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Research article

The synergistic effect of gentamicin and ceftazidime against *Pseudomonas fluorescens*

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With antibiotic resistance becoming a significant problem in recent years, methodologies to overcome resistance have quickly become a necessity. One such mechanism to overcome resistance is to use antibiotics in combination. Clinical advice recommends the use of gentamicin and ceftazidime in combination to treat severe *Pseudomonas aeruginosa* infections. However, there is little evidence to support this recommendation. This study proposed that this recommendation is due to a synergistic effect and aimed to determine the optimum combination treatment, using *Pseudomonas fluorescens* as a model organism. Potentially, this research could give reason to a medical recommendation and even instigate a change in this treatment strategy in a clinical setting. To find the minimal inhibitory concentration (MIC) of the antibiotics used singularly, varying concentrations of each antibiotic and *P. fluorescens* were placed in each well of a microtitre plate and incubated at 30°C for 24 h. Wells determined to have no growth were re-plated on nutrient agar and incubated at 30°C for 22 h for minimal bactericidal concentration (MBC) testing. When testing gentamicin and ceftazidime in combination, the checkerboard method was employed along with the fractional inhibitory concentration index (FICI) to test for synergy. A value of ≤0.5 defined synergy; 0.5 < FICI < 4 defined no interaction; ≥4 defined antagonism. No results of synergy were found; there were five results of no interaction and six results of antagonism. The MIC of ceftazidime was 3 μg/ml and the MBC was 4 μg/ml. The MIC of gentamicin was 0.25 μg/ml and the MBC was 3 μg/ml. The combination of gentamicin and ceftazidime is optimal at a volume ratio of 1:1, in this case 25 μl gentamicin/25 μl ceftazidime, where gentamicin has a concentration of 0.5 μg/ml and ceftazidime has a concentration of 0.25 μg/ml, when used against 50 μl of 1–2 × 10^6 colony forming units per millilitre of *P. fluorescens in vitro*. This study recommends that this combination therapy be studied in depth *in vivo*, and that clinicians understand that this combination of antibiotics does not have a synergistic effect when treating patients in this manner.

Key words: gentamicin, ceftazidime, *Pseudomonas*, combination therapy, synergy

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Introduction

Pseudomonas fluorescens is a common environmental bacterium, found in soil, in water and on plant surfaces (Guttman, Morgan and Wang, 2008). Although considered non-pathogenic to humans, in recent years several clinical strains of *P. fluorescens* have been found to be able to survive at 37°C (Chapalain *et al.*, 2007), despite being considered as psychrophile, with an optimum temperature of 25–30°C (Balachander and Vendan, 2007), leading to belief that human physiological temperature is not a barrier for the microorganism. There is evidence that a biofilm of *P. fluorescens* can be formed at 37°C (Donnarumma *et al.*, 2010), and that glial cells exposed to *P. fluorescens* react with marked changes in nucleus morphology, displaying typical changes of apoptotic mechanism (Picot *et al.*, 2001). This, along with recent clinical observations that *P. fluorescens* is a causative agent of nosocomial infections, illustrates that some strains of *P. fluorescens* could

behave as a pathogen (Picot *et al.*, 2001). There are several cases in which this bacterium has been found to be a human pathogen; however, in these cases, owing to the opportunistic nature of the bacterium, underlying conditions were present, for example, pathogenesis occurred in oncology and coronary care patients, as described by Benito *et al.* (2012), Gershman *et al.* (2008) and Hseuh *et al.* (1998). In these three cases, all patients recovered, some with the aid of antibiotic treatment, and Benito *et al.* (2012) demonstrate that the bacterium is susceptible to both gentamicin and ceftazidime. Furthermore, 54% of individuals with Crohn's disease were found to have a highly specific antigen of *P. fluorescens* (I2) in their serum, with a correlation of the severity of their condition and the level of circulating I2, illustrating an involvement in a multi-factorial disease (Madi *et al.*, 2010).

Although this study examines *P. fluorescens*, due to localized restrictions, this research could be extrapolated for use on the closely related, and more established pathogen, *Pseudomonas aeruginosa* (Dempsy, 2004). Although *P. aeruginosa* separated from other Pseudomonas species earlier than others, which allowed it to acquire a range of functions for it to become a dangerous opportunistic pathogen, *P. fluorescens* is a relatively close relative, being closer than both *Pseudomonas stutzeri*, and *Pseudomonas syringae* in terms of genetic differences (Guttman, Morgan and Wang, 2008).

Pseudomonas aeruginosa, although rare as a community-acquired pathogen (Huang, Lin and Wang, 2002), is among the most common pathogens involved in nosocomial infections (Giamarellou, 2002), being the most common organism (29%) to be isolated from postoperative wounds (Ranjan *et al.*, 2011), with its presence also being found in various other bodily sites (Aloush *et al.*, 2006). Its presence in various hospital sites such as hospital sinks, suction apparatus, air conditioning filters, operating tables and even on staff hands could play a role in its prevalence (Pal, Rodrigues, and Datta, 2010), leading to the bacteria often being named in the media for its role in the infection of immunocompromised patients (Hota *et al.*, 2009) and premature neonates (Badr *et al.*, 2011; BBC News Brisol, 2012; BBC News Northern Ireland, 2012). When presented with a patient suffering a *P. aeruginosa* infection, it is recommended to use the third-generation cephalosporin ceftazidime with an aminoglycoside such as amikacin, gentamicin or tobramcyin as treatment (American Society of Health-System Pharmacists, 2004). Ceftazidime hydrate (Fig. 1A) works by disrupting the synthesis of the peptidoglycan layer of the bacterial wall (Babic, Hujer and Bonomo, 2006) and has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria, including both *P. fluorescens* and *P. aeruginosa* (Richards and Brogden, 1985; Rains, Bryson and Peters, 1995). Likewise, gentamicin also has a broad spectrum of activity, but it works by inhibiting bacterial protein synthesis by binding to the 30S subunit of the ribosome (Liou *et al.*, 2006). In contrast to ceftazidime, however, gentamicin is made up of three major components: C_1 ($C_{21}H_{43}N_5O_7$), C_{1a} ($C_{20}H_{41}N_5O_7$) and C_2 ($C_{19}H_{39}N_5O_7$); usually <45% is C_1, <35% is C_{1a} and <30% is C_2 (Fig. 1B).

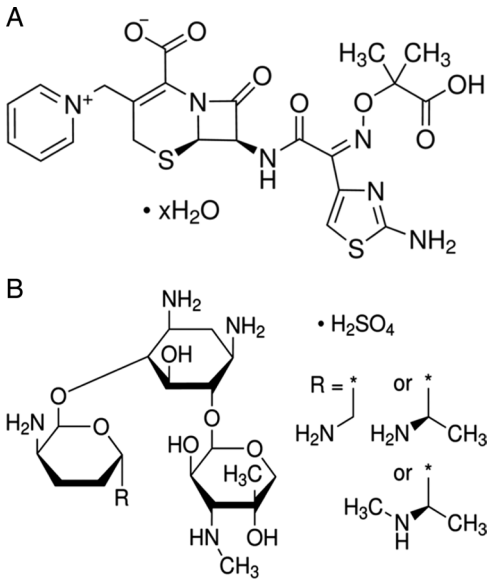

Figure 1. The chemical structure of (**A**) ceftazidime and (**B**) gentamicin. (Reproduced with permission from Sigma-Aldrich Co . LLC (Sigma-Aldrich, 2012, 2013)).

There is minimal research as to why ceftazidime and gentamicin should be used in conjunction when treating a *P. aeruginosa* infection; however, it is recommended in hospital settings. This study is designed to give reason for this recommendation. One meta-analysis of published studies explained that their analysis of *P. aeruginosa* bacteraemia showed significant benefits for patients using antibiotics in combination compared with patients receiving a single antibiotic, when looking at mortality rates. They found that there were more than double the amount deaths for patients receiving a single antibiotic, than patients receiving a combination of antibiotics. However, this was only true for *P. aeruginosa* infections. Furthermore, of the 17 studies meeting the inclusion criteria, not one used ceftazidime and gentamicin as their combination of antibiotics (Safdar, Handelsman and Makim, 2004).

Only a few studies could be found that had used ceftazidime and gentamicin in combination, *in vitro* against *P. aeruginosa*. Sputum samples from cystic fibrosis patients with chronic pulmonary colonization of *P. aeruginosa* underwent direct sputum sensitivity testing using the Epsilometer test (Etest) method for MIC determination. The study revealed that 44% of samples were susceptible to gentamicin and 53% of samples were susceptible to ceftazidime. When looking at combination therapy, 21% of gentamicin/ceftazidime combinations showed synergy, and in 20% of cases, this was the most efficient combination (Serisier *et al.*, 2012). Pruekprasert and Tunyapanit (2005) found that when testing 50 *P. aeruginosa* isolates with ceftazidime and gentamicin in combination, 38.9% of the isolates showed synergy, defined as a fractional inhibitory concentration index (FICI) ≤0.5. They also found that in 44.4% of isolates, an additive effect was observed ($0.5 < FICI < 1.0$), 16.7% of isolates exhibited an indifference effect ($1.0 < FICI < 2.0$),

and that no isolates showed an antagonistic effect (FICI > 2). Shibi, Tawfik and Ramadan (1997) examined the effect of a time interval between the administration of ceftazidime and gentamicin *in vitro* to treat *Pseudomonas aeruginosa* and found that adding the antibiotics at the same time had the best effect of lowering colony forming units (CFUs).

Most clinicians agree that it is better to treat endocarditis and meningitis, caused by *P. aeruginosa*, with a bactericidal antibiotic (Seth and Seth, 2009); however, it does not matter whether a bactericidal or bacteriostatic antibiotic is used for pneumonia or a urinary tract infection (Borton *et al.*, 2011). As a study that could be applied *in vivo*, both the MIC and MBC therefore need determining. The checkerboard method only tests for the MIC, for this study the MBC was also tested, by taking a sample from wells with no visible growth and plating it up on nutrient agar to see if growth returns (Igbinosa *et al.*, 2012).

The aim of this study is to determine whether the recommendation by clinicians to use gentamicin and ceftazidime in combination as a treatment strategy for a *P. aeruginosa* infection is due to these two antibiotics creating a synergistic effect, as there is little research to suggest why there is this recommendation. It is reasonable to hypothesize that this recommendation is due to a synergistic effect. A second aim is to determine what combination of the two antibiotics is most effective.

Methods

MIC and MBC tests

Using aseptic techniques throughout, the bacterial strain *P. fluorescens* NCIB 9046 was suspended in 0.85% saline solution (until equal to 0.5 McFarland solution). Then 1 ml of this solution was placed into 99 ml of Mueller Hilton broth, creating a bacterial suspension of $1–2 \times 10^6$ CFU/ml (Choi and Lee, 2012; Igbinosa *et al.*, 2012). To find the MIC of each antibiotic, 50 µl of 256–0 µg/ml of ceftazidime (Ceftazidime Hydrate, Sigma life science, C3809–1G, lot: SLBD0920V) and gentamicin (Gentamicin injectable, for IM or IV injection, 80 mg in 2 ml, Gentacin, 051365) was placed in triplicate wells of a gamma-irradiated 96-well U-bottom microtitre plate (separate plate for each antibiotic). Dilutions were created in the wells using Mueller Hilton broth as a diluent. To this, 50 µl of $1–2 \times 10^6$ CFU/ml of the aforementioned bacterial suspension was added to each well, and each plate had the lid replaced and sealed with tape before being placed in the incubator at 30°C for 24 h. The MIC was defined as the lowest dilution of antibiotic which completely inhibited the growth of *P. fluorescens*, with growth determined as a cream/white dot in the centre of a well (Igbinosa *et al.*, 2012). For MBC testing, a loop of solution from wells not containing growth after the initial incubation was spread onto nutrient agar and incubated for a further 22 h at 30°C. The MBC was determined as the lowest dilution of antibiotic in which no growth was seen on the agar plate, where growth was determined as cream/white colonies (Igbinosa *et al.*, 2012).

Test for synergy

Antibiotic combinations were evaluated by using the checkerboard method. Gentamicin was diluted across the gamma-irradiated 96-well U-bottom microtitre plate, in the wells, creating concentrations of 4, 2, 1, 0.5, 0.25, 0.125 and 0 µg/ml. Dilutions of ceftazidime were premade in sterile universal bottles with concentrations of 4, 2, 1, 0.5, 0.25, 0.125 and 0 µg/ml, both using Mueller Hilton broth as a diluent. A total of five microtitre plates were created with varying volumes of the two antibiotics, all with a total of 50 µl. Plate 1 contained 10 µl of gentamicin and 40 µl of ceftazidime; plate 2 contained 20 µl of gentamicin and 30 µl of ceftazidime; plate 3 contained 25 µl of gentamicin and 25 µl of ceftazidime; plate 4 contained 30 µl of gentamicin and 20 µl of ceftazidime; and plate 5 contained 40 µl of gentamicin and 10 µl of ceftazidime. Once the plates had the antibiotics combinations in position, 50 µl of $1–2 \times 10^6$ CFU/ml of bacterial suspension was pipetted into each well. The plates had their lids replaced and sealed with tape before being incubated at 30°C for 24 h. MBC testing was also completed, as previously described (Igbinosa *et al.*, 2012).

MIC results were analysed using a FICI. FICI was calculated as:

$$\frac{\text{MIC antibiotic A in combination}}{\text{MIC antibiotic A alone}} + \frac{\text{MIC antibiotic B in combination}}{\text{MIC antibiotic B alone}}.$$

A value of ≤0.5 defined synergy; a value of $0.5 < \text{FICI} < 4$ defined no interaction; a value of ≥4 defined antagonism (Mitsugui *et al.*, 2011).

Statistical tests

The statistical tests were selected after determining that the data were normally distributed (parametric), using SPSS version 18. A p value <0.05 was considered statistically significant. Using the data from the antibiotics used singularly, the independent samples Mann–Whitney U test was employed to test that the two antibiotics had the same concentration distribution. Further using this data, both the Pearson's correlation and Spearman's rho analyses were used to test whether there was a correlation between the amount of growth visible, after the initial incubation, and the strength of the antibiotics. To test whether there was a difference in the MIC values of antibiotics used singularly, a Chi-squared test was used. To test whether there was a difference in the amount of growth, after the initial incubation, between the five combination plates, a chi-squared test was employed.

Results

MIC and MBC tests

For the MIC test for ceftazidime used against *Pseudomonas fluorescens*, the MIC was determined as 3 µg/ml, as it was the lowest concentration to show no growth. For the MBC test

for ceftazidime used against *P. fluorescens*, the concentrations within the range of 256–3 μg/ml were plated on nutrient agar in triplicate. All three triplicate wells containing 3 μg/ml showed growth, and so the MBC was determined as 4 μg/ml. For the MIC test for gentamicin used against *P. fluorescens,* the MIC was determined as 0.25 μg/ml, as it was the lowest concentration to show no growth. Although 1 out of the 3 wells containing 0.75 μg/ml of gentamicin had growth present, this was considered as an anomalous result and disregarded. For the MBC test for gentamicin used against *P. fluorescens*, concentrations 256–0.25 μg/ml were plated on nutrient agar in triplicate. All three triplicate wells containing 0.25–2 μg/ml gentamicin showed growth, and so the MBC was determined as 3 μg/ml (Table 1).

As the concentration of both antibiotics increases, when used singularly, the amount of growth seen decreases; this illustrates that there is a negative correlation between the concentration of both antibiotics used singularly and the amount of growth, significant to the <0.01 level, when statistically tested by both the Pearson correlation and Spearman's rho. Furthermore, there is a statistically significant difference in the MICs of gentamicin and ceftazidime when used singularly, when statistically tested with the chi-squared test, with a significance of <0.01.

FICI values for antibiotics in combination

A value of ≤0.5 illustrates synergy; 0.5 < FICI < 4 illustrates no interaction between antibiotics; and ≥4 illustrates antagonism. Interpreted values will demonstrate whether a synergistic effect occurs, giving reason to clinical recommendations to use these two antibiotics in combination. Plate 1: The FICI values 8.17 and 4.3 indicate that the two antibiotics are antagonizing each other's antimicrobial activity and hence raising their MIC values. Plate 2: When looking at the FICI values, both 8.04 and 5.3 indicate that the two antibiotics are antagonizing each other's antimicrobial activity and hence raising their MIC values. Plate 3: When looking at the calculated FICI value of 2.04, it is illustrated that there is no interaction between the two antibiotics, in that the MIC values remain unaltered by the other, neither positively nor negatively. Plates 4 and 5: These two plates have matching FICI calculations. The well G4 has a calculated FICI value of 4.04 which indicated that the two antibiotics are antagonizing one another's antimicrobial activity and hence raising the MIC values. However, wells F5 and E6 have produced FICI values of 2.83 and 1.17, respectively, and this indicates that the antibiotics are neither positively nor negatively interacting with one another, to alter the MIC values of one another (Fig. 2 and Table 2). No results of synergy occurred; thus, clinical recommendations to use ceftazidime and gentamicin in combination to treat a *Pseudomonas* infection are not due to a synergistic effect.

Table 1. MIC and MBC results for gentamicin and ceftazidime

Antibiotic	MIC (μg/ml)	MBC (μg/ml)
Gentamicin	0.25	3
Ceftazidime	3	4

Ratio of antibiotics in combination analysis

There is a statistically significant difference in the amount of growth between the five combinations of gentamicin and ceftazidime used, after the initial incubation, with a significance of <0.01 when tested by a chi-squared test. Figure 3 illustrates that when looking at growth after the original incubation, as the volume of gentamicin increases and the ceftazidime decreases, the number of wells showing growth decreases. This shows that combinations of 30 μl gentamicin/20 μl ceftazidime and 40 μl gentamicin/10 μl ceftazidime have the greatest bacteriostatic effect against *P. fluorescens* as only 21 wells (43%) showed growth in comparison to 35 wells (71%) growth for the combination 10 μl gentamicin/40 μl ceftazidime. The combination 25 μl gentamicin/25 μl ceftazidime had the least amount of growth return, only one well (2%), when re-plated for the MBC test, illustrating that this combination had the best bactericidal effect on *P. fluorescens*. A combination of 30 μl gentamicin/20 μl ceftazidime and 40 μl gentamicin/10 μl ceftazidime had the most amount of re-growth when re-plated for the MBC test (20 and 22%, respectively). When looking at the total amount of growth, growth after the original incubation and further incubation on nutrient agar, the combination of 25 μl gentamicin/25 μl ceftazidime has the fewest number of wells, only 25, showing growth (51%) in comparison to 39 wells (79%) for the 10 μl gentamicin/40 μl ceftazidime combination. The optimal combination of antibiotics comes from the 25 μl gentamicin/25 μl ceftazidime plate, from well F5 where gentamicin has a concentration of 0.5 μg/ml and ceftazidime has a concentration of 0.25 μg/ml, when used against 1–2 × 10^6 CFU/ml of *P. fluorescens*.

Discussion

Pseudomonas fluorescens was susceptible to both gentamicin and ceftazidime as Benito *et al.* (2012) also found. Extensive searches for relevant research regarding these compounds and the effect of synergy have yielded little published research for *P. fluorescens*. These results can however be extrapolated to *Pseudomonas aeruginosa* as *P. fluorescens* is one of its closest relatives and, in addition, Richards and Brogden (1985) and Mikura *et al.* (2011) state that gentamicin and ceftazidime are also effective against the more established human pathogen, *P. aeruginosa*.

The MIC of ceftazidime was determined to be 3 μg/ml and the MBC to be 4 μg/ml against *P. fluorescens*. This MIC value is within the range of 2–128 μg/ml as stated by Tunney and Scott (2004) and within the range of 0.5–128 μg/ml for *P. aeruginosa*, stated by Richards and Brogden (1985) and Rains, Bryson and Peters (1995), who also state that the MBC values are either the same or less than twice the MIC, which the results in this study support.

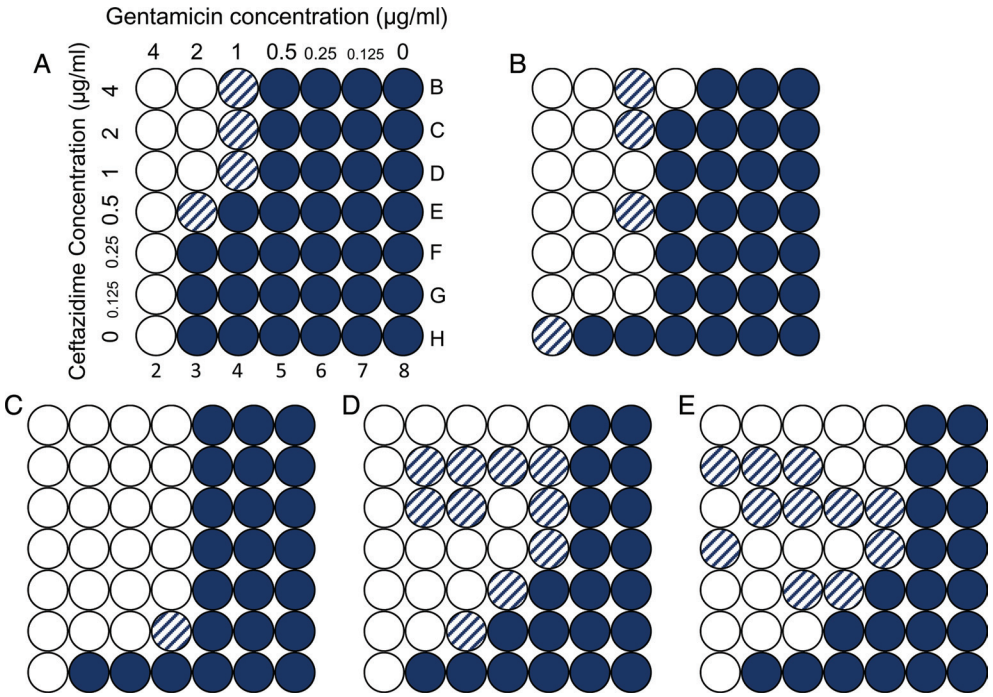

Figure 2. *Pseudomonas fluorescens* growth when gentamicin and ceftazidime are used in combination. (**A**) A volume of 10 µl of gentamicin and 40 µl ceftazidime for concentrations ranging from 4 to 0 µg/ml are in each well-concentrations only shown on (A) but are the same throughout the plates. (**B**) A volume of 20 µl of gentamicin and a 30 µl of ceftazidime of concentrations ranging from 4 to 0 µg/ml are in each well. (**C**) A volume of 25 µl of gentamicin and a 25 µl of ceftazidime of concentrations ranging from 4 to 0 µg/ml are in each well. (**D**) A volume of 30 µl of gentamicin and a 20 µl of ceftazidime of concentrations ranging from 4 to 0 µg/ml are in each well. (**E**) A volume of 40 µl of gentamicin and a 10 µl of ceftazidime of concentrations ranging from 4 to 0 µg/ml are in each well. To each well, 50 µl of 1–2 × 10^6 CFU/ml of *P. fluorescens* suspension is added. Filled circles represent wells where growth occurred after incubation at 30°C for 24 h. Open circles represent wells where no growth occurred after incubation at 30°C for 24 h. Growth was recognized as a white/cream dot in the centre of the well (MIC test). Wells showing no growth were plated onto nutrient agar for the MBC test. They were incubated at 30°C for 22 h. Circles with cross lines represents re-growth on nutrient agar, recognized as visible cream colonies on the agar (n = 1).

The MIC of gentamicin was determined to be 0.25 µg/ml and the MBC to be 3 µg/ml against *P. fluorescens*. This is lower than the range of 1–16 µg/ml for *P. aeruginosa* stated by Burgess and Nathisuwan (2002). The MBC is also lower than >8 µg/ml stated by Rukholm *et al.* (2006). This could mean that *P. fluorescens* is more susceptible to gentamicin than *P. aeruginosa*. However, Sheppard (unpublished data) found the same MIC value as this study, when using the same stock of gentamicin and same strain of *P. fluorescens*.

Safdar, Handelsman and Makim (2004) explain that from conducting a meta-analysis, the use of a combination of antibiotics is more beneficial on mortality rates than using an antibiotic singularly. During this study, no results of synergy were produced (FICI ≤ 0.5); however, results of no interaction (0.5 < FICI < 4) were produced. Considering that it is beneficial to use a combination therapy, the use of the 'no interaction' results could be used *in vivo*, with perhaps a beneficial effect on the patient, and this study has determined the best combination to use.

Balakumar, Rohilla and Thangathirupathi (2010) illustrated the nephrotoxic effects, and Zaske *et al.* (1981) and Gyselynck, Forrey and Cutler (1971) illustrated the ototoxic effects when high levels of gentamicin are used *in vivo*. However, Richards and Brogden (1985) and Rains, Bryson and Peters (1995) discussed that ceftazidime is eliminated quickly from the body via the urine, and for this reason, it has limited side effects. Due to the side effects of gentamicin and the quick elimination time of ceftazidime, it would be sensible to believe that using a combination in which the gentamicin level is lowest would be more desirable, for use *in vivo*, when looking at all of the combinations that produce a desired effect.

Seth and Seth (2009) describe that it is better to treat severe *P. aeruginosa* infections with a bactericidal antibiotic, which illustrates that the choice of the 25 µl gentamicin/25 µl ceftazidime plate is the best choice, as the lowest amount of re-growth occurred from this plate, and has a lower volume of gentamicin compared with 30 µl gentamicin/20 µl ceftazidime and 40 µl gentamicin/10 µl ceftazidime.

However, when considering that it is better to treat severe *P. aeruginosa* infections with a bactericidal antibiotic it would perhaps be beneficial to use combination of antibiotics found in well F5 rather than in G5. Well F5 (25 µl of 0.5 µg/ml gentamicin

and 25 µl of 0.25 µg/ml ceftazidime) would produce a FICI of 2.83, which would still demonstrate no interaction between antibiotics. This study shows that it may be best to suggest well F5 is more appropriate, because re-growth does not occur at this level, and it is the lowest concentration of gentamicin possible. Considering this, this study demonstrates that the most practical combination of gentamicin and ceftazidime is to use a volume ratio of 1:1, in this case 25 µl gentamicin/25 µl ceftazidime, where gentamicin has a concentration of 0.5 µg/ml and ceftazidime has a concentration of 0.25 µg/ml, when used against 1–2 × 10^6 CFU/ml of *P. fluorescens*.

Table 2. FICI calculation and interpretation from the checkerboard method results

Plate	Well	Calculation	Result	Interpretation
1	E3	2/0.25 + 0.5/3	8.17	Antagonism
	D4	1/0.25 + 1/3	4.3	Antagonism
2	G4	2/0.25 + 0.125/3	8.04	Antagonism
	B5	1/0.25 + 4/3	5.3	Antagonism
3	G5	0.5/0.25 + 0.125/3	2.04	No interaction
4	G4	1/0.25 + 0.125/3	4.04	Antagonism
	F5	0.5/0.25 + 0.25/3	2.83	No interaction
	E6	0.25/0.25 + 0.5/3	1.17	No interaction
5	G4	1/0.25 + 0.125/3	4.04	Antagonism
	F5	0.5/0.25 + 0.25/3	2.83	No interaction
	E6	0.25/0.25 + 0.5/3	1.17	No interaction

Furthest bottom right wells were selected for FICI analysis; in the case of plates 1, 2, 4 and 5, more than one well was chosen for analysis as a staggered result was created, as seen in Figure 2.

Antibiotic resistance has become more prevalent in recent years, leading to methodologies for treating infection without the concern of antibiotic resistance being needed more than ever. One such method for overcoming antibiotic resistance is to use existing antibiotics in combination. The aim of the study was to determine whether clinical recommendations to use ceftazidime and gentamicin in combination against *P. aeruginosa* are due to a synergistic effect. This study determines that a synergistic effect does not occur, and that this recommendation is due to evidence that combination therapy increases a patient's likelihood of survival compared with a single antibiotic treatment. Although synergy was not found in this study, it may prove beneficial to use this combination *in vivo*. The major limitation of this study is the lack of replication, and this study recommends this treatment be replicated *in vitro* and studied in depth *in vivo*.

Author biography

A.E.M. has just graduated with a First in BSc Biomedical Sciences from the University of Chester, where during her studies here she gained a passion for research, especially within the field of microbiology. For this reason, she is currently studying for a Master of Research (MRes) in Applied Science, at the same university, where her focus is on the Quorum sensing and quorum quenching of *Chromobacterium violaceum*. Her hopes for the future are to go on to complete a PhD and gain a career in research focusing on antibiotic resistance and alternative antimicrobial therapies.

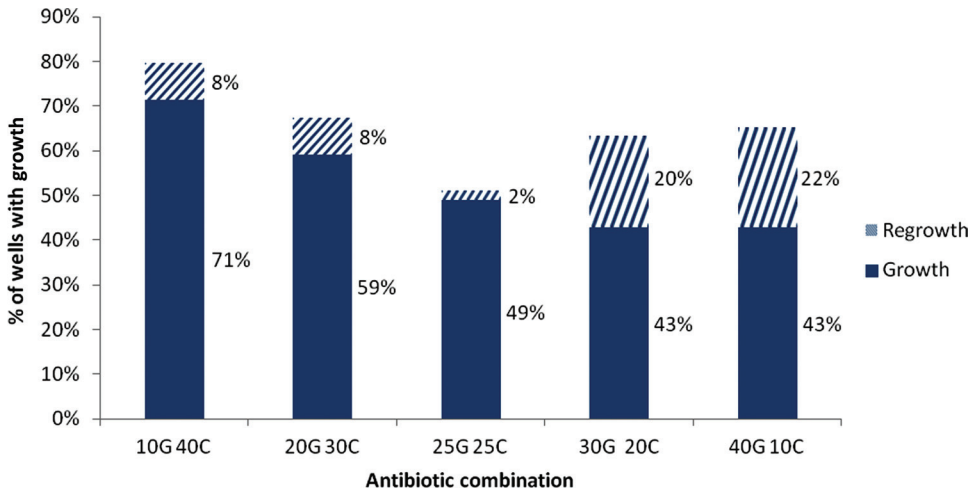

Figure 3. The total amount of growth, including original growth, and re-growth, for all five combinations of gentamicin and ceftazidime. G represents gentamicin, C represents ceftazidime. Each well contained a total of 50 µl of antibiotic and 50 µl of 1–2 × 10^6 CFU/ml of *Pseudomonas fluorescens* suspension. Filled boxes represent the number of wells that showed growth after the original incubation at 30°C for 24 h. Wells that showed no growth were plated onto nutrient agar for the MBC test. They were incubated at 30°C for 22 h. Boxes with lines represent the number of wells where re-growth occurred on nutrient agar (n = 1).

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