Title: LONGITUDINAL SURVEILLANCE AND COMBINATION ANTIMICROBIAL SUSCEPTIBILITY TESTING OF MULTIDRUG-RESISTANT *ACHROMOBACTER* SPP. FROM CYSTIC FIBROSIS PATIENTS

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Running title: Synergy Testing and diversity of *Achromobacter* spp. recovered from Cystic Fibrosis patients.

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Abstract

Background: *Achromobacter* spp. are recognized as an emerging pathogen in patients with Cystic Fibrosis (CF). Though recent works have established species-level identification using *nrdA* sequencing, there is a dearth in knowledge relating to species-level antimicrobial susceptibility patterns and antimicrobial combinations which hampers the use of optimal antimicrobial combinations for the treatment of chronic infections. The aims of this study were i) to identify at species-level referred *Achromobacter* isolates ii) to describe species-level antimicrobial susceptibility profiles iii) to determine the most promising antimicrobial combination for chronic *Achromobacter* infections.

Methods: A total of 112 multidrug-resistant (MDR) *Achromobacter* spp. isolates from 39 patients were identified using *nrdA* sequencing. Antimicrobial susceptibility and combination testing were carried out using the Etest method.

Results: We detected six species of *Achromobacter* and found that *A. xylosoxidans* was the most prevalent species. Interestingly, sequence analysis showed it was responsible for persistent infection (18/28 patients) followed by *A. ruhlandii* (2/3 patients). Piperacillin-tazobactam (70.27%) and cotrimoxazole (69.72%) were the most active antimicrobials. Differences were observed in species-level susceptibility to ceftazidime, carbapenems, ticarcillin-clavulanate, and tetracycline. Antimicrobial combinations with cotrimoxazole or tobramycin demonstrate the best synergy while cotrimoxazole gave the best susceptibility breakpoint Index values.

Conclusions: This study enriches the understanding of MDR *Achromobacter* spp. epidemiology, confirms prevalence and chronic colonization of *A. xylosoxidans* in CF

lungs. It presents *in vitro* data to support the efficacy of new combinations for use in the treatment of chronic *Achromobacter* infections.

Keywords: *Achromobacter* spp.; *A. xylosoxidans*; Cystic Fibrosis; Antimicrobial susceptibility testing; Synergy testing; Etest

1.0 Introduction

Several pathogens have been reported as causing chronic infections but *Achromobacter* spp. have increasingly been implicated as causal agents of infection and colonisation in CF individuals (1-7). National CF registries have reported slight increasing rates of *Achromobacter* spp. colonisation/infection in individuals (8) varying between 2 and 17 % (9).

Achromobacter spp. are aerobic, Gram-negative, catalase- and oxidase-positive, non-fermenting bacilli that are phenotypically similar but genetically distinct and are widely distributed in the environment (5). Innate and readily acquired adaptive resistance with antimicrobial exposure thereby altering expression of certain genes promote chronic infection and this has been extensively described in literature (3). This intrinsic resistance to multiple antimicrobials limits the therapeutic options for *Achromobacter* spp. infections (5, 6).

The clinical relevance of isolation of *Achromobacter* spp. in the sputum of CF patients is unclear. Some studies have proposed a link between decline of lung function and chronic infection by *Achromobacter xylosoxidans* especially in patients with chronic *Pseudomonas aeruginosa* infections (10). Others on the other hand postulate that the biofilm-forming ability of *Achromobacter xylosoxidans* correlates with poor lung function (11).

A. xylosoxidans is the most frequently isolated species from clinical samples but there are known difficulties associated with the species identification *of Achromobacter* isolates (9, 12). Conventional identification methods such as

biochemical test, or indeed mass spectrometry, were shown to lack the optimal discriminatory power needed to characterise *Achromobacter* isolates at species-level (1, 13, 14). However, Spilker et al. (15, 16) reported characterization of new *Achromobacter* species through sequencing of the *nrd*A housekeeping gene. Characterisation of *Achromobacter* clinical isolates at the species-level and its relationship with antimicrobial resistance profiles is expected to increase our understanding of the clinical relevance of colonisation by this seemingly CF-related bacterium (1).

The aims of this present study were:

i) To identify at species-level all *Achromobacter* spp. isolates referred to the Scottish CF antimicrobial susceptibility centre using *nrdA* sequencing.

ii) To describe species-level antimicrobial susceptibility profiles of *Achromobacter* spp.

iii) To determine most promising antimicrobial combination treatment for multidrug-resistant *Achromobacter* spp.

2.0 Results

2.1 Study population and geographic metadata

A total of 112 presumptive multidrug-resistant *Achromobacter* spp. were referred for extended antimicrobial susceptibility testing. Isolates were collected over an 18 year period (24 Sep 2001 to 09 Oct 2019) from 9 hospitals: 8 in Scotland and one in Belfast (North Ireland). Edinburgh had the highest patient population (43.59%) and

submitted 60/112 samples. Glasgow and Aberdeen with patient proportions of 17.95% and 12.82% submitted 10 and 17/112 samples respectively. Patient age ranged from 11 to 78 years with a median age at first referral of 28 years. Study population comprised of 17 (43.59%) and 22 (56.41%) male and female patients respectively. Of this population, 18 individuals had single isolate referrals whilst the remaining 21 individuals had multiple referrals (2-9 isolates per individual) with colonization periods of 1-10 years (Table 1).

2.2 Species prevalence

*nrd*A sequencing differentiated the 112 referred isolates into 6 different species. We observed that A*. xylosoxidans* (n=88/112, 78.57%) was the most prevalent species amongst our patients (n=28/39, 71.79%) while A*. insuavis* was the second most prevalent *Achromobacter* spp. (10%). To illustrate the degree of sustained colonisation at patient level we constructed a neighbour joining tree (Fig 1) which demonstrates that repeat isolates from patients (>2 submissions) were representatives of the patients' first referred isolates for 19 out of the 21 patients (90.48%).

2.3 Antimicrobial susceptibility testing

Using 18 antimicrobials susceptibility testing were performed on the 112 *Achromobacter* isolates. Though unequal sampling was carried out in our study, Fig 2 shows that most of the *Achromobacter* isolates were resistant (≥ 93.33%) to the aminoglycosides; amikacin, gentamicin, and tobramycin as well as the fluoroquinolones; ciprofloxacin and levofloxacin (83.04 - 91.96%). The most active

antimicrobials were piperacillin-tazobactam (70.27%), followed by co-trimoxazole (69.72%) and minocycline (62.39%). Aztreonam had no activity against *Achromobacter* spp. (For detailed information, see supplementary data). Thereafter, we grouped the first-referred study isolates and determined if differences existed in the antimicrobial susceptibility patterns (Fig 2). Statistical analysis showed that susceptibility differences exist for amikacin (p=0.032), gentamicin (p=0.002), tobramycin (p=0.007), ciprofloxacin (p=0.001), levofloxacin (p<0.001) doxycycline (p<0.001), minocycline (p=0.005) and ticarcillin/clavulanate (p=0.043). There was no statistical difference in the susceptibility of both *Achromobacter* groups to aztreonam, chloramphenicol, colistin, co-trimoxazole, ceftazidime or piperacillin-tazobactam.

2.4 Antimicrobial synergy testing

Antimicrobial synergy testing was conducted using the direct overlay Etest method and findings classified using the FICI and SBPI criteria. In summary, a total of 738 antimicrobial combinations were tested with a mean of 6.6 combinations per isolate.

2.4.1 FICI

Using FICI criteria, the rates of synergy and antagonism between pairs of antimicrobials were 10.57% and 2.30% respectively. Species grouping showed 2-fold higher rate of synergy (11.75%) in *A. xylosoxidans* (n=604) when compared with non-*xylosoxidans* (n=138) cumulatively (5.22 % synergy).

The rates of antimicrobial antagonism in *A. xylosoxidans* were 2.32% while in non-*xylosoxidans* 2.24% was observed. A summary of the most synergistic combinations for *Achromobacter* spp. were ceftazidime + imipenem (n=14, 50% synergy),

tobramycin + ceftazidime (n=23, 34.78% synergy) and tobramycin + imipenem (n=32, 21.88% synergy). The highest rate of antagonism was observed with ceftazidime + co-trimoxazole (n=24, 12.50 % antagonism) and minocycline + ticarcillin-clavulanate (n= 18, 11.11% antagonism). However when grouped as species; for *A. xylosoxidans* ceftazidime + imipenem combinations (n=13, 53.85%) as well as tobramycin with ceftazidime (n=18, 38.89%)/imipenem (n=26, 23.08%) were the most synergistic combinations. While for non-*xylosoxidans* the antimicrobial combination of tobramycin and ceftazidime (n=5/ 20%) was the most synergistic combination. Interestingly, for A*. xylosoxidans* (Table 2), cotrimoxazole combinations followed by tobramycin was the most prevalent synergistic combinations. While in non-*xylosoxidans* (Table 3) tobramycin combinations were the most prevalent synergistic combinations. This suggests that to achieve synergy, antimicrobial combinations with cotrimoxazole and tobramycin should be explored.

2.4.2 SBPI

But in the laboratory, there is uncertainty about which combinations might be synergistic *in vitro*, therefore our laboratory previously proposed use of the SBPI method. For the 738 antimicrobial combinations tested in all study *Achromobacter* spp., the median SBPI was 4.67 while the mean SBPI was 15.20. By species the median and mean values of 4.00 and 15.79 were observed for *A. xylosoxidans* while higher values (10.00 and 23.34) were observed for non- *xylosoxidans*.

The highest median SBPI for *Achromobacter* spp. was combinations of levofloxacin with piperacillin-tazobactam (12.00) or co-trimoxazole (9.63). Tobramycin when combined with either ceftazidime or imipenem gave the lowest median values. Table

2 demonstrates that highest median SBPI for *A. xylosoxidans* was combinations of cotrimoxazole with Ticarcillin-clavulanate (11.33) and imipenem (10.00). Similarly, for non- xylosoxidans (Table 3) a high SBPI value was obtained for co-trimoxazole combinations with levofloxacin (31.28) or ceftazidime (SBPI 22.44).

3.0 Discussion

Our study focused on *Achromobacter* spp. which has been reported as one of the emerging pathogens found in cystic fibrosis patients (4, 17, 18). As the Scottish CF antimicrobial reference laboratory we receive only multi and extensively drug resistant isolates from Scottish hospitals as well as Belfast for antimicrobial synergy testing. It is therefore difficult to show that our study is a representative picture of the Scottish CF population. However, like other studies (3-5, 19) our study reiterates the dominance of *A. xylosoxidans* (28 out of 39 patients) amongst the CF population. Also, with an *A. xylosoxidans* prevalence of 78.57% our study agrees with the estimated UK prevalence of 78.4% (3). Amoureux et al. (1) suggested that its dominant role might be due to either a higher natural abundance or the presence of favourable selective factors for example the possession of innate resistance to disinfectants such as quaternary ammonium compounds which ensures its ability to thrive in clinical samples. Also, *A. insuavis* was the second most patient carried *Achromobacter* spp. but the persistent colonization of *A. ruhlandii* in our CF population meant that the latter was the second most isolated species. Previous studies have demonstrated that persistent CF infections are mainly attributed to *A. xylosoxidans*, *A. insuavis* and *A. dolens* (5). This was also observed in our study, however longitudinal analysis of our data agrees with Gade *et al*. (6) that *A. ruhlandii*

is also capable of persisting in the CF airways. The mechanisms of persistence has not been fully established with several hypotheses postulated. Gade *et al*. (6) reports that inter-patient transmission might be possible while Edwards et al. (5) showed that *Achromobacter* spp. is patient specific and there was clearance in all but one patient when treated with oral cotrimoxazole. Also, Dupont et al. (4) reported that the environmental habitat might not play a role in the reseeding of isolates for patients with persistent infections. We hypothesize that these isolates may not be entirely eradicated from the CF airways during treatment and on the development of favourable conditions multiply and cause pulmonary exacerbations. This is because analysis of the antimicrobial susceptibility patterns of isolates from patients with repeated submissions show similar antimicrobial patterns while results from our *nrd*A sequencing demonstrate persistent infection. We also did not observe any evidence of potential transmission between individuals or shared geographical location. However, further epidemiological studies on these isolates is necessary to enrich our knowledge on the mechanisms of persistence.

Coward et al. (3) reported that there are no established guidelines for managing CF patients who persistently harbour *Achromobacter* spp. with antimicrobial susceptibility pattern/testing less defined. Indeed, there is a dearth in the knowledge of species-level antimicrobial susceptibility patterns as well as the most promising synergistic combinations. Similar to other studies (3, 19-21) the most active antimicrobial was piperacillin-tazobactam, and cotrimoxazole at 70% while minocycline was third at 62%. As expected, we observed a high resistance of our isolates to the aminoglycosides. This resistance reported by Bador et al. (22) is due

to the possession of AxyXY-OprZ efflux system which confer resistance to aminoglycoside in *Achromobacter* spp. especially *A. xylosoxidans, A. ruhlandii* and *A. insuavis* which make up 96% of this study. Similarly as observed by Amoureux *et al*. (19), *Achromobacter* spp. was more susceptible to imipenem (47%) compared to meropenem (37%) although our values (given that our samples were MDR and XDR strains) had a lower susceptibility percentage. Also, mirrored in our observation as described in most CF and environmental isolates were high ciprofloxacin (92%) and aztreonam (100%) resistance while for the newer β-lactam combinations such as ceftolozane-tazobactam (100%) and ceftazidime-avibactam (78%) our isolates were resistant to these drugs. This observation was also made by Coward et al. (3). But, few CF studies have described species antimicrobial susceptibility patterns of *Achromobacter* spp. We demonstrate that in *Achromobacter* spp. differences exist in the susceptibility of non-*xylosoxidans* compared with *A. xylosoxidans* to the carbapenems, cephalosporins or tetracyclines. The presence of resistance-nodulation-cell-division-type pumps in *A. xylosoxidans* such as AxyABM, AxyXY-OprZ and TetA confers the cell with the ability to pump cephalosporins, fluoroquinolones, aztreonam, chloramphenicol, carbapenems and tetracycline out of the cell (23, 24). Papalia et al. (25) reported that a homologue of AxyABM was present in *A. ruhlandii*, therefore conferring it with the ability to expel chloramphenicol. Further research is needed to determine the presence of efflux pumps and characterize if present in non-*xylosoxidans.*

Irrespective of isolate susceptibility, antibiotic exposure gives rise to the emergence of multi drug and extensively drug resistant strains causing limited therapeutic

options in patient management. Therefore to reduce toxicity and improve efficacy while preventing the emergence of drug resistance, multiple antibiotics thought to be effective as single agents are typically often prescribed in the clinic (26). But there is limited information on synergistic combination for the treatment of *Achromobacter* spp. infections. To the best of our knowledge this is the first time antimicrobial synergy results are described for *Achromobacter* spp. to species level. Analysis of our data showed that there were differences in the synergy observed in both *Achromobacter* groups when there was availability of the interpretative guidelines. For all and first- referred isolates, there was ~50% increase in synergy for *A. xylosoxidans* compared to non-*xylosoxidans* when two CLSI interpretative guidelines were known. Though not a remit of this study, more research would enhance knowledge on how the more resistant *A. xylosoxidans* is able to demonstrate more synergistic combinations than non-*xylosoxidans*.

At a genus level, our results demonstrate that for *Achromobacter* spp. combinations of ceftazidime + imipenem (50%) was the most synergistic combinations. This is in contrast with observations made by Saiman et al. (27) which stated that ciprofloxacin + meropenem combinations (9%) were the most synergistic combinations. It is worth noting that not all the combinations were tested at the same frequency and differences existed in the rates observed for both studies. But, Gómara et al. (26) reported that due to lack of standardization, differences exist in synergy reported using different methods. Indeed, Saiman used the checkerboard while our lab used the direct overlay E-test method. It might also be due to our cut off which analysed only combinations which had been tested more than 5 times. The unpredictability of

synergy and the non-correlation of synergy and clinical efficacy was the reason our lab had earlier proposed the use of SBPI (28) as a useful parameter for comparing *in vitro* effectiveness of combinations thereby ranking them. Our results suggest that combinations of cotrimoxazole with several antimicrobials are able to give a high SBPI values although these values do not predict a synergistic FICI. A major limitation of our data is the lack of information on the clinical outcomes of our combinations. Therefore, further investigation is required to assess if there is a correlation of SPBI values and clinical efficacy. In clinical practice however, there is a growing evidence showing the lack of effectiveness seen when guiding antimicrobial selection based on in vitro synergy testing. Indeed, results from a randomized, double-blind controlled trial demonstrated that there was no difference between groups and proposed synergy testing in CF patients should be stopped (29). However, this study was carried out using multiple-combination bactericidal test method and with various degrees of agreement in synergy methods, it is possible that others might be more clinically relevant. Finally, the selected study of multidrug-resistant strains may have overestimated our observation of persistence. It would be interesting to how the use of random selection would impact on our results.

In summary, the *Achromobacter* spp. remains a key emerging pathogen in CF individuals and has been implicated in pulmonary exacerbations. This research reiterates the prevalent MDR species that make up the *Achromobacter* genus and highlights their susceptibility profiles to several antimicrobials. It also attempts to give antimicrobial combinations which might be used in the treatment of chronic *Achromobacter* infections. With inconsistency reported in clinical outcomes of these

patients, accurate identification of *Achromobacter* spp. will undeniably play a vital role in approach taken during therapeutic management of CF patients.

4.0 Materials and method

4.1 Study Isolates

A total of 112 presumptive *Achromobacter* spp. identified by 8 Scottish and 1 Northern Ireland laboratories were collected over an 18 year period (24th September 2001 – 9th October 2019) when they were sent for extended susceptibility testing. Isolates were stored in the bacterial preservation system MICROBANK^{TM} (PRO-LAB DIAGNOSTICS Ontario, Canada) at -80^{o}C.

Isolates were plated on receipt onto Mueller-Hinton agar (MH), MacConkey agar, *Pseudomonas* Cetrimide agar and *Burkholderia cepacia* selective agar plates (All agar plates manufactured by Oxoid Ltd., Basingstoke, UK). Following 18-24 hr incubation at 35 ^{o}C in ambient air, plates were examined for purity and thereafter incubated a further 24 hr to confirm for purity. Oxidase testing (Oxoid Ltd., Basingstoke, UK) was performed as a confirmatory test on 18-24 hr colonies. Oxidase positive and non-lactose fermenting isolates were accepted as *Achromobacter* spp

4.2 *nrdA* Sequencing

Species identification of isolates was carried out by *nrdA* sequencing according the method described by Spilker et al. (15). Briefly, a single colony of *Achromobacter* spp. was suspended in 20 µl of lysis buffer composed of 0.25 % (v/v) sodium

dodecyl sulfate (Sigma-Aldrich, Irvine, UK) and 0.05 N NaOH (Sigma-Aldrich, Irvine, UK). On heating for 15 mins at 95 ^{o}C, 180 µl of high-pressure-liquid-chromatography grade water (Sigma-Aldrich, Irvine, UK) was added to the suspension. The solution was centrifuged at 13,300 rpm for 5 mins and the supernatants stored at -20 ^{o}C. Full length *nrdA* amplification and sequencing was carried out using the *nrdA*-specific forward (GAACTGGATTCCCGACCTGTTC) and reverse (TTCGATTTGACGTACAAGTTCTGG) primers as previously published (15). Amplified PCR products were sequenced by Eurofins genomics (GATC Biotech AG, Konstanz, Germany) and sequence chromatograms were visualized and edited using the SeqMan Pro (DNAStar, Madison, WI, USA). Allele numbers were assigned to each isolate using the *Achromobacter* MLST database (http://pubmlst.org/org/achromobacter/). Trimmed sequences were aligned using MegAlign Pro (DNAStar, Madison, WI, USA). Clustal W in MegAlign Pro was used to generate a neighbour joining tree with 1,000 bootstrap replications using default parameters. Study isolates were grouped as either *A. xylosoxidans* or non-*xylosoxidans* following *nrd*A identification.

4.3 Minimum Inhibitory Concentration (MIC) testing

MIC testing was performed on MH Agar using the Etest methodology according to manufacturer's instructions (Liofilchem, Abruzzi, Italy and BioMerieux, Basingstoke, UK). The antimicrobials tested were amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, aztreonam, ceftazidime, piperacillin/tazobactam, imipenem, meropenem, colistin, chloramphenicol, minocycline and co-trimoxazole. Data relating to susceptibility to ticarcillin/clavulanate which the service had stopped testing were

included in the analyses up to its stop date (2018). While susceptibility data of antimicrobials introduced by the service later than 2001, namely doxycycline (October 2003), Ceftazidime-avibactam (January 2018) and ceftolozane-tazobactam (January 2018) were included in the analyses from the time of introduction.

In this study, MIC values between the standard doubling dilution scale were rounded up to the next doubling dilution (e.g. 0.75 = 1.0 mg/L). The MICs for amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, aztreonam, ceftazidime, piperacillin/tazobactam, imipenem, meropenem, ticarcillin/clavulanate, doxycycline, chloramphenicol, minocycline and co-trimoxazole were interpreted as susceptible (S), intermediate (I) or resistant (R) according to the Clinical and Laboratories Standards Institute (CLSI) approved interpretive standards for non-enterobacteriaceae (30). Ceftazidime/avibactam, ceftolozane/tazobactam and colistin were interpreted as per CLSI standards for *Pseudomonas aeruginosa* (30). In this study, multidrug-resistance (MDR) was defined as acquired non-susceptibility to at least one agent in ≥3 antimicrobial groups (31).

4.4 Combination testing

Combination testing was performed using a minimum of six pairs of antimicrobials (A + B), as described previously (28). Briefly, MH agar plates were inoculated with two Etest strips (A and B) placed top to tail according to the manufacturer's instructions. After 1 hr to allow antimicrobial migration into the agar, each strip is removed and fresh Etest is placed in opposite orientation on the imprint (Etest A strip replaced with fresh Etest B strip and vice versa). Plates were further incubated for 22-24 hrs in ambient air at 35 ^{o}C.

4.4.1 Fractional inhibitory concentration index (FICI)

Indices derived from the combination MIC results were calculated using the MIC value read off the Etest strip and interpreted as described below.

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

Where an MIC was found to be greater than the antimicrobial range tested, the next doubling dilution above the highest value of the range tested was used to calculate the FICI (e.g. if an MIC of >256mg/L was found then the FICI was calculated using 512mg/L) (32). The indices were interpreted as: synergy - FICI ≤0.5, no interaction - FICI >0.5 and ≤4.0 and antagonism - FICI >4.0 (33).

Analyses of species susceptibility to double combinations of antimicrobials tested ≥5 times was carried out when CLSI breakpoints for non-enterobacteriaceae was known.

4.4.2 Susceptible breakpoint index (SBPI)

SBPI = (Susceptible breakpoint of antimicrobial A / MIC of antimicrobial A $_{combination}$) + (Susceptible breakpoint of antimicrobial B / MIC of antimicrobial B $_{combination}$) (28). The combination results were graded and reported in rank order of their SBPI results from highest to lowest SBPI which displays the effectiveness of the combination in decreasing order. Any combination found to be antagonistic (FICI >4.0), was not ranked and was not recommended for therapy irrespective of the SBPI result.

4.5 Statistical methods

Descriptive statistics were derived using Microsoft Office Excel 2013 and IBM SPSS statistics for windows, Version 24 (IBM Corp., Armonk, N.Y., USA). A two-tailed Mann Whitney test was performed using GraphPad Prism, Version 8.4.0 (GraphPad software, San Diego, California, USA).

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Figure Legends

Table 1. Characteristics of study population and demographics

P00, anonymized participant number; F, female; M, male; -, data unavailable

Fig 1. Neighbour joining tree illustrating the *nrd*A clustering of *Achromobacter* spp. isolates from patients with repeated submission.

P, patient; 01-38 anonymized number, a-i; repeated samples submitted by patient.

Fig 2. ***Achromobacter* spp. susceptibility patterns.** Percentage susceptibility of all-referred *Achromobacter* spp., first-referred isolates (*A. xylosoxidans* and non-*xylosoxidans*) to several antimicrobials. 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; CHL, chloramphenicol; DOX, doxycycline; MIN, minocycline; SXT, co-trimoxazole

[a] CLSI-approved interpretative standards for non-enterobacteriaceae
[b] CLSI-approved interpretative standards for *P. aeruginosa.* Colistin resistance may be over-estimated due to limitations of the diffusion method.

Table 2. Summary of antimicrobial combinations tested on *A. xylosoxidans* isolates

TOB, tobramycin; LVX, levofloxacin; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; TIM, ticarcillin-clavulanate; CHL, chloramphenicol; MIN, minocycline; SXT, co-trimoxazole
[a] Percentage active when used as a single agent
[b] Number of times the combinations were tested

Table 3. Summary of antimicrobial combinations tested on *Non- xylosoxidans* isolates

TOB, tobramycin; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; MIN, minocycline, SXT, co-trimoxazole; CHL, chloramphenicol

[a] Percentage active when used as a single agent

[b] Number of times the combinations were tested

Table 1. Characteristics of study population and demographics

Participant number	Sex	Location	Age at first submission (yrs)	No of samples submitted	Period of colonization (yrs)
P01	F	Inverclyde	12	5	4
P02	F	Glasgow	16	1	-
P03	F	Edinburgh	13	6	9
P04	F	Edinburgh	20	3	1
P05	M	Kilmarnock	11	4	3
P06	M	Edinburgh	22	1	-
P07	F	Edinburgh	18	8	10
P08	F	Dumfries	25	5	5
P09	M	Dundee	24	5	8
P10	M	Aberdeen	20	7	10
P11	F	Aberdeen	16	1	-
P12	M	Edinburgh	24	7	8
P13	F	Edinburgh	18	6	6
P14	F	Edinburgh	54	1	-
P15	F	Edinburgh	25	1	-
P16	F	Edinburgh	19	9	6
P17	M	Edinburgh	23	4	4
P18	M	Glasgow	13	4	4
P19	M	Edinburgh	28	1	-
P20	F	Dundee	33	1	-
P21	M	Edinburgh	30	3	3
P22	M	Edinburgh	18	2	2
P23	F	Aberdeen	21	4	1
P24	M	Glasgow	20	1	-
P25	M	Aberdeen	48	3	4
P26	F	Glasgow	20	1	-
P27	F	Aberdeen	30	2	3
P28	F	Glasgow	71	1	-
P29	M	Edinburgh	32	1	-
P30	F	Belfast	69	1	-
P31	F	Belfast	44	1	-
P32	F	Belfast	-	1	-
P33	F	Belfast	-	1	-
P34	M	Glasgow	21	1	-
P35	F	Glasgow	24	1	-
P36	F	Edinburgh	23	3	2
P37	M	Edinburgh	78	2	2
P38	M	Edinburgh	16	2	2
P39	M	Belfast	-	1	-

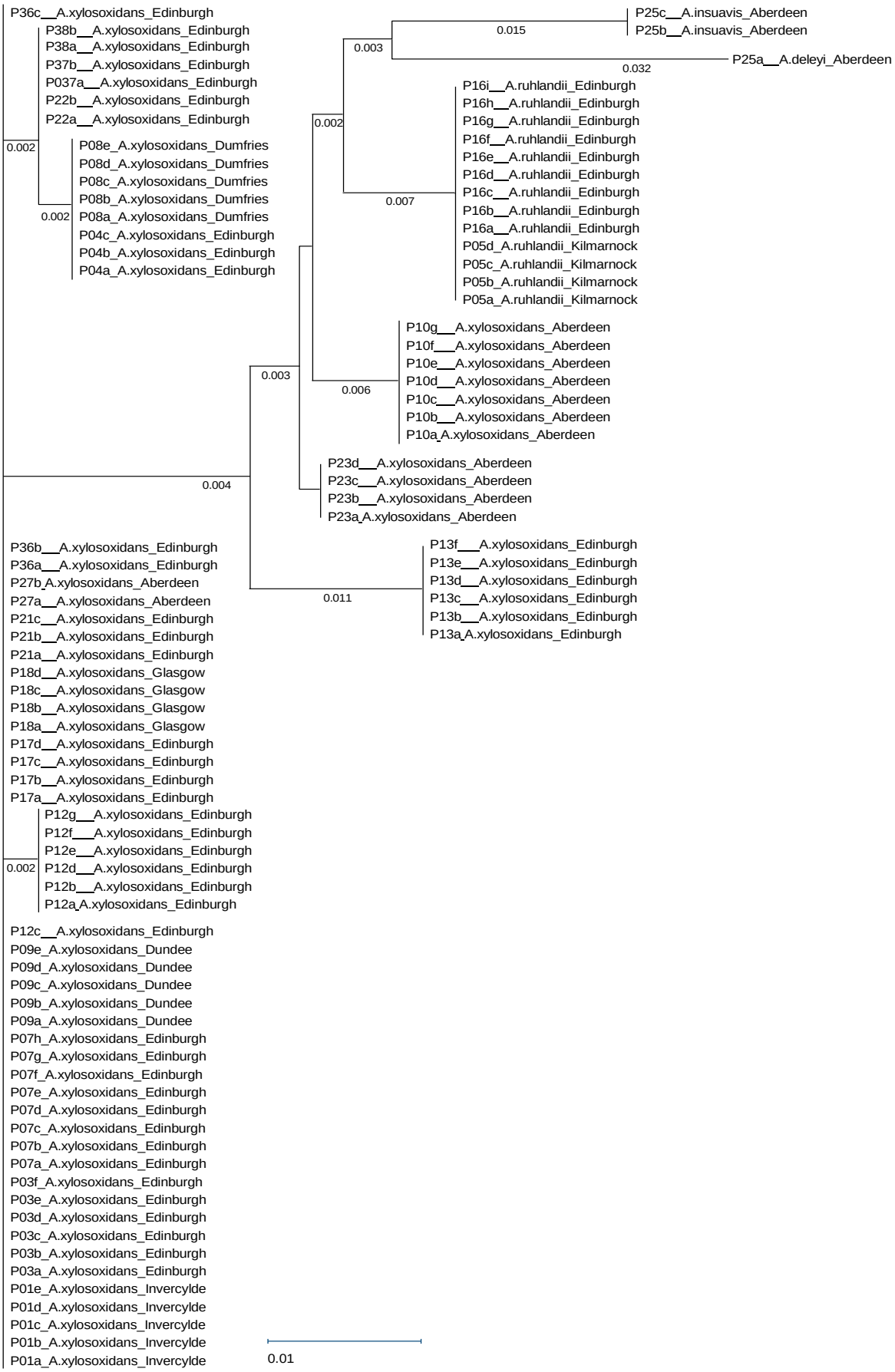

Fig 1. Neighbour joining tree illustrating the *nrdA* clustering of *Achromobacter* spp. isolates from patients with repeated submission.

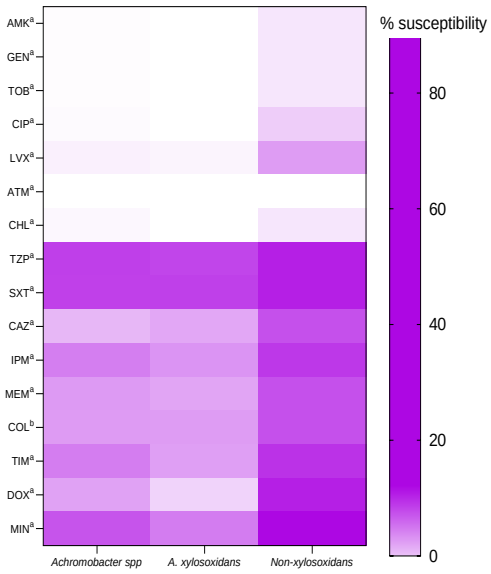

Fig 2. *Achromobacter* spp. susceptibility patterns.

Table 2. Summary of antimicrobial combinations tested on *A. xylosoxidans* isolates

Antimicrobial			Synergy		Antagonistic		SBPI	
First (%S[a])	Second(%S[a])	Number [b]	%	Rank	%	Rank	Median	Rank
CAZ (23)	IPM (46.6)	13	53.85	1			5.17	5
TOB (0)	CAZ (23)	18	38.89	2			3.17	13
TOB (0)	IPM (46.6)	26	23.08	3			3.33	10
IPM (46.6)	SXT (64.7)	10	20.00	4			10.00	2
CAZ (23)	SXT (64.7)	15	20.00	4	13.33	1	8.50	4
MIN (54.1)	SXT (64.7)	15	13.33	6	6.67	5	4.67	6
TOB (0)	MEM (36.8)	15	13.33	6			2.79	16
TOB (0)	TIM (49.3)	15	13.33	6			2.75	18
SXT (64.7)	CHL (2.4)	16	12.50	9			9.13	3
TOB (0)	SXT (64.7)	10	10.00	10	10.00	3	4.23	8
SXT (64.7)	TIM (49.3)	10	10.00	10			11.33	1
LVX (1.1)	MIN (54.1)	16					3.33	10
LVX (1.1)	TIM (49.3)	10					4.46	7
MIN (54.1)	CHL (2.4)	24					3.08	14
MIN (54.1)	CAZ (23)	19					3.33	10
MIN (54.1)	TIM (49.3)	18			11.11	2	3.08	14
MIN (54.1)	IPM (46.6)	11					3.67	9
TOB (0)	MIN (54.1)	13			7.69	4	2.79	16

Table 3. Summary of antimicrobial combinations tested on *Non- xylosoxidans* isolates

Antimicrobial			Synergy		Antagonistic		SBPI	
First (%S[a])	Second (%S[a])	Number [b]	%	Rank	%	Rank	Median	Rank
CAZ (37.5)	TOB (4.2)	5	20.00	1			3.42	8
LVX (20.8)	MIN (91.7)	6	16.67	2			6.67	3
TOB (4.2)	IPM (50)	6	16.67	3			4.33	6
CAZ (37.5)	SXT (87.5)	9		4	11.11	1	22.44	2
LVX (20.8)	SXT (87.5)	6		5			31.28	1
MIN (91.7)	CHL (4.2)	5		7			6.67	3
TOB (4.2)	MEM (37.5)	5					4.33	6
CAZ (37.5)	MIN (91.7)	5					6.46	5