



HOPITAL ERASME

ULB



Comparison of chromogenic and selective media for the detection of *S. aureus* and *P. aeruginosa* in respiratory samples from CF patients

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Background

Cystic fibrosis (CF)

- Chronic bacterial infections contribute to a decline in lung function
- *S. aureus* (SA)
 - 44% colonisation and infection in Belgium (multicentric study)¹
 - 4.2% (0-23%) small colony variants (SCV) ~ underdetection?
- *P. aeruginosa* (PA)
 - 20-70% in Belgium (multicentric study)²
 - Abnormal phenotypes (mucoid, SCV)
- SCV ~ persistent infection

¹Vergison A et al. National survey of molecular epidemiology of *Staphylococcus aureus* colonization in Belgian cystic fibrosis patients. J Antimicrob Chemother. 2007 May;59(5):893-9

² Scientific Institute of Public Health. The belgian cystic fibrosis registry. Annual report 2007, Brussels, 2009.

Objectives

- To compare the performance of chromogenic media with conventional media for the detection and the presumptive identification of *S. aureus* and *P.aeruginosa*
 - normal phenotype
 - variants phenotypes (SCV, mucoid)

Methods

■ Materials

- 159 respiratory samples from 64 CF patients in a CF reference centre
- COL, HAEM, MAN, MAC and BCSA usual plates
- SAID and PAID chromogenic plates
- Incubated at 35°C for 5 days, examined daily

■ Presumptive identification

- SAID: suspect colonies (characteristic growth, morphology and colour)
- PAID: all

■ Phenotypic identification

- SA: coagulase
- PA: oxidase, ADH, 42°, Kligler

■ Genotypic confirmation

- SA: *nuc* and *mecA* genes, 16S rRNA
- PA (if atypical biochemical profile): 16S rRNA sequencing

■ Antimicrobial susceptibility testing

- Normal SA: Vitek2
- SCV-SA: disk diffusion, Vancomycin agar screen
- PA: disk diffusion

Results

Results

Prevalence of *S. aureus* and *P. aeruginosa* colonization

	Number of sputum samples ^a (n=159)	Number of isolates	Number of CF patients ^b (%) (n=64)
<i>S. aureus</i>	48	79	33 (51.6)
- MRSA*		11	6 (9.4)
- SA-SCV		21	11 (17.2)
<i>P. aeruginosa</i>	72	133	33 (51.6)
- mucoid		54	19 (29.7)
- PA-SCV		33	19 (29.7)
SA+PA	22		17 (26.6)

• *All *mecA* +

• Note: SA= *S. aureus*; PA= *P. aeruginosa*; SCV= small colony variant; CF= cystic fibrosis

• ^aMean sputum samples per patient = 2.6; ^bMedian age = 30 years (range 5-60)

Results

Analytical performance of MAN and SAID media for *S. aureus* detection

Medium	MAN	SAID	
No. of SA isolates* (N=79)	64	71	
-SCV (n=21)	18	21	
Sensitivity (%)	81.0	89.9	<i>p=0.107</i>
Specificity (%)	77.1	67.2	
NPV (%)	85.9	92.0	
PPV (%)	70.3	61.2	

Note: CF= cystic fibrosis; SA= all *S. aureus*; SCV= small colony variant

* 2 only recovered Colombia blood agar

Results

Morphological aspect of SCV *S. aureus*

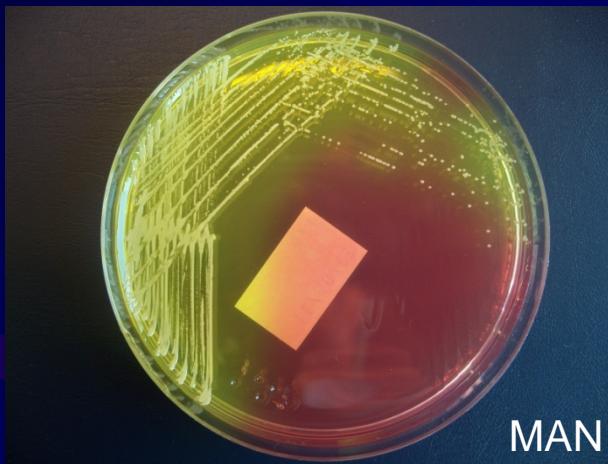
>24h



>24h



>24h



>48h



Antimicrobial resistance profile of SA (n=79) isolates

Antimicrobials*	PEN	FOX	VAN	CIP	GEN	SXT	LIN
N-SA (%) (n=58)	82.4	9.8 ^a	0	23.5	5.9	2.0	0
SCV-SA (%) (n=21)	76.0	28.6 ^a	0	42.9	9.5	95.2	0

Note: N-SA= normal phenotype *S.aureus*; SCV= small colony variant

•*PEN, penicillin; CEF, cefoxitin; VAN, vancomycin; CIP, ciprofloxacin; GEN, gentamicin; SXT, cotrimoxazole; LIN, linezolid

•^a All *mecA* +

Results

Analytical performance of MAC and PAID media for *P. aeruginosa* detection

	MAC	PAID	
No. of PA isolates* (N=133)	101	118	
-Mucoid (n=54)	53	49	
-SCV (n=33)	18	28	<i>p=0.025</i>
Sensitivity (%)	75.9	88.7	<i>p=0.013</i>
Specificity (%)	64.5	90.8	
PPV (%)	78.9	92.9	
NPV (%)	60.5	85.6	

Note: CF= cystic fibrosis; PA= all *P.aeruginosa*; SCV= small colony variant

* 2 only recovered on Haemophilus agar; 1 only on *Burkholderia cepacia* selective agar

Results

Morphological aspect of normal phenotype *P. aeruginosa*



>48h



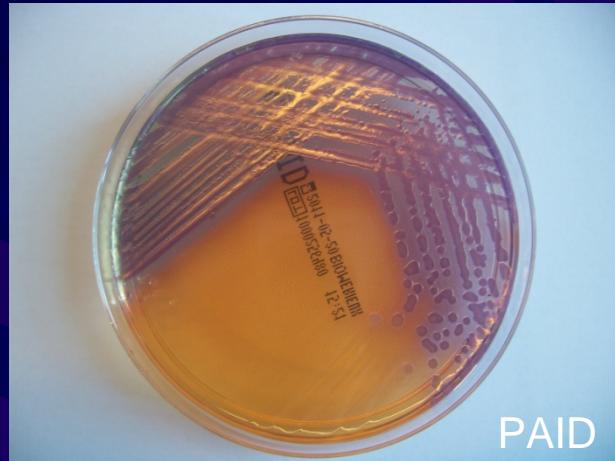
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Results

Morphological aspects of mucoid *P. aeruginosa*



MAC

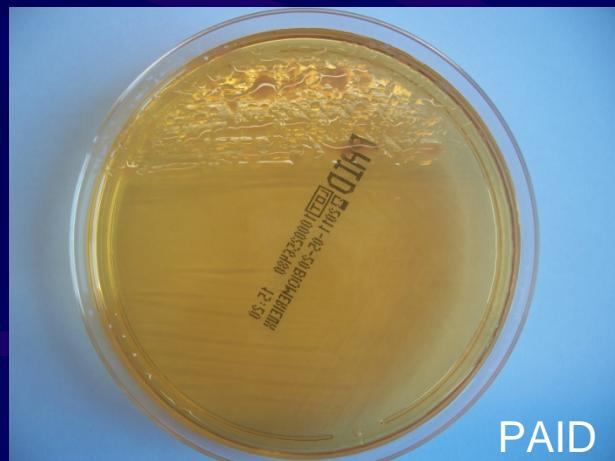


PAID



PAID

>48h

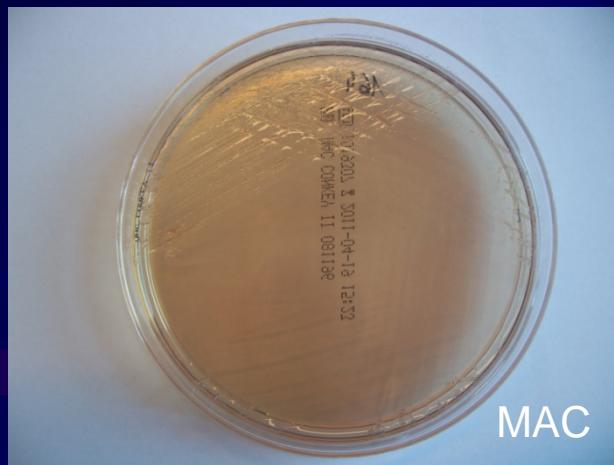


PAID

>48h

Results

Morphological aspect of SCV *P. aeruginosa*



>48h



>48h

Results

Antimicrobial resistance profile of PA (n=133) isolates

Antimicrobials *	MER	AKN	TOB	ATM	CAZ	CFP	TZP	CIP
N-PA (%) (n=46)	17.8	33.3	13.3	22.2	28.9	22.2	6.7	20.0
SCV-PA (%) (n=33)	29.4	44.1	23.5	38.2	41.2	35.3	20.6	29.4
Mucoid PA (%) (n=54-10) ^a	25.0	38.6	11.0	27.2	31.8	38.6	22.7	18.2

• Note: N-PA= normal phenotype *P.aeruginosa*; SCV= small colony variant

• * MER, meropenem; AKN, amikacin; TOB, tobramycin; ATM, aztreonam; CAZ, ceftazidime; CFP, cefepime; TZP, piperacillin-tazobactam; CIP, ciprofloxacin

•^a not available for 10 isolates

Conclusions

Conclusion –

- Prevalence of *S. aureus* and *P. aeruginosa* among CF patients: 52%
 - SA-SCV: 18%; MRSA: 10%
 - PA-SCV and mucoid PA: 30%
- PAID chromogenic media demonstrated better performances than conventional media
 - For detection of PA isolates
 - For recovery of SCV
 - But translucent colonies should be identified to exclude PA
- SAID had limited ability to differentiate SA from the polymicrobial flora in CF sputa (PPV=61.2%)
- *S. aureus* and *P. aeruginosa* SCVs were more resistant to antimicrobials than normal phenotype

Acknowledgements



Results



MAC



PAID

Achromobacter xylosoxydans plated onto MAC and PAID