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Short Communication

Antimicrobial susceptibility of non-fermenting Gram-negative pathogens isolated from cystic fibrosis patients

María Díez-Aguilar[a,b], Miquel Ekkelenkamp[c], María-Isabel Morosini[a,b], Irene Merino[a,b], Juan de Dios Caballero[a,b], Mark Jones[d], Mireille van Westreenen[e], Michael M. Tunney[f], Rafael Cantón[a,b,*], Ad C. Fluit[c]

[a] Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain
[b] Red Española de Investigación en Patología Infecciosa (REIPI), Madrid, Spain
[c] Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands
[d] Basilea Pharmaceutica, Basel, Switzerland
[e] Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands
[f] School of Pharmacy, Queen's University Belfast, Belfast, UK

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ABSTRACT

Non-fermenting Gram-negative bacteria (NFGNB) are increasingly cultured in respiratory samples from cystic fibrosis (CF) patients. This study determined the antimicrobial susceptibility of clinical CF respiratory isolates from distinct geographical regions. A total of 286 isolates (106 *Stenotrophomonas maltophilia*, 100 *Burkholderia* spp., 59 *Achromobacter* spp., 12 *Pandoraea* spp., 9 *Ralstonia* spp.) from the Netherlands, Northern Ireland, Spain, USA and Australia were tested. $MIC_{50/90}$ values and susceptibility categorisation were determined. Trimethoprim/sulfamethoxazole (SXT) was the most active compound for all micro-organisms (MIC_{50}, 0.12–4 mg/L; MIC_{90}, 1–16 mg/L). For *S. maltophilia*, 47% and 62% of isolates were susceptible to SXT according to CLSI and EUCAST breakpoints, respectively. Ceftazidime presented lower susceptibility (35%; MIC_{50}, 32 mg/L; MIC_{90}, 256 mg/L). MIC_{90} values for tobramycin and colistin were >128 mg/L and >16 mg/L, respectively. Regarding *Burkholderia*, 72%, 56% and 44% were susceptible to SXT, ceftazidime and meropenem, respectively. For both ceftazidime and meropenem, MIC_{50} and MIC_{90} values were within the intermediate or resistant category. The most active antibiotics for *Achromobacter* spp. were SXT (MIC_{50}, 0.5 mg/L; MIC_{90}, 8 mg/L) and imipenem (MIC_{50}, 2 mg/L; MIC_{90}, 8 mg/L). SXT, imipenem and ciprofloxacin were active against 12 *Pandoraea* spp. (MIC_{50}, 0.12–4 mg/L; MIC_{90}, 1–8 mg/L). Ciprofloxacin (MIC_{50}, 4 mg/L) and SXT (MIC_{50}, 1 mg/L) were the only active antibiotics for *Ralstonia* spp. There were no statistically significant differences in susceptibility rates between countries. NFGNB other than *Pseudomonas aeruginosa* are potential pathogens in CF. SXT was demonstrated to be the most active compound against these isolates.

1. Introduction

Cystic fibrosis (CF) patients are regularly colonised by opportunistic micro-organisms. In adult patients, *Pseudomonas aeruginosa* is the main pathogen, but in recent years other non-fermenting bacteria from different genera, such as *Stenotrophomonas, Burkholderia, Achromobacter, Ralstonia* and *Pandoraea*, are being increasingly isolated [1–3]. This could potentially be due to the aggressive antimicrobial therapy used against *P. aeruginosa*, the development of new techniques for bacterial identification, and improvement in life expectancy for people with CF [4].

In most cases, it has been reported that chronic colonisation with these micro-organisms is associated with a decline in pulmonary function; however, the pathogenic role of some of these species is not completely clear owing to their coexistence with other pathogens including *Staphylococcus aureus* and *P. aeruginosa* [4]. Unlike *P. aeruginosa*, for which antimicrobial treatment is standardised, treatment protocols are not in place for these non-fermenting micro-organisms and limited data are available regarding their susceptibility profiles. Studying the susceptibility patterns and epidemiology of these micro-organisms is therefore essential to improve the management of CF patients.

* Corresponding author.
E-mail address: rafael.canton@salud.madrid.org (R. Cantón).

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Table 1
MIC_{50} and MIC_{90} values (mg/L) for the tested antimicrobials and micro-organisms.

Micro-organism/antimicrobial	CAZ	MER	IPM	ATM	CIP	TOB	COL	SXT
Stenotrophomonas maltophilia ($n = 106$)								
MIC_{50}	32	>64	>128	>256	4	64	1	4
MIC_{90}	256	>64	>128	>256	32	>128	>16	16
Burkholderia spp. ($n = 100$)								
MIC_{50}	8	8	32	128	4	128	>16	2
MIC_{90}	128	32	128	>256	64	>128	>16	8
Achromobacter spp. ($n = 59$)								
MIC_{50}	8	2	2	>256	8	128	2	0.5
MIC_{90}	128	32	8	>256	32	>128	>16	8
Pandoraea spp. ($n = 12$)								
MIC_{50}	128	>64	2	>256	4	128	>16	0.12
MIC_{90}	256	>64	8	>256	8	>128	>16	1
Ralstonia spp. ($n = 9$)								
MIC_{50}	16	128	32	>256	4	128	>16	1

$MIC_{50/90}$, minimum inhibitory concentrations required to inhibit 50% and 90% of the isolates, respectively; CAZ, ceftazidime; MER, meropenem; IPM, imipenem; ATM, aztreonam; CIP, ciprofloxacin; TOB, tobramycin; COL, colistin; SXT, trimethoprim/sulfamethoxazole.

The aim of this study was to determine the susceptibility of non-fermenting Gram-negative bacteria (NFGNB) other than *P. aeruginosa* to a range of antimicrobial agents used in CF patients. This study is included within the objectives of the Innovative Medicines Initiative (IMI) iABC European project, which is mainly focused on the development of a new inhaled compound for CF patients.

2. Material and methods

A total of 286 isolates recovered from respiratory samples of CF patients in 2003–2016 from five different countries [Spain ($n = 103$), Northern Ireland ($n = 98$), the Netherlands ($n = 82$), the USA ($n = 2$) and Australia ($n = 1$)] were included in this study. Overall, 106 *Stenotrophomonas maltophilia*, 100 *Burkholderia* spp. (51 *Burkholderia multivorans*, 20 *Burkholderia cenocepacia*, 12 *Burkholderia contaminans*, 10 *Burkholderia vietnamiensis*, 4 *Burkholderia cepacia* and 3 *Burkholderia gladioli*), 59 *Achromobacter* spp. (53 *Achromobacter xylosoxidans*, 4 *Achromobacter* spp. and 2 *Achromobacter insolitus*), 12 *Pandoraea* spp. and 9 *Ralstonia* spp. isolates were studied. Bacteria were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) and the identification was confirmed by whole-genome sequencing. The species of *Burkholderia* was also confirmed by sequencing the *recA* gene as previously described [5].

Minimum inhibitory concentrations (MICs) were determined by the standard ISO broth microdilution method with frozen panels (Trek Diagnostic Systems, Westlake, OH). The antimicrobial agents and the concentration range tested were as follows: ciprofloxacin (CIP) (0.03–32 mg/L); tobramycin (TOB) (0.125–128 mg/L); ceftazidime (CAZ) (0.25–256 mg/L); meropenem (MER, 0.06–64 mg/L), imipenem (IPM) (0.125–128 mg/L); aztreonam (0.25–256 mg/L); trimethoprim/sulfamethoxazole (SXT) (0.06–32 mg/L); and colistin (COL) (0.25–16 mg/L).

MIC_{50} and MIC_{90} values (MICs required to inhibit 50% and 90% of the isolates, respectively) as well as susceptibility categorisation were assessed considering both European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints and the epidemiological cut-off (ECOFF) when available (Tables 1 and 2; Fig. 1). To analyse data by country, χ^2/Fisher's test with Bonferroni correction was used to compare susceptibility rates when an antimicrobial breakpoint was available (statistically significant when $P < 0.016$). To reflect differences in mechanisms of antimicrobial resistance between countries, MIC_{90} values were graphically represented (Supplementary Fig. S1).

Statistical analysis was performed using Stata Statistical Software for Windows: Release 11.0 (StataCorp LP, College Station, TX). Isolates categorised as intermediate and susceptible were grouped for data analysis.

3. Results

MIC_{50} and MIC_{90} values for all of the tested antimicrobial agents and micro-organisms are presented in Table 1. SXT was the most active compound for all of the micro-organisms, with an MIC_{50} range of 0.12–4 mg/L and an MIC_{90} range of 1–16 mg/L. All of the micro-organisms presented either an MIC_{50} or MIC_{90} to COL of >16 mg/L.

Analysing *S. maltophilia* isolates, 47% and 62% of the isolates were susceptible to SXT when considering CLSI [susceptible (S), ≤2 mg/L; resistant (R), ≥4 mg/L] and EUCAST (S, ≤4 mg/L; R, >4 mg/L) clinical breakpoints, respectively (Table 2; Fig. 1). Coinciding with the CLSI breakpoint, the EUCAST ECOFF for SXT is 2 mg/L, which means that 47% of the isolates were included in the wild-type population. CAZ presented a lower level of susceptibility [S, 35%; intermediate (I), 6%; R, 59%], placing both the MIC_{50} (32 mg/L) and MIC_{90} (256 mg/L) within the resistant population (Table 2; Fig. 1). There are no defined clinical breakpoints for CIP, but the MIC_{50} value (4 mg/L) was close to the EUCAST modal value (2 mg/L) in *S. maltophilia*. For both TOB and COL, MIC_{90} values were high (>128 mg/L and >16 mg/L, respectively).

Considering all of the *Burkholderia* spp., according to CLSI breakpoints 72%, 56% and 44% of isolates were susceptible to SXT (S, ≤2 mg/L; R, ≥4 mg/L), CAZ (S, ≤8 mg/L; I, 16 mg/L; R, ≥32 mg/L) and MER (S, ≤4 mg/L; I, 8 mg/L; R, ≥16 mg/L), respectively (Table 2). Both for CAZ and MER, the MIC_{50} and MIC_{90} values were included within the intermediate or resistant category, although the MIC_{90} was lower for MER (32 mg/L) than for CAZ (128 mg/L).

Analysing the antimicrobial activity of the different species of the *Burkholderia cepacia* complex (51 *B. multivorans*, 20 *B. cenocepacia*, 12 *B. contaminans*, 10 *B. vietnamiensis* and 4 *B. cepacia*), the lowest CAZ MIC_{90} was obtained for *B. vietnamiensis* (16 mg/L vs. 64–256 mg/L). *Burkholderia vietnamiensis* also presented lower IPM MIC_{50} (1 mg/L) and MIC_{90} (32 mg/L) values than the other species (IPM MIC_{50} range, 32–64 mg/L; IPM MIC_{90} range, 128–256 mg/L). The susceptibility profiles for the rest of the antimicrobials were similar for all of the *Burkholderia* spp.

Table 2
Clinical susceptibility of *Stenotrophomonas maltophilia* and *Burkholderia* spp. to ceftazidime (CAZ), meropenem (MER) and trimethoprim/sulfamethoxazole (SXT) according to Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.

Breakpoints	Micro-organism	CAZ			MER			SXT	
		%S	%I	%R	%S	%I	%R	%S	%R
CLSI	*S. maltophilia*	35	6	59	–	–	–	47	53
	Burkholderia spp.	56	5	39	44	25	31	72	28
EUCAST	*S. maltophilia*	– [a]	–	–	–	–		62	38
	Burkholderia spp.	–	–	–	2	98	–	–	–

S, susceptible; I, intermediate; R, resistant.
[a] –, indicates that clinical breakpoints have not been defined.

Neither CLSI nor EUCAST have established specific clinical breakpoints for *Achromobacter* spp. Different *Achromobacter* spp. were not analysed separately as the majority of isolates tested were *A. xylosoxidans* (89.8%). After SXT, the most active agent was IPM (MIC_{50}, 2 mg/L; MIC_{90}, 8 mg/L). The COL MIC had a bimodal distribution, with a first modal value of 1 mg/L and a second modal value of 32 mg/L.

SXT, IPM and CIP were active against the 12 *Pandoraea* spp. isolates tested, with a range of MIC_{50} and MIC_{90} values of 0.12–4 mg/L and 1–8 mg/L, respectively. Analysing *Ralstonia* spp., a high level of antimicrobial resistance was observed, with CIP (MIC_{50}, 4 mg/L) and SXT (MIC_{50}, 1 mg/L) as the only active antibiotics.

For *S. maltophilia*, there were no statistically significant differences between countries in CAZ and SXT susceptibility ($P > 0.016$) as shown in Supplementary Fig. S1, which shows the antibiotic MIC_{90} values for the separate countries.

Considering the origin of *Burkholderia* spp., *B. multivorans* was most frequently isolated species in Northern Ireland and the Netherlands (48.6% and 68.6%, respectively), whereas in Spain the most frequently isolated species was *B. contaminans* (39.3%). A lower rate of MER susceptibility was observed for *Burkholderia* isolates from Northern Ireland (54.3%) compared with those from Spain (78.6%) and the Netherlands (74.3%), although the difference was not statistically significant ($P = 0.02$). Curiously, similar MER MIC_{90} values were observed between these countries (4–8 mg/L) (Supplementary Fig. S1). There were no statistically significant differences in CAZ and SXT susceptibility by country, although CAZ MIC_{90} values for isolates from Spain were 2–3 dilutions lower than the CAZ MIC_{90} values found in the Netherlands and Northern Ireland. The SXT MIC_{90} values for isolates from Northern Ireland were 2–3 dilutions lower than those from Spain and the Netherlands.

For *Achromobacter* spp., the SXT MIC_{90} value reported from Northern Ireland was 4 dilutions lower than that from Spain.

4. Discussion

NFGNB other than *P. aeruginosa*, such as *B. cepacia* complex, *Stenotrophomonas, Achromobacter*, *Ralstonia* and *Pandoraea*, are increasingly isolated in respiratory samples from CF patients. These micro-organisms are in general intrinsically resistant to multiple antibiotics and treatment guidelines are not yet available, therefore clinicians judge each patient individually considering the in vitro antimicrobial susceptibility reports and clinical outcome after therapy. Furthermore, the available clinical breakpoints are intended for systemic therapy and may not be adequate for inhaled therapy. Inhaled therapy has the potential to achieve high pulmonary concentrations and may be able to inhibit micro-organisms with MICs above the breakpoints for systemic therapy [6].

Some studies have defined *S. maltophilia* as a coloniser, whilst others demonstrated that this micro-organism is capable of causing a deterioration in pulmonary function [7,8]. Nevertheless, the presence of *S. maltophilia* cannot be ignored in some patients as it is associated with an increased risk of pulmonary exacerbations, the need for lung transplantation and death [9]. Generally, SXT is the antibiotic of choice, but during the last years increasing rates of resistance have been reported in CF patients ranging from 16% to 45% [2,3,10,11]. In the current study, the SXT resistance rate was 38% and 53% following EUCAST and CLSI guidelines, respectively. Comparison between the EUCAST MIC distribution and that obtained in this study shows a clear displacement of the latter to higher concentrations. In fact, the majority of non-CF *S. maltophilia* isolates are susceptible to SXT (global rate of <10% resistance) [12]. Although CAZ and fluoroquinolones are considered as options for *S. maltophilia*, high rates of resistance to both compounds are also increasingly being reported. In the current study, ca. 60% of the isolates were resistant to CAZ, similar to rates previously published (80% [13] and 70% [2]). Newer fluoroquinolones such as moxifloxacin may have a better activity against *S. maltophilia* than CIP ($MIC_{90} = 32$ mg/L in this study) [12].

Isolation of *Burkholderia* spp. is particularly worrying in CF patients as it is related to a rapid decline in pulmonary function and high morbidity and mortality [14]. Similar to *S. maltophilia*, SXT is the antibiotic of choice for *Burkholderia* spp., but combinations are frequently used. A lower SXT resistance rate (28%) was observed than for *S. maltophilia*. Resistance rates for MER and CAZ were >30% for both antibiotics.

The clinical relevance of isolation of *Achromobacter* spp. in the sputum of CF patients is unclear. Some studies have demonstrated that its presence is associated with a risk of pulmonary exacerbation but not with a worsened long-term prognosis [15]. Also, *Achromobacter* isolated from patients with CF appear to be more virulent than those isolated from other sources [16]. The most active agents for *Achromobacter* were SXT and IPM. In contrast to previously published data, MER was less active than IPM [16]. CAZ, COL and TOB have been considered adequate for inhalation therapy [17]; however, in this study these antibiotics presented high MIC_{90} values.

The prevalence of *Ralstonia* and *Pandoraea* infection in CF is low. The pathogenic role of *Pandoraea* spp. appears to be due to the increase in the production of pro-inflammatory cytokines, but the clinical impact is still uncertain [18]. Although only a limited number of *Ralstonia* and *Pandoraea* isolates were tested, the current results demonstrated that IPM and SXT had good activity against *Pandoraea*, and CIP and SXT against *Ralstonia*, in agreement with previously published data [4,18,19].

As the scope of the initial research of the iABC project was to analyse *P. aeruginosa* susceptibility, a limitation of the current study is that some antibiotics suitable for non-fermenters other than *P. aeruginosa* were not included in the MIC panels. However, the findings of this study provide insights into the epidemiology and susceptible patterns of these micro-organisms from different geographical regions.

In conclusion, NFGNB other than *P. aeruginosa* are potential pathogens increasingly being isolated from respiratory samples of

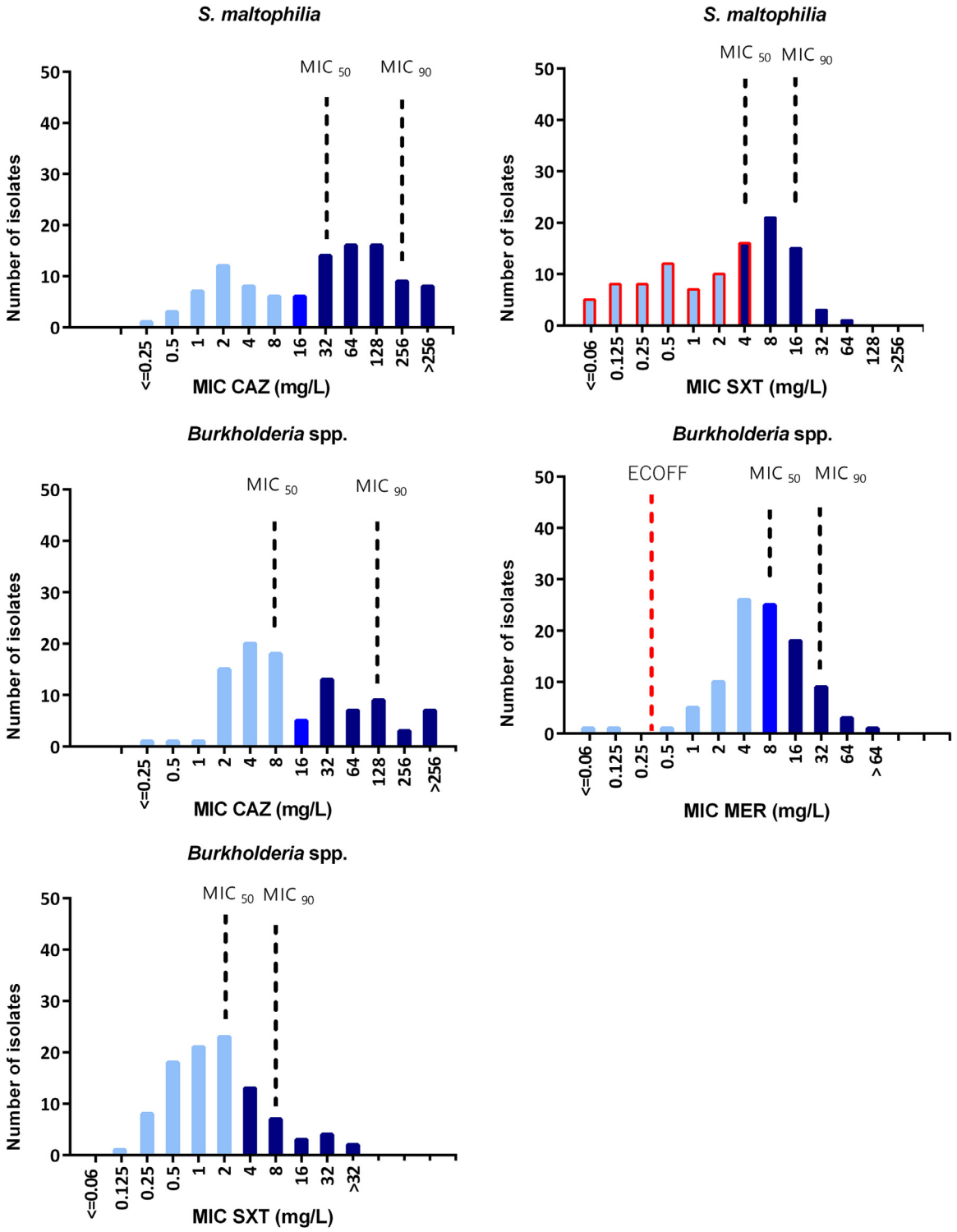

Fig. 1. Ceftazidime (CAZ), trimethoprim/sulfamethoxazole (SXT) and meropenem (MER) minimum inhibitory concentration (MIC) distribution for *Stenotrophomonas maltophilia* and *Burkholderia* spp. Susceptibility categorisation was performed using Clinical and Laboratory Standards Institute (CLSI) guidelines (susceptible, light blue; intermediate, medium-light blue; resistant, dark blue). For *S. maltophilia* and SXT, European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptible isolates are marked in red. In the case of *Burkholderia* spp. and MER, the epidemiological cut-off (ECOFF) is represented by a discontinuous red line. MIC50/90, MICs required to inhibit 50% and 90% of the isolates, respectively.

CF patients. Little is known about their epidemiology, clinical management and antimicrobial susceptibility. We provide susceptibility testing data to different antimicrobials to better define their antimicrobial susceptibility profile. Considering available clinical breakpoints, SXT was demonstrated to be the most active compound against all of the isolates tested.

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Declarations

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Competing Interests

None declared.

Ethical Approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.09.001.

References

[1] Magni A, Giordano A, Mancini C, Pecoraro C, Varesi P, Quattrucci S, et al. Emerging cystic fibrosis pathogens: incidence and antimicrobial resistance. New Microbiol 2007;30:59–62.
[2] de Dios Caballero J, del Campo R, Royuela A, Solé A, Máiz L, Olveira C, et al. Bronchopulmonary infection–colonization patterns in Spanish cystic fibrosis patients: results from a national multicenter study. J Cyst Fibros 2016;15:357–65. doi:10.1016/j.jcf.2015.09.004.
[3] Valenza G, Tappe D, Turnwald D, Frosch M, König C, Hebestreit H, et al. Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. J Cyst Fibros 2008;7:123–7. doi:10.1016/j.jcf.2007.06.006.
[4] Green H, Jones A. The microbiome and emerging pathogens in cystic fibrosis and non-cystic fibrosis bronchiectasis. Semin Respir Crit Care Med 2015;36:225–35. doi:10.1055/s-0035-1546752.
[5] Spilker T, Baldwin A, Bumford A, Dowson CG, Mahenthiralingam E, LiPuma JJ. Expanded multilocus sequence typing for *Burkholderia* species. J Clin Microbiol 2009;47:2607–10. doi:10.1128/JCM.00770-09.
[6] Ratjen A, Yau Y, Wettlaufer J, Matukas L, Zlosnik JEA, Speert DP, et al. In vitro efficacy of high-dose tobramycin against *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* isolates from cystic fibrosis patients. Antimicrob Agents Chemother 2015;59:711–13. doi:10.1128/AAC.04123-14.
[7] Waters V, Yau Y, Prasad S, Lu A, Atenafu E, Crandall I, et al. *Stenotrophomonas maltophilia* in cystic fibrosis: serologic response and effect on lung disease. Am J Respir Crit Care Med 2011;183:635–40. doi:10.1164/rccm.201009-1392OC.
[8] Goss CH, Mayer-Hamblett N, Aitken ML, Rubenfeld GD, Ramsey BW. Association between *Stenotrophomonas maltophilia* and lung function in cystic fibrosis. Thorax 2004;59:955–9. doi:10.1136/thx.2003.017707.
[9] Waters V, Atenafu EG, Lu A, Yau Y, Tullis E, Ratjen F. Chronic *Stenotrophomonas maltophilia* infection and mortality or lung transplantation in cystic fibrosis patients. J Cyst Fibros 2013;12:482–6. doi:10.1016/j.jcf.2012.12.006.
[10] Cantón R, Valdezate S, Vindel A, Sánchez Del Saz B, Maíz L, Baquero F. Antimicrobial susceptibility profile of molecular typed cystic fibrosis *Stenotrophomonas maltophilia* isolates and differences with noncystic fibrosis isolates. Pediatr Pulmonol 2003;35:99–107. doi:10.1002/ppul.10216.
[11] San Gabriel P, Zhou J, Tabibi S, Chen Y, Trauzzi M, Saiman L. Antimicrobial susceptibility and synergy studies of *Stenotrophomonas maltophilia* isolates from patients with cystic fibrosis. Antimicrob Agents Chemother 2004;48:168–71.
[12] Chang Y-T, Lin C-Y, Chen Y-H, Hsueh P-R. Update on infections caused by *Stenotrophomonas maltophilia* with particular attention to resistance mechanisms and therapeutic options. Front Microbiol 2015;6:1–20. doi:10.3389/fmicb.2015.00893.
[13] Wei C, Ni W, Cai X, Zhao J, Cui J. Evaluation of trimethoprim/sulfamethoxazole (SXT), minocycline, tigecycline, moxifloxacin, and ceftazidime alone and in combinations for SXT-susceptible and SXT-resistant *Stenotrophomonas maltophilia* by in vitro time–kill experiments. PLoS One 2016;11:1–9. doi:10.1371/journal.pone.0152132.
[14] Regan K, Bhatt J. Eradication therapy for *Burkholderia cepacia* complex in people with cystic fibrosis. Cochrane Database Syst Rev 2016:11:CD009876. doi: 10.1002/14651858.CD009876.pub3.
[15] Edwards BD, Greysson-Wong J, Somayaji R, Waddell B, Whelan FJ, Storey DG, et al. Prevalence and outcomes of *Achromobacter* species infections in adults with cystic fibrosis: a North American cohort study. J Clin Microbiol 2017;55:2074–85. doi:10.1128/JCM.02556-16.
[16] Filipic B, Malesevic M, Vasiljevic Z, Lukic J, Novovic K, Kojic M, et al. Uncovering differences in virulence markers associated with *Achromobacter* species of CF and non-CF origin. Front Cell Infect Microbiol 2017;7:224. doi:10.3389/fcimb.2017.00224.
[17] Wang M, Ridderberg W, Hansen CR, Høiby N, Jensen-Fangel S, Olesen HV, et al. Early treatment with inhaled antibiotics postpones next occurrence of *Achromobacter* in cystic fibrosis. J Cyst Fibros 2013;12:638–43. doi:10.1016/j.jcf.2013.04.013.
[18] Martina PF, Martínez M, Frada G, Alvarez F, Leguizamón L, Prieto C, et al. First time identification of *Pandoraea sputorum* from a patient with cystic fibrosis in Argentina: a case report. BMC Pulm Med 2017;17:1–5. doi:10.1186/s12890-017-0373-y.
[19] Prior AR, Gunaratnam C, Humphreys H. *Ralstonia* species—do these bacteria matter in cystic fibrosis? Paediatr Respir Rev 2017;23:78–83. doi:10.1016/j.prrv.2016.09.005.