

SUPPORTING INFORMATION

Variable susceptibility to gallium compounds of major cystic fibrosis pathogens

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Table S1. Characteristics of bacterial strains used in this study^a

Bacterial species	Strain	Country	Year	Source	Resistance	Reference
<i>Achromobacter xylosoxidans</i>	ATCC 27061 ^T	Japan	ns	ear discharge	ns	[1]
<i>A. xylosoxidans</i>	CF-2	Italy	2008-2010	respiratory secretion of CF patient	MDR	[2]
<i>A. xylosoxidans</i>	CF-3	Italy	2008-2010	respiratory secretion of CF patient	MDR	[2]
<i>A. xylosoxidans</i>	CF-4	Italy	2008-2010	respiratory secretion of CF patient	MDR	[2]
<i>Burkholderia cenocepacia</i>	LMG 16656 ^T	UK	1989	sputum of CF patient	ns	[3]
<i>B. cenocepacia</i>	FFC 0076	UK	ns	CF patient	ns	This study ^b
<i>Burkholderia dolosa</i>	LMG 18943 ^T	USA	ns	sputum of CF patient	ns	[4]
<i>B. dolosa</i>	FFC0305	USA	ns	CF patient	ns	This study ^b
<i>Burkholderia multivorans</i>	LMG 31010 ^T	Belgium	1992	CF patient	ns	[5]b
<i>B. multivorans</i>	454	Czech Republic	2002	CF patient	ns	This study ^b
<i>Haemophilus influenzae</i>	ATCC 49247	USA	1984	sputum of a pneumonia patient	ns	ATCC
<i>H. influenzae</i>	ATCC 9833	USA	ns	CF of patient with meningitis	ns	ATCC
<i>H. influenzae</i>	FC 89	Italy	2004-2009	CF patient	AMP, IPM	[6]
<i>H. influenzae</i>	FC 104	Italy	2004-2009	CF patient	ns	[6]
<i>Mycobacterium abscessus</i>	ATCC 19977 ^T	USA	1953	Knee infection	MDR	[7]
<i>M. abscessus</i>	ISS6	Italy	ns	unknown	MDR	This study ^b
<i>M. abscessus</i>	ISS7	Italy	ns	unknown	MDR	This study ^b
<i>M. abscessus</i>	ISS9	Italy	ns	unknown	MDR	This study ^b
<i>Pseudomonas aeruginosa</i>	ATCC 15692 (PAO1)	ns	ns	infected wound	ns	ATCC
<i>P. aeruginosa</i>	TR1	Italy	2009	respiratory secretion of CF patient	ns	[8]
<i>P. aeruginosa</i>	FM12	Italy	ns	respiratory secretion of CF patient	ns	[9]
<i>P. aeruginosa</i>	FM13	Italy	ns	respiratory secretion of CF patient	ns	[9]
<i>Staphylococcus aureus</i>	ATCC 25923	USA	1945	human clinical	ns	ATCC
<i>S. aureus</i>	BG-1	Italy	2016	CF patient	ns	This study ^b
<i>S. aureus</i>	BG-6	Italy	2016	CF patient	MRSA	This study ^b
<i>S. aureus</i>	BG-7	Italy	2016	CF patient	MRSA	This study ^b
<i>Stenotrophomonas maltophilia</i>	ATCC 13637 ^T	ns	ns	OP region of patient with mouth cancer	ns	ATCC
<i>S. maltophilia</i>	K279a	UK	ns	blood of non-CF patient	MDR	[10]
<i>S. maltophilia</i>	OBGTC23	Italy	2003-2005	sputum of CF patient	MDR	[11]
<i>S. maltophilia</i>	OBGTC26	Italy	2003-2005	sputum of CF patient	MDR	[11]
<i>Streptococcus pneumoniae</i>	ATCC 33400 ^T	ns	ns	unknown	ns	ATCC
<i>S. pneumoniae</i>	PFC-01	Italy	2010	CF patient	ns	[12]
<i>S. pneumoniae</i>	PFC-02	Italy	2010	CF patient	ns	[12]
<i>S. pneumoniae</i>	PFC-04	Italy	2010	CF patient	ns	[12]

^a Abbreviations: ATCC, American type culture collection; AMP, ampicillin; CF, cystic fibrosis; CSF, cerebrospinal fluid; IPM, imipenem; MDR, multi-drug resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; OP, Oropharyngeal; ns, not specified. ^T type strain.

^b Identification of strains as member of *B. cepacia* complex was carried out by a triphasic analysis: (i) growth on the selective *Burkholderia cepacia* agar base (ThermoFisher); (ii) positive identification using the commercial system API 20NE (bioMérieux, Marcy l'Etoile, France); (iii) molecular identification by *recA*-PCR-Restriction Fragment Length Polymorphism as previously described¹³. *M. abscessus* was isolated on Lowenstein-Jensen solid medium and identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics); *S. aureus* strains were identified by both manual (API-STAPH System; bioMérieux, Marcy-L'Etoile, France) and automate (VITEK 2 System, bioMérieux) biochemical test-based system

Table S2. Iron concentration in culture media used for Ga(III) susceptibility testing

Medium	Iron (μM)
CAMHB	3.305 ± 0.001
ID-CAMHB	0.143 ± 0.006
HTM	8.237 ± 0.008
DHTM	0.284 ± 0.001
THYB	17.136 ± 0.011
DTHYB	5.298 ± 0.002
ASM	3.880 ± 0.002
RPMI-CAA	0.210 ± 0.001
RPMI-HS-CAA	1.820 ± 0.002

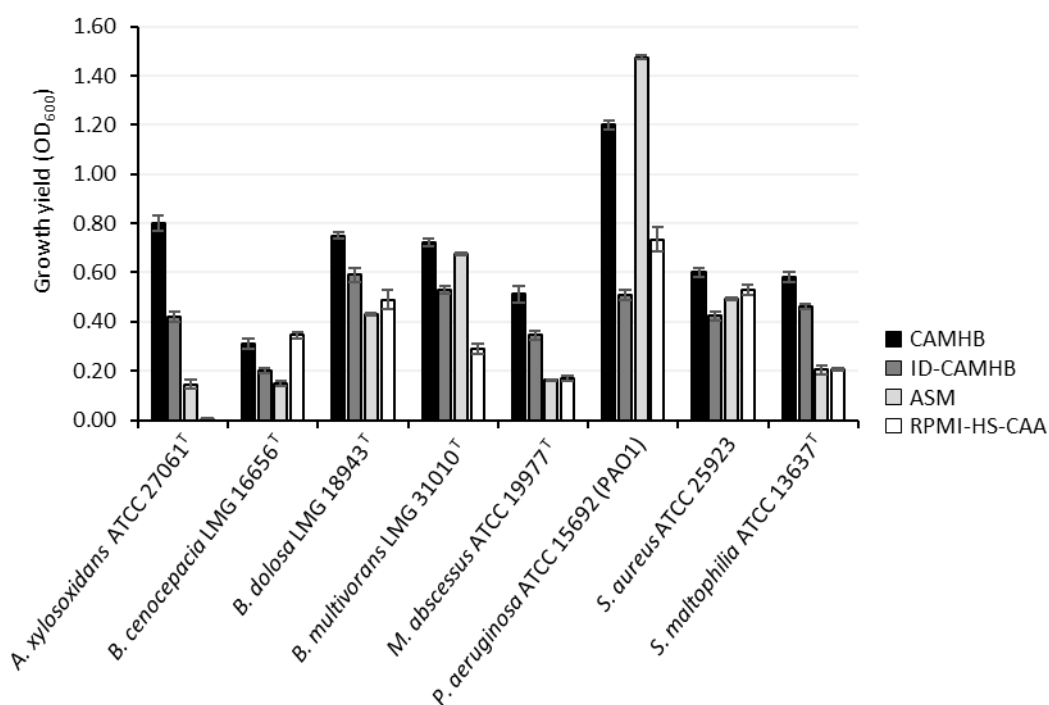


Figure S1. Growth of reference non-fastidious CF pathogens in CAMHB, ID-CAMHB, ASM and RPMI-HS-CAA. Individual bacterial strains were inoculated (*ca.* 5×10^5 CFU/ml) into 96-well microtiter plates containing CAMHB (black), ID-CAMHB (dark grey), ASM (light grey) and RPMI-HS-CAA (white). The OD₆₀₀ was determined after 24-h incubation at 37 °C for all strains except *M. abscessus* ATCC 19977^T which required 72-h incubation. Data are the means of triplicate experiments \pm standard deviation.

References

- [1] Yabuuchi, E., Oyama, A. (1971) *Achromobacter xylosoxidans* n. sp. from human ear discharge. *Jpn. J. Microbiol.* 15, 477-481. DOI: 10.1111/j.1348-0421.1971.tb00607.x.
- [2] Trancassini, M., Iebba, V., Citerà, N., Tuccio, V., Magni, A., Varesi, P., De Biase, R.V., Totino, V., Santangelo, F., Gagliardi, A., Schippa, S. (2014) Outbreak of *Achromobacter xylosoxidans* in an Italian Cystic fibrosis center: genome variability, biofilm production, antibiotic resistance, and motility in isolated strains. *Front. Microbiol.* 5, 138. DOI: 10.3389/fmicb.2014.00138.
- [3] Govan, J.R., Brown, P.H., Maddison, J., Doherty, C.J., Nelson, J.W., Dodd, M., Greening, A.P., Webb, A.K. (1993) Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. *Lancet* 342, 15-19. DOI: 10.1016/0140-6736(93)91881-l.
- [4] Coenye, T., LiPuma, J.J., Henry, D., Hoste, B., Vandemeulebroecke, K., Gillis, M., Speert, D.P., Vandamme, P. (2001) *Burkholderia cepacia* genomovar VI, a new member of the *Burkholderia cepacia* complex isolated from cystic fibrosis patients. *Int. J. Syst. Evol. Microbiol.* 51, 271-279. DOI: 10.1099/00207713-51-2-271.
- [5] Vandamme, P., Holmes, B., Vancanneyt, M., Coenye, T., Hoste, B., Coopman, R., Revets, H., Lauwers, S., Gillis, M., Kersters, K., Govan, J.R. (1997) Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. *Int. J. Syst. Bacteriol.* 47, 1188-1200. DOI: 10.1099/00207713-47-4-1188.
- [6] Cardines, R., Giufrè, M., Pompilio, A., Fiscarelli, E., Ricciotti, G., Di Bonaventura, G., Cerquetti, M. (2012) *Haemophilus influenzae* in children with cystic fibrosis: antimicrobial susceptibility, molecular epidemiology, distribution of adhesins and biofilm formation. *Int. J. Med. Microbiol.* 302, 45-52. DOI: 10.1016/j.ijmm.2011.08.003.
- [7] Moore, M., Frerichs, J.B. (1953) An unusual acid-fast infection of the knee with subcutaneous, abscess-like lesions of the gluteal region; report of a case with a study of the organism, *Mycobacterium abscessus*, n. sp. *J. Invest. Dermatol.* 20, 133-69. DOI: 10.1038/jid.1953.18.
- [8] Bragonzi, A., Paroni, M., Nonis, A., Cramer, N., Montanari, S., Rejman, J., Di Serio, C., Döring, G., Tümmler, B. (2009) *Pseudomonas aeruginosa* microevolution during cystic fibrosis lung infection establishes clones with adapted virulence. *Am. J. Respir. Crit. Care Med.* 180, 138-145. DOI: 10.1164/rccm.200812-1943OC.
- [9] Massai, F., Imperi, F., Quattrucci, S., Zennaro, E., Visca, P., Leoni, L. (2011) A multitask biosensor for micro-volumetric detection of N-3-oxo-dodecanoyl-homoserine lactone quorum sensing signal. *Biosens. Bioelectron.* 26, 3444-3449. DOI: 10.1016/j.bios.2011.01.022.
- [10] Avison, M.B., Higgings, C.S., von Heldreich, C.J., Bennett, P.M., Walsh, T.R. (2001) Plasmid location and molecular heterogeneity of the L1 and L2 β -lactamase genes of *Stenotrophomonas maltophilia*. *Antimicrob. Agents Chemother.* 45, 413-419. DOI: 10.1128/AAC.45.2.413-419.2001.
- [11] Di Bonaventura, G., Prosseda, G., Del Chierico, F., Cannavacciuolo, S., Cipriani, P., Petrucca, A., Superti, F., Ammendolia, M.G., Concato, C., Fiscarelli, E., Casalino, M., Piccolomini, R., Nicoletti, M., Colonna, B. (2007) Molecular characterization of virulence determinants of *Stenotrophomonas maltophilia* strains isolated from patients affected by cystic fibrosis. *Int. J. Immunopathol. Pharmacol.* 20, 529-537. DOI: 10.1177/039463200702000311.
- [12] Pimentel de Araujo, F., D'Ambrosio, F., Camilli, R., Fiscarelli, E., Di Bonaventura, G., Baldassarri, L., Visca, P., Pantosti, A., Gherardi, G. (2014) Characterization of *Streptococcus pneumoniae* clones from paediatric patients with cystic fibrosis. *J. Med. Microbiol.* 63, 1704-1715. DOI: 10.1099/jmm.0.072199-0.
- [13] Mahenthalingam, E., Bischof, J., Byrne, S.K., Radomski, C., Davies, J.E., Av-Gay, Y., Vandamme, P. (2000) DNA-Based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J. Clin. Microbiol.* 38, 3165-3173. DOI: 10.1128/JCM.38.9.3165-3173.2000.