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Short Communication

A comparative analysis between next-generation sequencing and conventional culture method to detect empyema-associated microorganisms: A systematic review

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Abstract

Empyema poses a significant global health concern, yet identifying responsible bacteria remains elusive. Recent studies question the efficacy of conventional pleural fluid culture in accurately identifying empyema-causing bacteria. The aim of this study was to compare diagnostic capabilities of next-generation sequencing (NGS) with conventional pleural fluid culture in identifying empyema-causing bacteria. Five databases (Google Scholar, Science Direct, Cochrane, Research Gate, and PubMed) were used to search studies comparing conventional pleural fluid culture with NGS for identifying empyema-causing bacteria using keywords. Positive results identified through conventional pleural fluid culture and NGS were extracted. In addition, bacterial profiles identified by NGS were also documented. Joanna-Briggs Institute (JBI) critical appraisal tool was employed to assess quality of included studies. Descriptive analysis was employed to present outcome of interests. From five databases, three studies, with 354 patients, were included. Findings from three studies showed that NGS outperformed conventional pleural fluid culture in detecting empyema-causing bacteria even in culture-negative samples. Moreover, dominant bacterial profiles identified through NGS included *Streptococcus pneumoniae, Staphylococcus aureus*, and anaerobic bacteria. In conclusion, NGS outperforms conventional pleural fluid culture in detection empyema-causing bacteria, yet further studies with larger samples and broader bacterial profiles are needed to increase confidence and urgency in its adoption over conventional pleural fluid culture.

Keywords: Empyema, culture, metagenomic, next-generation sequencing, diagnostic

Introduction

Empyema, a pleural infection caused by microbial proliferation, poses a significant global health concern [1]. Its incidence rates have surged over the past decade, with an increase from 6.44 to 8.38 per 100,000 cases and a mortality rate of 14% [2]. Empyema is a leading cause of pleural effusion, constituting up to 25.1% of cases, with the highest prevalence observed in older patients,

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accounting for 62% of cases [3]. As immunity response decreases with age, risk of mortality due to empyema increases in older patients [3,4]. A statistically significant rise in the polymicrobial etiology of empyema (p<0.0001) was seen in the study conducted in 2023, which examined the epidemiology of pulmonary empyema during the COVID-19 pandemic [5].

Clinicians encountered challenges in empyema treatment due to patients' characteristics, high incidence, mortality rates, and treatment duration [2-4]. Furthermore, deciding between medication and surgical intervention presents further obstacles in treating empyema [6]. Tailoring antimicrobial therapy to empyema is crucial, yet identifying responsible bacteria remains elusive [7,8].

Conventional pleural fluid culture is widely used for microbial identification due to its affordability, sensitivity, and widespread adoption. One persistent issue with traditional microbe culture for pathogenic microorganism identification is low sensitivity and specificity. Due to certain bacterial species being uncultivable or requiring certain culture conditions, up to 40% of the samples may be culture-negative [9-13]. Recent studies questioned the efficacy of conventional pleural fluid culture in accurately identifying empyema-causing bacteria [4,14-17]. Therefore, metagenomics techniques, such as next-generation sequencing (NGS), have gained prominence in identifying empyema-causing bacteria [15,16,18]. The aim of this study was to compare diagnostic capabilities of NGS with conventional pleural fluid culture in identifying empyema-causing bacteria.

Methods

Search strategy

Literature searching was carried out as of December 20, 2024. Five databases were used (Google Scholar, Science Direct, Cochrane, Research Gate, and PubMed) to search the studies. The keywords of 'empyema', 'next-generation sequencing', and their synonyms were used. Protocol in the present study followed the guideline of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [19] and was registered in Prospective Register of Systematic Reviews (PROSPERO) with registered number: CRD42023469839.

Study selection

Inclusion criteria in the present systematic review adopted Population, Intervention, Control, Outcome, and Study design (PICOS) framework, which included studies that had following criteria: (a) Population: empyema patients; (b) Intervention: NGS; (c) Control: conventional pleural fluid culture; (d) Outcome: positive results identified through conventional pleural fluid culture and NGS, and bacterial profile identified through NGS; and (e) Study design: observational study. Included studies were limited to studies conducted within the past ten years. All studies published in language other than English and Indonesia, not reporting one of outcomes of interest, and all review articles, conference abstracts, case report, case series, editorials, commentary, thesis, and erratum were excluded.

Data extraction

From included studies, the study characteristics were extracted: first author, publication year, country, study design, and sample size. Positive results identified through conventional pleural fluid culture and NGS also were extracted. In addition, bacterial profiles identified by NGS were documented.

Data screening

Screening was carried out by two independent authors (YI and AMS). Duplicates were immediately removed using Mendeley. First-layer screening process was conducted based on the title and abstract, and subsequently refined through full-text screening according to inclusion and exclusion criteria.

Quality assessment

Joanna-Briggs Institute (JBI) critical appraisal tool was employed to assess quality of included studies. JBI critical appraisal tool encompasses six domains: study design, methodology,

participant selection, data collection, analysis, and interpretation. Each item within these domains was assessed, categorized as either 'yes' or 'no'. Overall score was then expressed as a percentage (%). A cut-off score exceeding 70% was deemed indicative of low risk of bias.

Statistical analysis

Descriptive analysis was employed to present outcome of interests. Continuous variables are presented using mean and standard deviation, while dichotomous variables are reported as frequencies or percentages.

Results

The literature searches, screening process workflow, and number of studies obtained from each step are presented in **Figure 1**. The searches yielded 7,205 studies and after removing duplicates, 7,175 studies were screened for eligibility. A total of the 7,168 studies were excluded since the titles and abstracts were not matched with the aim of the study leaving seven studies. Out of the seven studies, four studies were excluded since the the whole genome sequencing was conducted not using NGS, resulting three studies included in the further analysis.

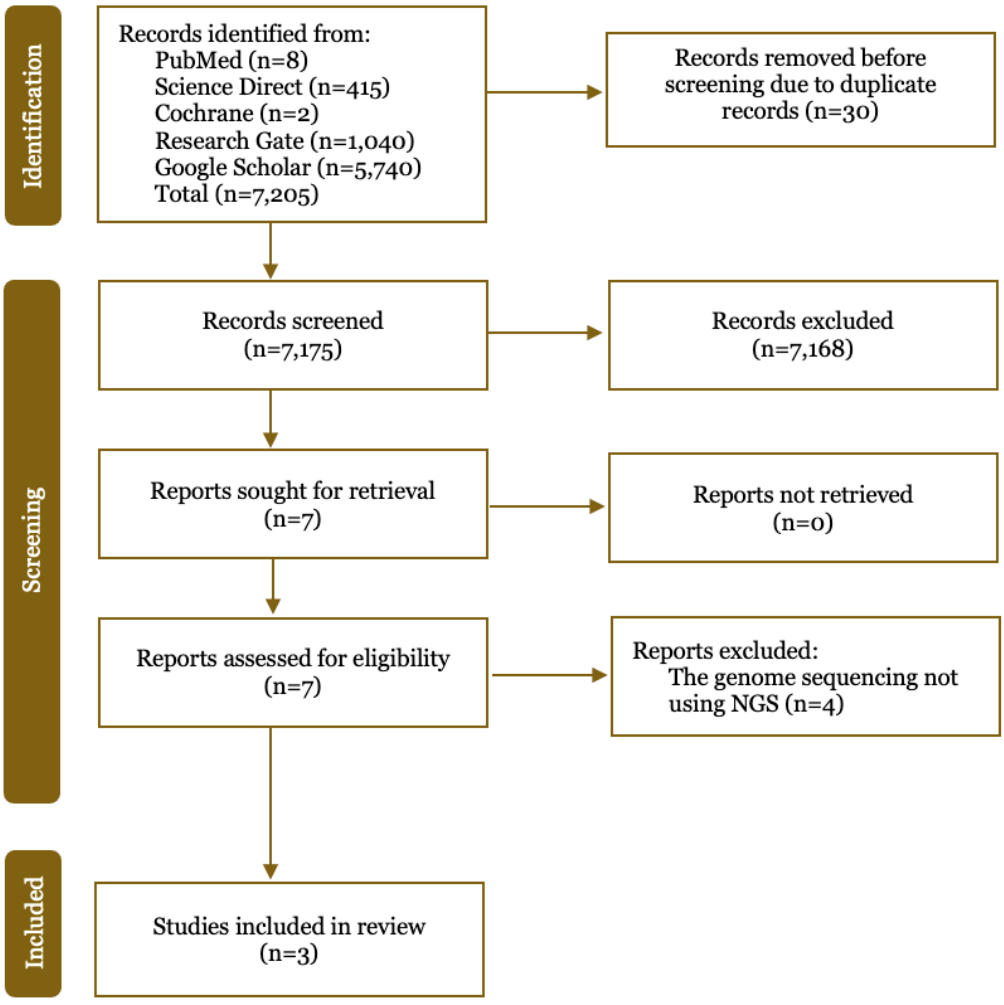

Figure 1. PRISMA flowchart of included studies.

Characteristics of included studies

A total of three studies were included and their characteristics are summarized in **Table 1**. The studies covered 354 empyema patients from three countries (Norway, China and United Kingdom). Despite different metagenomic techniques, all three studies employed NGS, comparing it with conventional pleural fluid culture.

Table 1. Characteristic of included studies comparing next-generation sequencing (NGS) and conventional culture method to detect empyema-causing bacteria (n=3)

Author, year	Country	n	Method	Culture-positive n (%)	NGS-positive n (%)	NGS-positive, culture-negative n (%)
Dyrhovden *et al.*, 2018 [8]	Norway	385	Metagenomic	38 (10%)	87 (22.5)	39 (61)
Chen *et al.*, 2021 [6]	China	45	NGS	12 (26.7%)	45 (100)	Not available
Kanellakis *et al.* 2022 [9]	United Kingdom	245	NGS	55 (22%)	245 (100)	190(77.5)

Comparison of conventional pleural fluid culture and next-generation sequencing (NGS)

Findings from three studies suggested that NGS outperformed conventional pleural fluid culture in detecting empyema-causing bacteria even in culture-negative samples (**Table 1**). Dyrhoyden *et al.* [8] found that NGS identified empyema-causing bacteria in 62.11% of samples versus 10% with conventional pleural fluid culture. Kanellakis *et al* [9] similarly demonstrated NGS detecting empyema-causing bacteria in 100% sample versus 22% with conventional pleural fluid culture.

Bacterial profile from next-generation sequencing (NGS)

As presented in **Table 2**, various empyema-causing bacteria were detected by NGS from empyema patients. Dyrhoyden *et al.* [8] highlighted *Streptococcus pneumoniae* as the main cause, while Chen *et al.* [6] reported *Staphylococcus aureus* predominance. Kanellakis *et al.* [6] found anaerobic bacteria dominate, comprising 55% of the patients.

Table 2. Bacterial profile identified by next-generation sequencing (NGS) from the included studies (n=3)

Bacteria species	Dyrhovden *et al.* 2018 (n=385 cases)	Chen *et al.* 2021 (n=45 cases)	Kanellakis *et al.* 2022 (n=245 cases)
Anaerobics	Not detected	Not detected	55
Enterobacteriaceae	Not detected	Not detected	14
Staphylococcus aureus	5	36	1
Streptococcus pneumonia	8	Not detected	1
Pseudomonas aeruginosa	2	Not detected	Not detected
Streptococcus pyogenes	1	Not detected	Not detected
Escherichia coli	1	Not detected	Not detected
Klebsiella pneumoniae	1	Not detected	Not detected
Mycobacterium tuberculosis	Not detected	Not detected	1
Other Gram positive	Not detected	Not detected	59
Other Gram negative	Not detected	Not detected	110

Risk of bias

Using the JBI critical appraisal tool, the average bias score for two included studies [8,9] exceeded 70%, indicating low risk of bias. One study [6] had moderate risk. However, all three studies were included due to limitation of the available studies (**Table 3**).

Table 3. Detailed score for each domain of Joanna-Briggs Institute (JBI) critical appraisal for included studies (n=3)

First author, year	Joanna-Briggs Institute (JBI) critical appraisal domain								Bias
	1	2	3	4	5	6	7	8	
Dyrhovden *et al.*, 2018 [8]	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Low risk
Chen *et al.*, 2021 [6]	Unclear	Unclear	Yes	Yes	No	No	Yes	Yes	Moderate
Kanellakis *et al.* 2022 [9]	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Low risk

Discussion

Comparing NGS with conventional pleural fluid culture to determine empyema-causing bacteria has revealed intriguing insights into the diagnostic landscape of empyema. In the present study, only three studies compared NGS with conventional pleural fluid culture, making it difficult to assess NGS superiority. Few studies focus on empyema-causing bacteria, mirroring challenges in culture-based diagnosis. Similarly, our hospital experience revealed complexities in culturing empyema cases, leading to limited reported empyema-causing bacteria in literature.

In the present study, NGS consistently yielded more apparent empyema-causing bacteria findings than conventional pleural fluid culture when examining empyema samples. NGS ability to identify empyema-causing bacteria, even in culture-negative cases, underscores its superiority over culture-based methods in empyema diagnosis. In conventional pleural fluid culture, low bacterial counts leading to false negatives, especially for anaerobic bacteria [13,14]. In addition, overall poor sensitivity rates of conventional pleural fluid culture ranging from 18% to 60% [3,4]. Blood culture bottles are used to tackle this issue, yet limitations persist, particularly in extreme environments [14,15,20]. False negatives are common in tests due to weaknesses in the conventional pleural fluid culture, including issues with antibiotic use, sample collection, and sample transportation [14,21-23]. Prolonged waiting times for bacterial growth often led to patient improvement or deterioration before empirical therapy, and method's ability to distinguish between similar bacterial strains is limited [14,24,25]. Therefore, when comparing the capabilities of NGS compared to culture, the ability of NGS is able to detect DNA or RNA and has a shorter time than specimen culture which takes time and is highly dependent on the skills of the specimen stylist to get more accurate results.

Furthermore, bacterial profile obtained using NGS is notably diverse, enhancing prospects for comprehensive etiological identification in empyema cases. Metagenomics offers advantages by detecting diverse microorganisms and novel genes from DNA or RNA fragments in a single analysis, surpassing conventional pleural fluid culture's limitations [16]. This advancement holds promise for facilitating accurate antibiotic therapy, thereby mitigating morbidity and mortality associated with empyema.

To the best of our knowledge, the present study is the first to suggest NGS's potential to overcome limitation of conventional pleural fluid culture in detecting empyema-causing bacteria. Further studies with larger samples and and broader bacterial profiles are needed to confirm NGS's superiority over culture in identifying empyema-causing bacteria, emphasizing its urgent role in improving clinical outcomes. With the capability for better detection of microorganisms up to the genetic stage, it opens the opportunity for patients with empyema to get accurate antibiotic therapy leading to have better outcomes.

Conclusion

NGS outperforms conventional pleural fluid culture in detection empyema-causing bacteria and bacterial profile obtained using NGS is notably diverse, yet further studies with larger samples and broader bacterial profiles are needed to increase confidence and urgency in its adoption over culture.

Ethics approval

Not required.

Acknowledgments

None.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available as part of the article.

How to cite

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