Title: Trends of antimicrobial resistance and combination susceptibility testing of cystic fibrosis multidrug-resistant *Pseudomonas aeruginosa:* A ten-year update

Running title: A ten year update on synergy testing of multidrug resistant *Pseudomonas aeruginosa* recovered from cystic fibrosis patients.

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Abstract

Background: Antimicrobial combination therapy is a time/resource- intensive procedure commonly employed in the treatment of cystic fibrosis (CF) pulmonary exacerbations caused by *P. aeruginosa.* Ten years ago the most promising antimicrobial combinations were proposed, but there has since been the introduction of new β-lactam+β-lactamase inhibitor antimicrobial combinations. The aims of this study were i) to compare *in vitro* activity of these new antimicrobials with other anti-pseudomonals agents and suggest their most synergistic antimicrobial combinations. ii) to determine antimicrobial resistance rates and study inherent trends of antimicrobials over ten years.

Methods: A total of 721 multidrug-resistant *P. aeruginosa* isolates from 183 patients were collated over the study period. Antimicrobial susceptibility and combination testing were carried out using the Etest method. The results were further assessed using the fractional inhibitory concentration index (FICI) and the susceptible breakpoint index (SBPI).

Results: Resistance to almost all antimicrobial agents maintained a similar level during the studied period. Colistin (p<0.001) and tobramycin (p=0.001) were the only antimicrobials with significant increasing isolate susceptibility while an increasing resistance trend was observed for levofloxacin. The most active antimicrobials were colistin, ceftolozane/tazobactam, ceftazidime/avibactam, and gentamicin. All combinations with β-lactam+β-lactamase inhibitors produced some synergistic results. Ciprofloxacin+ceftolozane/tazobactam (40%) and amikacin+ceftazidime (36.7%) were the most synergistic combinations while colistin combinations gave the best median SPBI (50.11).

Conclusions: This study suggests that effective fluoroquinolone stewardship should be employed for CF patients. It also presents *in vitro* data to support the efficacy of novel combinations for use in the treatment of chronic *P. aeruginosa* infections.

Keywords: *Pseudomonas aeruginosa*; Cystic Fibrosis; Antimicrobial susceptibility testing; Synergy testing; Etest

1.0 Introduction

In cystic fibrosis (CF) patients, *Pseudomonas aeruginosa* is the most commonly isolated pathogen and more than 70% of CF patients are colonized with this bacterium by the age of 25 (1, 2). *P. aeruginosa* is the primary cause of acute respiratory exacerbations in CF patients with persistent infections leading to a progressive decline in pulmonary function (3). It has been established that the presence of *P. aeruginosa* in respiratory cultures is a major predictor of mortality and morbidity (2). Therefore, in clinical practice to improve life expectancy and the quality of life especially for patients awaiting lung transplantation aggressive antimicrobial treatment is employed (1-3). But the cumulative lifetime treatment of CF patients with antibiotics leads to the development of multidrug-resistant (MDR) *P. aeruginosa* (4). For this reason, various treatment approaches are employed in patient management to delay the development of multidrug-resistant strains. These approaches include combination therapy and the use of modified dosing strategies to optimize antimicrobial pharmacokinetic/pharmacodynamics (PK/PD) parameters. To serve as a guide, ten years ago our lab published that the most promising *in vitro* antimicrobial combinations for use in the treatment of MDR *P. aeruginosa* infections were based on amikacin and ceftazidime combinations (5). However in recent times, there has been the development of novel antipseudomonal agents such as ceftolozane/tazobactam (C/T) and ceftazidime/avibactam (4). As single agents, ceftolozane and ceftazidime have been reported as the most active antipseudomonal agents. However, coupling these antimicrobials with tazobactam and avibactam extends the susceptibility pattern of these antimicrobials to include the extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (4). *In vitro* investigations reported in BSAC data (UK report) state that ceftolozane/tazobactam is a potent antipseudomonal antibiotic with higher susceptibility rates than other β-lactam/β-lactamase inhibitor combinations, carbapenems and fluoroquinolones. Susceptibility rates have been consistently high over the 9 years analysed (2010–18), with 100%, 99.5%, 99.4%, 99.4-100%, 99%, 100%, 90-100%, 100% and 100% respiratory isolates susceptible to ceftolozane/tazobactam for each year (6). Similarly, susceptibility rates of ceftazidime/avibactam were 98.6% for the 2016–17 period and 100% for the 2017–18 period. As a result, these new β-lactam combinations are effective against many Gram-negative bacilli, including MDR *P. aeruginosa* associated with urinary tract infections, nosocomial pneumonia, and complicated intraabdominal infections as well as in the treatment of acute pulmonary exacerbations in cystic fibrosis (7-9). However, recent studies including ours (10) have shown that there can be the development of resistance to these new antimicrobial agents.

The purpose of the current study was to compare the *in vitro* activity of ceftolozane/tazobactam and ceftazidime/avibactam with other antimicrobials on CF MDR *P. aeruginosa* and propose an up-to-date

most promising antimicrobial combination for the treatment of CF MDR *P. aeruginosa* infections. A secondary objective was to determine the antimicrobial resistance rates of CF MDR *P. aeruginosa* and study inherent trends of these antimicrobials over ten years. This would provide empirical evidence in the treatment of pulmonary exacerbations.

2.0 Materials and method

2.1 Study Isolates

Between 13 January 2009 and 02 April 2020, 721 CF-MDR *Pseudomonas aeruginosa* identified by 10 British laboratories were collected over 10 years when they were sent for extended antimicrobial susceptibility testing. Isolates were stored in the bacterial preservation system MICROBANK^{TM} (PRO-LAB DIAGNOSTICS Ontario, Canada) at -80^{o}C and were plated on receipt onto Mueller-Hinton agar (MH), MacConkey agar, *Pseudomonas* Cetrimide agar and *Burkholderia cepacia* selective agar plates (All agar plates were manufactured by Oxoid Ltd., Basingstoke, UK). After 18-24 hr incubation in ambient air at 35^{o}C, plates were verified for culture purity. As a confirmatory test, oxidase testing (Oxoid Ltd., Basingstoke, UK) was performed on 18-24 hr colonies. Isolates were accepted as *Pseudomonas aeruginosa* when they were oxidase-positive and non-lactose fermenting. In this study, multidrug resistance was defined as acquired non-susceptibility to at least one agent in ≥3 antimicrobial groups (11). These isolates were referred to as MDR3 while MDR2 and MDR1 referred to isolates with resistance to two and one antimicrobial groups respectively.

2.2 Minimum Inhibitory Concentration (MIC) testing

MIC testing was performed on MH Agar using the Etest methodology according to the manufacturer's instructions (Liofilchem, Abruzzi, Italy and BioMerieux, Basingstoke, UK). The antimicrobials tested were the aminoglycosides (amikacin, gentamicin, and tobramycin), fluoroquinolones (ciprofloxacin and levofloxacin), lipopeptides (colistin), and the β-lactams. Of the β-lactams, mono-agents tested were monobactams (aztreonam), cephalosporins (ceftazidime), and carbapenems (imipenem and meropenem) while combinations tested were piperacillin/tazobactam, ticarcillin/clavulanate, ceftazidime/avibactam, and ceftolozane/tazobactam. Susceptibility of ticarcillin/clavulanate included in the analyses was up to its stop date (2017) while ceftazidime/avibactam and ceftolozane/tazobactam were included in the analyses from the time of introduction (Jan 2018).

In this study, MIC values between the standard doubling dilution scale were rounded up to the next doubling dilution. The MICs for all tested antimicrobials were interpreted as susceptible (S), intermediate (I) or resistant (R) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) approved interpretive standards for *P. aeruginosa* (12). Due to changes in EUCAST

breakpoints during the studied period, isolate susceptibility patterns were according to the year of submission.

2.3 Combination testing

Antimicrobial combination testing for each isolate was performed using a minimum of six pairs of antimicrobials as previously described (5). Briefly, a saline suspension of 0.5 McFarland standard (1.0 for mucoid strains) from 24hr cultures was inoculated onto MH agar plates according to the EUCAST guidelines for the disk diffusion plate inoculation. Two Etest strips (A and B) were placed top-to-tail according to the manufacturer's instructions. After 1hr to allow antimicrobial diffusion into the agar, each strip was removed and replaced with a fresh Etest (i.e. Etest A strip replaced with fresh Etest B strip and vice versa). Plates were further incubated for 18±2hr in ambient air at 35±1°C.

2.3.1 Fractional inhibitory concentration index (FICI)

Synergy MIC was expressed using the FICI and calculated as described below.

FICI = (MIC A $_{combination}$ / MIC A $_{single}$) + (MIC B $_{combination}$ / MIC B $_{single}$).

If an MIC value was greater than the antimicrobial range tested, the next doubling dilution above this value of the range tested was used to calculate the FICI (e.g. if an MIC of >32mg/L was found then the FICI was calculated using 64mg/L) (13). These indices were interpreted as synergy - FICI ≤0.5, no interaction - FICI >0.5 and ≤4.0, and antagonism - FICI >4.0 (14).

Analyses of species susceptibility to synergy combinations (≥10 replicates) of tested antimicrobials were carried out when EUCAST breakpoints for *P. aeruginosa* was known.

2.3.2 Susceptible breakpoint index (SBPI)

The SBPI was used to describe synergy analysis and calculated as described below.

SBPI = (Susceptible breakpoint of antimicrobial A / MIC of antimicrobial A $_{combination}$) + (Susceptible breakpoint of antimicrobial B / MIC of antimicrobial B $_{combination}$) (5). These combination results were categorised in rank order of their decreasing SBPI results. All antagonistic (FICI >4.0) combinations irrespective of the SBPI result were not ranked nor recommended for therapy.

2.4 Statistical methods

Statistical analysis of categorical and continuous variables were carried out using Microsoft Office Excel 2013 and IBM SPSS statistics for windows, Version 24 (IBM Corp., Armonk, N.Y., USA). The One-way ANOVA with Duncan post hoc test was used for continuous data while the Kruskal Wallis test was used for comparing categorical data.

3.0 Results

3.1 Study Isolates

During the study period, 721 MDR *P. aeruginosa* isolates from 104 female and 79 male CF patients were referred for extended susceptibility testing from 8 Scottish hospitals while others were from York and Belfast. The median age at first referral was 27 years (range 7-69 years) and with a median of 3 samples, between 1 and 20 isolates were submitted per patient during the study period.

Figure 1 shows that 69% (496/721 isolates) of the submitted isolates were resistant to the three groups of antimicrobials (MDR3) tested while 22% (158/721) of submitted isolates were resistant to only two groups (MDR2). Of the latter, 81% (129/158) of MDR2 isolates showed resistance to the fluoroquinolones and β-lactams.

3.2 Antimicrobial Susceptibility profile

The results of MIC tests (Figure 2) carried out on 721 isolates showed that the most active antimicrobial agents were colistin (R=7%), followed by the new β-lactam combinations; ceftolozane/tazobactam (R=37%) and ceftazidime/avibactam (R=47%). Interestingly, *P. aeruginosa* isolates were resistant to the β-lactam combinations; piperacillin/tazobactam (67%) and ticarcillin/clavulanate (86%). Most of the *P. aeruginosa* isolates were resistant to the fluoroquinolones-ciprofloxacin (89%) and levofloxacin (93%) while <70% resistance was observed for the aminoglycosides with lower resistance rates in gentamicin (36%). In summary, 20% of isolates were susceptible while 63.9% were resistant to all tested antimicrobials. The fluoroquinolones had the most resistant isolates (90.83%) followed by β-lactam (67.88%) and aminoglycosides (56.68%).

3.3 Antimicrobial Resistance trend

When the annual mean MIC values for each antimicrobial agent were analysed (Table 1), colistin was the only antimicrobial which showed a downward trend (R^2=0.48) while upward trends were observed for the fluoroquinolones especially for levofloxacin (R^2=0.44). Similarly, an upward trend was observed in the β-lactams group of which meropenem (R^2=0.4967) and piperacillin/tazobactam (R^2=0.3007) demonstrated the greatest increase. The trends for the aminoglycosides during the study period were level ($R^2 \leq 0.005$).

We analysed our data to determine if there were any statistically significant differences in the annual means for each antimicrobial. Analysis using the one-way ANOVA showed there was a statistically significant difference in the annual mean MICs of all tested antimicrobials except tobramycin (p=0.52), ceftazidime (p=0.19), and ceftazidime/avibactam (p=0.19).

Therefore, we investigated whether observed increases in annual antimicrobial MICs corresponded to temporal increases in annual resistant strains by assessing time-based differences in resistance to each tested antimicrobial. Table 2 shows that amongst the aminoglycosides, there were statistically significant differences (p=0.001-0.041) in the decrease of resistant isolates with tobramycin exhibiting the sharpest decrease (R^2=0.5633). In contrast, levofloxacin (R^2=0.472) showed an upward trend but

this was not statistically significant. For the β-lactams group (except imipenem), a statistically significant resistance increase to meropenem (p=0.01), piperacillin/tazobactam (p<0.001), and ticarcillin/clavulanate (p=0.024) were observed while statistically significant resistance decrease to ceftazidime (p=0.017) and aztreonam (p=0.024) in resistance rates ($R^2 \leq 0.1$) were observed. Interestingly, longitudinal analyses of isolates for colistin resistance showed that there was a statistically significant continuous decrease (R^2=0.6881, p<0.001) in resistant isolates during the study period.

3.4 Antimicrobial Synergy testing

A total of 4062 antimicrobial combinations tests were performed using different antimicrobial pairs. Overall, 0.01% antagonism and 9.97% synergy were observed for all the tested combinations. In the antimicrobial groups, 10.31% synergy was observed for aminoglycosides (n=1290), 9.30% for fluoroquinolones (n=774), and 10.20% for β-lactams (n=2196) while low synergy rates (3.84%) were observed for colistin (n=964). Of these, the β-lactam (cephalosporin) with aminoglycoside (n=281) as well as β-lactam+β-lactamase inhibitor antimicrobials (n=19) with another β-lactam (carbapenems) gave the highest synergy values 20.64 and 26.32% respectively. Table 3 shows that the highest synergy was observed with antimicrobial combinations of ciprofloxacin and ceftolozane/tazobactam (n=15, 40% synergy) followed by amikacin and ceftazidime (n=60, 36.7% synergy). Similarly, combinations with ceftazidime were synergistic in 6/7 tested combinations. No synergy was observed when antimicrobial combinations of colistin with levofloxacin/ceftazidime or imipenem with tobramycin/ciprofloxacin were tested. In addition, table 3 shows that synergy was observed in all the tested combinations with the β-lactam+β-lactamase inhibitor antimicrobials (n=12) with ceftolozane/tazobactam combinations the most synergistic. Indeed, this antimicrobial combination gave the highest synergy rate (n=82, 23.17% synergy). Synergy rates for ceftazidime/avibactam were not analysed as only one combination was synergistic.

4.0 Discussion

The use of antimicrobials has been demonstrated to greatly improve the life expectancy of CF patients (15). However, a major drawback of this management approach is the development of antimicrobial resistance due to exposure to several multiple antimicrobial cocktails (1-4, 16). To manage infective pulmonary exacerbations, CF patients are treated with antimicrobial combinations of which one/both are generally effective as single agents and there is a lack of evidence guiding the clinician to decide the best antimicrobial combination that would give a positive treatment outcome (5). Our study focused on *P. aeruginosa*, Bullington et al. (17) reported that 62% of healthcare providers and 56% of people living with CF are concerned about antimicrobial-resistant infections from *P.*

aeruginosa and *Burkholderia* spp. This study analysed the multi and extensively drug-resistant isolates received by our CF antimicrobial reference laboratory, and hence does not provide a representative picture of the general CF population. Nonetheless, as previously reported by studies sampling CF patients (5) we observed colistin (93% susceptible) was the most active antimicrobial. These results should be interpreted with care because for colistin susceptibility testing, it is advised that the use of micro broth dilution should be employed (12) but our lab used the Etest method. In keeping with the same study (5) ciprofloxacin was the most active fluoroquinolone. However, we show that a steady upward trend in annual MIC values was observed for the quinolone antimicrobial class. This predominance of fluoroquinolone-resistant isolates in our study population may be linked to the use of ciprofloxacin for first isolates or patients chronically infected with *P. aeruginosa* as per European guidelines (18). Fluoroquinolones are used in the treatment of a range of infections due to its safety, oral bioavailability, and broad-spectrum activity (19, 20). Despite several guidelines to limit the use of fluoroquinolones in human and veterinary medicine, quinolone-resistance in all species targeted by this antimicrobial class has been growing steadily (19-23). Also, our data suggest that for the aminoglycosides (especially tobramycin) and colistin there was an increase in *P. aeruginosa* susceptibility rates but in contrast, for the fluoroquinolones, we observed that there was a ~50% upward trend in the resistance to levofloxacin. Therefore, we agree with Cogen et al., (15) who reported that although antimicrobial stewardship in this patient population is challenging, its role and impact would enrich patient management and care.

In this study, ceftolozane/tazobactam and ceftazidime/avibactam were observed as the most susceptible β-lactam antimicrobials tested. However, our susceptibility rates was lower in contrast with previous studies which reported *in vitro* activity of ceftolozane/tazobactam (85.1%) against *P. aeruginosa* as comparable with the activity of colistin (89.4%) (24). Gramegna et al. (25) working on 120 CF-derived *P. aeruginosa* isolates demonstrated that the lowest percentage of *in vitro* drug resistance was observed using ceftolozane/tazobactam with 84.2% susceptibility rates. A plausible explanation of the difference in susceptibility rates might be the study isolate population; their study was composed of 55% susceptible strains therefore increasing the susceptibility rates. Indeed, Zamudio et al. (10) reported lower susceptibility values (50%) and Finklea et al. (26) agreed that lower susceptibility values (30%) were observed if the isolate population differed. Similarly, Mirza et al. proposed that previous studies had reported a susceptibility rate of 65.4 - 94% for ceftolozane/tazobactam and 51.8 to 92% for ceftazidime/avibactam in meropenem-non-susceptible isolates (27). Several resistance mechanisms have been proposed, for example, our laboratory characterising resistance mechanisms in *P. aeruginosa* showed it is due to mutation in the AmpC β-lactamase, loss of outer membrane porin D (OprD) while ceftolozane/tazobactam and

ceftazidime/avibactam double resistance is associated with AmpD β-lactamase variations (10). However, more research is important to determine other resistance mechanisms that would help develop effective strategies to cope with drug resistance and for epidemiological studies.

To improve efficacy while preventing the emergence of drug resistance, antimicrobial combinations are often prescribed in the management of CF patients (5). However, the selection of an optimal combination remains a continual clinical challenge. In a previous work published by our laboratory (5), antimicrobial combination of amikacin+ceftazidime was stated as the most synergistic combination. This present study reiterates the dominance of this combination as one of the most synergistic combination. Interestingly, Nazli et al. (28) demonstrated a 15% synergy using amikacin+ceftazidime antimicrobial combinations. Furthermore, our analysis demonstrate that combinations with β-lactam combinations were synergistic. Indeed, newer β-lactam combinations with ciprofloxacin, tobramycin, and meropenem showed promising results (>25% synergy). The most promising antimicrobial combination in the present study was ciprofloxacin+ceftolozane/tazobactam. On the basis of our data suggesting *in vitro* effectiveness of ciprofloxacin antimicrobial combinations with ceftolozane/tazobactam, we propose that this combination is explored in clinical care particularly on the backdrop of restrictions in fluoroquinolone usage. The use of this combination therapy may reduce the likelihood of the emergence antimicrobial resistance and achieve multi-target engagements through inhibition of DNA replication and cell wall biosynthesis. The use of SBPI was proposed earlier (5) as index for ranking *in vitro* effectiveness of combinations. Our results suggest that combinations of colistin with several antimicrobials can give high SBPI values while not predicting synergism as measured by FICI. Though the reason for this is unclear, we hypothesize that while both indices use the combination MIC, SBPI compares it with the organisms' susceptible breakpoint while FICI employs the single agent MIC.

We acknowledge several limitations to this study, the study population consisting of mainly multidrug-resistant isolate population might have impacted our observations. Also, the choice of antimicrobials and its combination cut-off (≥10 times) might have impacted on our results. For example, it would have made our data richer if other newer combinations such as cefiderocol which has low affinity for AmpC β-lactamases and active against carbapenem-non-susceptible isolates were used in susceptibility/synergy testing.

In summary, this research reiterates the upward trend in fluoroquinolones resistance and the increase in susceptibility to colistin and aminoglycosides in CF isolates suggesting effective antimicrobial stewardship for these antimicrobial agents. It also gives empirical *in vitro* evidence that antimicrobial combinations with β-lactam+β-lactamase inhibitors may be the best synergistic antimicrobial combinations to use in the treatment of chronic *P. aeruginosa* infections.

5.0 Acknowledgements

The authors would like to thank the laboratories and clinicians who use the Cystic Fibrosis Antibiotics Susceptibility testing service (CFASS) for their support in sending samples. CFASS is an adult patient testing facility funded by the National Services Division of the Common Services Agency. IMG serves as a consultant to and/ speaker to Pfizer and MSD. All other authors declare no competing interests.

6.0 References

[1.] **López-Causapé C, Rojo-Molinero E, Macia MD and Oliver A.** 2015. The problems of antibiotic resistance in cystic fibrosis and solutions. Expert Review of Respiratory Medicine. **9:**73-88. https://doi.org/10.1586/17476348.2015.995640

[2.] **Forrester JB, Steed LL, Santevecchi BA, Flume P, Palmer-Long GE and Bosso JA** 2018. In Vitro Activity of Ceftolozane/Tazobactam vs Non-fermenting, Gram-Negative Cystic Fibrosis Isolates. In *Open Forum Infectious Diseases* **5**(7):ofy158. https://doi.org/10.1093/ofid/ofy158

[3.] **Garazzino S, Altieri E, Silvestro E, Pruccoli G, Scolfaro C and Bignamini E.** 2020. Ceftolozane/Tazobactam for Treating Children with Exacerbations of Cystic Fibrosis Due to *Pseudomonas aeruginosa*: A Review of Available Data. *Frontiers in Pediatrics* **8:** https://doi.org/10.3389/fped.2020.00173

[4.] **Alvarez-Buylla A, Allen M, Betts D, Bennett S, Monahan I, Planche T and INVICTUS study group Auckland Cressida Bowker Karen Chesterfield Helen Dall'antonia Martino Diggle Mathew El Sakka Noha Elamin Wael Hussain Abid Lambourne Jon Perry John Planche Timothy Pryzbylo Michael Wilson Peter Wootton Mandy.** 2020. Multicentre study of the in vitro activity of ceftolozane/tazobactam and other commonly used antibiotics against *Pseudomonas aeruginosa* isolates from patients in the UK. *JAC-Antimicrobial Resistance.* **2**(2)**:**dlaa024. https://doi.org/10.1093/jacamr/dlaa024

[5.] **Milne K and Gould IM.** 2010. Combination testing of multidrug-resistant cystic fibrosis isolates of Pseudomonas aeruginosa: use of a new parameter, the susceptible breakpoint index. *Journal of Antimicrobial Chemotherapy* **65 (**1)**:**82-90. https://doi.org/10.1093/jac/dkp384

[6.] **BSAC.** BSAC Respiratory Resistance Surveillance Programme, Respiratory Data http://www.bsacsurv.org/reports/respiratory#results

[7.] **Kollef MH, Nováček M, Kivistik Ü, Réa-Neto Á, Shime N, Martin-Loeches I, Timsit J, Wunderink RG, Bruno CJ, Huntington JA and Lin G.** 2019. Ceftolozane–tazobactam versus meropenem for treatment of nosocomial pneumonia (ASPECT-NP): a randomised, controlled, double-blind, phase 3, non-inferiority trial. *The Lancet Infectious Diseases* **19**(12)**:**1299-1311. https://doi.org/10.1016/S1473-3099(19)30403-7

[8.] **Wagenlehner F M, Umeh O, Steenbergen J, Yuan G, and Darouiche RO.** 2015. Ceftolozane-tazobactam compared with levofloxacin in the treatment of complicated urinary-tract infections, including pyelonephritis: a randomised, double-blind, phase 3 trial (ASPECT-cUTI). *The Lancet* **385**(9981)**:**1949-1956. https://doi.org/10.1016/S0140-6736(14)62220-0

[9.] **Lucasti C, Hershberger E, Miller B, Yankelev S, Steenbergen J, Friedland I, and Solomkin J.** 2014. Multicentre, double-blind, randomized, phase II trial to assess the safety and efficacy of ceftolozane-tazobactam plus metronidazole compared with meropenem in adult patients with complicated intra-abdominal infections. *Antimicrobial Agents and Chemotherapy* **58** (9)**:**5350-5357 https://doi.org/10.1128/AAC.00049-14

[10.] **Zamudio R, Hijazi K, Joshi C, Aitken E, Oggioni MR, and Gould IM.** 2019. Phylogenetic analysis of resistance to ceftazidime/avibactam, ceftolozane/tazobactam and carbapenems in

piperacillin/tazobactam-resistant *Pseudomonas aeruginosa* from cystic fibrosis patients. *International Journal of Antimicrobial Agents* **53**(6)**:**774-780 https://doi.org/10.1016/j.ijantimicag.2019.02.022

[11.] **Basak S, Singh P, and Rajurkar M.** 2016. Multidrug resistant and extensively drug resistant bacteria: A study. *Journal of Pathogens* **2016:** 1-5 https://doi.org/10.1155/2016/4065603

[12.] **EUCAST.** 2020. The European Committee on Antimicrobial Susceptibility Testing. "European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0,2020 http://www.eucast.org"

[13.] **MacKenzie FM, Smith SV, Milne KE, Griffiths K, Legge J, and Gould IM.** 2004. Antibiograms of resistant Gram-negative bacteria from Scottish CF patients. *Journal of Cystic Fibrosis* **3**(3)**:**151-157. https://doi.org/10.1016/j.jcf.2004.03.009

[14.] **Odds FC** 2003. Synergy, antagonism, and what the chequerboard puts between them. *Journal of Antimicrobial Chemotherapy* **52**(1)**:**1 https://doi.org/10.1093/jac/dkg301

[15.] **Cogen JD, Kahl BC, Maples H, McColley SA, Roberts JA, Winthrop KL, Morris AM, Holmes A, Flume PA, and VanDevanter DR.** 2020. Finding the relevance of antimicrobial stewardship for cystic fibrosis. *Journal of Cystic Fibrosis* **19**(4):511-520 https://doi.org/10.1016/j.jcf.2020.02.012

[16.] **Romano MT, Premraj S, Bray JM, and Murillo LC.** 2020. Ceftolozane/tazobactam for pulmonary exacerbation in a 63-year-old cystic fibrosis patient with renal insufficiency and an elevated MIC to *Pseudomonas aeruginosa*. *IDCases* **21:**e00830. https://doi.org/10.1016/j.idcr.2020.e00830

[17.] **Bullington W, Hempstead S, Smyth AR, Drevinek P, Saiman L, Waters VJ, Bell SC, VanDevanter DR, Flume PA, and Elborn S.** 2020. Antimicrobial resistance: Concerns of healthcare providers and people with CF. *Journal of Cystic Fibrosis.* https://doi.org/10.1016/j.jcf.2020.05.009

[18.] **Allen P, Borick J, and Borick J.** 2020. Acute and Chronic Infection Management in CF. In: Lewis, MD, FAAFP D. (eds) *Cystic Fibrosis in Primary Care.* pp. 69-87 Springer, Cham https://doi.org/10.1007/978-3-030-25909-9_8

[19.] **Kim ES, and Hooper DC.** 2014. Clinical importance and epidemiology of quinolone resistance. *Infection & Chemotherapy* **46**(4)**:**226-238. https://doi.org/10.3947/ic.2014.46.4.226

[20.] **Aldred KJ, Kerns RJ, and Osheroff N.** 2014. Mechanism of quinolone action and resistance. *Biochemistry* **53**(10)**:**1565-1574. https://doi.org/10.1021/bi5000564

[21.] **Redgrave LS, Sutton SB, Webber MA, and Piddock LJ.** 2014. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends in Microbiology* **22** (8)**:**438-445. https://doi.org/10.1016/j.tim.2014.04.007

[22.] **Hooper DC, and Jacoby GA.** 2016. Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. *Cold Spring Harbor Perspectives in Medicine* **6:**a025320 https://doi.org/10.1101/cshperspect.a025320

[23.] **World Health Organization.** 2014. Antimicrobial resistance: Global Report on Surveillance. World Health Organization

https://apps.who.int/iris/bitstream/handle/10665/112642/9789241564748_eng.pdf

[24.] **Gherardi G, Linardos G, Pompilio A, Fiscarelli E, and Di Bonaventura G.** 2019. Evaluation of in vitro activity of ceftolozane-tazobactam compared to other antimicrobial agents against *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Diagnostic Microbiology and Infectious Diseases* **94**(3)**:**297-303 https://doi.org/10.1016/j.diagmicrobio.2019.01.012

[25.] **Gramegna A, Millar BC, Blasi F, Elborn JS, Downey DG, and Moore JE.** 2018. In vitro antimicrobial activity of ceftolozane/tazobactam against *Pseudomonas aeruginosa* and other non-fermenting Gram-negative bacteria in adults with cystic fibrosis. Journal of Global Antimicrobial Resistance. **14:**224-227. https://doi.org/10.1016/j.jgar.2018.03.002

[26.] **Finklea JD, Hollaway R, Lowe K, Lee F, Le J, and Jain R.** 2018. Ceftolozane/tazobactam sensitivity patterns in Pseudomonas aeruginosa isolates recovered from sputum of cystic fibrosis patients. *Diagnostic Microbiology and Infectious Diseases* **92** (1)**:**75-77 https://doi.org/10.1016/j.diagmicrobio.2018.05.002

[27.] **Mirza HC, Hortaç E, Koçak AA, Demirkaya MH, Yayla B, Güçlü AÜ, and Başustaoğlu A.** 2020. In vitro activity of ceftolozane–tazobactam and ceftazidime–avibactam against clinical isolates of meropenem-non-susceptible *Pseudomonas aeruginosa*: A two-centre study. Journal of Global Antimicrobial Resistance. **20:**334-338. https://doi.org/10.1016/j.jgar.2019.09.016

[28.] **Nazli E, Zer Y, and Eksi F.** 2015. In vitro efficacy of various antibiotic combinations against *Pseudomonas aeruginosa isolates*. *Journal of International Medical Research* **43**(2)**:**217-225. https://doi.org/10.1177/0300060514553490

7.0 Figure Legends

Figure 1. Resistance profile of study isolates to antimicrobial groups. Antimicrobial agents in the aminoglycoside group are Amikacin, Gentamicin and Tobramycin. Levofloxacin and Ciprofloxacin are grouped as fluoroquinolones while Aztreonam, Ceftazidime, Meropenem, Imipenem are grouped as the β-lactams. Also included in this group are β-lactams combinations; Piperacillin/Tazobactam, Ceftazidime/Avibactam, Ticarcillin/Clavulanate and Ceftolozane/tazobactam.

Figure 2. *Pseudomonas aeruginosa* MIC susceptibility patterns to tested antimicrobials. Percentage of susceptible isolates are represented by green bars while orange and blue bars represent Intermediate and resistant isolates.

* Pip/Tazo, Piperacillin/Tazobactam; Tic/Clav, Ticarcillin/Clavulanate; Ceft/Tazo, Ceftolozane/tazobactam; Cef/Avi, Ceftazidime/Avibactam.

Table 1. Temporal variations in MIC values for CF derived *P. aeruginosa* (n=721)

* Pip/Tazo, Piperacillin/Tazobactam; Tic/Clav, Ticarcillin/Clavulanate; Ceft/Tazo, Ceftolozane/tazobactam; Cef/Avi, Ceftazidime/Avibactam.

ND: Not determined
NS: Non significant

Table 2. Temporal differences of antimicrobial resistance of CF derived MDR *Pseudomonas aeruginosa*

[a] AMK, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; ATM, aztreonam; CAZ, ceftazidime; TZP, piperacillin/tazobactam; IPM, imipenem; MEM, meropenem; COL, colistin; TIM, ticarcillin/clavulanate; CZA, ceftazidime/avibactam; C/T, ceftolozane/tazobactam

[b] Percentage of resistant isolates

ND: Not determined
NS: Non significant

Table 3. Summary of results for combinations tested ≥10 times for CF-derived MDR *Pseudomonas aeruginosa*

[a] AMK, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; ATM, aztreonam; CAZ, ceftazidime; TZP, piperacillin/tazobactam; IPM, imipenem; MEM, meropenem; COL, colistin; TIM, ticarcillin/clavulanate; CZA, ceftazidime/avibactam; C/T, ceftolozane/tazobactam

[b] Percentage susceptible when used as a single agent

[c] Number of times the combinations were tested