



Early View

Original article

The microbiology of pleural infection in adults: a systematic review

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

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Contribution details: JPC, IP and NMR conceived the review. EH and MH performed the literature search. MH, JPC, RA, RMM and NMR discussed and agreed on inclusion and exclusion criteria. MH, TC, RA and EB screened publications for eligibility. MH and TC extracted, combined and analysed data. MH wrote the first manuscript. NMR, JPC and 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, 75 papers reporting on 10,241 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 were 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 PROSPERO 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: $\text{mean} = [(2 * \text{median}) + \text{minimum} + \text{maximum}] / 4$. [11]

The absolute numbers of isolated organisms/groups from each study were summated. The sum of the numbers per organism/group 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 40⁰), sub-tropical regions

(between 23.5° and 40° North and South of the equator), and tropical regions (between 23.5° North and 23.5° 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 remaining 136 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 Streptococci* 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 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%). Four of these studies provided details about the polymicrobial results. In 75% of the instances anaerobic bacteria were isolated, they were found to be mixed with other organisms; either aerobic or anaerobic. Forty nine percent of the isolates from the *Viridans* group were retrieved from polymicrobial cultures in comparison to 28.5% for the *Staph. aureus* isolates. Notably, none of the *Pneumococci* isolates grew in mixed cultures.

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 data on the methicillin sensitivity of the *Staph. aureus* isolates. Figure S1 presents the proportions 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 half of the instances, the incidence of polymicrobiality in culture-positive samples can be assumed to be double that figure; viz. about 23%. In a metagenomic study by Dyrhovden et al., massive parallel sequencing of bacterial DNA on 44 samples of pleural infection identified polymicrobial 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-parapneumonic 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. The aforementioned study found that *Pneumococci* are exclusively isolated from monomicrobial cultures in contrast to the *Viridans Streptococci* that are commonly mixed with anaerobes.[84] This is similar to the findings of this review and further confirms that there is more than one pathobiologic pathway for the development of pleural infection.

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, and 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 Streptococci*. 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. This should be reflected in the choice of empirical antimicrobial treatment to address the preponderance of certain microbes and prevalence of antibiotic resistance which should always be supported by data on local resistance patterns.

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

Age (mean of means \pm SD of 56 studies) <i>years</i> *	54.4 \pm 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 \pm SD age by study cohort <i>years</i>	
Medical	58.8 \pm 9.2
Surgical	49.6 \pm 9.6
Percent positivity of microbial culture	
Mean \pm SD	56 \pm 21%
Weighted mean	52.4%

* Studies with mixed adult and paediatric populations were not included

IQR; interquartile range, SD; standard deviation

Figure legends:

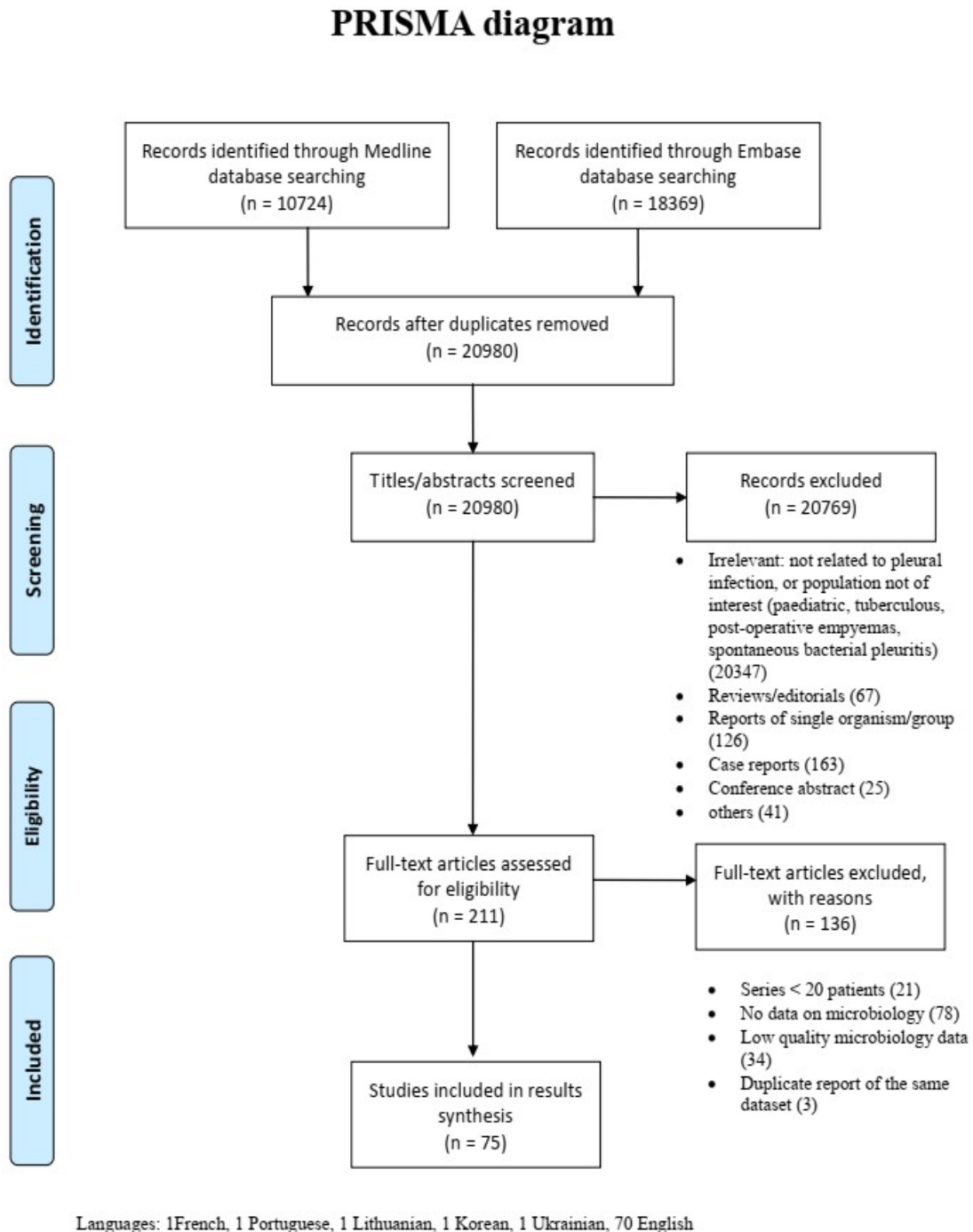
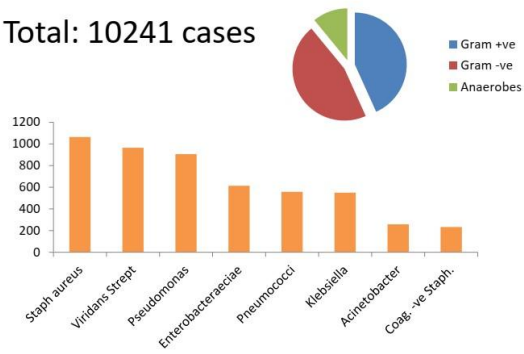


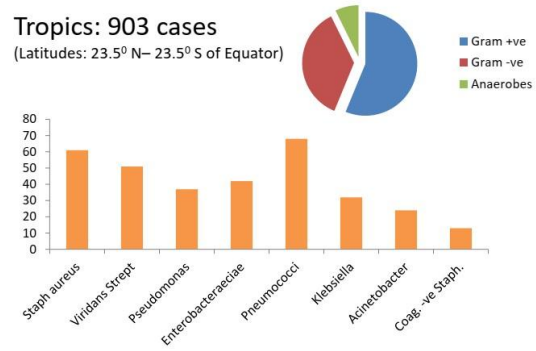
Figure 1: PRISMA flowchart of the systematic review

Total: 10241 cases



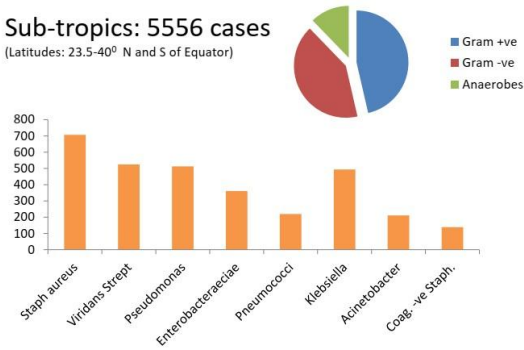
Tropics: 903 cases

(Latitudes: 23.5° N– 23.5° S of Equator)



Sub-tropics: 5556 cases

(Latitudes: 23.5-40° N and S of Equator)



Temperate: 3782 cases

(Latitudes: 40°– 66.5° N and S of Equator)

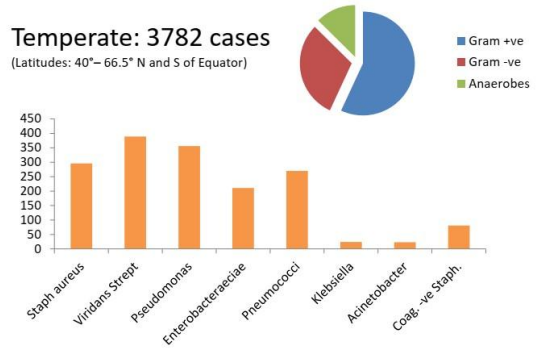


Figure 2: The relative contribution of organism categories (pie charts) and specific organisms (bar charts) in culture results of infected pleural fluid. Top left: worldwide; top right: temperate regions; bottom left: subtropics; and bottom right: tropics.

1523 subjects from 11 studies

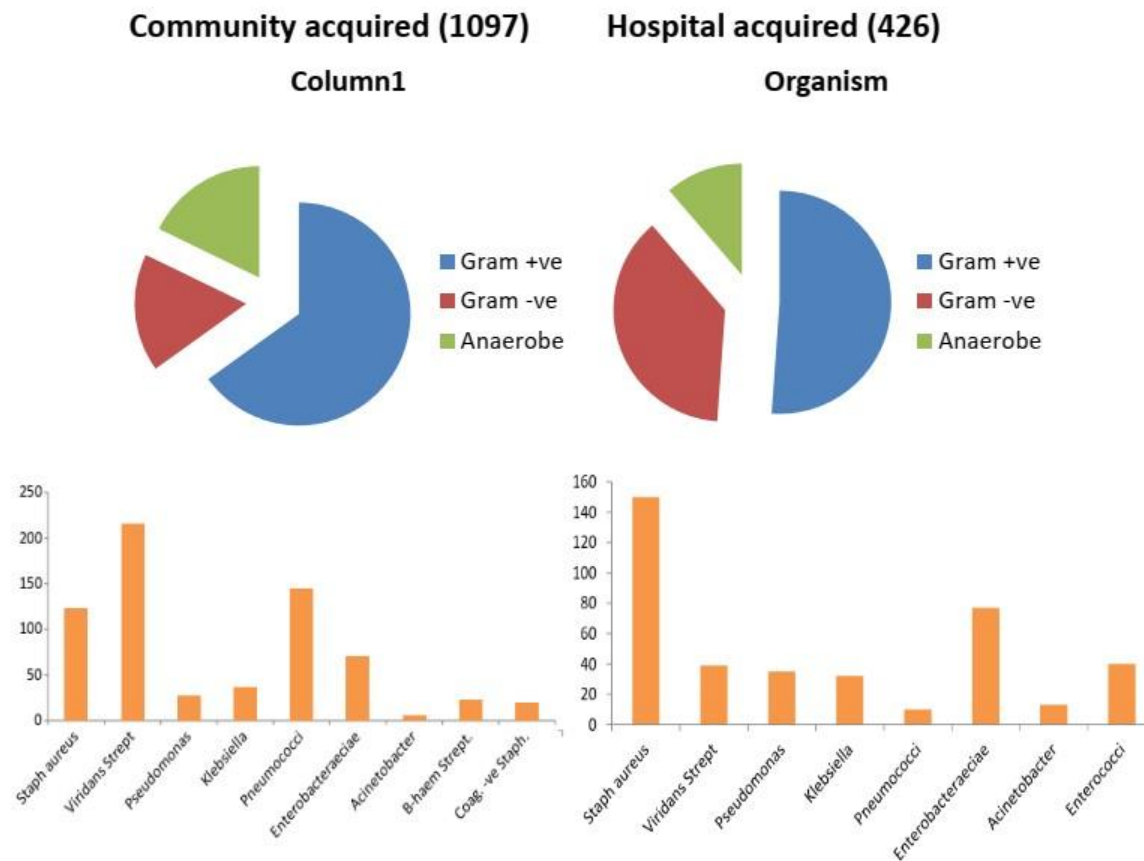


Figure 3: The relative contribution of organism categories (pie charts) and specific organisms (bar charts) in culture results of infected pleural fluid. Left: community-acquired infection; right: hospital-acquired infection.

Supplement figures

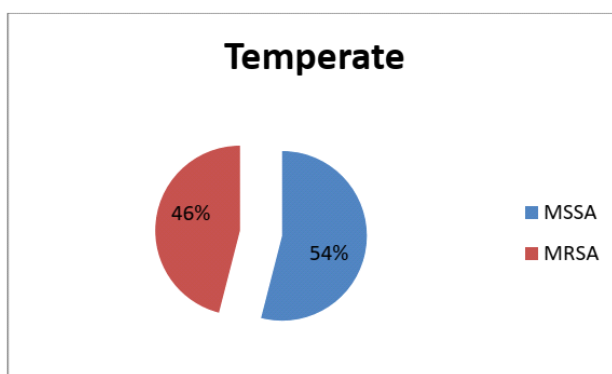
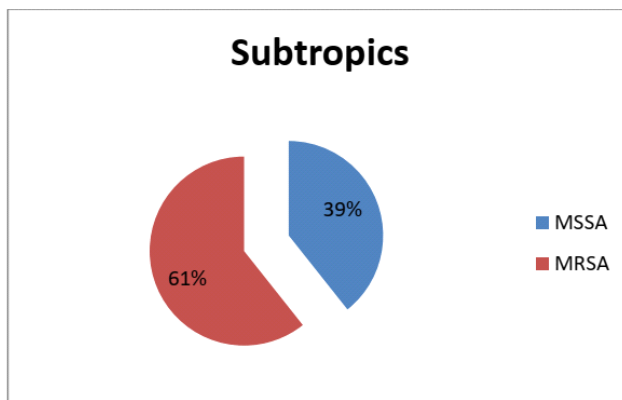
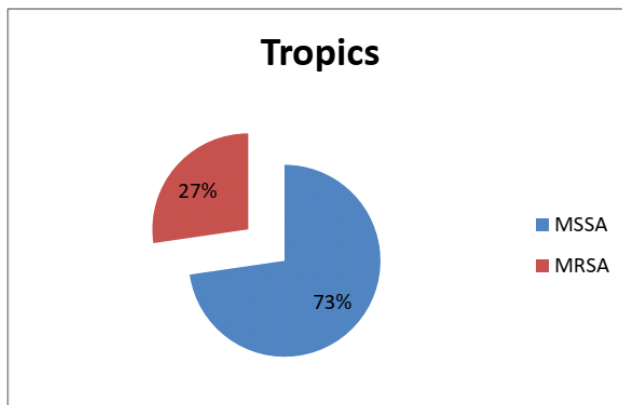


Figure S1: Pie charts show percentages of methicillin-sensitive (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) in culture results from papers from tropics (top), sub-tropics (middle) and temperate (bottom) regions.

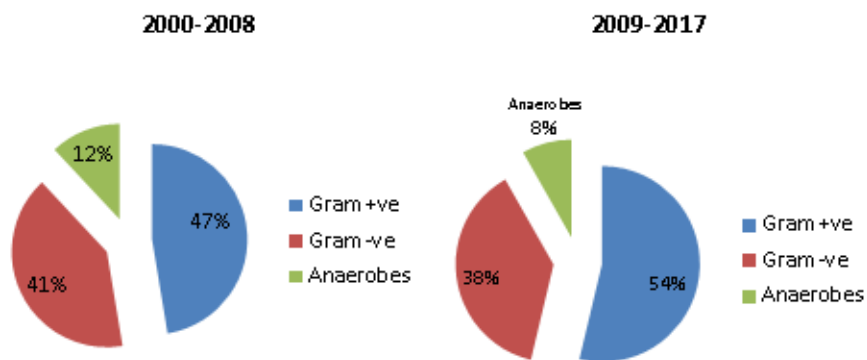


Figure S2: Pie charts show the percentages of isolates from the three bacteriologic groups in papers published between 2000-2008 (left) and papers published between 2009-2017 (right).

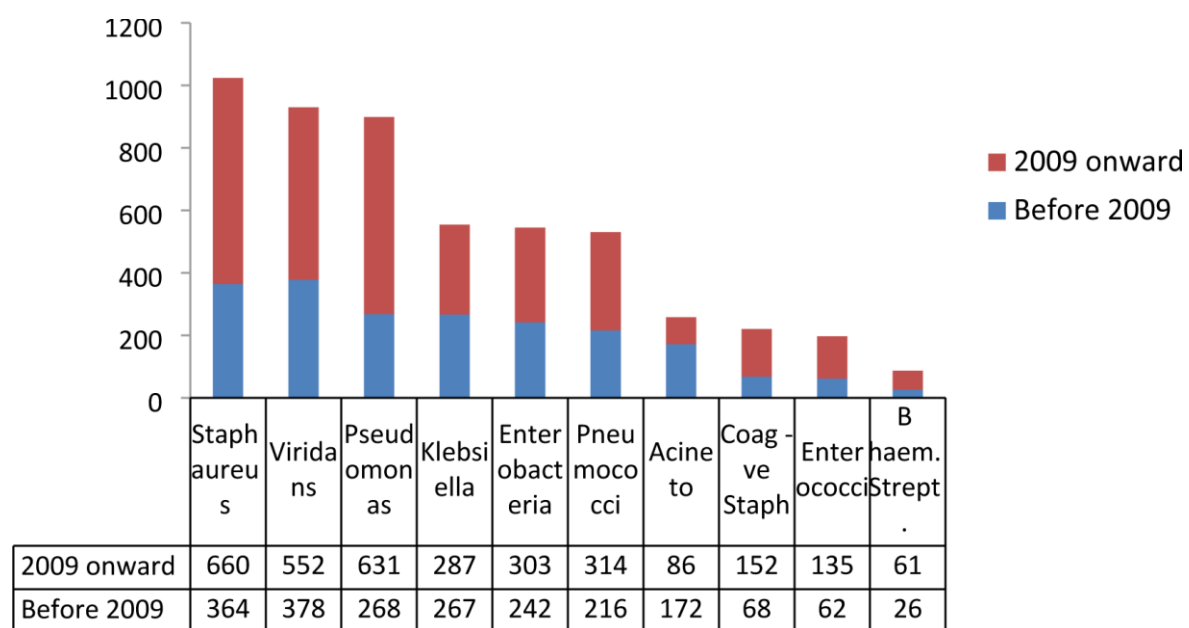


Figure S3: Bar graph shows numbers reported for the ten most common microbes/groups from papers before (blue parts) and after 2009 (red parts).

Methicillin resistance in *Staphylococcus aureus*

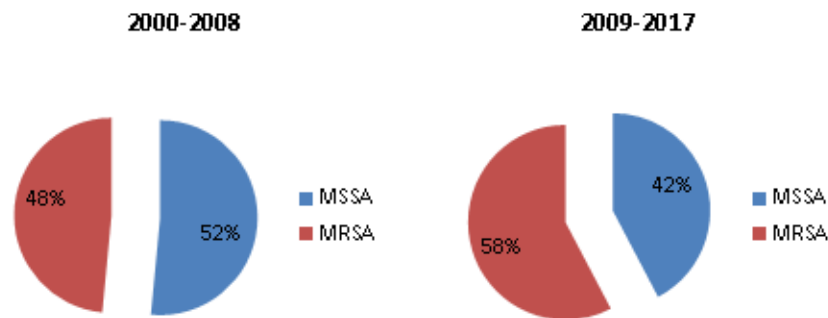


Figure S4: Pie charts show percentages of methicillin-sensitive (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) in culture results from papers published between 2000-2008 (left) and papers published between 2009-2017 (right).

Search strategy

The following strategy was used to search Ovid Medline

1. Adult.mp. or middle aged.sh. or age:.tw.
2. Pleura empyema/
3. Pleura effusion/
4. Exp pleurisy/
5. Empyem*.ti,ab.
6. Parapneumon*.ti,ab.
7. Pyothorax.ti,ab.
8. Pleuritis.ti,ab.
9. (Pleur* ADJ4 effus*).ti,ab.
10. (Pleur* ADJ4 infect*).ti,ab.

11. (Pleur* ADJ4 suppurat*).ti,ab.
12. (Pleur* ADJ4 collect*).ti,ab.
13. OR/2-12
14. 1 AND 13
15. Exp animals/ not humans.sh.
16. 14 NOT 15
17. Limit 16 to yr="2000-2017"