AABIP JOURNAL CLUB

MORE THAN MEETS THE CULTURE: USING NEXT GENERATION SEQUENCING TO UNDERSTAND THE BACTERIOLOGY OF PLEURAL INFECTION

THE CLINICAL QUESTION

What does the microbiome of pleural infection look like, and does the bacterial pattern have an impact on one year survival of patients?

STUDY CONCLUSION

Most pleural infections (79%) are polymicrobial. Pleural infections involving anaerobes or bacteria from the Streptococcusanginosus group tended to be community acquired and had a better survival profile than infections involving Staphylococcus aureus or Enterobacteriaceae which tended to be hospital acquired with lower rates of survival.

STUDY BACKGROUND

Pleural infection can lead to significant morbidity and mortality. Current management includes timely drainage coupled with empiric antibiotic coverage. Correct identification of pleural pathogens is crucial for guiding treatment of pleural space infections. Tailoring antimicrobial treatment is often challenging as pleural fluid cultures historically have a low rate of microbial detection. Sequencing of the bacterial 16S ribosomal nucleic acid gene has previously been used to increase the rate of bacterial identification in pleural infections but is not currently widely available.

CURRENT PRACTICE

The British Thoracic Society 2010 guidelines for pleural infection and the American Association for Thoracic Surgery (AATS) 2017 guidelines for empyema both recommend obtaining pleural fluid gram stain and culture for bacterial identification. The AATS guidelines specifically recommend inoculating aerobic and anaerobic

CURRENT PRACTICE CONT.

blood culture bottles with pleural fluid. This has previously been shown to increase yield by approximately 20% though the rate of bacterial detection was still only 58%.
DNA sequencing can be more reliable than traditional culture for bacterial identification. A prior study comparing culture to capillary sequencing of bacterial DNA allowed for a decrease in undiagnosed pathogens from 42% to 26%. More recently, next generation sequencing has been found to be even more reliable than capillary sequencing. Next generation sequencing has only been studied in pleural infections once and had a relatively small sample size of 64 patients. The current study aimed to use a larger sample size to re-evaluate the performance of next generation sequencing of the 16S rRNA gene for bacterial pathogen identification in pleural space infections

STUDY DESIGN

Type of trial: Prospective follow up of the PILOT trial

Randomization, blinding, controls: non-randomized, non-blinded, 20 non-infectious pleural fluid samples and 10 PCR grade water samples used as negative controls

N= 243

Study groups: Experimental group consisted of 243 pleural fluid samples with previously identified pleural infection. 20 additional pleural fluid samples from non-infectious causes used as negative controls. 10 non-template control samples with PCR grade water were also used as negative controls to assess for background contamination.

Settings: Participating UK centers

Enrollment: May 1, 2013 – Jan 1, 2017

Treatment period: N/A

Follow up: There was no follow up

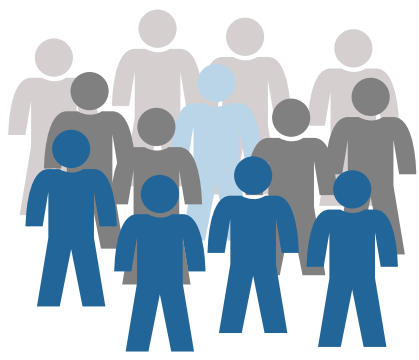
Primary outcome: characterization of pathogens in pleural infections and the impact of different pathogens or patterns on one year survival.

Secondary outcomes:
1) Association of pleural fluid bacterial patterns with:
-Duration of hospitalization
-3-month need for surgery
2) Bacterial patterns of hospital and community acquired infections
3) Dental hygiene and dominance of anaerobes
4) Comparison of culture versus molecular techniques for pathogen identification.

INTERVENTIONS

16S rRNA next generation sequencing

POPULATION



Inclusion criteria: pleural fluid specimens previously enrolled in the PILOT clinical trial
Exclusion criteria: Not previously enrolled in the PILOT clinical trial
Baseline Characteristics of pleural infection participants (as reported in the PILOT clinical trial) N=243

-Median Age: 61years
-Male: 178 (73%), Female 71(25%)
-Community acquired infection: 221 (91%)
-Hospital acquired infection: 17 (7%)
-Polymicrobial infection: 192 (79%)
-Median hospitalization duration: 14 days
-Positive culture-based microbiology: 55 (22%)
-Surgical drainage: 63 (26%)
-Comorbidities present: 153 (63%)
-Current smoker: 64 (27%)
-Former smoker: 107 (44%)
-Poor dental hygiene: 113 (47%)

Baseline Characteristics of the pleural fluid control group: N=20
-Median Age: 71 years
-Male: 11 (73%)
-Female 9 (25%)
-Malignant pleural effusion: 10
-Effusion from heart failure: 7
-Effusion from hepatic failure: 3
-Median White cell count (109 cells/L): 5.3
-Median CRP (mg/L): 11.6

TAKE HOME MESSAGE

Pleural infection can cause significant morbidity and mortality. Most pleural infections are polymicrobial with anaerobes and gram-negative bacteria being the most common culprits. Traditional pleural fluid cultures are often unreliable for bacterial pathogen identification which can impede proper antibiotic selection. 16S rRNA sequencing is more reliable for bacterial identification however is not widely available and may have a cost that is prohibitive. Other culture independent technologies such as polymerase chain reaction are also emerging and may be more immediately available than gene sequencing.

FUNDING

UK Medical Research Council, National Institute for Health Research Oxford Biomedical Research Centre, Wellcome Trust, Oxfordshire Health Services Research Committee, Chinese Academy of Medical Sciences, and John Fell Fund.

OUTCOMES

Primary outcomes:

-245 different bacterial species identified in the 243 pleural samples

-Anaerobic bacteria had the highest mean abundance (33.5%)

-Anaerobic bacteria found in 68% of all samples

-79% of pleural infections were polymicrobial

-S pneumoniae was most abundant pathogen in monomicrobial infections

Secondary outcomes:

-91% of infections were community acquired

-Higher abundance of S. pneumoniae in community acquired infection (p=0.049)

-3x higher abundance of Enterobacteriaceae (p=0.043) and 5x higher abundance of S. aureus in hospital acquired infections

-Similar abundance of anaerobic and gram-negative infections in community versus hospital acquired infections

-Community acquired infections had higher bacterial variability with 233 different species versus 55 different species in hospital acquired infections

-Culture data was obtained from 22% of samples

-Mean number of pathogens identified from positive cultures was 1.1 compared to mean of 3.2 in next generation sequencing

-Independent of the predictive score for pleural infection survival (RAPID Score)

oPresence of anaerobes and S anginosus group had better 1-year survival compared to when these were absent

oPresence of S aureus was associated with poorer survival (p<0.0001)

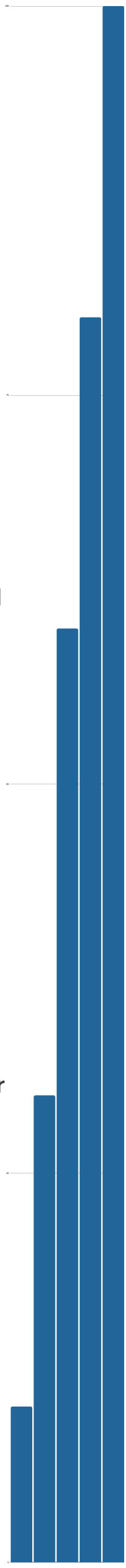
-Dominance of S aureus or Enterobacteriacea were associated with poorer survival compared to when they were present but not dominant

-Bacterial group was not associated with a significant difference in 3-month need for surgery or duration of hospitalization

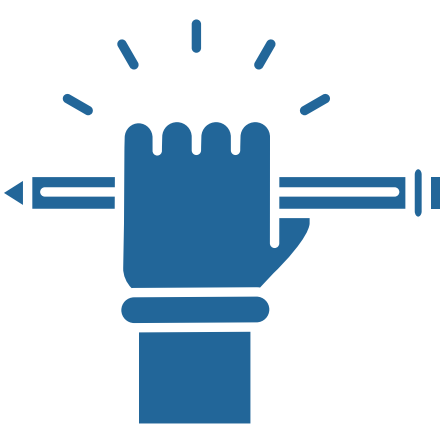
-Hospital acquired infection had higher mortality than community acquired infection (p=0.003)

-Oral hygiene was not associated with predominance of anaerobes

Adverse events: none



STUDY STRENGTHS



This study provides important characterization of pleural infections that have the potential to change practice patterns and clinical outcomes.

Results from the study suggest that previous estimates on the rates of polymicrobial and gram-negative infections are underestimated.

STUDY LIMITATIONS

Potential for selection bias exists as the patients enrolled in the original PILOT study were recruited from UK centers. This geographic location may impact pleural infection patterns.

The study only focuses on bacterial causes of pleural infection, viral and fungal etiologies are not addressed.

SUGGESTED READING

1. Corcoran JP, Psallidas I, Gerry S, Piccolo F, Koegelenberg CF, Saba T, Daneshvar C, Fairbairn I, Heinink R, West A, Stanton AE. Prospective validation of the RAPID clinical risk prediction score in adult patients with pleural infection: the PILOT study. European Respiratory Journal. 2020 Nov 1;56(5).

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ARTICLE CITATION

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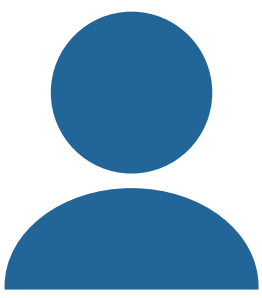

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