Approaches to measure the fitness of *Burkholderia cepacia* complex isolates

C. F. Pope,¹ S. H. Gillespie,¹,² J. E. Moore³,⁴ and T. D. McHugh¹

¹Centre for Clinical Microbiology, University College London, Rowland Hill Street, London NW3 2QG, UK
²Regional Microbiology Network, Health Protection Agency, Holborn Gate, London WC1V 7PP, UK
³Department of Bacteriology, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB, UK
⁴School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine BT52 1SA, UK

Members of the *Burkholderia cepacia* complex (Bcc) are highly resistant to many antibacterial agents and infection can be difficult to eradicate. A coordinated approach has been used to measure the fitness of Bcc bacteria isolated from cystic fibrosis (CF) patients with chronic Bcc infection using methods relevant to Bcc growth and survival conditions. Significant differences in growth rate were observed among isolates; slower growth rates were associated with isolates that exhibited higher MICs and were resistant to more antimicrobial classes. The nucleotide sequences of the quinolone resistance-determining region of gyrA in the isolates were determined and the ciprofloxacin MIC correlated with amino acid substitutions at codons 83 and 87.

Biologically relevant methods for fitness measurement were developed and could be applied to investigate larger numbers of clinical isolates. These methods were determination of planktonic growth rate, biofilm formation, survival in water and survival during drying. We also describe a method to determine mutation rate in Bcc bacteria. Unlike in *Pseudomonas aeruginosa* where hypermutability has been detected in strains isolated from CF patients, we were unable to demonstrate hypermutability in this panel of *Burkholderia cenocepacia* and *Burkholderia multivorans* isolates.

INTRODUCTION

Until recently, the *Burkholderia cepacia* complex (Bcc) contained nine species that are opportunistic pathogens, causing lung infection in cystic fibrosis (CF) patients (Coeyne et al., 2001; Isles et al., 1984; LiPuma, 1998; Mahenthiralingam et al., 2002). However, *Burkholderia ubonensis* and an additional five novel species have also now been recognized as members of the complex, which now contains 15 species (Vanlaere et al., 2008). The complex also contains taxon K: this group contains at least two novel species which have recently been recognized as *Burkholderia contaminans* and *Burkholderia lata* (Vanlaere et al., 2009).

Species within the Bcc are phylogenetically distinguishable but are phenotypically indistinguishable and have previously been referred to as genomovars. A varying course of infection occurs and may be due to differences in pathogenicity and transmissibility of species and strains (Conway et al., 2002). Infection with *Burkholderia cenocepacia* is associated with a worse outcome than infection with other species although the effect of the less commonly isolated species on prognosis is unclear. *Burkholderia multivorans* and *B. cenocepacia* are most commonly isolated from CF patients (Mahenthiralingam et al., 2001, 2002, 2005). In contrast the prevalence of *B. cepacia*, *Burkholderia stabilis*, *Burkholderia anthina* and *Burkholderia pyrrocinia* (Mahenthiralingam et al., 2002; Vandamme et al., 2002) among CF patients is low, while *Burkholderia vietnamiensis* and *Burkholderia ambifaria* are rarely found (Mahenthiralingam et al., 2002, 2005; Willmott & Maxwell, 1993). Antibiotics that usually have activity against *B. cepacia* include semisynthetic penicillins, carbapenems, fluoroquinolones, ceftazidime and cotrimoxazole (Mahenthiralingam et al., 2005).

Members of the Bcc are ubiquitous organisms in the environment and outbreaks have been attributed to contamination of disinfectants, antiseptics, nebulizers and medical devices (Hutchinson et al., 1996; Oie & Kamiya, 1996). The ability of bacteria to survive drying allows their environment and outbreaks have been attributed to contamination of disinfectants, antiseptics, nebulizers and medical devices (Hutchinson et al., 1996; Oie & Kamiya, 1996). The ability of bacteria to survive drying allows their contamination of disinfectants, antiseptics, nebulizers and medical devices (Hutchinson et al., 1996; Oie & Kamiya, 1996). The ability of bacteria to survive drying allows their contamination of disinfectants, antiseptics, nebulizers and medical devices (Hutchinson et al., 1996; Oie & Kamiya, 1996). The ability of bacteria to survive drying allows their contamination of disinfectants, antiseptics, nebulizers and medical devices (Hutchinson et al., 1996; Oie & Kamiya, 1996). The ability of bacteria to survive drying allows their...
The objective of this work was to optimize and set out a method to investigate the existence of hypermutability in the Bcc. The gene was sequenced to assess the contribution of gyrase A (gyrA) to mutation rates. Oliver et al. isolated from lungs of CF patients exhibited an increased mutation rate. Studies have shown that strains of Pseudomonas aeruginosa growing in the lungs of CF patients are likely to be hypermutable if resistant colonies were observed to three or more of the topoisomerase genes (Chen & Lo, 2003; Drlica & Malik, 2003). Ciprofloxacin-resistant mutants of B. cepacia have been shown to contain mutations within gyrA (Pope et al., 2008) and therefore the QRDR region of this gene was sequenced to assess the contribution of gyrA nucleotide polymorphisms to fluoroquinolone MIC in clinical isolates of the Bcc.

High mutation rate can lead to rapid adaptation and bacteria that have a higher spontaneous mutation rate than the majority of the population are more likely to gain resistance via mutation in chromosomal genes. It can be beneficial, therefore, for the bacterial population to be heterogeneous, increasing the probability that a subset of cells will survive. This ‘bet-hedging’ may be a useful strategy in uncertain environments and may promote survival. However, few mutations are beneficial, most are deleterious and therefore any advantage is likely to be short lived. Therefore, the mutation rate that has evolved represents a compromise between allowing adaptation and avoiding excessive deleterious mutations. Previous studies have shown that strains of Pseudomonas aeruginosa isolated from lungs of CF patients exhibited an increased rate of mutation (Oliver et al., 2000). Mutators were found in the lungs of 37% of CF patients and in these patients 43% of the total P. aeruginosa population exhibited elevated mutation rates. Oliver et al. (2004) have also shown that hypermutable strains of P. aeruginosa exist in the CF lung before initiation of antimicrobial therapy. Thus resistant mutants can readily occur in this setting before or during treatment. The environment of the CF lung is characterized by changing host immune responses and varying antibiotic levels. Although conditions for B. cepacia growing in the lungs of CF patients are likely to be similar to those for P. aeruginosa, this is the first study to investigate the existence of hypermutability in the Bcc.

The objective of this work was to optimize and set out a comprehensive series of methods that can be used to measure the fitness, screen for elevated mutation rates and characterize fluoroquinolone resistance of Bcc clinical isolates. These methods have been used to investigate a small panel of B. cenocepacia and B. multivorans strains and can now be applied to investigate a larger number of isolates to elucidate mechanisms leading to resistance.

**METHODS**

**Strains and culture conditions.** Clinical strains of Bcc bacteria including isolates of B. multivorans (n=2) and B. cenocepacia (n=6) were isolated from sputum samples from eight adult CF patients with well-characterized, chronic Bcc infection that attended a CF clinic at Belfast City Hospital. Expectorated sputum (50:50 Sputasol:sputum) was inoculated onto B. cepacia selective agar (Mast Diagnostic). An oxidase reaction was performed and an API NH test (bioMérieux) was set up on all characteristic colonies. Bcc isolates were characterized by recA sequence typing to confirm Bcc species status (Mahenthiralingam et al., 2000). Where we have assigned the same species to more than two isolates, we have not characterized these isolates further, as we believe epidemiologically that all B. cenocepacia isolates belonging to the recA IIIa lineage are clonal. This is due to spread amongst patients, antibiogram profile, clinical manifestation pattern and epidemiological pattern of acquisition. We believe all other Bcc isolates to be non-clonal and represent unique species, as their clinical picture would suggest. Isolates were stored at −70 °C using cryogenic beads (Pro-lab Diagnostics). For each mutation rate estimation experiment and fitness assay, the culture was initiated by inoculating a bead, from a stored culture, onto Columbia blood agar (Oxoid) then incubating at 37 °C for 18 h.

**MIC determination.** The MIC of the following antibiotics was determined for the isolates by E-test (AB Biodisk) using the manufacturer’s instructions: amoxicillin–clavulanic acid, ceftazidime, ciprofloxacin, colistin, gentamicin, meropenem, piperacillin–tazobactam, temocillin, tetracycline, tigecycline and trimethoprim–sulfamethoxazole. Briefly, organisms were suspended in 3 ml sterile distilled water to a turbidity of 0.5 MacFarland. A cotton wool swab was used to inoculate Isosensitest agar (Oxoid). E-test strips were placed onto the plates, which were incubated at 37 °C for 18 h. The point of intersection of the inhibition ellipse on the E-test strip was recorded.

**Hypermutability.** Hypermutability of clinical Bcc isolates was assessed using E-test as previously described (Macia et al., 2004). This method is based on the assumption that resistant colonies which appear within the E-test inhibition ellipse will only occur in bacterial populations that are hypermutable. Strains were defined as hypermutable if resistant colonies were observed to three or more of the antibiotics tested (Macia et al., 2004). The mutation rate was also estimated by a fluctuation test (Lea and Coulson method of the median; Lea & Coulson, 1949).

To estimate mutation rate (μ) by fluctuation test, approximately 10^6 exponentially growing cells were independently inoculated into 28 microcentrifuge tubes containing 1 ml Mueller–Hinton broth and incubated at 37 °C for 22 h on an orbital shaker at 250 r.p.m. This achieved a cell density of approximately 10^9 c.f.u. ml^-1. Cells were harvested by centrifugation (13 000 g, 3 min), the supernatant was removed and the pellet was suspended in residual Mueller–Hinton broth (approx. 40 μl). The bacterial suspension was spread evenly onto Mueller–Hinton agar plates containing 2× MIC of ciprofloxacin.
The number of culturable cells, from three aliquots (approx. 10% of the independently inoculated cultures), was determined using the method of Miles and Misra in order to determine total cell numbers (Billington et al., 1999; Miles & Misra, 1938). Plates were incubated at 37 °C for 18 h and the proportion of cultures with mutant colonies was recorded. The mutation rate was determined using the method of Youmans and Youmans to determine total cell numbers (Youmans & Youmans, 1949; Pope et al., 2008). The BACTEC 9240 continuous blood culture system with standard aerobic medium (Plus Aerobic/F) was used. Aliquots of 100 μl of the diluted overnight culture (1/10 and 1/1000) were removed using a 0.5 ml syringe and a needle and were aseptically inoculated into duplicate culture vials. The vials were then loaded immediately into the system. The length of time to detection (time to positivity) was measured for all strains. The growth rate constant k can be determined using the equation k=\log(A−logB)/t, where A is the largest inoculum employed, B is the smallest inoculum and t is the difference in time to positivity in hours. This experiment was repeated in triplicate.

Survival in water. Survival in water was assessed by adapting the method of Sanchez et al. (2002) (Pope et al., 2008). Overnight cultures were pelleted by centrifugation, washed three times in PBS and concentrated 10-fold to a final cell concentration of approximately 1 x 10^9. Aliquots (10 μl) of the concentrated culture were spotted into duplicate wells of a 96-well flat-bottomed microtitre plate and allowed to dry at room temperature. Survival was determined in triplicate and the survival curve was obtained by plotting the mean culturable count, using GraphPad Prism.

Survival on dry surfaces. Survival on dry surfaces was assessed by adapting the method of Sanchez et al. (2002) (Pope et al., 2008). Overnight cultures were pelleted by centrifugation, washed three times in PBS and concentrated 10-fold to a final cell concentration of approximately 1 x 10^9. Aliquots (10 μl) of the concentrated culture were spotted into duplicate wells of a 96-well flat-bottomed microtitre plate and allowed to dry at room temperature. Survival was determined (at 0, 1, 2, 3, 4, 5, 6, 7 and 8 h) by suspending bacteria in 100 μl PBS containing 0.25% (v/v) Triton X-100. Numbers of culturable bacteria in this suspension were determined as described above. Each experiment was repeated in triplicate and the survival curve was obtained by plotting the mean culturable count, using GraphPad Prism.

Fluoroquinolone resistance: genotyping mutants. A loopful of Bcc culture was emulsified in 0.5 ml sterile distilled water and heated at 95 °C for 10 min. Cellular debris was removed by centrifugation (13000 g, 3 min). The supernatant was removed, transferred to a microcentrifuge tube and diluted 1:10 in sterile distilled water. Ten microlitres was added to each PCR. The mutants were characterized by sequencing of the QRDR of gyrA using primers previously described (gyrA F, ATCTCGATTACGCGATGAGC; and gyrA R, GCGTTGTAGCAGCGGTT) (Pope et al., 2008).

PCR conditions. Primers were designed using the sequenced genome of B. cenocepacia AU 1054 chromosome 1 (accession no. CP000378) to amplify gyrA (Markowitz et al., 2006; Pope et al., 2008). The optimum PCR mastermix contained 1.3 mM MgCl₂ in KCl buffer (Bioline), 10 μl of each primer at a final concentration of 20 pmol, 100 μM deoxynucleoside triphosphates and 1 U Taq polymerase (Invitrogen). Cycling conditions consisted of 95 °C for 3 min followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. This was followed by strand elongation at 72 °C for 7 min (GeneAmp PCR system 9700).

PCR product purification. PCR products were purified using the MinElute Purification kit (Qiagen), according to the manufacturer’s instructions.

Cycle sequencing. Forward and reverse cycle sequencing reactions were performed in duplicate using the BigDye Terminator Cycle Sequencing Ready Reaction DNA sequencing kit v2.0. (Applied Biosystems), according to the manufacturer’s instructions. Approximately 40 ng purified PCR product was added per cycle sequencing reaction. Each reaction consisted of 1 μl Ready Reaction mix, 10.8 μl PCR-quality water and 3.2 μl of 1 mmol l⁻¹ primer. PCR products were sequenced using an ABI PRISM 377 DNA sequencer. Sequences were analysed using Bionumerics version 2.0 (Applied Maths).

RESULTS

Antibiotic susceptibility

Isolates BCH 2, BCH 3, BCH 5, BCH 6, BCH 7 and BCH 8 were identified as B. cenocepacia. BCH 1 and BCH 4 were identified as B. multivorans. In this study, isolates were resistant to colistin. All isolates were also resistant to trimethoprim–sulfamethoxazole, tetracycline and gentamicin. Variable susceptibility was found for tigecycline,
piperacillin–tazobactam and temocillin. The most active antibiotics were meropenem, ciprofloxacin and ceftazidime. Antibiotic susceptibility results are shown in Table 1.

**Hypermutability**

Using the E-test methodology, we did not identify any strains with resistant colonies growing within the inhibition ellipse for any antibiotic tested. This was confirmed by results obtained by fluctuation analysis: estimated mutation rates of the B. multivorans and B. cenocepacia isolates ranged from $1.9 \times 10^{-8}$ to $5.0 \times 10^{-8}$. When comparing mutation rates, a change of $\geq$sixfold is considered a significant difference and therefore as all isolates had estimated mutation rates within this range it is unlikely that any of the isolates had elevated mutation rates.

**Fitness assays**

Significant differences were found in growth rates of the B. multivorans and B. cenocepacia isolates. Youmans & Youmans (1949) measured the difference in time to positivity of serial dilutions of a culture of M. tuberculosis to calculate the growth constant $k$ and generation time. We used a liquid-based automated culture system to determine growth rate and generation time, avoiding time-consuming and laborious standard growth curve techniques. We have adapted the method of Youmans & Youmans (1949) for use as a measure of growth rate of Bcc clinical isolates using the MB/BacT ALERT/3D system and have correlated inoculum size with time to positivity. This has proved to be a reliable, reproducible and simple method for measurement of growth rate.

Isolates with the slowest growth rates exhibited resistance to a greater number of antibiotics than those with faster growth rates. B. cenocepacia isolate BCH 6 has the longest generation time ($120.7 \pm 1.13$), was resistant to all antibiotics tested and was also the most resistant of the eight strains studied. Both B. multivorans isolates BCH 1 and BCH 4 have short generation times ($54.8 \pm 0.61$, $54.7 \pm 1.12$) as does B. cenocepacia isolate BCH 5 ($54.6 \pm 0.63$). These three isolates are also the most antibiotic-susceptible strains. The full antibiotic susceptibility data are shown in Table 1.

One B. multivorans (BCH 1) isolate demonstrated an increased propensity to form biofilms compared to the other isolates, shown in Fig. 1. For isolates BCH 2–BCH 8 differences in biofilm formation were not significant, determined by one-way ANOVA using GraphPad Prism software.

A decrease in viability over time was observed for all isolates but there were no statistically significant differences between the isolates in their ability to survive in water (Fig. 2) or to survive drying (Fig. 3). For survival in water, the difference in c.f.u. between isolates at time points was found to be not significant ($P$-value 0.0714) by two-way ANOVA, using GraphPad Prism. For survival during drying, no significant differences were found by $F$ test of survival curves, using GraphPad Prism.

### Table 1. Antibiotic susceptibilities (mg l$^{-1}$) of clinical Bcc isolates (six B. cenocepacia isolates and two B. multivorans isolates) and association between generation times of clinical Bcc isolates (min $\pm$ SEM) and antibiotic resistance

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg l$^{-1}$) BCH 1</th>
<th>MIC (mg l$^{-1}$) BCH 2</th>
<th>MIC (mg l$^{-1}$) BCH 3</th>
<th>MIC (mg l$^{-1}$) BCH 4</th>
<th>MIC (mg l$^{-1}$) BCH 5</th>
<th>MIC (mg l$^{-1}$) BCH 6</th>
<th>MIC (mg l$^{-1}$) BCH 7</th>
<th>MIC (mg l$^{-1}$) BCH 8</th>
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<tr>
<td>Amoxicillin–clavulanic acid</td>
<td>$&gt;256$</td>
<td>$&gt;256$</td>
<td>$&gt;256$</td>
<td>$&gt;256$</td>
<td>$&gt;256$</td>
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<td>$&gt;256$</td>
<td>$&gt;256$</td>
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<tr>
<td>Ceftazidime</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>64</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6</td>
<td>128</td>
<td>64</td>
<td>1.5</td>
<td>3</td>
<td>32</td>
<td>256</td>
<td>32</td>
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<tr>
<td>Clinafloxacin</td>
<td>2</td>
<td>16</td>
<td>8</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>2</td>
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<tr>
<td>Colistin</td>
<td>128</td>
<td>$&gt;1024$</td>
<td>$&gt;1024$</td>
<td>$&gt;1024$</td>
<td>192</td>
<td>$&gt;1024$</td>
<td>$&gt;1024$</td>
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<tr>
<td>Gentamicin</td>
<td>24</td>
<td>96</td>
<td>96</td>
<td>16</td>
<td>32</td>
<td>$&gt;256$</td>
<td>$&gt;256$</td>
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<td>Meropenem</td>
<td>2</td>
<td>4</td>
<td>12</td>
<td>2</td>
<td>0.75</td>
<td>$&gt;32$</td>
<td>12</td>
<td>$&gt;32$</td>
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<tr>
<td>Piperacillin–tazobactam</td>
<td>64</td>
<td>$&gt;256$</td>
<td>3</td>
<td>16</td>
<td>16</td>
<td>$&gt;256$</td>
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<tr>
<td>Temocillin</td>
<td>12</td>
<td>4</td>
<td>32</td>
<td>24</td>
<td>32</td>
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<td>8</td>
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<tr>
<td>Tetracycline</td>
<td>$&gt;32$</td>
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<tr>
<td>Tigecycline</td>
<td>6</td>
<td>8</td>
<td>24</td>
<td>128</td>
<td>32</td>
<td>24</td>
<td>8</td>
<td>6</td>
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<td>Trimethoprim–sulfamethoxazole</td>
<td>$&gt;32$</td>
<td>$&gt;32$</td>
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<td>$&gt;32$</td>
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<tr>
<td><strong>Generation time</strong> (min $\pm$ SEM)</td>
<td>54.8 ($\pm$ 0.61)</td>
<td>76.4 ($\pm$ 0.93)</td>
<td>83.1 ($\pm$ 0.48)</td>
<td>54.7 ($\pm$ 1.12)</td>
<td>54.6 ($\pm$ 0.63)</td>
<td>120.7 ($\pm$ 1.13)</td>
<td>71.6 ($\pm$ 0.76)</td>
<td>85.07 ($\pm$ 1.25)</td>
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</tbody>
</table>
Sequence of the QRDR of gyrA

The identity of amino acids within the QRDR correlated with ciprofloxacin MIC. Nucleotide sequences are shown in Fig. 4. Ciprofloxacin MIC values are shown in Table 1. *B. multivorans* isolate BCH 1 was classed as resistant with an MIC of 6 mg l\(^{-1}\). *B. multivorans* isolate BCH 4 and *B. cenocepacia* isolate BCH 5 were classed as intermediate. Of the eight clinical organisms examined, *B. cenocepacia* isolate BCH 7 exhibited the highest level of resistance at 256 mg l\(^{-1}\). This isolate contains Thr83 and Gly87.

**DISCUSSION**

Hypermutability was not observed in the panel of *B. cenocepacia* and *B. multivorans* isolates investigated by E-test or estimation of mutation rate by fluctuation test. These isolates represent only a small number of strains and the possibility that other CF Bcc isolates exhibit elevated mutation rates cannot be excluded. Macia *et al.* (2004) used E-test and disc diffusion to detect hypermutable strains of *P. aeruginosa* isolated from sputum of CF patients. It is assumed that resistant mutant colonies which appear within the E-test inhibition ellipse are unlikely to occur in bacterial populations which are non-hypermutable. This also allows MIC determination of both the susceptible and resistant subpopulations. Hypermutable strains are associated with higher MICs (Macia *et al.*, 2004). This mutation rate estimation method can be applied to screen for elevated mutation rates in a larger number of Bcc isolates.

In this paper, we demonstrated that differences in planktonic growth rates of the *B. cenocepacia* and *B. multivorans* isolates were statistically significant, as measured by a modified Youmans & Youmans (1949) method. Although it appears that the most resistant isolates have the slowest growth rates, it is not possible to demonstrate an association between generation time and the relative resistance of each isolate due to the small numbers involved. Caraher *et al.* (2006) demonstrated that generation times in Bcc bacteria ranged from 70 to 186 min and that there was no association between generation time and species status. These data demonstrate that multidrug resistance in Bcc may incur fitness costs. However, the limitation of this paper is that only a small number of non-isogenic clinical isolates were included, therefore the relative fitness of each in relation to observed resistance is difficult to assess. It is also not possible to determine whether there is a relationship between species status and growth rate.

Isolates were comparable in the amount of biofilm formed as measured by the crystal violet assay. However, *B. multivorans* isolate BCH 1 produced extensive biofilm. The reasons for the propensity of this isolate for forming biofilms are unclear. The extent of biofilm formation varies...
between species of the Bcc. Conway et al. (2002) also demonstrated differences in ability to form biofilms and that B. multivorans and B. cenocepacia are capable of greater biofilm formation than B. cepacia, B. stabilis and B. vietnamiensis, and found that there was no correlation between the ability to form biofilms and growth rate. B. dolosa has since been demonstrated to be comparable to B. cenocepacia and B. multivorans in biofilm formation (Caraher et al., 2006).

B. cenocepacia recA type A organisms form more biofilms than type B organisms (Conway et al., 2002) but explanations for increased biofilm formation in B. multivorans are unclear. As demonstrated here, when measuring fitness via multiple models, the fittest strain, as measured by one assay, may not be the fittest in another assay. This emphasizes the need for multiple models to build a complete picture of the fitness landscape.

The transmissibility of B. cepacia isolates may be affected by the ability to survive in water and survive during drying. No significant difference was found in environmental survival between the clinical isolates. Drabick et al. (1996) demonstrated significant strain-to-strain differences in survival on environmental surfaces and differences in survival on different surfaces with the greatest survival occurring on PVC for Bcc bacteria. Isolates survived longer if suspended in sputum (Drabick et al., 1996). It is possible that very small differences in environmental survival may not be detected by the fitness assays described here.

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**Fig. 3.** Comparison between the fitness of different Bcc isolates using survival during drying as a measure. BCH 2, BCH 3, BCH 5, BCH 6, BCH 7 and BCH 8 are isolates of B. cenocepacia. BCH 1 and BCH 4 are isolates of B. multivorans. Error bars represent the standard error of the mean.

**Fig. 4.** Amino acid sequences of QRDRs of gyrA in clinical isolates. BCH 2, BCH 3, BCH 5, BCH 6, BCH 7 and BCH 8 are isolates of B. cenocepacia. BCH 1 and BCH 4 are isolates of B. multivorans. Polymorphisms are found at codon 83 (shown in bold and underlined) and 87 (shown in italic and underlined). Isolate BCH 2 contains an alanine residue at position 83. Isolates BCH 1, BCH 6, BCH 7 and BCH 8 contain a threonine residue at this position while isolates BCH 3, BCH 4 and BCH 5 contain a serine residue. Isolates BCH 2 and BCH 7 contain a glycine residue at codon 87, while BCH 1, BCH 3, BCH 4, BCH 5, BCH 6 and BCH 8 contain an aspartic acid residue at this position.
In Gram-negative bacteria, fluoroquinolone-resistance mutations within gyrA are most commonly found at codons 83 and 87 (Drlica & Malik, 2003; Hooper, 2003). Susceptible isolates contain serine or threonine at codon 83 and aspartic acid at codon 87. Clinical isolates of the Bcc contain polymorphisms within the QRDR at these positions and there is an association between the MIC and the identity of amino acids at codons 83 and 87. The interpretative breakpoints used for ciprofloxacin as recommended by the NCCLS were: ≤1 mg l⁻¹, susceptible; 2 mg l⁻¹, intermediate; and ≥4 mg l⁻¹, resistant. B. multivorans isolate BCH 1 was classed as resistant, with an MIC of 6 mg l⁻¹, while B. multivorans isolate BCH 4 and B. cenocepacia isolate BCH 5 were classed as intermediate. Isolates BCH 4 and BCH 5 contained Ser83 and Asp87 within the QRDR, the most common genotype in susceptible isolates. However, isolate BCH 1 contained Thr83 and Asp87, and had a higher MIC for ciprofloxacin (6 mg l⁻¹) than BCH 4 (1.5 mg l⁻¹) and BCH 5 (3 mg l⁻¹). It is likely that other resistance mechanisms, e.g. increased expression of efflux pumps, are contributing to the observed reduced susceptibility to ciprofloxacin in this strain. Mutations in gyrA initially result in substitution of Ser83 and subsequently substitution of Asp87 (Drlica & Malik, 2003; Hooper, 2003; Tavio et al., 1999). The altered gyrase reduces binding of fluoroquinolones, even if only one resistance mutation is present (Willmott & Maxwell, 1993). The isolate exhibiting the highest MIC (BCH 7) at 256 mg l⁻¹ contained Thr83 and Gly87. Isolates of P. aeruginosa and Campylobacter jejuni are 10-fold less susceptible to fluoroquinolones than wild-type Escherichia coli because they contain a threonine residue rather than serine at codon 83 and this reduces the ability of fluoroquinolones to bind to gyrase (Chen & Lo, 2003; Tavio et al., 1999). The BCH 2 isolate contained an alanine residue at codon 83 and was highly resistant to ciprofloxacin (128 mg l⁻¹). This resistance mutation is not common but has been documented in fluoroquinolone-resistant E. coli (Tavio et al., 1999). Isolate BCH 2 also contained a Gly87 mutation at codon 87. The two B. multivorans isolates (BCH 1 and BCH 4) were the most susceptible of the panel of clinical isolates. However, it is not possible to comment on whether differences in species affect fluoroquinolone susceptibility due to the low numbers of isolates investigated. The mechanisms conferring resistance to other antimicrobial classes have not been investigated.

Single-step fluoroquinolone-resistance mutations occur at low or no cost in B. cepacia and other bacteria and confer low-level resistance (Pope et al., 2008; Kugelberg et al., 2005). Isolates containing these mutations may remain in the bacterial population in the absence of an antibiotic selective pressure. When fluoroquinolone selective pressure is reapplied, the resistant mutants can replicate to take over the bacterial population.

Bcc bacteria exhibit differences in growth rate. We have shown that there is a correlation between the identity of amino acids within the QRDR of gyrA and fluoroquinolone resistance in this training set of B. cenocepacia and B. multivorans isolates. Only a small number of Bcc isolates have been investigated by these methods; however, the techniques set out can now be applied to a larger number of Bcc isolates.

ACKNOWLEDGEMENTS

The authors would like to thank AB Biodisk for providing free-of-charge E-test strips.

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