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ORIGINAL ARTICLE

The bacteriology in adult patients with pneumonia and parapneumonic effusions: increased yield with DNA sequencing method

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Abstract

The aim of this study was to use a 16S rDNA sequencing method in combination with conventional culture in patients with parapneumonic effusions (PPE) to evaluate the methods, study the microbiological spectrum, and examine the presence of bacteria within the different stages of PPE. Adults with community-acquired pneumonia (CAP) and PPE ($n = 197$) admitted to the Departments of Infectious Diseases at four hospitals in Stockholm County during 2011–2014 were prospectively studied. All patients underwent thoracentesis. Twenty-seven non-infectious pleural effusions were used as controls. The pleural samples were analyzed with culture, 16S rDNA sequencing, pH, glucose, and lactate dehydrogenase. Microbiological etiology was found in 99/197 (50%) of the patients with mixed infections in 20 cases. The most common pathogens were viridans streptococci ($n = 37$) and anaerobic bacteria ($n = 40$). Among the 152 patients with both methods performed, 26/152 (17%) and 94/152 (62%) had bacteria identified with culture and 16S rDNA sequencing respectively ($p < 0.001$). In 24/26 (92%) culture-positive cases, the same organism was identified by 16S rDNA. All controls were negative in both methods. Among the patients with complicated PPE and complete sampling, bacteria were found in 69/74 patients (93%), all detected with 16S rDNA sequencing, compared to 23/74 (31%) culture-positive samples ($p < 0.001$). Compared with culture, 16S rDNA sequencing substantially improved the microbiological yield, a microbiological diagnosis was achieved in almost all patients with complicated PPE, and the specificity seemed to be high. 16S rDNA sequencing should be used together with culture in patients with PPE to guide antibiotic therapy.

Keywords Pneumonia · Etiology · PCR · Parapneumonic effusion · Pleuritis

Summary of the article's main point
Among patients with parapneumonic effusions, bacteria were often detected in pleural fluid, with viridans streptococci and anaerobic species as the most frequently findings. Compared with culture, 16S rDNA sequencing improved the microbiological yield and the specificity seemed to be high.

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Introduction

In patients with community-acquired pneumonia (CAP), development of parapneumonic effusion (PPE) is common, occurring in up to 57% [1, 2]. A majority of these effusions resolve with antibiotics alone and do not require drainage [2]. Effusions that resolve with antibiotics alone are often called simple parapneumonic effusions (SPPE) and are characterized by a LDH level less than half of that in the serum, normal pH, and normal glucose levels [2]. It has earlier been thought that SPPE do not contain bacterial organisms [2, 3]. However, if appropriate antibiotic treatment is not commenced, a SPPE may progress into a fibrinopurulent stage (complicated PPE or empyema), indicating translocation of bacteria into the pleural space. This is a severe condition with high morbidity and mortality [4–6]. Antibiotic therapy is thus an essential part of the treatment [7, 8], and fast, accurate diagnostic methods as well as knowledge of the microbiological spectrum are crucial.

According to previous studies, the etiology in patients with PPE seems to differ compared to that in patients with CAP without effusions, and it has also been suggested that polymicrobial etiology is more common [2, 5, 9]. Identifying the microbiological etiology in PPE has however been challenging. The yield of conventional culture in pleural fluid samples considered infected has been low, varying between 18 and 33% [10].

During the last two decades, several pediatric studies have used PCR methods for identification of bacteria in infected pleural fluid in children [11–17], showing high diagnostic yield compared with conventional culture. A few studies have also used PCR to determine the etiology in adults with pleural infections [10, 18]. However, to our knowledge, no previous study has prospectively investigated the microbiology by using both culture and molecular methods in adult patients admitted to hospital with pneumonia and with different stages of parapneumonic effusion. The incidence of mixed infections in patients with PPE has neither been thoroughly investigated earlier.

The microbiological laboratory at Karolinska University Hospital has implemented a 16S rDNA sequencing analysis for detection of up to three different pathogens in a single sample [19]. The aim of this study was to evaluate the 16S rDNA sequencing method and compare it with conventional culture in patients with different stages of PPE. In addition, we aimed to study the microbiological spectrum including mixed infections and examine the presence of bacteria within different stages of parapneumonic effusion (simple PPE, complicated PPE, and empyema).

Material and methods

Patients: intervention group

This prospective study was conducted at the Departments of Infectious Diseases (DIDs) at four hospitals in Stockholm County: the Karolinska University Hospital Solna, the Karolinska University Hospital Huddinge; and the community teaching hospitals Danderyd Hospital and Södersjukhuset. All admitted patients at the DIDs that underwent thoracentesis for pleural culture during 2011–2014 were evaluated for inclusion in the study by two of the study investigators (NJ, JH).

The inclusion criteria were (1) a clinical presentation of acute lower respiratory tract infection together with (2) chest X-ray or CT scan showing new infiltrates and pleural effusion and (3) performance of thoracentesis for culture of the pleural fluid. Written informed consent was obtained from enrolled patients or from their nearest relative. During a 4-year period (Jan 01, 2011 to Dec 31, 2014), 197 patients were included in the study. Of those who fulfilled the inclusion criteria during this period, no patient or nearest relative denied participation in the study. The study was approved by the regional ethics committee, Stockholm, Sweden, nr 2010/2066-31.

Patients: control group

The control group consisted of 27 patients, admitted to the Lung-Allergy Clinic at Karolinska University Hospital Solna during 2016, with pleural effusion and in need of a thoracentesis. The most common cause of the pleural fluid was malignancy; 20 patients had lung cancer, one patient had another malignancy with malignant pleural cells, three patients had cardiac heart failure, and three patients had other lung-associated diseases.

None of the patients had clinical signs of ongoing infection.

Specimen collection

In addition to culture, the pleural fluids in the intervention group were also analyzed for 16S rDNA sequencing and chemistry analyses including pH, glucose, and lactate dehydrogenase (LD).

Pleural fluid cultures were obtained for all 197 patients. Totally, 152/197 patients had pleural fluid analyzed with 16S rDNA and chemistry analyses were performed in 139 cases. Information of the macroscopic appearance of the pleural fluid was collected from the medical records.

All patients in the control group had pleural fluid analyzed by culture and 16S rDNA sequencing.

Microbiological evaluation

Culture procedure

Samples (about 3 ml pleural fluid/bottle from each patient) were inoculated in BacT/ALERT (bioMérieux SA, F-69280 Marcy l'Etoile, France) aerobic (FA) and anaerobic (FN) blood cultivation bottles. The samples were inoculated for 10 days before negative results were reported. Positive bottles were subjected to initial gram staining and thereafter present pathogens were identified by standard cultivation procedures, biochemical identifying methods, and/or MALDI-TOF mass spectrometry. No initial microscopy was done before positive culture results.

16S rDNA sequencing analysis

A modified protocol for 16S rDNA sequencing analysis was used [20]. In brief, a ~460-bp region of 16S rRNA gene for the bacteria was amplified by real-time PCR followed by a chemical purification protocol (ExoProStar) and standard dideoxy nucleotide sequencing based on the Big Dye® Terminator v.3.1 Cycle Sequencing Kit and the Big Dye® XTerminator™ Purification kit. Obtained labeled sequences were separated by capillary electrophoresis technology in ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

The primers used for PCR and sequencing reactions were forward 5′>CGGCCCAGACT-CCTACGGGAGGCA GCA<3′ and reverse 5′>GCGTGGACTACCAGGGTATC TAAT CC<3′.

For bacterial identification and typing, the obtained DNA sequences were analyzed by standard BLAST database search and RipSeq 16S sequence analysis [19].

Chemistry evaluation

Pleural fluid was analyzed at the local hospital laboratory within 2 h after thoracentesis with a panel containing pH (ABL800, Radiometer A/S), glucose, and LD (Cobas8000, Roche). pH was analyzed in 131, glucose in 139, and LD in 135 of the 197 cases.

Definitions

In the interpretation of the results from pleural culture and 16S rDNA sequencing, bacteria were only included when the laboratory could identify the species. Coagulase-negative staphylococci, *Corynebacterium* spp., and *Micrococcus* spp. were interpreted as contaminations from the skin and excluded from further analysis. The other microbiological findings by culture and 16S rDNA were judged as pathogens.

Complicated parapneumonic effusion (CPPE) was defined with at least one of the following criteria: (1) pleura-pH < 7.2 or, if pH was not analyzed pleura-glucose < 3.4 mmol/l or LDH > 17 microkat/l (corresponding to 1000 IU/l); (2) Frank pus; (3) thorax surgery with decortication [21]. If none of these criteria were fulfilled, the effusion was defined as SPPE.

Statistical analysis

For univariate analysis of categorical variables, Fisher's exact test (two-tailed test) was used.

Results

Patients' characteristics

In total, 197 patients were included in the study. The proportion male/female was 130/67. The mean age was 66 years (18–98 years). The comorbidity of the population is shown in Table 1.

Almost all parapneumonic effusions were community-acquired; only two patients were infected in-hospital. The in-hospital mortality was 5.6% (11 patients) and the 1-year mortality was 16.8% (33 patients).

A total of 90.9% (179 patients) were on antibiotic treatment when thoracentesis for etiologic diagnosis was performed. At the time for thoracentesis, the patients had been treated with antibiotics between 0 and 64 days, median 4 days, and mean 5.4 days.

Table 1 Comorbidity of the 197 included patients

Comorbidity	Number of patients	Percent
Any chronic disease[a]	143	73
Hypertension	44	22
Cardiac disease	42	21
Neurologic disease	27	14
Pulmonary disease	24	12
Diabetes mellitus	20	10
Alcohol abuse	13	7
Malignancy	10	5
Liver disease	9	5
Renal disease	8	4
Dementia	6	3

Data are presented as number (%), unless otherwise indicated

[a] Only chronic diseases found in at least five of the patients are shown

Microbiologic etiology of PPE: all patients

In total, 125 bacterial pathogens were found in pleural samples by culture and/or with 16S rDNA sequencing (Table 2). The most common findings were viridans streptococci, *Fusobacterium* spp., and *Streptococcus pneumoniae*. Anaerobic bacteria were found in 40 patients.

In 99/197 (50%) patients, bacteria were identified with at least one of the two methods and at least two pathogens were seen in 20 patients. In 17 patients, two different bacteria were found, and three different bacteria were detected in 3 patients.

Table 2 Bacterial findings in pleural fluid in the whole study population; analyses by conventional culture ($n = 197$) and 16S rDNA sequencing method ($n = 152$)

Microorganism	Number of positive samples, *N* (% of total positive samples)
Viridans streptococci	37 (30)
Streptococcus anginosus group	32
Streptococcus viridans	1
Viridans streptococci spp.	4
Fusobacterium spp.	18 (14)
Fusobacterium nucleatum	12
Streptococcus pneumoniae	14 (11)
Porphyromonas spp.	6 (5)
Porphyromonas gingivalis	2
Porphyromonas endodontalis	1
Prevotella spp.	6 (5)
Prevotella oris	1
Streptococcus pyogenes	6 (5)
Staphylococcus aureus	5 (4)
E. coli	4 (3)
Haemophilus influenzae	3 (2)
Parvimonas micra	3 (2)
Enterococcus faecalis	2 (1.5)
Klebsiella pneumoniae	2 (1.5)
Neisseria meningitidis	2 (1.5)
Peptostreptococci spp.	2 (1.5)
Aggregatibacter spp.	2 (1.5)
Aggregatibacter aphrophilus	1
Aggregatibacter segnis	1
Streptococcus dysgalactiae	2 (1.5)
Other bacteria[a]	11
Total number of bacterial findings	125 (100)

[a] The following bacteria were only found once (*Bacillus* spp., *Bacteroides fragilis*, *Campylobacter gracilis*, *Enterobacter aerogenes*, *Francisella tularensis*, *Haemophilus haemolyticus*, *Haemophilus parainfluenzae*, *Lactobacillus catenaformis*, *Pasturella multocida*, *Pseudomonas aeruginosa*, *Streptococcus* spp.)

Diagnostic yield with different microbiological methods: patients with pleural fluid analyzed with both conventional culture and 16S rDNA PCR

Figure 1 presents the findings from pleural fluid among the 152 cases analyzed with both microbiological methods. In 26/152 (17%) and 94/152 (62%) patients, bacteria were identified by culture and 16S rDNA PCR, respectively ($p < 0.0001$). At least two different bacterial species were found by culture in 4 cases (3%) compared to 18 cases (12%) with 16S rDNA sequencing ($p = 0.003$).

All 26 culture-positive samples had bacteria detected in pleural fluid with 16S rDNA sequencing. In 24 of these 26 cases (92%), the same organism was identified by both methods. Among these 24 cases with concordant findings between the methods, an additional microorganism was identified in five samples with PCR, and in two samples by culture. In two cases, different pathogens were found with the two methods. A comparison of the results obtained with the two methods is shown in detail in supplemental data table.

Among patients with ongoing antibiotic therapy at the time for pleural puncture, 18/138 (13%) had positive pleural fluid culture compared to 8/14 (57%) ($p = 0.0004$) culture-positive cases among those without antibiotic treatment at the time for sampling. In total, 11 of these 14 patients had never been treated with antibiotics during the current disease episode before thoracentesis (antibiotic treatment-naïve cases) of which culture was positive in 6 (55%) cases. The corresponding numbers of PCR-positive cases were 83/138 (60%) and 11/14 (79%) for patients with and without antibiotic exposure respectively ($p = 0.25$).

In the whole population, PCR analysis was not performed in 45 cases. In these patients, pleural fluid culture was positive in 5 cases: viridans streptococci (3), *Staphylococcus aureus* (1), *Escherichia coli* (1).

Diagnostic yield with different microbiological methods in patients with complicated vs. simple PPE

CPPE was identified in 87/197 (44%) patients while 68/197 (35%) had a SPPE as defined above. In 42/197 (21%) of the patients, classification was not possible due to absence of chemistry analyses (Table 3).

For patients with CPPE, at least one bacteria was identified in 72/87 (83%) cases; identification was made by culture in 26/87 (30%) cases and with 16S rDNA sequencing in 69/87 (79%) cases.

For patients with SPPE, a bacterial pathogen was found in 17/68 (25%) of the cases, all identified with 16S rDNA sequencing. One patient with SPPE was culture-positive.

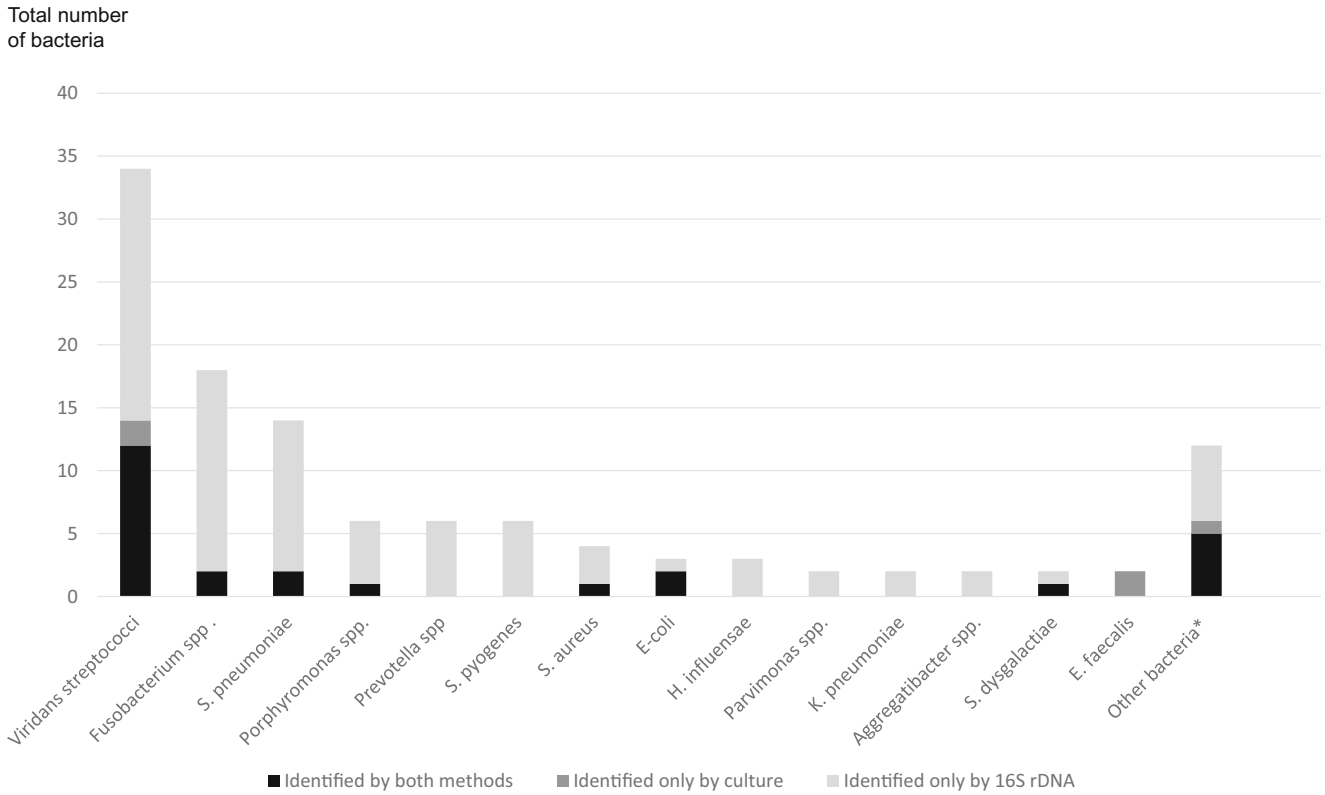

Fig. 1 Microbiological yield by culture and 16S rDNA sequencing in 152 patients tested by both methods. The asterisk indicates that the following bacteria were only found once: *Bacillus* spp., *Bacteroides fragilis*, *Campylobacter gracilis*, *Enterobacter aerogenes*, *Francisella tularensis*, *Haemophilus haemolyticus*, *Haemophilus parainfluenzae*, *Lactobacillus catenaformis*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Streptococcus* spp., *Peptostreptococcus* spp.

Table 3 Bacterial findings in pleural fluid with culture and DNA sequencing in different parapneumonic stages

Stage of parapneumonic effusion	Total bacterial findings (%)	Bacterial findings with culture (%)	Bacterial findings with 16S rDNA (%)	*p* value
Complicated parapneumonic effusion ($n = 87$)	72 (83)	26 (30)	69 (79)	
Pus ($n = 20$)[a]	17 (85)	11 (55)	15 (75) [b]	
Clear fluid, no surgery but pathological chemistry ($n = 66$)	55 (83)	15 (23)	54 (82) [c]	
Thoracic surgery ($n = 2$)[a]	0[d]			
Complicated parapneumonic effusion in patients with complete sample collection ($n = 74$)[e]	69 (93)	23 (31)	69 (93)	$p < 0.0001$[f]
Pus ($n = 16$)	15 (94)	9 (56)	15 (94)	
Clear fluid, no surgery but pathological chemistry ($n = 58$)	54 (93)	14 (24)	54 (93)	
Thoracic surgery ($n = 0$)				
Simple parapneumonic effusion ($n = 68$)[g]	17 (25)	1 (1.5)	17 (25)	
Simple parapneumonic effusion in patients with complete sample collection ($n = 59$)[e]	17 (29)	1 (1.7)	17 (29)	$p < 0.0001$[f]

[a] One patient had findings of pus and thoracic surgery intervention

[b] Four patients were not tested with 16srRNA

[c] Nine patients were not tested with 16srRNA

[d] None of the two patients were tested with 16srRNA

[e] Samples analyzed by both microbiological methods together with biochemistry analyses

[f] Fischer exact test used as statistical method

[g] Nine patients were not tested with 16srRNA

Diagnostic yield with different microbiological methods in patients with CPPE vs. SPPE and complete sample collection

Among the 87 patients with CPPE, 74 patients had a complete sample collection, i.e., samples analyzed by both microbiological methods together with biochemistry analyses (Table 3). Bacterial agents were found in 69/74 (93%) cases with 16S rDNA sequencing, compared to 23/74 (31%) culture-positive samples ($p < 0.0001$).

In patients with CPPE with pus, culture was positive in 9/16 (56%) compared to 14/58 (24%) ($p = 0.03$) in CPPE patients without pus. The bacterial yield with 16S rDNA was similar in these two groups: 15/16 (94%) vs. 54/58 (93%) ($p = 1.0$).

Among the nine patients without antibiotic therapy at the time for the thoracentesis (all but one were antibiotic treatment-naïve cases), 6/9 (67%) had positive cultures compared to 17/65 (26%) ($p = 0.02$) culture-positive results among those with ongoing antibiotics when pleural samples were obtained. The corresponding numbers for positive PCR analysis were 9/9 (100%) and 60/65 (92%) ($p = 1.0$) among patients without vs. with antibiotic treatment.

Among the patients with SPPE, 59 patients had pleural fluid analyzed with both microbiological methods. Bacteria were identified in 17/59 (29%) of these cases, all recognized with 16S rDNA. One patient (1.7%) ($p < 0.0001$) was culture-positive (Table 3).

Microbiological yield in the control group

No pathogens were detected by any of the two methods among the 27 patients in the control group.

Discussion

The main findings of this study were:

1. Half of the patients had findings of bacteria in pleura with viridans streptococci spp. being the most frequently identified species, and mixed bacterial infections were common.
2. The microbiological yield with culture was low (17%) but increased significantly (62%) by use of 16S rDNA.
3. The 16S PCR was positive in 92% of culture-positive samples.
4. A bacterial pathogen was detected in almost all patients with CPPE, but also in one third of the patients with an SPPE.

After viridans streptococci, the most common findings were *Fusobacterium* spp. and *Streptococcus pneumoniae*. Altogether, anaerobic bacteria were the most frequent findings, detected in one third of all patients with an identified etiology. Only one Scandinavian study has earlier investigated the etiology in patients with pleural infection [5]. In that study, there were less findings of anaerobic bacteria (20%) and more findings of *Staphylococcus aureus* (14%) compared with the present study. The study had different diagnostic methods design though; only cases positive with conventional culture were included, with almost one third of the cases nosocomial infected. An additional aspect that might have contributed to the differences in the microbiological yield is that in the Meyer study only 67% of the patients were pre-treated with antibiotics with a median time of 2 days of treatment when pleural sampling was performed compared to 91% pre-treated patients in the present study with a median treatment time of 4 days. Compared with studies that used similar methodology [10, 18], the bacterial findings were more comparable although less pneumococci and more cases of *Fusobacterium* spp. were found in the present study. However, also these two latter studies had differences in study design compared with the present study. Both studies included all patients with suspected pleural infection regardless if it was PPE or had another pathogenesis. In the Insa study, pleural fluid samples received by the laboratory for culture were analyzed by 16S PCR followed by a review of clinical records to obtain patient data. In the Maskell study, the patients were prospectively enrolled, but only those who fulfilled the criteria for a suspected bacterial infection (pleural fluid with findings of pus, positive gram stain or culture, or pH < 7.2) were studied. Only 8% of the patients in the present study with an identified etiology had findings of enteric gram-negative bacteria, a number comparable with findings in other studies [10, 18]. All these patients also had coexisting illnesses indicating that enteric gram-negative bacteria are rarely found in community-acquired PPE, especially in earlier healthy individuals.

We found mixed infections in 20% of all patients with an identified etiology, a higher amount compared to an earlier study [18]. The most common copathogens found were different species of anaerobic bacteria, common in the oral cavity.

Only 16% of all pleural fluid cultures were positive with few findings of pneumococci (1%) and anaerobic bacteria (2.5%), compared to the yield with 16S rDNA—9% and 15% respectively of tested patients in the whole study population. These results are in accordance with a previous study by Kawanami et al. [22] and could be due to the high number of patients with ongoing antibiotic therapy when the culture was obtained, and to the fact that both fusobacteria and pneumococci are very sensitive to antibiotics with rapid killing of the bacteria [14]. This is also supported by the Meyer study in which pneumococci were identified in 13% of positive pleural cultures when sampling was performed before antibiotics had been administered compared to 4% when antibiotics already were started at the time of sampling [5].

Among the patients tested with both culture and 16S rDNA, the concordance between these two methods was very high, with a false-negative PCR rate of only 8% compared to culture. These results are in agreement with that by the study by Insa et al. [10], but much higher compared with that by the study by Maskell et al. [18] where the corresponding correlation only was 35%. This difference may, at least in part, be explained by the use of fresh pleural fluids in the present study and the Insa study, whereas the Maskell et al. used frozen fluids. All 16S rDNA-negative results in the present study were negative in the corresponding culture, and the 27 patients in the control group all tested negative for both culture and 16S rDNA. Thus, although we are not able to estimate the exact specificity, these data point toward a high correctness of the 16S rDNA sequencing analysis for identification of bacteria in PPE.

There was no difference in the bacterial yield with PCR among those with vs. without ongoing antibiotic therapy when pleural fluid was collected. These results are also equivalent to those of another small study [13] and probably due to the fact that the PCR methods also enable detection of dead bacteria.

Mixed infections were also frequently detected with this PCR method, identifying three times as many cases of at least two pathogens compared to culture, results similar to those of previous studies [10, 13, 18].

In a small pediatric study by Menezes-Martins et al. investigating the bacteriology of PPE with a study design similar to that of the present study, cultures from pleural fluid in children with CPPE were positive in only one third of the cases, comparable to the findings in the present study [13]. However, in the present study, more than half of the patients with empyema had bacteria identified by culture compared to only one fourth of those categorized to CPPE but without frank pus. The higher yield for culture in patients with empyema could be attributable to a more severe stage with a higher bacterial load [23].

Among the patients with an expected bacterial pleural infection, i.e., CPPE, and complete sample collection, bacteria was identified with our 16S rDNA method in 93% of the cases, a number similar to that of the study by Menezes-Martins et al. [13] but higher compared to that of other studies which used PCR [10, 12, 18]. There was no difference in the yield with 16S rDNA in patients with different stages of CPPE or the relation to ongoing antibiotic treatment. This suggests that the 16S rDNA analysis has an ability to detect an etiological agent in most patients with bacterial PPE.

Interestingly, in patients with SPPE, i.e., patients not expected to have bacteria found in pleura, 16S rDNA identified bacterial agents in 29% of the cases. These data are also in agreement with the Menezes-Martins study [13]. The interpretation of these findings is challenging; a small amount of dead or alive bacteria may translocate from the site of the pulmonary infection to the pleura but without any regular inflammatory response, and nevertheless still be detectable with 16S rDNA. However, several of these cases had complicated courses of medical care with pleural fluid difficult to drain and repeated thoracentesis. Another possibility is that bacteria are detected in the beginning of a formation of a CPPE, where the bacterial burden still is low and the inflammatory response in pleura has not yet started. Many of these patients had on the other hand been treated with antibiotics for several days when the pleural drainage occurred, still with findings of bacterial DNA. In contrast, in an earlier etiological CAP study [24], we found that bacterial DNA in the airways were cleared within a few days after start of antimicrobial therapy. The difference may be explained by the fact that the pleural space is a secluded compartment implicating an increased difficulty in bacterial component clearance.

A limitation in the present study is that the sample analysis among the enrolled patients was not complete. This issue is inherent in most prospective trials involving respiratory tract infections. Comparison of data in the group with complete sampling vs. the whole study population showed similar results, and no patient or nearest relative denied participation in the study. Selection bias in the collection of samples is thus unlikely. Further, after this study was performed (2011–2014), new generation sequencing (NGS) has been implemented as a method in the microbiology laboratory. However, we do not believe that the result in the present study would have differed substantially if we had used NGS, apart from a possibility to detect more than three pathogens in a few cases. Finally, due to the lack of a golden standard, the clinical relevance of bacterial DNA findings is difficult to interpret in all clinical sequencing trials, also in those where the samples are collected from a sterile location. However, the fact that the concordance between positive culture and DNA findings in PPE was high and that the 16S rDNA analyses were negative in the control group would support the opinion that a majority of the DNA findings had clinical significance.

In conclusion, we found that among antibiotic-treated patients with CAP and PPE, bacterial DNA were often detected in pleural fluid, even in some patients with SPPE. Viridans streptococci and anaerobic bacteria were the most frequently occurring pathogens, and gram-negative enteric bacteria were rare. In comparison with culture, 16S rDNA sequencing analysis substantially improved the microbiological yield with a low false-negative PCR rate compared with culture. Based on these findings, it can be proposed that in these patients, apart from culture, pleural fluid should also be investigated with 16S rDNA to guide antibiotic therapy.

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Compliance with ethical standards

Written informed consent was obtained from enrolled patients or from their nearest relative. The study was approved by the regional ethics committee, Stockholm, Sweden, nr 2010/2066-31.

Conflict of interest The authors declare that they have no conflict of interest.

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