Title: The microbiology of pleural infection in adults: a systematic review

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RMM critically reviewed the manuscript. All authors reviewed and approved the final manuscript.

Abstract

Background and objectives: Pleural infection is a major cause of morbidity and mortality among adults. Identification of the offending organism is key to appropriate antimicrobial therapy. It is not known whether the microbiological pattern of pleural infection is variable temporally or geographically. This systematic review aimed to investigate available literature to understand the worldwide pattern of such microbiology and the factors that might affect such pattern.

Data sources and eligibility criteria: Ovid Medline and Embase were searched between 2000 and 2018 for publications that reported on the microbiology of pleural infection in adults. Both observational and interventional studies were included. Studies were excluded if the main focus of the report was paediatric population, tuberculous empyema, or post-operative empyema.

Study appraisal and synthesis methods: studies of 20 or more patients with clear reporting of microbial isolates were included. The numbers of isolates of each specific organism/group were collated from the included studies. Besides the overall presentation of data, subgroup analyses by geographical distribution, infection setting (community vs. hospital), and time of the report was carried out.

Results: From 20,980 reports that the initial search returned, 74 papers reporting on 9644 patients were included in the data synthesis. The most common organism reported worldwide was Staphylococcus aureus. Geographically, Pneumococci and Viridans Streptococci were the most commonly reported isolates from tropical and temperate regions, respectively. The microbiological pattern was considerably different between community- and hospital-acquired infections where more gram-negative and drug-resistant isolates reported in the hospital-acquired infections. The main limitations of this systematic review were the heterogeneity in the method of reporting of certain bacteria and the predominance of reports from Europe and South East Asia.

Conclusions: In pleural infection, the geographical location and the setting of infection have considerable bearing on the expected causative organisms. This should be reflected in the choice of empirical antimicrobial treatment.

Systematic review registration number: CRD42017076418

Keywords: bacterial infection, pleural disease, empyema, respiratory infection

INTRODUCTION

Pleural infection is a common disease worldwide with considerable morbidity and mortality.[1] Despite improvements in healthcare quality, the incidence of pleural infection has been on the rise in the last two decades.[1] With the evidence supporting the use of fibrinolytics plus deoxyribonuclease (DNase) in management[2, 3] and widespread availability of less invasive (video-assisted thoracoscopic) surgery, the average cost per hospitalisation is currently estimated to be 4,400 USD.[4]

The cornerstones of treating pleural infection are prompt drainage of the infected fluid and timely initiation of antimicrobial treatment.[5] Antimicrobials are almost always started empirically with broad spectrum coverage until microbial culture results inform more directed therapy. Commonly, the use of more focussed and less broad spectrum antibiotics is not achievable, due to the yield of conventional cultures which is in the vicinity of 40-60%, [6]. Knowledge of the predominant organisms that cause pleural infection is a key and necessary step to achieve successful empiric coverage.

The so called "atypical" pathogens that commonly cause pneumonia do not have any significant role in pleural infection,[7, 8] probably due to differences in the milieu between the lung parenchyma and the pleura. Recent reviews report the '*milleri'* group (more recently termed 'Streptococcus anginosus' group) of the *Streptococcus* genus as the most common culprit of pleural infection,[6] but this is mainly based on data from Europe, North America and Australia. However, reports from Taiwan and South Korea cite *Klebsiella* species as the most common organism isolated in community-acquired pleural infections.[9, 10]

This systematic review aimed to search the existing literature on the microbiology of pleural infection and determine the worldwide overall pattern of such microbiology. The primary research question was to assess the most common organisms/groups responsible for pleural infection in adults worldwide. The secondary research questions were to address 1) if there were different profiles of organisms according to geographical region, 2) the differences between the microbiology of hospital-acquired and community-acquired pleural infections and 3) the average yield of microbial cultures in pleural infection.

METHODS

Protocol and registration

The systematic review was carried out in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and its protocol was registered in the PROSEPRO database (CRD42017076418).

Search strategy and selection criteria

This is a systematic review of papers published between 2000 and 2017 that report the microbiology of bacterial pleural infection in adults. No language restrictions were applied and non-English publications were included as long as reasonable translation could be obtained. All publications including data on microbiological results on pleural infection in adults were included. Studies with mixed adult and paediatric population but with predominance of adult patients (more than 75%) were also included. Both observational (prospective and retrospective) and interventional studies were included provided that microbiological results were reported clearly. Reviews, conference abstracts and small case series reporting less than twenty subjects were excluded.

This systematic review focused on bacterial pleural infections. Reports describing pleural infection complicating thoracic surgery and spontaneous bacterial pleuritis were excluded as they were not felt to represent the typical microbiological pattern seen in the majority of cases with pleural infection. Papers with the main focus on tuberculous or fungal pleural infections were excluded as these represent a different process to that seen in bacterial pleural infection. Papers reporting exclusively on paediatric pleural infection or a single causative organism/group were also excluded.

The initial electronic search was carried out on both Ovid Embase and Medline databases for publications between 2000 and 2017 and was performed on 31/7/2017. The search was repeated on 26/7/2018 to include any new published papers. The search terms used were ‘empyema’, ‘pleural infection’ and ‘pleuritis’. The terms were intentionally broad to capture all publications. The full search strategy is available in the supplementary material.

Screening and data management

Due to the very large number of entries that the initial search returned, primary screening for relevant titles/abstracts was split between two authors (MH and TC) without duplication. The exclusion criteria of this phase are mentioned above. The second phase of screening to choose papers containing useful microbiological details was carried out independently by two reviewers (MH and EB). Excluded papers at this stage were papers with no microbiological results. An extraction spreadsheet was used to collect data from all included papers. This included the number of patients, mean/median age, the percentage of positivity of pleural fluid culture, and the absolute number of positive culture results for each organism/bacterial group.

Streptococcus milleri group was combined with the *Viridans Streptococci* group to avoid overlap. *Enterobacteriaceae* were grouped under a single heading except for *Klebsiella* spp. as these were noted to be a site of difference between reports from different regions. All anaerobic bacteria were reported as a single group to avoid breaking down the categories into very small numbers.

Data analysis

There was no formal assessment for risk of bias in the studied papers given that the main area of the review was based on observational data and not treatment effects. As a quality measure, papers with poorly reported microbiological results were excluded from the final dataset used to synthesise results. This was carried out independently by two reviewers (MH and RA) who analysed the full dataset to appraise the quality of the reporting of the microbiology results. Papers were judged to have good quality data if culture results clearly reported the names of the organism or group and there was an account of results of anaerobic cultures (whether positive or negative). Any disagreement on the list of included papers was settled by discussion between reviewers.

Data regarding the age and number of study participants were reported as mean/median. Where appropriate, the mean of some variables was estimated from the median and range using the following formula: mean = [(2*median) + minimum +maximum]/4.[11]

The absolute numbers of isolated organisms/groups from each study were summated. The sum of the numbers per organism/group across all studies was expressed as a percentage of the grand total of all organisms/groups across all studies.

Subgroup analyses

A pre-hoc subgroup analysis of relative contribution of each organism was carried out according to:

a. The latitude of the city where the publication came from. This was divided into 3 regions; temperate regions (North or South of latitude 40^0), sub-tropical regions (between 23.5^0 and 40^0 North and South of the equator), and tropical regions (between 23.5^0 North and 23.5^0 South of the equator).[12]

b. The setting of infection: relative contribution of different organisms in community-acquired versus hospital-acquired infections.
c. Time trend: Reports were divided into two groups according to publication date. The first period was 2000-2008 and the second period was 2009-2017 to examine for changing trends in the microbiology by time.

RESULTS

Screening results

The initial search identified 20,980 publications. Titles/abstracts of these publications were screened for eligibility resulting in the exclusion of 20,705 publications due to reasons detailed in the PRISMA diagram (Figure 1). Two hundred and eleven full papers were downloaded and assessed for eligibility. Of these, 75 papers[2, 8–10, 13–83] were judged to have good quality previously unpublished microbiological data and were included in the data synthesis. The reasons for exclusion of the remaining136 papers are detailed in Figure 1. The repeat search between 2017 and 2018 yielded 536 publications, of which two were deemed potentially relevant but on full paper analysis were excluded.

Study characteristics

The total number of patients reported from the 75 studies combined was 10241. The mean of mean ages reported from the 56 studies that exclusively included adult patients was 54.4±9.5 years. The characteristics of included studies are presented in Table 1.

Synthesis of results

Combining numbers of organisms described in all studies, a total of 6202 bacterial isolates were reported; 50.4% (95% CI 48 – 50.6%) were gram positive aerobic organisms, 37.5% (95% CI 37.2 – 39.6%) were gram negative aerobic organisms and 12.1% (95% CI 11.4 – 13.1%)were anaerobes. All studies used conventional culture techniques except for a single study[36] where nucleic acid tests were used. The mean diagnostic yield of bacterial culture was 56% (95% CI 50.6 – 61.4%).

The most common aerobic isolates were: *Staphylococcus aureus*(20.7%), Viridans *Streptococci* group (18.7%), *Pseudomonas* species (17.6%), *Enterobacteriaceae* group (11.9%), *Streptococcus pneumoniae* (10.8%), *Klebsiella* species (10.7%), , , *Acinetobacter* species (5%) and coagulase negative *staphylococci* (4.5%) (Figure 2). Twenty four papers (reporting on 3842 patients) presented culture results separately for Strept. *milleri* and Viridans streprococi group. From a total of 531 isolates, 309 (58.3%) were from the *milleri* group.

Some of the studies reporting unselected results of pleural infection included results of cultures positive for mycobacterium tuberculosis (TB) or fungi. The pooled numbers from these reports suggest that the incidence of TB as an aetiology of suspected bacterial infection was 8.8% (in a total of 2074 cases from 15 studies), while that of fungi was 3% (in a total of 3003 cases from 20 studies).

Twenty four of studies indicated the proportion of cultures that yielded more than one organism from the same sample . The median percentage of polymicrobial results from the overall cultured samples was 12.9% (IQR 8.0 to 17.9%).

Subgroup analyses

Geographical differences

The majority of publications from the tropics: came from the following countries: Thailand, Mexico, Singapore, Saudi and Cameroon. Most of the studies from the subtropics came from the Northern Hemisphere (96%) and these were from South East Asia, Southern US, the Middle East and Southern Europe. All studies from temperate regions came from Europe and North America.

Figure 2 shows the relative contribution of the different microbial groups and the preponderant aerobic organisms in each of the three geographical regions (the tropics, sub-tropics and temperate regions). The sub-tropics had higher incidence of gram negative organisms in comparison to the two other regions. *Staphylococcus aureus* was the most preponderant culture result, followed by *Klebsiella*, *Pseudomonas spp*. and the Viridans group which were all more common than *Pneumococci*. Both the tropics and temperate regions had a higher incidence of gram positive organisms. Viridans *Streptococci* were the most widely reported organisms from temperate regions while *Streptococcus pneumoniae* were the most commonly reported organisms from the tropics (Figure 2).

Twenty five papers (reporting on 4285 patients) presented date on the methicillin sensitivity of the Staph. *aureus* isolates. Figure S1 presents the proprortions of the methicillin-sensitive (MSSA) to the methicillin-resistant Staph. *aureus* (MRSA) isolate from the three geographical regions.

In terms of geographical differences in non-bacterial results, positive TB culture results were reported in 37/406 patients (9%) in the largest study reported from the United Kingdom [36] while positive TB cultures were reported in 148/511 patients (29%) in the largest two reports from India[38, 62].

Community- versus hospital-acquired

Eleven studies reporting data on 1523 patients identified the setting of the pleural infection. Figure 3 shows the combined numbers of culture isolates from these studies. In community-acquired infections, gram-positive aerobes (65.1%) were the predominant group followed by anaerobes (17.8%) and then gram negative aerobes (17.1%). The most common aerobic isolates were the Viridans group (32%), *Pneumococci* (22%) followed by *Staphylococcus aureus* (18.5%). In hospital-acquired infections, gram negative aerobes had a larger share (37.5%), with less anaerobic isolates (11%). The most common aerobic isolates were *Staphylococcus aureus* (37.8%) followed by the Enterobacteriaceae group, *Pseudomonas spp.* and *Klebsiella spp*. which combined made up 26.7% of aerobic isolates. Information on resistance to methicillin in *Staphylococcus aureus* isolates was included in these eleven studies. In community-acquired infections, 67% of the *Staphylococcus aureus* isolates were methicillin-sensitive, while in the hospital-acquired group, 42% of the isolates were methicillin-sensitive.

Time trends

Figure S2 shows the contribution of the three main microbial groups in the aetiology of pleural infection in the earlier half (2000-2008) and the latter half (2009-2017) of the study period. The total number each of the ten most common organisms was isolated in the two study periods is presented in figure S3. Figure S4 shows the proportion of methicillin-sensitive and -resistant *Staphylococcus aureus* isolates according to the time period.

DISCUSSION

To our knowledge, this study is the first systematic review of microbiology of pleural infection in adults. The results demonstrate that the mean diagnostic yield of bacterial cultures on pleural fluid is 56% (Table 1), which means that in more than two fifths of cases, the organism(s) remains unknown and antimicrobial treatment is entirely empirical. The information obtained from our data should inform clinical care, and specifically choice of empirical therapy by region.

In total, 12.9% of cultures demonstrated more than one isolate. Given that cultures identified the causative organisms in only about have of the instances, the incidence of polymicrobiality in culture-positive samples can be assumed to be around 23%. In a metagenomic study by Dyrhovden et., massive parallel sequencing of bacteria DNA on 44 samples of pleural infection identified poylmicrobial infection in 25% of the parapneumonic and 59% of the primary pleural infection samples.[84]

The role of oropharyngeal flora, composed of strict or facultative anaerobes as pathogens causing pleural infection, is supported in this study. The viridans group and *Pneumococci* are consistently among the most common isolates in different regions, and particularly in community-acquired infection. Isolates from the '*salivarius*' and '*mutans*' groups (which fall in the viridans group) were reported, and beta-haemolytic *streptococci*, was the sixth commonest isolate in community-acquired infections, stressing the significant role played by oropharyngeal flora in the likely pathogenesis of pleural infection. In the aforementioned metagenomic study, *Streptococcus intermedius* and *Fusobacterium nucleatum* (both commonly implicated in dental/periodontal infection) were the most common pathogens identified, particularly in non-parapenumonic pleural infections.[84] The exact mechanisms whereby oral flora gain access to the pleural space are incompletely understood, but this pattern seems independent of geography.

Strictly anaerobic bacteria are found in 12.8% of all culture-positive cases, and in 17.8% in community-acquired infections alone. Anaerobic organisms are known to be difficult to culture, with specific culture methods required [85], meaning that our data is likely an underestimate of the true contribution. In a study addressing anaerobic organisms in pleural infection where ideal culture methods were undertaken, anaerobic organisms were isolated in 74% of culture-positive pleural effusions[85], with micro-aerophilic *Streptococci* (from the viridans group) included in these numbers. Anaerobic bacteria were found to be mixed with aerobic bacteria in almost two thirds of cases, and three or more organisms were isolated from 14% of the samples.[85] These data highlight the importance of anaerobic treatment in empirical regimes.

The preponderance of oropharyngeal flora in culture results might be explained in cases of hospital-acquired pleural infection due to a presumed high risk of aspiration in this cohort of patients. However, the results of this review demonstrate that anaerobes were isolated relatively *more* commonly in community-acquired infections which may be related to poor dental hygiene[86] with spread to the pleura via the haematogenous route. The tropism of anaerobes to the pleura is thought to be due to the favourable environment of the space[1] which is very different from the environment of the lung parenchyma which has high oxygen tension.

Staphylococcus aureus is by far the most common organism isolated regardless of study or setting, but is particularly prevalent in hospital-acquired disease. Although many studies included are somewhat old, it is alarming that methicillin-resistant isolates, represent one third of positive cultures in community-acquired infection. This has important implications on the initial choice of empiric antibiotics.

A clear geographic variation in pleural infection was seen, although the precise reasons behind this strong signal are unclear. There is a large economic, particularly health economic, variation between the groups of countries making up the three regions, with different hospital level number, availability and complexity of patient care with different local antibiotic prescribing practices. Besides climatic variations, all these factors potentially contribute to variation to patterns of microbiology and antibiotic resistance.

In tropical regions, the profile was strongly gram positive, with *Pneumococci* being the most common isolate. We speculate that this may be due to the generally younger populations affected by pleural infection in these regions, and relatively higher rates of human immune deficiency virus (HIV) infection (particularly in Africa) which are both associated with higher incidence of pneumococcal pneumonia. The profile in the temperate regions was also strongly gram-positive with the highest proportion attributable to viridans *streptoccoci*. This group is particularly associated with oral/dental infections and poor oral hygiene[84] which could be related to higher alcohol-related disease burden in North America and Western Europe (where most of the studies from temperate regions originate) in comparison to South East Asia region.[87],()

In the subtropics, a higher proportion of gram negative isolates was seen. The results are however confounded by a large number of reports from this region originating in Taiwan and South Korea, where life expectancy is among the highest in the world, translating to a higher comorbidity burden and hence higher hospitalisations and hospital-acquired infections which could account for the difference in the prevalence of gram negative pleural infections in this region The peculiarity of the higher incidence of *Klebsiella spp* from this region is an extension of an observed, and not completely explained, trend of increased propensity of the *Klebsiella spp* to cause pyogenic infections in other parts of the body.[88] There is also high prevalence of Pseudomonas *spp*. Infection, which is mainly driven by data from two studies from the Indian subcontinent.[43, 62] There is data to suggest that higher temperatures are associated with increased incidence of Pseudomonas *spp*. and other gram negative bacterial blood stream infections.[89]

The analysis by the publication year shows an increase in role played by gram positive bacteria in last few years. In particular, *Staph. aureus* overtakes the viridans *streptococci* as the most common isolate, and the proportion of methicillin resistant isolates increase from 48 to 58%. This pattern is not uniform across regions, as methicillin resistance appears highest in the subtropics.

There are limitations to this study; there existed a large heterogeneity in reporting bacterial groups, particularly organisms from the *Streptococcus* genus. Despite attempts to exclude paediatric patients and those with post-operative pleural infections, several reports included some patients from these categories which decrease the accuracy of the data to a degree. Information on the site of acquiring infection (community vs. hospital) was not available in many of the included studies which could have contributed to a degree to the temporal and regional variations noticed. The majority of the included papers relied on conventional cultures in delineating the microbial aetiology, which means that the burden of infection caused by difficult-to-culture bacteria is likely to be underestimated. Finally, there was predominance of reports from Europe and Far East and relative paucity of reports from Africa and the South America which affects to a degree the reliability of the findings.

Conclusion

In pleural infection, the geographical location and the setting of infection seem to have a bearing on the expected causative organisms and this should be reflected in the choice of empirical antimicrobial treatment to address preponderance of certain microbes and prevalence of antibiotic resistance which should always be supported by data on local resistance patterns

References

1. Bedawi EO, Hassan M, Rahman NM. Recent developments in the management of pleural infection: a comprehensive review. *The Clinical Respiratory Journal* [Internet] 2018 [cited 2018 Jul 14]; Available from: http://doi.wiley.com/10.1111/crj.12941.

2. Rahman NM, Maskell NA, West A, Teoh R, Arnold A, Mackinlay C, Peckham D, Davies CWH, Ali N, Kinnear W, Bentley A, Kahan BC, Wrightson JM, Davies HE, Hooper CE, Lee YCG, Hedley EL, Crosthwaite N, Choo L, Helm EJ, Gleeson FV, Nunn AJ, Davies RJO. Intrapleural use of tissue plasminogen activator and DNase in pleural infection. *N. Engl. J. Med.* 2011; 365: 518–526.

3. Piccolo F, Pitman N, Bhatnagar R, Popowicz N, Smith NA, Brockway B, Nickels R, Burke AJ, Wong CA, McCartney R, Choo-Kang B, Blyth KG, Maskell NA, Lee YCG. Intrapleural tissue plasminogen activator and deoxyribonuclease for pleural infection. An effective and safe alternative to surgery. *Ann Am Thorac Soc* 2014; 11: 1419–1425.

4. Shen H-N, Lu C-L, Li C-Y. Epidemiology of pleural infections in Taiwan from 1997 through 2008: Pleural infections in Taiwan. *Respirology* 2012; 17: 1086–1093.

5. Davies HE, Davies RJO, Davies CWH, on behalf of the BTS Pleural Disease Guideline Group. Management of pleural infection in adults: British Thoracic Society pleural disease guideline 2010. *Thorax* 2010; 65: ii41–ii53.

6. Lisboa T, Waterer GW, Lee YCG. Pleural infection: Changing bacteriology and its implications: Pleural infection: changing bacteriology. *Respirology* 2011; 16: 598–603.

7. Wrightson JM, Wray JA, Street TL, Chapman SJ, Gleeson FV, Maskell NA, Peto TEA, Rahman NM, Crook DWM. Absence of Atypical Pathogens in Pleural Infection. *Chest* 2015; 148: e102–e103.

8. Falguera M, Carratala J, Bielsa S, Garcia-Vidal C, Ruiz-Gonzalez A, Chica I, Gudiol F, Porcel JM. Predictive factors, microbiology and outcome of patients with parapneumonic effusion. *European Respiratory Journal* 2011; 38: 1173–1179.

9. Tu C-Y, Hsu W-H, Hsia T-C, Chen H-J, Chiu K-L, Hang L-W, Shih C-M. The changing pathogens of complicated parapneumonic effusions or empyemas in a medical intensive care unit. *Intensive Care Medicine* 2006; 32: 570–576.

10. Lin Y-C, Tu C-Y, Chen W, Tsai Y-L, Chen H-J, Hsu W-H, Shih C-M. An Urgent Problem of Aerobic Gram-Negative Pathogen Infection in Complicated Parapneumonic Effusions or Empyemas. *Internal Medicine* 2007; 46: 1173–1178.

11. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005; 5: 13.

12. American Meteorological Society. Meteorology Glossary [Internet]. Available from: http://glossary.ametsoc.org/wiki/Main_Page.

13. LeMense GP, Strange C, Sahn SA. Empyema thoracis. Therapeutic management and outcome. *Chest* 1995; 107: 1532–1537.

14. Wait MA, Sharma S, Hohn J, Dal Nogare A. A randomized trial of empyema therapy. *Chest* 1997; 111: 1548–1551.

15. Lim TK, Chin NK. Empirical treatment with fibrinolysis and early surgery reduces the duration of hospitalization in pleural sepsis. *Eur. Respir. J.* 1999; 13: 514–518.

16. Davies CW, Kearney SE, Gleeson FV, Davies RJ. Predictors of outcome and long-term survival in patients with pleural infection. *Am. J. Respir. Crit. Care Med.* 1999; 160: 1682–1687.

17. Odeh M, Sabo E, Oliven A, Srugo I. Role of tumor necrosis factor-alpha in the differential diagnosis of parapneumonic effusion. *Int. J. Infect. Dis.* 2000; 4: 38–41.

18. Chen KY, Hsueh PR, Liaw YS, Yang PC, Luh KT. A 10-year experience with bacteriology of acute thoracic empyema: emphasis on Klebsiella pneumoniae in patients with diabetes mellitus. *Chest* 2000; 117: 1685–1689.

19. Kalaajieh WK. Etiology of exudative pleural effusions in adults in North Lebanon. *Can. Respir. J.* 2001; 8: 93–97.

20. Chu MW, Dewar LR, Burgess JJ, Busse EG. Empyema thoracis: lack of awareness results in a prolonged clinical course. *Can J Surg* 2001; 44: 284–288.

21. Tuncozgur B, Ustunsoy H, Sivrikoz MC, Dikensoy O, Topal M, Sanli M, Elbeyli L. Intrapleural urokinase in the management of parapneumonic empyema: a randomised controlled trial. *Int. J. Clin. Pract.* 2001; 55: 658–660.

22. Hoth JJ, Burch PT, Bullock TK, Cheadle WG, Richardson JD. Pathogenesis of posttraumatic empyema: the impact of pneumonia on pleural space infections. *Surg Infect (Larchmt)* 2003; 4: 29–35.

23. Anstadt MP, Guill CK, Ferguson ER, Gordon HS, Soltero ER, Beall AC, Musher DM. Surgical versus nonsurgical treatment of empyema thoracis: an outcomes analysis. *Am. J. Med. Sci.* 2003; 326: 9–14.

24. Petrakis IE, Kogerakis NE, Drositis IE, Lasithiotakis KG, Bouros D, Chalkiadakis GE. Video-assisted thoracoscopic surgery for thoracic empyema: primarily, or after fibrinolytic therapy failure? *The American Journal of Surgery* 2004; 187: 471–474.

25. Diacon AH, Theron J, Schuurmans MM, Van de Wal BW, Bolliger CT. Intrapleural Streptokinase for Empyema and Complicated Parapneumonic Effusions. *American Journal of Respiratory and Critical Care Medicine* 2004; 170: 49–53.

26. Banga A, Khilnani G, Sharma S, Dey A, Wig N, Banga N. A study of empyema thoracis and role of intrapleural streptokinase in its management. *BMC Infectious Diseases* [Internet] 2004 [cited 2018 Apr 13]; 4Available from: http://bmcinfectdis.biomedcentral.com/articles/10.1186/1471-2334-4-19.

27. Luh S-P, Chou M-C, Wang L-S, Chen J-Y, Tsai T-P. Video-assisted thoracoscopic surgery in the treatment of complicated parapneumonic effusions or empyemas: outcome of 234 patients. *Chest* 2005; 127: 1427–1432.

28. Khwaja S, Rosenbaum DH, Paul MC, Bhojani RA, Estrera AS, Wait MA, DiMaio JM. Surgical treatment of thoracic empyema in HIV-infected patients: severity and treatment modality is associated with CD4 count status. *Chest* 2005; 128: 246–249.

29. Tsai T-H, Jerng J-S, Chen K-Y, Yu C-J, Yang P-C. Community-Acquired Thoracic Empyema in Older People: THORACIC EMPYEMA IN OLDER PEOPLE. *Journal of the American Geriatrics Society* 2005; 53: 1203–1209.

30. Lin F, Chen Y, Chen F, Chang S. Cytokines and fibrinolytic enzymes in tuberculous and parapneumonic effusions. *Clinical Immunology* 2005; 116: 166–173.

31. Cheng G, Vintch JRE. A retrospective analysis of the management of parapneumonic empyemas in a county teaching facility from 1992 to 2004. *Chest* 2005; 128: 3284–3290.

32. Ozol D, Oktem S, Erdinc E. Complicated parapneumonic effusion and empyema thoracis: Microbiologic and therapeutic aspects. *Respiratory Medicine* 2006; 100: 286–291.

33. Bilgin M, Akcali Y, Oguzkaya F. BENEFITS OF EARLY AGGRESSIVE MANAGEMENT OF EMPYEMA THORACIS. *ANZ Journal of Surgery* 2006; 76: 120–122.

34. Smolikov A, Smolyakov R, Riesenberg K, Schlaeffer F, Borer A, Cherniavsky E, Gavriel A, Gilad J. Prevalence and clinical significance of pleural microbubbles in computed tomography of thoracic empyema. *Clinical Radiology* 2006; 61: 513–519.

35. Ahmed RA, Marrie TJ, Huang JQ. Thoracic Empyema in Patients with Community-Acquired Pneumonia. *The American Journal of Medicine* 2006; 119: 877–883.

36. Maskell NA, Batt S, Hedley EL, Davies CWH, Gillespie SH, Davies RJO. The Bacteriology of Pleural Infection by Genetic and Standard Methods and Its Mortality Significance. *American Journal of Respiratory and Critical Care Medicine* 2006; 174: 817–823.

37. Jiménez D, Díaz G, García-Rull S, Vidal R, Sueiro A, Light RW. Routine use of pleural fluid cultures. Are they indicated? *Respiratory Medicine* 2006; 100: 2048–2052.

38. Malhotra P, Aggarwal AN, Agarwal R, Ray P, Gupta D, Jindal SK. Clinical characteristics and outcomes of empyema thoracis in 117 patients: A comparative analysis of tuberculous vs. non-tuberculous aetiologies. *Respiratory Medicine* 2007; 101: 423–430.

39. Levinson GM, Pennington DW. Intrapleural fibrinolytics combined with image-guided chest tube drainage for pleural infection. *Mayo Clin. Proc.* 2007; 82: 407–413.

40. Tsang KY, Leung WS, Chan VL, Lin AWL, Chu CM. Complicated parapneumonic effusion and empyema thoracis: microbiology and predictors of adverse outcomes. *Hong Kong Med J* 2007; 13: 178–186.

41. Kim Y-J, Cha S-I, Kwon J-S, Yoo S, Jun H-J, Kim E-J, Kim CH, Park J, Jung T-H. Treatment Results and Prognostic Factors of Complicated Parapneumonic Effusion and Empyema. *Tuberculosis and Respiratory Diseases* 2007; 63.

42. Chen C-H, Hsu W-H, Chen H-J, Chen W, Shih C-M, Hsia T-C, Tu C-Y. Different Bacteriology and Prognosis of Thoracic Empyemas Between Patients With Chronic and End-Stage Renal Disease. *Chest* 2007; 132: 532–539.

43. Mohanty S, Kapil A, Das BK. Bacteriology of parapneumonic pleural effusions in an Indian hospital. *Trop Doct* 2007; 37: 228–229.

44. Potaris K, Mihos P, Gakidis I, Chatziantoniou C. Video-Thoracoscopic and Open Surgical Management of Thoracic Empyema. *Surgical Infections* 2007; 8: 511–518.

45. El Solh AA, Alhajjhasan A, Ramadan FH, Pineda LA. A Comparative Study of Community- and Nursing Home-Acquired Empyema Thoracis: EMPYEMA THORACIS. *Journal of the American Geriatrics Society* 2007; 55: 1847–1852.

46. Liang SJ, Chen W, Lin Y-C, Tu C-Y, Chen H-J, Tsai Y-L, Shih C-M. Community-acquired thoracic empyema in young adults. *South. Med. J.* 2007; 100: 1075–1080.

47. Wozniak CJ, Paull DE, Moezzi JE, Scott RP, Anstadt MP, York VV, Little AG. Choice of First Intervention is Related to Outcomes in the Management of Empyema. *The Annals of Thoracic Surgery* 2009; 87: 1525–1531.

48. Lin M-C, Chen Y-C, Wu J-T, Ko Y-C, Wang C-C. Diagnostic and Prognostic Values of Pleural Fluid Procalcitonin in Parapneumonic Pleural Effusions. *Chest* 2009; 136: 205–211.

49. Chalmers JD, Singanayagam A, Murray MP, Scally C, Fawzi A, Hill AT. Risk factors for complicated parapneumonic effusion and empyema on presentation to hospital with community-acquired pneumonia. *Thorax* 2009; 64: 592–597.

50. Chen C-H, Chen W, Chen H-J, Yu Y-H, Lin Y-C, Tu C-Y, Hsu W-H. Transthoracic ultrasonography in predicting the outcome of small-bore catheter drainage in empyemas or complicated parapneumonic effusions. *Ultrasound Med Biol* 2009; 35: 1468–1474.

51. Chen W, Lin Y-C, Liang S-J, Tu C-Y, Chen H-J, Hang L-W, Hsu W-H, Shih C-M. Hospital-acquired thoracic empyema in adults: a 5-year study. *South. Med. J.* 2009; 102: 909–914.

52. Mitra S, Mukherjee S, Das S, Kundu S. Adult thoracic empyema: A comparative analysis of tuberculous and nontuberculous etiology in 75 patients. *Lung India* 2010; 27: 196.

53. Zablockis R, Petruskeviciene R, Nargela RV. [Causes and risk factors of pleural empyema and complicated parapneumonic pleural effusion]. *Medicina (Kaunas)* 2010; 46: 113–119.

54. Metin M, Yeginsu A, Sayar A, Alzafer S, Solak O, Ozgul A, Erkorkmaz U, Gürses A. Treatment of multiloculated empyema thoracis using minimally invasive methods. *Singapore Med J* 2010; 51: 242–246.

55. Bar I, Stav D, Fink G, Peer A, Lazarovitch T, Papiashvilli M. Thoracic Empyema in High-Risk Patients: Conservative Management or Surgery? *Asian Cardiovascular and Thoracic Annals* 2010; 18: 337–343.

56. Herrera-Kiengelher L, Báez-Saldaña R, Salas-Hernández J, Avalos-Bracho A, Pérez-Padilla R, Torre-Bouscoulet L. Frequency of adverse events and mortality in patients with pleural empyema in a public referral hospital in Mexico City. *Int. J. Tuberc. Lung Dis.* 2010; 14: 1187–1192.

57. Meyer CN, Rosenlund S, Nielsen J, Friis-Møller A. Bacteriological aetiology and antimicrobial treatment of pleural empyema. *Scandinavian Journal of Infectious Diseases* 2011; 43: 165–169.

58. Monteiro R, Alfaro T, Correia L, Simao A, Carvalho A, Costa J. [Lung abscess and thoracic empyema: retrospective analysis in an internal medicine department]. 2011; 24 Suppl 2.

59. Marks DJB, Fisk MD, Koo CY, Pavlou M, Peck L, Lee SF, Lawrence D, Macrae MB, Wilson APR, Brown JS, Miller RF, Zumla AI. Thoracic Empyema: A 12-Year Study from a UK Tertiary Cardiothoracic Referral Centre. Wilkinson RJ, editor. *PLoS ONE* 2012; 7: e30074.

60. Pefura Yone EW, Kuaban C, Afane Ze E, Simo L. Caractéristiques cliniques, bactériologiques et évolutives de la pleurésie purulente non-tuberculeuse chez les patients adultes séropositifs et séronégatifs au VIH à Yaoundé, Cameroun. *Revue de Pneumologie Clinique* 2012; 68: 225–232.

61. Považan A, Vukelić A, Kurucin T, Hadnadjev M, Považan D. The most common isolates from pleural infections. *Acta Microbiologica et Immunologica Hungarica* 2012; 59: 375–385.

62. Sonali J. EMPYEMA THORACIS: Bacteriological analysis of pleural fluid from the largest chest hospital in Delhi. *IOSR Journal of Dental and Medical Sciences* 2013; 3: 46–51.

63. White HD, White BAA, Song J, Fader R, Quiroga P, Arroliga AC. Pleural Infections: A 9-Year Review of Bacteriology, Case Characteristics and Mortality. *The American Journal of the Medical Sciences* 2013; 345: 349–354.

64. O'Connor JV, Chi A, Joshi M, DuBose J, Scalea TM. Post-traumatic empyema: Aetiology, surgery and outcome in 125 consecutive patients. *Injury* 2013; 44: 1153–1158.

65. Kim S-K, Kang CU, Song SH, Cho DG, Cho KD, Kim CH. Factors predictive of the failure of medical treatment in patients with pleural infection. *The Korean Journal of Internal Medicine* 2014; 29: 603.

66. Chen K-C, Chen H-Y, Lin J-W, Tseng Y-T, Kuo S-W, Huang P-M, Hsu H-H, Lee J-M, Chen J-S, Lai H-S. Acute thoracic empyema: Clinical characteristics and outcome analysis of video-

assisted thoracoscopic surgery. *Journal of the Formosan Medical Association* 2014; 113: 210–218.

67. Ohuchi M, Inoue S, Ozaki Y, Fujita T, Igarashi T, Ueda K, Hanaoka J. Single-trocar thoracoscopy under local anesthesia for pleural space infection. *General Thoracic and Cardiovascular Surgery* 2014; 62: 503–510.

68. Okiror L, Coltart C, Bille A, Guile L, Pilling J, Harrison-Phipps K, Routledge T, Lang-Lazdunski L, Hemsley C, King J. Thoracotomy and decortication: impact of culture-positive empyema on the outcome of surgery. *European Journal of Cardio-Thoracic Surgery* 2014; 46: 901–906.

69. Hajjar W, Ahmed I, Al-Nassar S, Alsultan R, Alwgait W, Alkhalaf H, Bisht S. Video-assisted thoracoscopic decortication for the management of late stage pleural empyema, is it feasible? *Annals of Thoracic Medicine* 2016; 11: 71.

70. Mehta HJ, Biswas A, Penley AM, Cope J, Barnes M, Jantz MA. Management of Intrapleural Sepsis with Once Daily Use of Tissue Plasminogen Activator and Deoxyribonuclease. *Respiration* 2016; 91: 101–106.

71. Kim J, Park JS, Cho Y-J, Yoon HI, Lee JH, Lee C-T, Lim H-J, Kim DK. Predictors of prolonged stay in patients with community-acquired pneumonia and complicated parapneumonic effusion: Delay in hospital stay in CPE patients. *Respirology* 2016; 21: 164–171.

72. Tsai C-H, Lai Y-C, Chang S-C, Chang C-Y, Wang W-S, Yuan M-K. Video-assisted thoracoscopic surgical decortication in the elderly with thoracic empyema: Five years' experience. *Journal of the Chinese Medical Association* 2016; 79: 25–28.

73. Tronina EY, Shipulin PP, Baydan VI, Severgin VE, Baydan VV, Ageyev SV, Kirilyuk AA, Kozyar ON, Polyak SD, Agrakhari A. [POSSIBILITIES OF MODERN TECHNOLOGIES IN THE TREATMENT OF PLEURAL EMPYEMA]. *Klin Khir* 2016; : 46–48.

74. Chubar IV. [ANALYSIS OF MICROFLORA OF PLEURAL CAVITY IN PLEURAL EMPYEMA]. *Klin Khir* 2016; : 47–49.

75. Wong D, Yap E. Pleural infection in a New Zealand centre: high incidence in Pacific people and RAPID score as a prognostic tool: Pleural infection in New Zealand. *Internal Medicine Journal* 2016; 46: 703–709.

76. Park C-K, Oh H-J, Choi H-Y, Shin H-J, Lim JH, Oh I-J, Kim Y-I, Lim S-C, Kim Y-C, Kwon Y-S. Microbiological Characteristics and Predictive Factors for Mortality in Pleural Infection: A Single-Center Cohort Study in Korea. Trottein F, editor. *PLOS ONE* 2016; 11: e0161280.

77. Majid A, Kheir F, Folch A, Fernandez-Bussy S, Chatterji S, Maskey A, Fashjian M, Cheng G, Ochoa S, Alape D, Folch E. Concurrent Intrapleural Instillation of Tissue Plasminogen Activator and DNase for Pleural Infection. A Single-Center Experience. *Annals of the American Thoracic Society* 2016; 13: 1512–1518.

78. Porcel JM, Esquerda A, Vives M, Bielsa S. Etiology of Pleural Effusions: Analysis of More Than 3,000 Consecutive Thoracenteses. *Archivos de Bronconeumología (English Edition)* 2014; 50: 161–165.

79. Tantraworasin A, Thepbunchonchai A, Siwachat S, Ruengorn C, Khunyotying D, Kaufman AJ, Taioli E, Saeteng S. Factors associated with recurrent bacterial empyema thoracis. *Asian Journal of Surgery* [Internet] 2017 [cited 2018 Apr 15]; Available from: http://linkinghub.elsevier.com/retrieve/pii/S1015958416304985.

80. Hsiao C-H, Chen K-C, Chen J-S. Modified single-port non-intubated video-assisted thoracoscopic decortication in high-risk parapneumonic empyema patients. *Surgical Endoscopy* 2017; 31: 1719–1727.

81. Popowicz N, Bintcliffe O, De Fonseka D, Blyth KG, Smith NA, Piccolo F, Martin G, Wong D, Edey A, Maskell N, Lee YCG. Dose De-escalation of Intrapleural Tissue Plasminogen Activator Therapy for Pleural Infection. The Alteplase Dose Assessment for Pleural Infection Therapy Project. *Annals of the American Thoracic Society* 2017; 14: 929–936.

82. Asai N, Suematsu H, Hagihara M, Nishiyama N, Kato H, Sakanashi D, Koizumi Y, Yamagishi Y, Mikamo H. The etiology and bacteriology of healthcare-associated empyema are quite different from those of community-acquired empyema. *Journal of Infection and Chemotherapy* 2017; 23: 661–667.

83. Koma Y, Inoue S, Oda N, Yokota N, Tamai K, Matsumoto Y, Okada N, Otsuka A, Nakashima N, Masuya D, Matsuoka H, Yoshimatsu H, Suzuki Y. Clinical characteristics and outcomes of patients with community-acquired, health-care-associated and hospital-acquired empyema: Clinical characteristics and outcomes of empyema. *The Clinical Respiratory Journal* 2017; 11: 781–788.

84. Dyrhovden R, Nygaard RM, Patel R, Ulvestad E, Kommedal Ø. The bacterial aetiology of pleural empyema. A descriptive and comparative metagenomic study. *Clinical Microbiology and Infection* [Internet] 2018 [cited 2019 Apr 23]; Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X18307833.

85. Boyanova L, Vladimir Djambazov, Gergova G, Dragomir Iotov, Petrov D, Osmanliev D, Minchev Z, Mitov I. Anaerobic microbiology in 198 cases of pleural empyema: a Bulgarian study. *Anaerobe* 2004; 10: 261–267.

86. Corcoran JP, Wrightson JM, Belcher E, DeCamp MM, Feller-Kopman D, Rahman NM. Pleural infection: past, present, and future directions. *The Lancet Respiratory Medicine* 2015; 3: 563–577.

87. James SL, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, Abbastabar H, Abd-Allah F, Abdela J, Abdelalim A, Abdollahpour I, Abdulkader RS, Abebe Z, Abera SF, Abil OZ, Abraha HN, Abu-Raddad LJ, Abu-Rmeileh NME, Accrombessi MMK, Acharya D, Acharya P, Ackerman IN, Adamu AA, Adebayo OM, Adekanmbi V, Adetokunboh OO, Adib MG, Adsuar JC, Afanvi KA, Afarideh M, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories,

1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 2018; 392: 1789–1858.

88. Lin Y-T, Chen T-L, Siu LK, Hsu S-F, Fung C-P. Clinical and microbiological characteristics of community-acquired thoracic empyema or complicated parapneumonic effusion caused by Klebsiella pneumoniae in Taiwan. *European Journal of Clinical Microbiology & Infectious Diseases* 2010; 29: 1003–1010.

89. Richet H. Seasonality in Gram-negative and healthcare-associated infections. *Clinical Microbiology and Infection* 2012; 18: 934–940.

Table 1: Characteristics of the papers included in the systematic review and the reported cohorts in the papers

Age (mean of means ±SD of 56 studies) *years**	54.4±9.5
Number of cases per publication (median, range)	83 (20 – 601)
IQR	(47.5 – 160.75)
Total number of cases	10,241
Study type (observational/interventional)	45 (59%) / 30 (41%)
Study nature (medical/surgical) n (%)	58 (78%) / 16 (22%)
Mean±SD age by study cohort *years*	
Medical	58.8±9.2
Surgical	49.6±9.6
Percent positivity of microbial culture	
Mean±SD	56±21%
Weighted mean	52.4%

* Studies with mixed adult and paediatric populations were not included

IQR; interquartile range, SD; standard deviation

Figure legends:

Figure 1: PRISMA flowchart of the systematic review

Figure 2: The relative contribution of organism categories (pie charts) and specific organisms (bar charts) in culture results of infected pleural fluid. Top left: worldwide; top right: temperate regions; bottom left: subtropics; and bottom right: tropics.

Figure 3 The relative contribution of organism categories (pie charts) and specific organisms (bar charts) in culture results of infected pleural fluid. Left: community-acquired infection; right: hospital-acquired infection.