Detection and Identification of CF Pathogens

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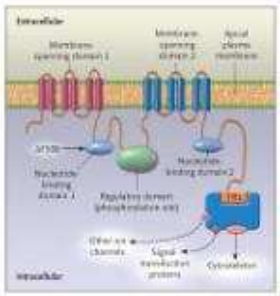
Pathophysiology of CF lung disease



CFTR gene defect

↓

Defective ion transport

↓

Abnormal mucus

↓

Defective mucociliary clearance

↓

Microbial development

Local inflammation

Alteration of endobronchial epithelium

↓

Deterioration of lung function

Microbial communities in the CF lung

Abiotrophia defectiva
Acetobacter indonesiensis
Achromobacter xylosoxidans
Acinetobacter baumannii
Acinetobacter sp.
Actinomyces sp.
Alcaligenes faecalis
Bergeyella sp.
Brevundimonas diminuta
Burkholderia cenocepacia
Burkholderia cepacia
Burkholderia dolosa
Burkholderia gladioli
Burkholderia group AD
Burkholderia group AT
Burkholderia multivorans
Burkholderia pyrrocina
Burkholderia stabilis
Burkholderia vietnamiensis
Capnocytophaga sp.
Carnobacterium sp.
Chromobacter violaceum
Chryseobacterium meningiseptica
Citrobacter freundii
Comamonas testosteroni
Dialister pneumosintes
Dolosigranulum pigrum
Eikenella corrodens
Enterobacter sp.
Escherichia coli
Gemella haemolysans
Gemella sanguinis
Gemella morbillorum
Granulicatella adiacens
Haemophilus influenzae
Haemophilus parainfluenzae
Herbaspirillum frisingense
Herbaspirillum huttiense
Herbaspirillum putei
Herbaspirillum seropedicae
Herbaspirillum sp.
Inquilinus limosus
Kingella denitrificans
Kingella oralis
Klebsiella pneumoniae
Kluyvera ascorbata
Lactobacillus delbrueckii
Lachnospiracene genomosp.
Moraxella catarrhalis
Morganella morganii
Mycobacterium abscessus
Mycobacterium avium complex
Mycobacterium chelonae
Mycobacterium gordonae
Mycobacterium simiae
Mycobacterium tuberculosis
Neisseria sp.
Ochrobactrium anthropi
Pandorea apista
Pandorea pnomenusa
Pandorea pulmonicola
Pandorea sputorum
Peptostreptococcus sp.
Porphyromonas sp.
Prevotella denticola
Prevotella melaninogenica
Prevotella oris
Prevotella salivae
Prevotella sp.
Proteus mirabilis
Proteus vulgaris
Providencia sp.
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas huttiensis
Pseudomonas putida
Ralstonia basilensis
Ralstonia gilardi
Ralstonia insidiosa
Ralstonia mannitolytica
Ralstonia metallidurans
Ralstonia pickettii
Ralstonia respiraculi
Rhizobium radiobacter
Rothia mucilaginosa
Salmonella Typhimurium
Segniliparus rugosus
Selenomonas infelix
Selenomas noxia
Selenomas sp.
Serratia marcescens
Sphingobacterium multivorum
Sphingobacterium spiritivorum
Staphylococcus aureus
Staphylococcus sp.
Stenotrophomonas maltophilia
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus constellatus
Streptococcus cristatus
Streptococcus genomospecies
Streptococcus gordonii
Streptococcus iniae
Streptococcus mitis
Streptococcus parasanguis
Streptococcus pneumoniae
Streptococcus salivarius
Streptococcus sanguinis
Streptococcus sp.
Tannerella forsythensis
Veillonella atypica
Veillonella sp.
Xanthomonas kyacinthi
Yersinia pseudotuberculosis
...
Fungi
Viruses
...

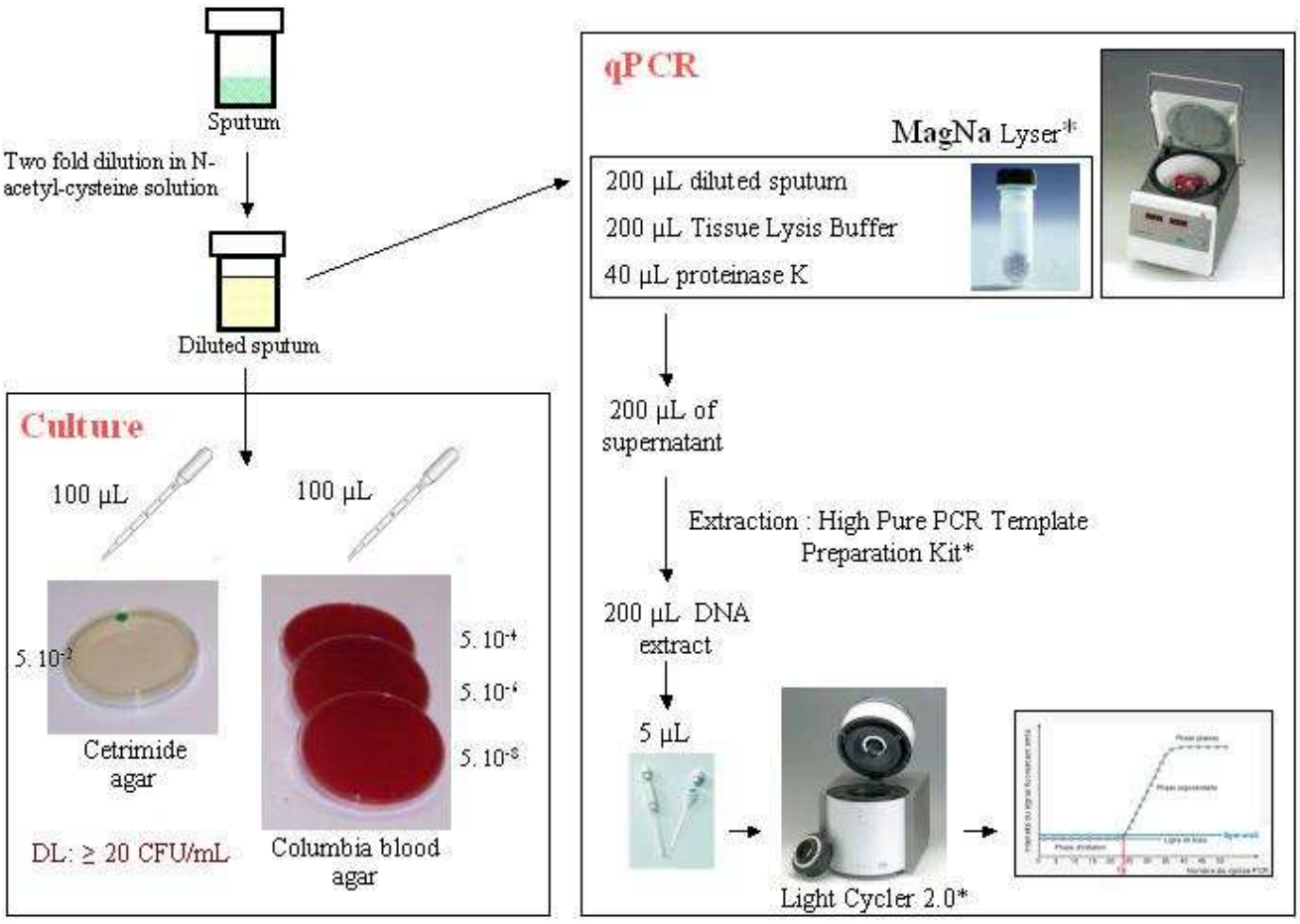
Bacterial populations in the CF lung

- ✓ Increase quantitatively and qualitatively with patient's age
- ✓ Are composed of commensal and environmental species some being unique to CF
- ✓ Fluctuate over time
- ✓ Are heterogenously distributed in the airways
- ✓ Are patient specific
- ✓ Colonize the mucus rather than the endobronchial epithelium
- ✓ Generate chronic inflammation of the respiratory tree through still poorly known factors and specific strain-patient interactions

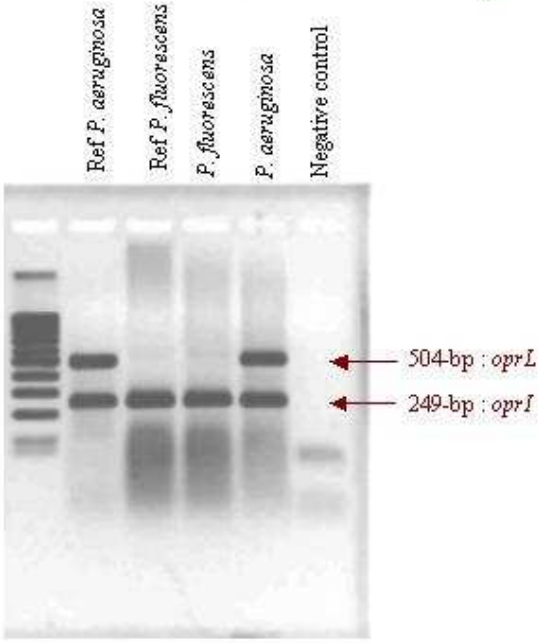
Pathogenicity of microorganisms

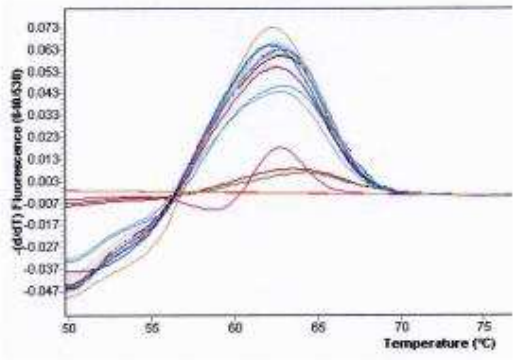
Organisms	Frequency of isolation (%)	CF population	Role in CF lung disease
Pseudomonas aeruginosa	59	all	proven
Staphylocccus aureus	48	mostly children and adolescents	proven
Burkholderia cepacia complex	3	mostly adolescents and adults	proven
Stenotrophomonas maltophilia	10	mostly adolescents and adults	not proven
Achromobacter xylosoxidans	9	mostly adolescents and adults	unlikely
Burkholderia gladioli	<1	mostly adolescents and adults	unlikely
Ralstonia sp.	<1	mostly adolescents and adults	unlikely
Pandoraea sp.	<1	mostly adolescents and adults	possible
Mycobacterium sp.	13	mostly adolescents and adults	proven
Haemophilus influenzae	15	children	likely
Streptococcus pneumoniae	5	children	unknown
Enterobacteriaceae	<5	children	unknown
Aspergillus fumigatus	9	all	proven
RSV	unknown	children	proven
Influenza virus	unknown	all	proven

Gilligan P. et al. Cumitech 43, ASM



Detection of the *oprL* gene by qPCR





Quantitation of *Pseudomonas aeruginosa* in wound biopsy samples: from bacterial culture to rapid 'real-time' polymerase chain reaction

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Crit Care 2000, 4:295–291

De Vos D. et al. *J. Clin Microbiol*. 1997

Xu J. et al. *Ann. Clin. Microb. Antimicrob*. 2004

Positivity of qPCR in chronically colonized patients

(62 sputum samples from 27 patients)

		CULTURE		Total
		NEG	POS	
PCR	NEG	0	1	1
	POS	2	59	61
Total		2	60	62

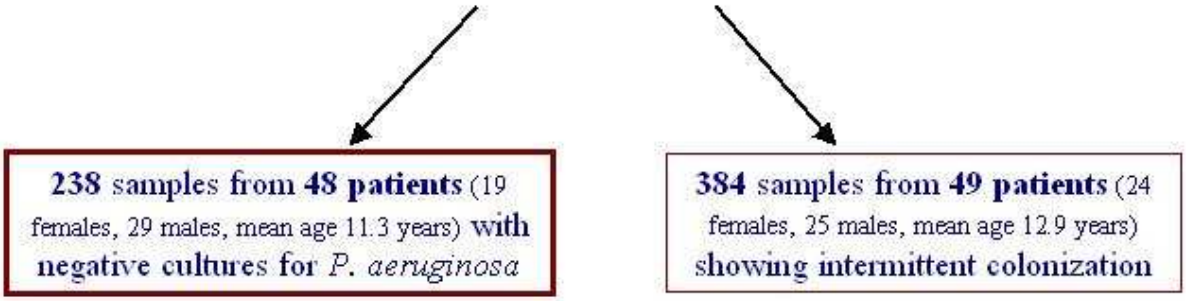
95% concordant results

> 98% detection with qPCR

Design of the study

From June 2003 to February 2009

622 sputum samples



48 culture negative (CN) patients

(238 samples)

No of samples	1	2	3	4	5	6	7	8	9	10	11-15	16-20	>20
No of patients	6	10	10	3	6	3	4	1	0	1	2	1	1

		CULTURE		Total
		NEG	POS	
	PCR inhibition	2	0	2
PCR	NEG	215	1	216
	POS	12	8	20
	Total	229	9	238

13 discordant results (5.5 %)

Culture negative (CN) patients

(13 samples)

Patient	Culture CFU/mL	PCR Copies/mL	Therapy	Subsequent detection of *P. aeruginosa*
MAR	< 100	-	Cipro/colistin	NC 50 months later
BUG	-	$< 10^3$	Caz/tobra	NC 3 months later
CHA	-	$< 10^3$	No	NC 28 months later
CIN	-	$< 10^3$	No	NC 51 months later
HAL	-	$< 10^3$	No	PC 6 months later
LEZ	-	$< 10^3$	No	NC 12 months later
M'BA	-	$2.\ 10^3$	No	PC 29 months later
MIT	-	$< 10^3$	No	NC 65 months later
US	-	10^2	No	NC 56 months later
VER	-	10^2	No	NC 59 months later
	-	$2.\ 10^3$	No	NC 10 months later
	-	$< 10^3$	No	NC 9 months later
GER	-	$< 10^3$	No	PC 1 month later

Very low bacterial loads

3/11 patients became CP

Design of the study

From June 2003 to February 2009

622 sputum samples

238 samples from **48 patients** (19 females, 29 males, mean age 11.3 years) with negative cultures for *P. aeruginosa*

384 samples from **49 patients** (24 females, 25 males, mean age 12.9 years) showing intermittent colonization

49 Patients with intermittent colonization

(384 sputum samples)

No of samples	1	2	3	4	5	6	7	8	9	10	11-15	15 -20	> 20
No of patients	1	3	5	1	5	3	5	8	6	5	5	1	2

		CULTURE		Total
		NEG	POS	
PCR	NEG	297	6	303
	POS	30	51	81
	Total	327	57	384

36 discordant results (9.4 %)

CP patients with intermittent colonization

(6 samples)

Patient	Culture CFU/mL	PCR copies/mL	Therapy	Subsequent detection of *P. aeruginosa*
BLA	< 100	-	No change	3 months later : Culture - / PCR -
ROG	< 100	-	No	3 months later : Culture + / PCR -
	< 100	-	No	3 months later : Culture - / PCR +
PAQ	< 100	-	Initiation	3 months later : Culture - / PCR -
LOM	< 100	-	Initiation	3 months later : Culture - / PCR ND
HAL	< 100	-	No change	3 months later : Culture - / PCR -

Very low bacterial loads

1/5 patient remained CP or PCR +

CN patients with intermittent colonization, treated

(11/30 samples)

Patient	Culture CFU/mL	PCR Copies/mL	Therapy	Subsequent detection of *P. aeruginosa*
FON	-	< 10^3	No change	3 months later : Culture + / PCR +
	-	4 .10^3	Change	3 months later : Culture - / PCR -
JAS	-	10^3	Change	1 month later : Culture - / PCR ND
SEM	-	10^3	No change	3 months later : Culture + / PCR ND
	-	10^4	No change	1 month later : Culture + / PCR +
	-	10^4	No change	1 month later : Culture - / PCR +
	-	< 10^3	Change	2 months later : Culture - / PCR -
	-	< 10^3	Change	3 months later : Culture - / PCR -
BIS	-	< 10^3	No change	1 month later : Culture + / PCR ND
BON	-	< 10^3	No change	3 months later : Culture + / PCR ND
SOC	-	< 10^3	No change	1 month later : Culture + / PCR ND

Very low bacterial loads

3/3 patients with therapeutic adaptation became CN

CN patients with intermittent colonization, not treated

(19/30 samples)

Patient	Culture CFU/mL	PCR Copies/mL	Therapy	Subsequent detection of *P. aeruginosa*
ANC	-	< 10^3	Initiation	3 months later : Culture + / PCR ND
BLA	-	< 10^3	No	3 months later : Culture - / PCR -
	-	< 10^3	No	3 months later : Culture - / PCR +
	-	< 10^3	No	3 months later : Culture -/ PCR+
BOS	-	7		lture - / PCR +
	-	<		lture - / PCR +
	-	<		lture +/ PCR +
DEL	-			lture - / PCR -
BRU	-	<		lture - / PCR -
EL H	-	<		ture - / PCR +
	-	<		ure + / PCR +
GER	-	< 10^3	No	1 month later : Culture + / PCR +
JAS	-	< 10^3	No	1 month later : Culture + / PCR ND
MER	-	< 10^3	Initiation	3 months later : Culture - / PCR -
ROG	-	< 10^3	No	3 months later : Culture - / PCR -
SOS	-	< 10^3	No	3 months later : Culture - / PCR -
	-	< 10^3	No	3 months later : Culture + / PCR ND
VAL	-	< 10^3	No	3 months later : Culture - / PCR -
	-	< 10^3	No	3 months later : Culture + / PCR +

Not treated		Initiation	
CN	CP	CN	CP
3	5	2	2

Conclusion

- ✓ qPCR does not seem to be useful for follow up of patients chronically colonized with *P. aeruginosa*
- ✓ qPCR is complementary to culture for early detection of *P. aeruginosa* in patients with previous NC
- ✓ Study by Xu J. et al showed that *oprL* qPCR was positive 4-17 months prior culture in 5 out of 10 CN patients
- ✓ Discordant results between qPCR (+) and culture (-) can be attributed to very low bacterial loads, to quiescent or non viable bacteria
- ✓ qPCR positive results in previously CN patients should be taken into account by clinicians for preventing development of colonization

Xu J. et al. *Ann. Clin. Microb. Antimicrob.* 2004, 3:21

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