiotic resistance *of Pseudomonas aeruginosa* (PA) in cystic fibrosis (CF), either qualitatively or quantitatively, to inform patient management. The aim of this study was to develop a simple method by which resistance can be quantified by calculating a relative resistance index (RRI), and to assess correlation of RRIs with clinical variables.
Methods: In our model, RRIs were calculated based on resistance to aztreonam, ceftazidime, ciprofloxacin, colistin, meropenem, tazocin, temicillin and tobramycin. Eighty-five adults with CF and chronic PA colonisation were identified. For each, all PA cultures were allocated a score of 0 for susceptible, 0.5 for intermediate resistance or 1 for resistance for each antibiotic listed above, and the RRI calculated by dividing the sum of these by the number of antibiotics, giving a maximum score of 1. The mean RRIs for all cultures were correlated with key clinical variables monitored in CF patients (including age, FEV_1, IV antibiotic days and BMI).
Results: RRIs for non-mucoid PA exhibited moderate positive correlation with total number of IV days ($r = 0.405$; $p < 0.001$) and moderate negative correlation with FEV_1 % predicted ($r = -0.437$; $p < 0.001$). RRIs were not significantly correlated with duration of colonisation, typing (clonal vs other strain) or BMI. Median RRIs were significantly higher for females (0.26, IQR 0.13–0.54) than males (0.18, IQR 0.07–0.37) for non-mucoid PA only ($p = 0.03$).
Conclusions: RRI is an easily calculated measure that correlates with other clinical variables in CF patients and enables quantitative monitoring of resistance.

Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disorder characterised by defective mucociliary clearance and chronic airway infection [1]. The most commonly isolated pathogen from CF airways is the opportunistic Gram-negative bacterium *Pseudomonas aeruginosa* (PA) [2]. Chronic PA infection is associated with significant morbidity and mortality in CF patients [3] and necessitates multiple antibiotic courses, which increase the risk of the development of antibiotic resistance [2]. Many of the PA strains isolated from CF airways are of the mucoid phenotype, capable of producing large quantities of alginate which enable the formation of a matrix in which microcolonies can exist. These microcolonies are thought to provide protection against the host immune response and antibiotic therapy; conversion of PA through mutation in patients to the mucoid phenotype is associated with increased morbidity and mortality [4]. Typically, initial colonisation is with non-mucoid forms of PA, with the mucoid form predominating later.

Antibiotic resistance in PA may be driven by inappropriate use of antibiotics in either oral, nebulised or intravenous formulations, as well as by the use of biocides which might also drive increased antibiotic resistance [5]. Development of novel virulence model systems in PA may help predict clinical outcomes in patients with CF [6]. There is a need to measure antibiotic susceptibility (AS) of PA, either qualitatively or quantitatively, to inform patient management. AS in PA is generally reported by classifying resistance to individual antibiotics qualitatively (sensitive (S), intermediate (I) or resistant (R)) or quantitatively (minimum inhibitory concentration (MIC)). PA strains can thus be grouped by the presence or absence of multi-drug resistance, the definition of which varies considerably in biomedical literature [7]. Multiple antibiotic resistant PA has previously been associated with a lower FEV_1 % predicted [8].

The aim of this study was to develop a method by which antibiotic resistance in PA can be quantified by calculating a relative resistance index (RRI). This would

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provide a measure of the total antibiotic resistance of an organism at a given time point, using multiple antibiotic classes (and multiple agents within each of those classes). The RRI would also allow tracking of changes in AS over time. To determine the potential clinical relevance of the RRI, correlation with important clinical variables in CF patients, including FEV_1 % predicted, number of IV (intravenous) antibiotic days and BMI (body mass index) were assessed.

Materials and methods

Patients attending the Regional Adult CF Centre in Northern Ireland (NI) were identified who met the Leeds definition [9] for chronic colonisation with PA between 2010 and 2011. PA was isolated from CF patients' fresh expectorated sputum in a UKAS accredited NHS Clinical Microbiology laboratory, in accordance with laboratory methodologies, as defined in the UK CF Trust guidelines [Laboratory Standards for Processing Microbiological Samples from People with CF] [10]. Patients concomitantly colonised with *Burkholderia cepacia* complex were excluded from the analysis. For each patient, the following information was obtained from laboratory data and the Northern Ireland Port CF registry: date of birth, date of first culture of PA, date first defined as chronic PA (Leeds definition), FEV_1 % predicted at annual review 2011, BMI at annual review 2011, total number of IV antibiotic days for 2010 and 2011. Annual review values were used instead of other hospital encounters as these were felt to provide values that were more reflective of the patients' baselines and less likely to be influenced by pulmonary exacerbations. Patients were also classified as to whether they cultured a distinct clonal type commonly isolated in NI, as defined by service genotyping analysis performed by Public Health England, or another unique genotype strain of PA.

Patients with CF, especially those from whom organisms are persistently isolated, are seen often at the clinic and sputum is sent to the laboratory at every visit. Antibiotic sensitivities, normally performed on one mucoid and one non-mucoid isolate per calendar month, were estimated using the disc method. For each antibiotic, a report of sensitive (S), intermediate resistance (I) or resistant (R) was issued. During the study period 2010 to 2011, Clinical and Laboratory Standards Institute (CLSI) zone size criteria were applied [11].

For each patient, all cultures of PA for which antibiotic sensitivity data were available during the study period (2010–2011) were allocated a score of 0 for susceptible, 0.5 for intermediate resistance or 1 for resistant for each of the antibiotics listed above. An overall RRI was calculated by adding the means scores for each antibiotic together and dividing by the number of antibiotics (eight in our analysis), giving a minimum RRI of 0 (fully sensitive) and a maximum RRI of 1 (pan-resistant). For the purpose of the study, for each patient we used the mean value of all cultures over the two-year period, to maximise data utilisation and provide a summary measure of resistance over the two-year period.

All statistical analyses were performed using SPSS v. 19.0 (IBM Corp., Armonk, NY, USA). Differences between median RRIs between 2010 and 2011 were assessed using Wilcoxon signed rank test. Differences in median RRIs between (i) mucoid and non-mucoid PA cultures (ii) strain type (LES [Liverpool Epidemic Strain] or unique strain) and (iii) gender were assessed using the Mann-Whitney test. Correlation of RRIs with clinical variables (age, duration of chronic PA colonisation, number of IV days 2010–2011, FEV_1 % predicted and BMI at annual review 2011) was assessed using Spearman's Rank correlation.

Results

Eighty-five patients met the inclusion criteria. As illustrated in Figure 1, the median RRIs were significantly higher for non-mucoid (0.24, IQR 0.10–0.43) than mucoid (0.06, IQR 0.01–0.13) PA ($p < 0.001$). Median RRIs did not differ significantly between 2010 and 2011 for non-mucoid ($p = 0.14$) or mucoid PA ($p = 0.87$). Median RRIs were significantly higher for females (0.26, IQR 0.13–0.54) than males (0.18, IQR 0.07–0.37) for non-mucoid PA only ($p = 0.03$).). There was no significant difference between median RRIs in those identified as having the distinct clonal type compared to those with unique PA strains for non-mucoid ($p = 0.14$) or mucoid PA ($p = 0.55$). In patients with the aforementioned clonal type, RRIs ranged from 0.00 to 0.75.

There was a moderate positive correlation of RRIs with age and number of IV days for non-mucoid PA, as detailed in Table 1(A) and (B), whilst in comparison, this was not the case for mucoid strains. Figure 2 shows a scatterplot of mean relative resistance indices over time period 2010–2011 against FEV_1 % predicted at annual
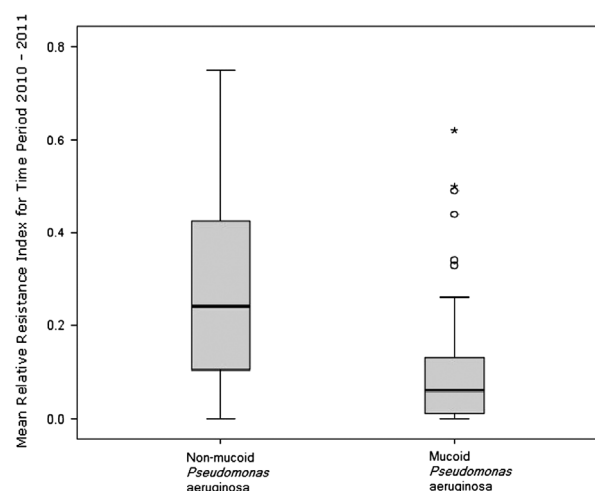

Figure 1. Box plot of mean relative resistance indices for non-mucoid compared with mucoid *Pseudomonas aeruginosa* for cultures taken over 2010–2011.

Table 1.

A. Correlation between RRIs and clinical variables for non-mucoid and mucoid *Pseudomonas aeruginosa*.

	Non-mucoid PA RRI 2010–2011	Mucoid PA RRI 2010–2011
Age	0.24 (0.04)*	0.036 (0.77)
Duration of chronic PA colonisation	0.06 (0.62)	0.090 (0.47)
Number of IV antibiotic days 2010–2011	0.41 (<0.001)**	0.122 (0.33)
FEV1 % predicted 2011 (annual review)	−0.44 (<0.001)**	−0.014 (0.91)
BMI 2011 (annual review)	−0.09 (0.46)	−0.081 (0.53)

B. Multiple linear regression model for predicting mean relative resistance index for non-mucoid *Pseudomonas aeruginosa* in 2011.

	Unstandardised co-efficient (B)	*t*-test statistic	Significance
Gender	0.960	1.850	0.072
Age	0.063	1.823	0.076
Typing (clonal strain or other)	−0.694	−1.304	0.200
Duration of chronic PA colonisation	0.000	0.687	0.496
Total IV days 2010–2011	0.018	2.393	0.021*

C. Correlation between resistance scores for individual antibiotics and FEV$_1$ % predicted 2011 and total intravenous antibiotic days 2010–2011.

		FEV1 % predicted 2011	Total IV antibiotic days 2010–2011
Resistance score for individual antibiotics non-mucoid PA 2010–2011	Aztreonam	−0.27 (0.02)*	0.35 (0.001)**
	Ceftazidime	−0.44 (<0.001)**	0.43 (<0.001)**
	Ciprofloxacin	−0.13 (0.298)	0.14 (0.21)
	Colistin	−0.16 (0.196)	0.09 (0.41)
	Meropenem	−0.52 (<0.001)**	0.41 (<0.001)**
	Tazocin	−0.37 (0.001)**	0.41 (<0.001)**
	Temocillin	−0.20 (0.10)	0.29 (0.011)*
	Tobramycin	−0.42 (<0.001)**	0.26 (0.03)*

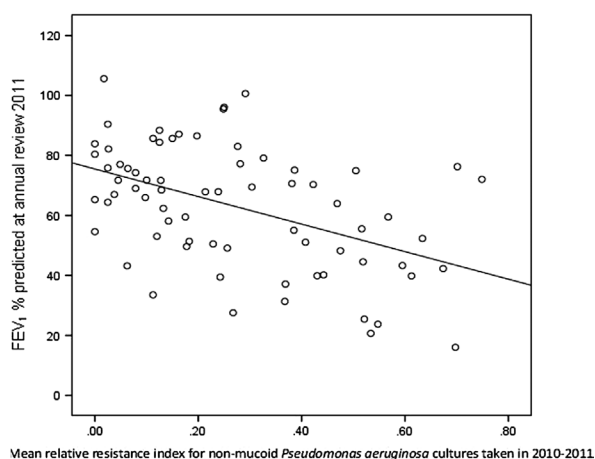Data in A and C are spearman's correlation coefficent and p value.

Figure 2. Scatterplot of mean relative resistance indices over time period 2010–2011 against FEV$_1$ % predicted at annual review 2011.

review 2011. RRI was not significantly correlated with duration of colonisation with chronic PA, typing (clonal vs other strain) or BMI. RRIs for mucoid PA did not correlate significantly with any of the clinical variables examined.

Table 1 (C) depicts the correlation of resistance scores for individual antibiotics in non-mucoid PA against FEV$_1$ % predicted and number of IV antibiotic days. Resistance to meropenem had the strongest negative correlation with FEV$_1$ % predicted ($r = −0.52$, $p < 0.001$), followed by ceftazidime ($−0.44$, $p < 0.001$). Resistance to ceftazidime had the strongest positive correlation with number of IV antibiotic days (0.42, $p < 0.001$), followed by meropenem (0.41, <0.001). Although overall scores for mucoid PA did not correlate with FEV$_1$ % predicted, significant

negative correlation was observed with the score for ceftazidime, meropenem, tazocin and tobramycin in mucoid PA, the strongest and most significant being for tobramycin ($−0.37$, $p = 0.003$), followed by meropenem ($−0.31$, $p = 0.017$).

Discussion

Chronic infections behave very differently. Although AS is widely used in treating acute infections, there have been few attempts to develop algorithms in CF and elsewhere, which define AS in terms of (i) the totality of antibiotic resistance of an organism at a given time point, over multiple classes/agents within each class or (ii) the change in AS over time reflecting an evolving resistome of PA. But what metrics are optimal for fulfilment of these criteria of such a novel marker/index? In our RRI model, we have proposed the equal weighting of AS across eight antibiotic agents from four antibiotic classes.

Correlations with clinical parameters were as follows. As expected, RRIs were higher for non-mucoid PA, as strains with mucoid phenotype are protected from the selective pressure imposed by antibiotics. Consequently, it seemed appropriate to analyse correlation with clinical variables separately for mucoid and non-mucoid PA. Our findings show that RRI for PA displays significant correlation with other clinical variables in CF, especially for non-mucoid PA. Total RRIs (i.e. against all antibiotics tested) were positively correlated with number of IV antibiotic days. Figure 2 shows the relationship when RRI is plotted against FEV1 values. It should be noted that from these data, we cannot state that poorer lung function is

caused by increasing RRI values and consequently this is not a cause and effect relationship. We are not proposing that RRI is a predictor or cause of lung function, but that the two show a moderate association, most likely due to those with poorer lung function requiring more antibiotic therapy which in turn drives antibiotic resistance, with a resulting increase in RRI values. One surprising finding was the lack of an association between duration of chronic PA colonisation and RRIs, with the number of IV antibiotics days appearing to be more important contributor to resistance than duration. Median RRIs were higher for females than males. This is unsurprising as female patients had more IV antibiotic days, which is likely to have driven antibiotic resistance. Although not recorded in our study, females are also likely to have received more intensive nebulised and oral antibiotic therapy, due to the observation that females tend to experience earlier PA infection and poorer outcomes [12]. RRIs did not differ significantly between patients with the clonal strain compared to those with unique strains, and there was a wide range of RRIs within those with the clonal strain, suggesting considerable heterogeneity in its resistome.

As regards resistance to individual antibiotics, when scores for individual antibiotic resistance are analysed, it appears that meropenem resistance is most strongly associated with a lower FEV_1, followed by ceftazidime. The reason for the strong association of meropenem resistance with adverse FEV_1 % predicted is unclear; however, it is most likely that this is a reflection of meropenem being reserved for more unwell individuals who may have a lower FEV_1 % predicted, leading to these individuals developing greater meropenem resistance. This relationship requires further understanding and is currently being investigated. As carbapenem resistance is a particularly concerning finding, it may be pertinent to consider weighting meropenem resistance more highly in future RRI models.

Alternative indices could be adopted, such as $RRI_{3/3}$ or $RRI_{6/12}$, which may yield higher quality information for targeted questions, and tailored accordingly in terms of its design, depending on what information is required. Measurement of the gradient of the linear line of best fit, would give an indication of the rate of change in AS (ΔAS), which is driven by acquisition of antibiotic resistance and related antibiotic resistance mutation frequency, thus the higher the ΔAS value, the faster the development of resistance. Such a marker could be extrapolated to potentially estimate future antibiotic resistance development and thus chart certain windows of opportunity, for example, escalation of treatment.

Overall, it is probable that individuals with lower FEV1% predicted, who may be more unwell, require more IV days and, therefore, develop more resistant forms of PA. However, it should also be considered that more resistant strains of PA may be predictive of poorer outcomes. Regardless, it appears that greater antibiotic resistance is a marker of lower FEV1 % predicted. In a study of over 4000 CF patients, Ren et al. [8] found that mean FEV1 decline did not change significantly after Multiple antibiotic resistant *Pseudomonas aeruginosa* (MARPA) detection. Consequently, they perceived that multi-antibiotic resistance was a marker of severe disease, but did not in itself contribute to more rapid decline in FEV1.

In summary, the advantages of objectively and quantitatively examining AS over time via the RRI marker, within the context of chronic PA colonisation include the ability to (i) succinctly define with a single value how resistant an organism is in its entirety over several classes of antibiotics, at a given time point. Such information would be useful to clinicians in helping to determine suitable antibiotic management strategies, e.g. antibiotic cycling, (ii) monitor the rate at which antibiotic resistance is developing, (iii) provide evidence relating to the introduction/elimination of particular strains of PA, as seen with markedly different RRI scores appearing/disappearing, with implications for infection control/antibiotic management, A rapidly changing RRI could also indicate the need to re-type the PA to assess if a new strain has been introduced. This could provide guidance as to when typing is warranted, as most centres do not regularly type PA due to financial restraints, and (iv) estimate windows of opportunity where antibiotic resistance is important e.g. deteriorating lung health and antibiotic allergy.

This work represents an advance in biomedical science because to date, no such cumulative approach has been described. This novel RRI marker may be a useful tool in forming a cumulative determination of antimicrobial susceptibility over several antibiotics through time, with useful benefits in quantifying antimicrobial resistance to clinicians and other health care professionals.

Summary table

What is known about this subject:
- Antimicrobial resistance has now emerged as a major threat to public health at a national and international level.
- Routine clinical microbiology laboratories test antimicrobial resistance individually for each antibiotic.
- There is no laboratory biomarker or algorithm that attempts to link cumulative antimicrobial susceptibility for several antibiotics simultaneously into a single holistic marker, for ease of interpretation amongst clinical staff.

What this paper adds:
- The proposed RRI is a means of combining single antimicrobial susceptibilities into a single index, that is easily interpreted by clinical staff.
- RRI provides a flexible and quantitative measure of resistance which allows statistical correlation of resistance with other clinical variables. In the patients in our study, RRI showed a moderate association with lung function and number of IV antibiotic days.
- Within CF, where antimicrobial resistance has a major influence on patient morbidity and mortality, the RRI could be extrapolated to potentially estimate future antibiotic resistance development and thus chart certain clinical windows of opportunity, for example, escalation of treatment.

Disclosure statement

No potential conflict of interest was reported by the authors.

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