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Early View

Original article

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Original article

Title page

Chest physiotherapy enhances detection of *Pseudomonas aeruginosa* in non-expectorating CF children

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Take home message:

Sputum collected after a chest physiotherapy session strongly enhances the detection of *P. aeruginosa* in non-expectorating CF children compared to the commonly used oropharyngeal swabs. Oropharyngeal swabs collected after chest physiotherapy may be an alternative.

Abstract

Lung damage in Cystic Fibrosis (CF) is strongly associated with lower airway infections. Early treatment of *Pseudomonas aeruginosa* is recommended. Pathogen detection requires sampling of lower airway secretions, which remains a challenge in non-expectorating patients. Our hypothesis was that chest physiotherapy would improve the quality of airway secretion samples and increase the rates of pathogens detected in non-expectorating patients.

This prospective multicentre study compared three successive methods for sampling airway secretions applied through a same session: 1) oropharyngeal swab (OP); 2) sputum collected after chest physiotherapy (CP-SP); and 3) oropharyngeal swab 2 performed after chest physiotherapy(CP-SP-CP-OP). *Haemophilus influenzae*, *Staphylococcus aureus* and *P. aeruginosa* (Pa) growth cultures were assessed. Accuracy tests and an equivalence test was performed to compare the three successive methods of collection. Three-hundred non-expectorating children with CF were included. Pa was detected cumulatively in 56 (18.9%) children and according to the collection techniques in 28 (9.8%), 37(12.4%) and 44 (15%) children by using CP-SP and CP-OP, respectively. Compared to OP, the increased detection rate was +22% for CP-OP, p=0,029 and +57% for CP-SP, p=0.003. CP-SP had the best positive predictive value (PPV) (86.3%) and negative PV (96%) for *Pa* compared to the overall detection. The results of this adequately powered study show differences in the rates of pathogens detected according to the sampling method used. Chest physiotherapy enhanced detection of *P. aeruginosa* in non-expectorating children with CF.

Introduction

Cystic fibrosis (CF) is a genetic disease characterised by altered lower airway clearance and a recurrent lower airway infections, which both concourse to CF related lung disease progression. Patients' follow-up should target the early detection of lower airway colonisation by potentially pathogenic microorganisms. Thus, current guidelines recommend sampling airway secretions for microbiological culture and analysis at least four times per year and proposing adapted antibiotic treatment(1).

Indeed, early detection of *P. aeruginosa* (Pa) in CF is crucial as early treatment is a key to delay *Pa*-related chronic bronchial infection (2). *Pa* colonisation may be present in young children who are not yet able to expectorate spontaneously, i.e. 17% in 4 year aged children(3). The accuracy of the evaluation of microbiological status of CF patients depends on the quality of both airway samples and microbiological procedures. Thus, it is crucial to optimise sampling of lower airway secretions in CF. Currently, bronchoalveolar lavage (BAL) is considered as the gold standard but cannot however be performed repeatedly. Moreover, a five-year BAL- versus oropharyngeal swab (OP) -directed therapy trial has been conducted for treating exacerbations in young CF children. This showed similar clinical and radiological outcomes in both groups (4). When patients are able to expectorate, results obtained in spontaneous sputum are considered to reflect the microbiological status of the lower airways. However, many children are unable to expectorate as they are not able or do not want enough or have not enough secretions to expectorate (5, 6).

Currently, oropharyngeal swab is a common sampling method. Nevertheless, available validation studies display notable differences in positive predictive values (PPV) and to a lesser extent in negative predictive values (NPV) compared to other methods (7-11). Moreover, sputum cultures are likely to be better indicators of the bronchial microbiological flora than OP swabs, as reported by at least two studies in expectorating (10, 12) and one in

non-expectorating patients (13). Since these studies, induced sputum collection by nebulised hypertonic saline solution has been proposed (13-21) with a good microbiological yield, but is consuming. In contrast, chest physiotherapy (CP) has rarely been reported (16) as a reliable method for obtaining sputum in non-expectorating children, although it is already used on a daily basis to improve airway clearance in CF.

In this present study, we focused on the methods used to sample airway secretions, and formulated the hypothesis that sputum collected after a chest physiotherapy course provided more accurate samples than oropharyngeal swabs in non-expectorating children, as previously suggested in CF patients with productive cough (22).

Patients and Methods

This prospective multicentre study was conducted in 16 French tertiary CF centre from 01/01/2006 to 01/01/2008. The included patients fulfilled the following criteria: (1) confirmed diagnosis of CF, (2) regularly followed up in a tertiary paediatric CF centre, (3) aged 18 years or less, (4) unable to spontaneously expectorate either routinely or during a pulmonary exacerbation. The study was approved by the Ethics Committee of Haute-Normandie (CCP-SPHN), and informed consent was obtained from all parents and children when relevant.

Study design: Demographic data, history of the microbiological status of the children and ability to expectorate were collected. During the appointment at the CF centre, the chest physiotherapist collected 3 airway secretion samples during the dedicated session by using three successive methods: 1. An oropharyngeal swab was first applied (OP); 2. A chest physiotherapy session followed by a provoked cough to obtain sputum (CP-SP); 3. A second oropharyngeal swab (CP-OP) was collected after chest physiotherapy.

Airway secretion sampling: The chest physiotherapists all applied a standardised operating procedure across the participating CF centres. The child was maintained in a sitting position and nasal lavage was first performed carefully by flushing 5ml isotonic saline serum in each nostril until the liquid returned clear. The nasal fluid was evacuated either through the other nostril or was aspirated by introducing a suction catheter (6F, Vygon Corp., France) through each nostril as far as the nasopharynx. Oral lavage was performed in older children with sterile water. OP swabs were rubbed on the tonsils and pharynx without touching the buccal mucosa. Then, the chest physiotherapists applied at least four series of 15 expiratory flow ventilations to the infant or child chest with an empty stomach, and drained the bronchial secretions to the lower pharynx. Cough at the end of chest physiotherapy was either spontaneous or gentle provoked by pressing the thumb on the trachea in younger patients in order to collect CP-SP spontaneously whenever possible, or quickly suctioned into a sterile vial (catheter AM10610P, Cair LGL Corp., France).

Microbiological analyses: The airway secretion samples were transported to the microbiology laboratory within two hours and each sample was processed separately. A standardised operating procedure based on the French standard operating procedures (23) was used in all CF centres. Pure and diluted liquefied sputum samples as well as oropharyngeal swabs were inoculated and incubated onto several non-selective and selective media isolation, notably for *Hi, Sa* and *Pa* detection and quantification. All media were incubated aerobically at 37°C for five days, and monitored daily. All different morphotypes of bacterial colonies were identified. For sputum samples only, quantification was conducted based on the Colony Forming Unit per millimeter (CFU/mL) counts and the dilution ratio of the plates. The study of antibiotic resistance was carried out according to the CA-SFM/EUCAST guidelines(24). Quantitative cultures were only done from sputum obtained after chest physiotherapy. Other identified bacteria were noted as recommended, but only HI, Pa and Sa were accounted for the comparison of the three methods.

The number of patients needed was calculated according to an expected prevalence of 20% of *Pa in the studied population*, and a true detection of 87.5% of *Pa* and *Sa* and, 69.2% of *Hi* (10). The expected difference between the methods of sputum collection was set to 10% (14, 15). A total number of 300 non-expectorating patients were required to achieve a power of 90% for equivalence between the studied methods.

Statistical analysis: The results of the three methods were compared by using the McNamar test (Cochran Mantel Haenszel for dichotomy variables). An equivalence test was performed: the three methods for collecting secretions were considered as equivalent if the difference of true detection was ≤ 10%, i.e. the 90%CI of this difference was comprised within -10%;+10%. The analyses were also displayed with a 95%CI. Then, accuracy tests (predictive values, specificity and sensitivity) were calculated with reference to sputum collection (CP-SP), and according to positive results (OP+CP-SP+CP-OP). All the tests were performed at a bilateral risk of α=0.05, with SAS® 8.2 software, SAS Institute, NC, Cary, USA.

Results

Three-hundred children were included, but the collection of secretions failed in a child. Therefore, data from 299 children were analysed (Table I). Nasal and oral lavage were performed prior to sampling in 216 (73.5%) and 198 (68%) children, respectively. The acceptability of the procedures is reported in table 2 and was comparable between the three methods. No respiratory distress occurred. The results for sampling airway secretions and microbiology cultures are displayed in table 2. *Sa*, *Hi* and *Pa* were detected in at least one sample in 188 (63.7%), 77(26.1%) and 56 (19.0%) children, respectively. The results of growth cultures were concordant for the three methods in 176 (58.8%) patients (n=106 for positive cultures and n= 70 for negative cultures).

Equivalence tests of equivalence for the three methods: The results for the 3 bacteria are presented for both $CI_{90\%}$ (primary endpoint) and $CI_{95\%}$ (Table 3). Positive cultures obtained after CP-SP and CP-OP versus OP differed according to the studied bacteria. Although close to the $_{90\%}$CI equivalence for *Pa*, CP-SP appeared the most efficient method for the 3 bacteria. Conversely, OP and CP-OP; CP-SP and CP-OP appeared to be equivalent methods. The only difference was a better identification of *Sa* with CP-OP compared to OP. The results for $_{95\%}$CI indicated that CP-SP is a better method than CP-OP, which is a better one than OP for detecting these 3 germs.

Accuracy of analyses of microbiological growths obtained by the three methods:

CP-SP detected the three bacteria more frequently than OPs (p=0.013, Table I). *Pa* was detected in 56 (18.9%) children. Overall, the use of CP-SP, CP-OP and OP contributed to detect *Pa* infection in 44 (78.6%), 37 (66%) and 28 (50%) children, respectively. Thus, the use of CP-SP and CP-OP augmented the overall detection of *Pa* of +57% (p=0.003) and +22% (p=0.029), respectively compared to OP. In detail, CP-SP and CP-OP enabled the additional identification of 22 and 13 infected children who were not by OP, respectively (figure 1). Conversely, CP-SP compared to OP or CP-OP failed to detect positive *Pa* growths in 11 (19.6%) children. *Pa* was detected with both OP and 0P2 in 3 samples, with

OP only in 3 samples, with CP-OP only in 5 samples. All *Pa* strains detected by both OP and CP-SP had similar antibiotic susceptibilities.

Hi was detected in 77 (26.1%) children. In samples collected after CP, CP-SP and CP-OP, Hi was cultured in 59 (20%) and 50 (17. 1%) children, respectively compared to 43 (14.6%) children with OP. Therefore, the use of CP-SP identified 75.3% of the infected children leading to an additional detection rate of 34.9% (p=0.01) *Hi* compared to the use of OP. *Hi* carriage was detected with only OP and only CP-OP sampling in 11 (14.2%) and 15 (19.4%) infected children, respectively (figure2).

Sa grew in 181 (61%) children, and no significant difference was shown in the detection of additional patients irrespective of the chosen methods (figure 3).

Sensitivity, specificity, predictive values and accuracy of OP and CP-OP were calculated by referring to CP-SP results (Table 4). The OP methods had a weak sensitivity for *Pa* identification compared to CP-SP. The predictive values of each method were then analysed by referring to all bacterial isolation obtained from all three sampling methods: OP+CP-SP+CP-OP (Table 5). CP-SP provided the best PPV and NPV for *Pa and Sa*.

Additional effects of age, cough and symptoms, current antibiotics were analysed. None of them had any relationship with the results of the current analyses.

Acceptability of chest physiotherapy and sampling. Overall, chest physiotherapy was well accepted in 75.5% of in this young population, 55% were agitated, and 86.1% cried mostly before starting the technique. The acceptability of sampling was also good (72.2%) and nausea was observed in 13 (16.7%) children. No respiratory worsening or distress were reported.

Discussion

In this present study, we focused on the methods used to sample airway secretions, and two different statistical approaches were applied in this study. The first considered equivalence tests at both $_{90\%}$CI and $_{95\%}$CI, the second tested the accuracy of the methods used and reflected the assessment at an individual level. The equivalence tests showed that oropharyngeal swab before chest physiotherapy, and sputum collected after chest physiotherapy provided different results. Our results clearly sustained the hypothesis that sputum provided a higher yield for the three studied bacteria. Considering *Pa* results alone, one third of the children infected with this pathogen would not have been identified by using oropharyngeal swab cultures.

Few studies have validated methods for sampling airway secretions in non-expectorating children and those have mainly compared them with bronchoalveolar lavage, considered as the gold standard. Existing data in the literature regard the results of the 3 common potentially pathogenic bacteria in CF (7-10). The OP sensitivity, specificity, positive and negative predictive values varied widely from a study to another; the ranges for *Pa* were of 46-75%, 80-97%, 55-83% and 70-97%, respectively. The variations in these results might depend on various parameters, and mainly on the size of the tested population for each bacteria and consequently on their relevant number of positive and negative growths for statistical analyses. For instance, an overwhelming number of negative culture growths will overestimate the negative predictive value and consequently bias correct estimation of the positive predictive value. The up-to date largest Australian study compared 690 paired OP and BAL sampling cultures in 181 young children with a prevalence of 7.8% Pa infections (11). Oral swabs compared to BAL had a very low sensitivity and PPV for detecting Pa: 23% and 18.2%, respectively.

~~Therefore,~~ We powered our study to prevent any errors related to the size of the populations, according to the expected results of less powered studies, which compared sputum growth samples with samples obtained using other methods. Moreover, sputum analyses allowed quantification of

pathogens and the detection limit of culture growths was 10^2CFU/mL. In expectorating patients, bronchoalveolar lavage was better correlated with sputum than OP culture growths (10) and sputum has been shown to provide more sensitive airway material for microbiological cultures than OP (12). Seven studies (13-15, 17, 18, 20, 21) have explored the benefit of induced sputum cultures in smaller sized populations (19 to 125 children) with a range from 29 to 167 samplings. The additional yield of induced sputum compared to oropharyngeal swabs or spontaneous sputum collection varied from none to + 175% pathogen growths, and conversely enabled the identification of new pathogens in the seven studies. This underlined the risk of false negative cultures from oropharyngeal swabs. The recent large study compared the results of 169-paired samples of oropharyngeal swabs by coughing and induced sputum from 103 CF children. They identified at least one pathogen in 38% of induced sputum compared with 14% of swabs samples (21) . Ninety-two percent of the pathogens were isolated from sputum contrasting with 31% from samples collected with oropharyngeal swab by coughing. Their findings were independent of the presence of symptoms and age, as our own study. In a molecular study, Zemanick *et al* (25) observed a marked underestimation of the detected bacterial strains in OP compared to sputum samples. Few studies focused on the non-expectorator children. In one study (14), 42% of the 20 non-expectorating children were shown to carry new bacteria. Zampoli *et al* (20) reported in infants a higher yield of induced sputum (+81%) than oropharyngeal swabs growth cultures. Besides, the small prevalence of positive growth samples in these previous studies prevented specific conclusions according to identified bacteria. The present study support the use of CP-SP for detecting additional *Pa*, which is known as a prognostic factor in CF. The benefit of induced sputum to detect additional *Pa* was found not relevant in three cohorts (18, 20), although it increased the screening of 54 % in another small one (19). Our results also suggests that a swab applied after chest physiotherapy is an acceptable alternative method. The qualitative effect of chest physiotherapy on swab culture was previously suggested with an achieved two-fold increase in detection of *Pa* (26), and doubling sensitivity of sputum compared to BAL (16).

Although the microbiological analyses of collected sputum during a chest physiotherapy session allowed screening 78.5% of the infected children with *Pa,* the PPVs of sputum growths did not achieve 100%. In fact, 2% of *Pa* infected children were screened solely with OP. The lack of concomitant positive sputum growths has previously been underlined, and remains discussed. The growing interest for upper airway infection and the need to treat it (27), agreed with that isolated upper airway *Pa* growths might reflect sinus infection(28), considered as a bacterial reservoir (29, 30). Others defined them as false positive results with no necessary treatment (11, 31).

This study has some strengths and limitations. The strengths are the adequately powered study on the number of detected *Pa* for preventing the bias in the measures of test accuracy. These results are those expected through the routine follow-up of CF paediatric cohort, as 96% of the collections were performed during routine visits. Attention was given to provide training and guidelines for a similar application in all CF centres of identical physiotherapy practices and microbiological methods, with a detection of bacteria at a low level of 10^2 CFU/mL. No centre effect was identified through the analyses. There are also some limitations. The techniques of chest physiotherapy for caring CF patients have progressed. The current used airway clearance technique in France is the autogenic drainage or the concept of flow and breathing level modulation, as recommended by ECFS(32). A similar acceptability was reported for each sampling means. Most cries started before the séance in this young population. Thus no specific adverse effect might be attributed to chest physiotherapy, a routine care at home in CF. The aim of this study was to test the growth results in real life and unfortunately, no molecular analysis of *Pa* strains was performed. However, antibiotic susceptibility testing was constantly similar for an individual patient. This suggest that same strains were found in upper and lower airways, as previously demonstrated (33). Not the entire population benefitted from of a nasal lavage or oral washing, without observing a difference in the proportion of the identified pathogens. Since this study, the MALDI-TOF MS identification tool became available. This would not significantly improve the identification of *Pa, Hi or Sa* in CF compared with the usual phenotypic method (34).

In conclusion, this prospective, powered, multicentre study has demonstrated that sputum collected after chest physiotherapy in non-expectorating CF children provides the most sensitive samples for *Pseudomonas aeruginosa* screening. Oropharyngeal swab applied after chest physiotherapy remains an acceptable alternative. These results may enhance both patients' care and end-points for research trials. A study by using induced sputum, a method of a growing interest, and/or by using PCR diagnosis, will be further clinically useful (21, 35, 36). Given the microbial diversity and the emergence of other pathogenic species (i.e. *Achromobacter* spp., *Stenotrophomonas* spp., it would be interesting to extend the study to a larger panel of biomarker bacteria, or even to make the comparison by metagenomics (21)

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Table 1: Description of the studied population		
Number of patients		299
Age (years)		7.2 ± 5.7
Boys		162 (54.4)
Age at diagnosis (months)		11.7 ± 24.9
Diagnosis circumstances		
	Neonatal Screening	151 (50.7)
	Meconium Ileus	48 (16.0)
	Symptoms	110 (37.0)
CFTR Genotypes		
	ΔF508/ΔF508	148 (49.5)
	ΔF508/other	110 (36.7)
	Other	41 (13.7)
History of microbiological status		
Haemophilus influenzae		176 (59.1)
Pseudomonas aeruginosa		136 (45.6)
Frequency of detection	Only once	64 (47.1)
	Intermittent	51 (37.5)
	Chronic	21 (15.4)
Staphylococcus aureus		243 (81.5)
Frequency of detection	Only once	31 (13.0)
	Intermittent	104 (43.5)
	Chronic	103 (43.1)
Clinical status at the visit		
	Exacerbations	54 (18.1)
	Routine control	244 (81.9)
Under antibiotic at the visit		119 (39.8)
	Azithromycin	51 (43.2)
	Antibiotic targeting HI	10 (8.5)
	Antibiotic targeting PA	58 (28.8)
	Antibiotic targeting SA	34 (49.2)

Results are expressed as mean±SD or n (%)

HI: Haemophilus influenzae; Pa: Pseudomonas aeruginosa; Sa: Staphylococcus aureus

Table 2 : Collection of airway secretions and growth results

	OP n =299	CP-SP n =299	CP-OP n =299
Successful collection of samples	299 (100)	296 (98.5)	299 (100)
Aspiration		160 (54.1)	
expectoration		127 (42.9)	
Missing data		9 (3.0)	
Occurrence of Cough	105 (36.3)	299[1] (100)	113 (39.4)
Safety			
Overall acceptability	212 (72.9)	210 (72.4)	206 (71.5)
Nausea/vomiting	20 (25.3)	13 (16.7)	21 (25.9)
Crying	51 (66.7)	68 (86.1)	57(71.3)
Agitation	40 (51.3)	44 (55.7)	34 (42.5)
Other	-	2 (2.7)	-
Microbiological analyses			
Negative for the three bacteria	86	49	66
Positive for at least one bacteria	209	246	227
Haemophilus influenzae			
+ve	43 (14.6)	59 (20)	50 (17.1)
-ve	252(85.4)	236 (80)	243 (82.9)
missing data	4	4	6
Pseudomonas aeruginosa			
+ve	28 (9.5)	44 (15)	37 (12.6)
-ve	267 (90.5)	249 (85)	257 (87.4)
missing data	4	6	5
Staphylococcus aureus			
+ve	144 (48.8)	156 (52.9)	152 (51.5)
-ve	150 (50.8)	139 (47.1)	143 (48.5)
missing data	5	4	4

Results are expressed as n(%) : +ve= positive; -ve= negative; 1: provoked cough is part of CP-SP to obtain airway secretions.

OP: oropharyngeal swab 1; CP-OP: oropharyngeal swab 2; CP-SP: Chest Physiotherapy

Table 3 : Equivalence tests for the three methods and comparison of the paired results (McNemar test)

Bacteria	Methods	McNemar test (p)	Difference	CI90%	CI95%
H.influenzae	OP / CP-SP	**0.014**	-5.4%	**[-10.5% ; -0.3%]***	[-11.5% ; 0.7%]*
	OP / CP-OP	0.144	-2.5%	[-7.4% ; 2.5%]	[-8.4% ; 3.4%]*
	CP-SP / CP-OP	0.194	2.9%	[-2.3% ; 8.2%]	[-3.3% ; 9.2%]*
P. aeruginosa	OP / CP-SP	**0.003**	-5.5%	**[-10.0% ; -1.1%]***	[-10.8% ; -0.2%]*
	OP / CP-OP	**0.029**	-3.1%	[-7.3% ; 1.1%]	[-8.1% ; 2.0%]*
	CP-SP / CP-OP	0 .144	2.4%	[-2.2% ; 7.1%]	[-3.1% ; 8.0%]*
S. aureus	OP / CP-SP	0.159	-3.7%	**[-10.5% ; 3.0%]***	[-11.8% ; 4.3%]*
	OP / CP-OP	0.262	-2.4%	[-9.1% ; 4.4%]	[-10.4% ; 5.7%]*
	CP-SP / CP-OP	0.466	1.4%	[-5.4% ; 8.1%]	[-6.7% ; 9.4%]*

* Confidence interval (CI) ranging out [-10%; 10%] (CI90%) signifying that the methods are not equivalent. The calculation was also displayed for the CI between [-5%; 5%] (CI95%).

OP: oropharyngeal swab 1; CP-OP: oropharyngeal swab 2; CP-SP: Chest Physiotherapy

Table 4: Accuracy bacteria detection by OP and CP-OP versus CP-SP.

Bacteria	vs CP-SP	Sp (%)	Se (%)	PPV (%)	NPV (%)	Accuracy (%)
H.influenzae	OP	95.3	55.0	74.4	89.5	87.3
	CP-OP	93.6	60.3	70.0	90.6	87.1
S. aureus	OP	76.6	81.7	82.5	76.0	79.1
	CP-OP	84.8	83.2	86.0	81.9	84.0
P. aeruginosa	OP	97.6	50.0	78.6	91.7	90.4
	CP-OP	96.8	65.1	77.7	94.1	92.1

Sp: specificity; Se: sensitivity; PPV: positive predictive value; NPV: negative predictive value.

OP: oropharyngeal swab 1; CP-OP: oropharyngeal swab 2; CP-SP: Chest Physiotherapy

Table 5: Predictive values of each sampling method with reference to pooled positive cultures.

	H. influenzae		*S. aureus*		*P. aeruginosa*	
	n=77		n=181		n=56	
%	PPV	NPV	PPV	NPV	PPV	NPP
OP	n=43		n=141		n=28	
	55.8	86.5	77.9	74.0	51.9	90.1
CP-OP	N=50		N=150		N=36	
	64.9	89.7	82.8	79.2	66.7	92.7
CP-SP	N=59		N=153		N=44	
	76.6	92.4	84.5	80.4	86.3	96.0

PPV: positive predictive value; NPV: negative predictive value.

OP: oropharyngeal swab 1; CP-OP: oropharyngeal swab 2; CP-SP: Chest Physiotherapy

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Legends

Figure 1: Number of *Pseudomonas aeruginosa* growths in the airway secretions of 295 non-expectorating CF patients using three methods: CP-SP and CP-OP significantly increased the screening of patients with Pa compared to OP. OP= oropharyngeal swab before chest physiotherapy, CP-SP = induced sputum after chest physiotherapy, CP-OP = oropharyngeal swab after chest physiotherapy. The results of positive airway secretion cultures collected by each method are compared by paired.

Figure 2: Number of *Haemophilus influenzae* growths (a) and Staphyloccocus aureus (b) in the airway secretions of 295 non-expectorating CF patients using three methods: CP-SP augmented the detection number of Hi carriers compared to OPs. No other significant differences within the other comparisons. OP= oropharyngeal swab before chest physiotherapy, CP-SP = induced sputum after chest physiotherapy, CP-OP = oropharyngeal swab after chest physiotherapy. The results of positive airway secretion cultures collected by each method are compared by paired.

Figure 1

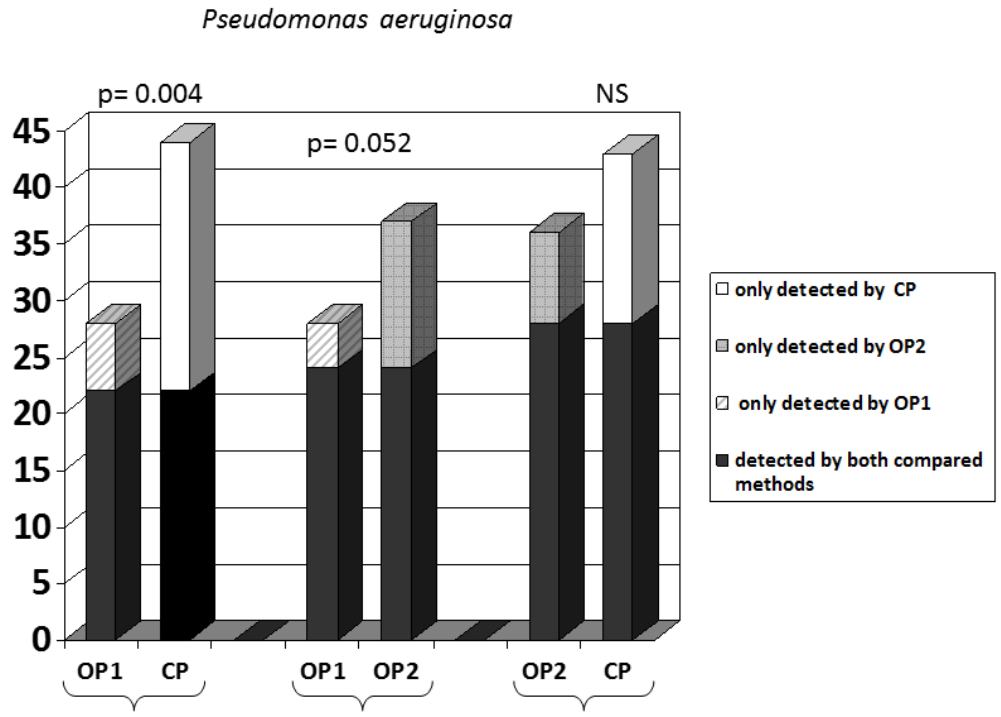
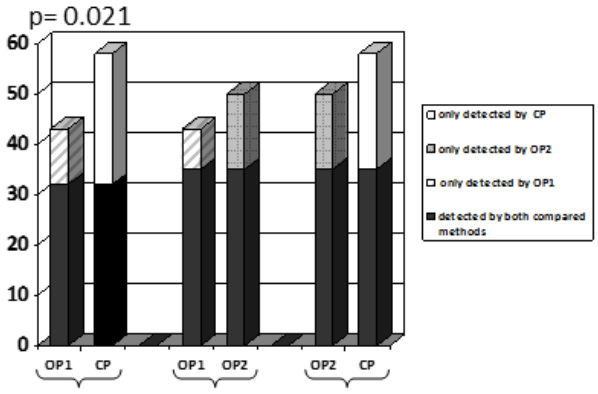

Pseudomonas aeruginosa
p= 0.004
NS
p= 0.052
45
40
35
30
25
20
15
10
5
0
OP1
CP
OP1
OP2
OP2
CP
only detected by CP
only detected by OP2
only detected by OP1
detected by both compared methods

Figure 2

a) Haemophilus influenzae



b) Staphyloccocus aureus