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Innovative Diagnostic Approach for Bloodstream Infections: Rapid Extraction and Filtration Method for Gram-Negative Bacteria

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Abstract

Sepsis presents a significant challenge in healthcare, with increasing incidence rates globally. This study addresses the pressing need for timely diagnosis and intervention by evaluating the efficacy of a rapid extraction and filtration method for identifying Gram-negative bacteria in blood samples. The research, conducted at Taipei Cathay General Hospital between July 1, 2023, and March 20, 2024, analyzed 191 positive blood bottle samples. The experimental group utilized Gram staining followed by rapid extraction and filtration, while the control group underwent traditional subculture. Results showed a high identification agreement rate of 94% (177/188) between the experimental and control groups. Furthermore, drug susceptibility testing exhibited a high level of consistency, with an Essential agreement (EA) and Category agreement (CA) of 98% (2694/2738) each. While the rapid method met acceptance criteria and demonstrated lower error rates, improvements are still warranted. This study underscores the potential of rapid diagnostic methods in improving sepsis management, with implications for enhancing patient care and healthcare efficiency. Future research should focus on broader clinical trials to further validate and optimize these diagnostic approaches.

Key Words: Bloodstream infection, Gram-negative bacteria, Rapid diagnostic method, Clinical microbiology, MALDI-TOF

Introduction

Based on data from our country's Ministry of Health and Welfare, sepsis was among the top 20 causes of death in 2022, claiming the lives of 2,345 individuals. This figure highlights a troubling trend: the increasing prevalence of sepsis patients in recent years. Contributing factors include the aging population, a rise in chronic diseases, immunocompromised individuals, and the growing use of invasive medical procedures. This trend is not unique to our country but is mirrored globally. A retrospective analysis in the United States from 1998 to 2009 showed a significant surge in sepsis and septic shock incidence, from 13 to 78 cases per 100,000 people[1]. Moreover, international studies emphasize the substantial global health burden of sepsis, with 48.9 million cases reported in

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2017[2].

Statistics from the National Development Council indicate that Taiwan's elderly population, aged over 65, reached 4,296,985 in 2023. Projections suggest that by 2026, seniors will make up 20.8% of the total population, transforming Taiwan into a super-aged society. This demographic shift is expected to increase the incidence and mortality rates of sepsis, adding strain to the healthcare system. Additionally, advancements in medical technology have led to more invasive treatments like surgery and infusion therapies, which, while improving disease management, also raise the risk of sepsis development.

In addition to demographic shifts and medical advances, the misuse of antibiotics and the rise of drug-resistant pathogens significantly contribute to the increasing incidence of sepsis. Multidrug-resistant strains, such as Gram-negative bacilli, present particular challenges to effective sepsis treatment, having developed resistance to conventional antibiotics and necessitating stronger therapeutic interventions[3].

Various studies have highlighted the prevalence of Gram-negative bacteria, especially Gram-negative bacilli (GNB), in bloodstream infections. Data from the National Healthcare Safety Network show that around one-quarter of central venous catheter-associated bloodstream infections between 2009 and 2010 were attributed to Gram-negative bacilli[4]. Similarly, a multicenter study in Brazil involving 2563 patients with nosocomial bacteremia found that 58.5% of infections were caused by Gram-negative bacteria[5]. Furthermore, Gram-negative bacteremia is more common in elderly individuals in the community, with a retrospective analysis showing Gram-negative bacteria as the causative agent in 36% of cases in patients aged over 65[6]. These findings underscore the significant role of Gram-negative bacteria, particularly in the elderly population, in contributing to the burden of sepsis.

The effectiveness of treatment relies on completing multiple interventions within a specified time frame, highlighting the importance of timely diagnosis and intervention. Currently, the testing process takes 48 to 72 hours on average to complete bacterial strain identification and drug sensitivity testing reports. Timely diagnosis and intervention are critical in managing sepsis, as delays can significantly affect patient outcomes[7]. Research has demonstrated that for septic shock patients, each hour of delay in administering appropriate antibiotic therapy is associated with a 7.6% decrease in survival rate[8,9]. This statistic underscores the urgent need for rapid diagnostic methods that can expedite the identification of pathogens and enable prompt, targeted treatment.

According to Tsuchida et al. (2018), using an improved in-house lysis-filtration (IH) method can significantly increase the accuracy of MALDI-TOF MS identification of pathogens in positive blood culture bottles, especially for Gram-positive bacteria. Compared to the Sepsityper Kit, the IH method achieved 98% accuracy for Gram-negative bacteria and 98.5% for Gram-positive bacteria. These data suggest that the improved lysis-filtration method can significantly enhance the speed and accuracy of pathogen identification, thus reducing clinical diagnosis time and improving treatment outcomes[10].

This study aims to evaluate the efficacy of the rapid extraction and filtration method for Gram-negative bacteria-positive blood bottles and to develop a streamlined process suitable for routine clinical application. The goal is to transcend traditional frameworks, expedite reporting timelines, and assist clinicians in promptly administering appropriate drug treatments, thereby reducing mortality and drug resistance in sepsis patients.

Method

Sample Collection and Preparation:

During the study period, we collected all positive blood bottle samples tested at Taipei Cathay General Hospital between July 1, 2023, to March 20,

2024. Each sample was divided into experimental and control groups. (Figure 1)

In the experimental group, Gram staining was initially conducted to identify a single strain of Gram-negative bacteria as the target sample. Subsequently, 0.5 ml of cell lysis buffer (OctylPhenoxypolyethoxyethanol, Triton X-100) from the DTC SepsiFilt Kit (DiaTech Technology Co. Ltd., Taiwan) was added to 3 ml of the blood sample to be tested. The lysis buffer in the DTC SepsiFilt Kit is specifically designed to lyse bacterial cells and release intracellular components for subsequent analysis. It contains surfac-

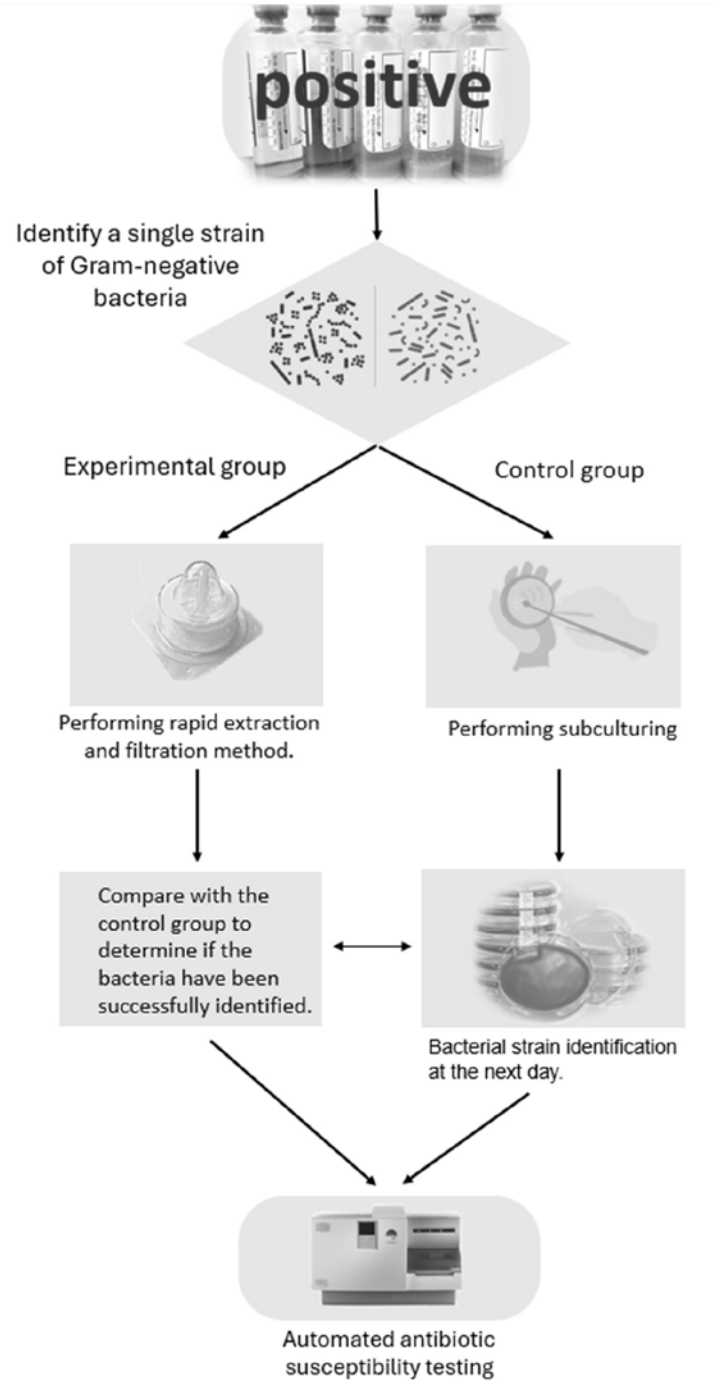

Figure 1.

tants and other agents that facilitate the breakdown of bacterial cell walls. The mixture was then placed on a Vortex Mixer for 10 seconds to ensure thorough mixing and complete action of the cell lysis solution.

Afterward, 1.5 ml of the mixture was filtered into a microcentrifuge tube using a filter with a 3 μm pore size (Nylon membrane manufactured by DiaTech Technology Co. Ltd.). The choice of a 3 μm pore size filter is based on its effectiveness in trapping non-bacterial components, such as red and white blood cells, while allowing smaller bacterial cells and proteins (less than 1 μm in size) to pass through. This ensures that the filtrate contains primarily bacterial cells and proteins. The filtrate was then centrifuged at 12,000g for 1 minute to collect the precipitate as the target test specimen (Figure 2). Since MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) can detect fine protein signals and utilize protein molecular sizes to establish spectra for bacterial identification, these test specimens, rich in bacterial cells and proteins, can be accurately identified for bacterial strains using MALDI-TOF. Following identification, the bacterial strains will undergo automated antibiotic susceptibility testing. All these procedures will be completed within the same day.

In the control group, parallel samples taken from the same positive blood culture bottles in the experimental group were sub-cultured to isolate single colony for subsequent strain identification by MALDI-TOF and drug susceptibility testing. Compared to the control group, the experimental group omitted the time for subculture, enabling the report to be sent to the clinical end approximately 24 hours earlier.

Analysis:

Our primary endpoint is to initially compare the results of the experimental and control groups and evaluate the consistency of test specimen identification to confirm whether the strains identified by the experimental group using rapid extraction and filtration are consistent with those identified by standard subculture in the control group. If the strains identified by the experimental group match those of the control group, it indicates that the results of the strain identification test and automated antibiotic susceptibility testing equipment conducted by the experimental group on the previous day can be trusted. However, if the strains identified by the experimental group differ from those cultured by the control group the next day, and the results of the strain identification test and automated antibiotic susceptibility testing equipment conducted by the experimental group on the previous day cannot be trusted, the standard process will continue, and the report will be sent to the clinical end.

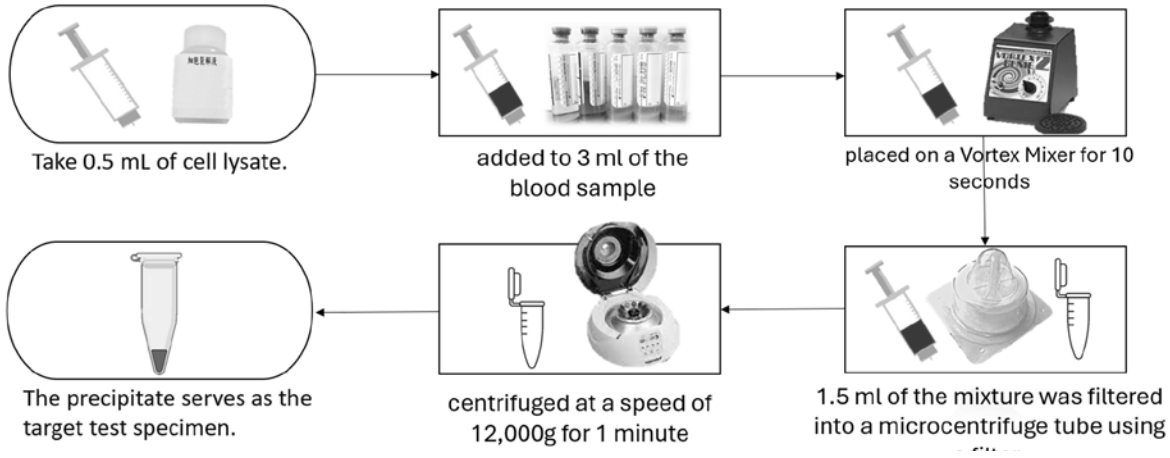

Figure 2.

Table 1.
Definition :

Term	abbr.	Definition
Essential agreement	EA	MICs are either consistent or differ by a dilution factor of one.
Category agreement	CA	Consistent SIR
Major error	ME	One result indicates sensitivity, while the other indicates resistance.
minor error	mE	One result indicates intermediate susceptibility, while the other indicates sensitivity or resistance

The secondary endpoint is to evaluate the accuracy of drug susceptibility testing in the experimental and control groups, and according to the standards outlined in "Cumitech 31A: Validation and Verification of Clinical Microbiology Laboratory Procedures [11]." This includes ensuring that Category agreement (CA) and Essential agreement (EA) are both greater than or equal to 90%, Major error (ME) is less than 5%, and the sum of Major error and minor error (mE) is less than 10%. (Table 1)

Rationale for Methodology:

The choice of using a rapid extraction and filtration method is supported by previous research that has shown its efficacy in improving the accuracy and speed of bacterial identification. Tsuchida et al. (2018) demonstrated that the improved in-house lysis-filtration method significantly increased the accuracy of MALDI-TOF MS identification of pathogens in positive blood culture bottles. The method was particularly effective for Gram-negative bacteria, achieving an accuracy rate of 98% [10]. This supports the use of the DTC SepsiFilt Kit and the procedures outlined above as an effective approach for rapid diagnosis.

Through this method, our aim is to simplify the process of identifying Gram-negative bacterial strains in blood samples and assessing their antibiotic susceptibility. By comparing the results of the experimental and control groups, our study aims to validate the effectiveness and accuracy of the rapid extraction and filtration method in a clinical setting and provide a practical method and basis for future clinical testing.

Results

In this study, we collected a total of 191 positive blood bottle samples between July 1, 2023, and March 20, 2024. However, three samples couldn't be successfully identified by traditional methods and were consequently excluded from statistical analysis. Out of the remaining 188 tested samples, we observed a high identification agreement rate of 94% (177/188) in the primary endpoint analysis. (Table 2). This indicates that the rapid extraction and filtration method allowed for successful identification of most Gram-negative bacteria, enabling reports to be sent to the clinical end 24 hours earlier. For the eleven samples that did not achieve consistency, we were able to resort to bacterial culture, identification, and drug susceptibility testing through traditional methods without compromising patient safety. In the observation of secondary endpoints, aside from two anaerobic strains that were originally unavailable for drug susceptibility testing at our hospital, we compared the rest results of 175 drug susceptibility tests conducted using both the rapid extraction and filtration method and the traditional approach. We found a high level of consistency between them (table 3). Specifically, the Essential agreement (EA) reached 98% (2694/2738), while the Category agreement (CA)

Table 2.

Specimen	Quantity	Strain identification		
		Traditional	Rapid filtration	Consistency rate
Escherichia coli	94	94	94	100%
Klebsiella pneumoniae	37	37	35	94%
Klebsiella oxytoca	3	3	3	100%
Klebsiella aerogenes	2	2	2	100%
Serratia marcescens	10	10	9	90%
Aeromonas hydrophila	2	2	2	100%
Aeromonas sobria	3	3	2	67%
Acinetobacter baumannii complex	2	1	1	100%
Acinetobacter nosocomialis	2	2	2	100%
Acinetobacter ursingii	2	2	0	0%
Achromobacter denitrificans	1	1	1	100%
Bacteroides fragilis	1	1	1	100%
Chryseobacterium indologenes	2	0	0	-
Citrobacter koseri	1	1	1	100%
Enterobacter cloacae	4	4	2	50%
Elizabethkingia anophelis	2	2	2	100%
Pantoea agglomerans	1	1	0	0%
Parabacteroides distasonis	1	1	1	100%
Proteus mirabilis	4	4	4	100%
Pseudomonas aeruginosa	4	4	4	100%
Raoultella ornithinolytica	1	1	1	100%
Salmonella spp.	4	4	4	100%
Stenotrophomonas maltophilia	7	7	5	71%
Vibrio vulnificus	1	1	1	100%
Total	191	188	177	94%

reached 98% (2673/2738). However, although the rapid extraction and filtration method met the acceptance criteria and showed a lower Major error (ME) rate of only 4.3% (22/513) and a lower minor error (mE) rate of 1.6% (43/2738), there is still some room for improvement in this aspect.

Based on the above results, this study verifies the effectiveness and accuracy of the rapid extraction and filtration method for rapid identification and drug susceptibility testing of Gram-negative bacterial infections in clinical settings. These findings hold important implications for enhancing clinical

Table 3.

Species	Cumitech 31A Criteria EA≥90 CA≥90 ME<5 ME+mE<10				
	Amount	EA	CA	ME	mE
Escherichia coli	94	99%	98%	3%	1.4%
Klebsiella pneumoniae	35	98%	99%	2.6%	1.0%
Klebsiella oxytoca	3	98%	98%	0%	0%
Klebsiella aerogenes	2	100%	100%	0%	0%
Serratia marcescens	9	97%	95%	1.5%	3.9%
Aeromonas hydrophila	2	93%	93%	33%	0%
Aeromonas sobria	2	89%	89%	50%	0%
Acinetobacter baumannii complex	1	100%	100%	0%	0%
Acinetobacter nosoomialis	2	100%	100%	0%	0%
Achromobacter denitrificans	1	92%	92%	0%	8.3%
Citrobacter koseri	1	100%	100%	0%	0%
Enterobacter cloacae	2	100%	100%	0%	0%
Elizabethkingia anophelis	2	96%	88%	5.5%	8.3%
Proteus mirabilis	4	97%	91%	7.4%	6.3%
Pseudomonas aeruginosa	4	98%	100%	0%	0%
Raoultella ornithinolytica	1	100%	100%	0%	0%
Salmonella spp.	4	99%	99%	5.9%	0%
Stenotrophomonas maltophilia	5	60%	80%	28.6%	0%
Vibrio vulnificus	1	92%	92%	0%	0%
Total	175	98%	98%	4.3%	1.6%

microbiology laboratory processes and improving the quality of care. Therefore, following the completion of this study, we continue to utilize rapid extraction and filtration methods alongside traditional methods in our hospital to achieve early report issuance while ensuring patient safety.

Discussion

Sepsis and bloodstream infections have posed significant global health challenges, with their incidence rising and resulting in considerable mortality. This challenge is exacerbated by shifting demo-

graphics, medical advancements, and the emergence of drug-resistant pathogens. Given these concerns, enhancing diagnostic efficiency is paramount for improving patient outcomes and reducing healthcare costs.

Previous studies have demonstrated that molecular techniques like MALDI-TOF can truncate bacterial species identification time from 24 to 48 hours to merely 4 to 18 hours. This furnishes clinicians with swifter and more accurate diagnostic insights, facilitating the precise and prompt administration of antibiotics. For instance, Maggie J Box, et al.'s study observed that expeditious diagnosis of bacteremia led to a significantly reduced mean time to targeted antibiotic therapy. This underscores the significance of rapid identification methods. Furthermore, their research revealed that early diagnosis was correlated with a decreased median length of hospital stay and median overall hospitalization costs [12.13]. These findings further underscore the potential economic and clinical benefits of early diagnosis in mitigating healthcare resource utilization and enhancing patient outcomes.

Our study builds upon existing inspection methodologies. In cases of bloodstream infections caused by Gram-negative bacteria, we significantly reduced the diagnosis time by approximately 24 hours, emphasizing the crucial role of timely diagnosis in sepsis management. In our investigation, by directly extracting bacteria from positive blood bottles, this innovative approach eliminated the time-consuming subculture process, substantially shortening reporting time and facilitating the prompt initiation of appropriate antibiotic therapy. This rapid extraction and filtration technique demonstrates comparable accuracy, but increased efficiency compared to traditional testing methods for Gram-negative bacteria in positive blood bottles, promising expedited diagnosis and streamlined clinical workflows. This is particularly critical in severe illnesses like sepsis, where prompt intervention can profoundly impact patient outcomes.

In addition to Gram-negative bacteria, Gram-positive bacteria also play a significant role in bloodstream infections. According to Tsuchida et al. (2018), an improved in-house lysis-filtration method achieved 98.5% accuracy for Gram-positive bacteria compared to 76.1% for the Sepsityper kit[10]. This suggests that our rapid extraction and filtration method may also be effective for Gram-positive bacterial identification, which warrants further investigation. In this study, we chose to prioritize the detection of Gram-negative bacteria due to the challenges associated with Gram-positive bacteria detection, particularly in terms of accuracy and efficiency. By initially focusing on Gram-negative bacteria, we ensured that our detection methods met the desired standards of accuracy and reliability. These preliminary results provide a solid foundation for future research, where we plan to implement Gram-positive bacteria detection and further validate and optimize these diagnostic methods. Our goal is to enhance the overall efficiency and accuracy of clinical microbiology testing.

Our research bolsters the burgeoning evidence base supporting the adoption of rapid diagnostic technologies and early detection of bloodstream infections, ultimately bolstering patient care and healthcare efficiency. Nevertheless, despite encouraging results, room for improvement persists. Although our method exhibited high consistency and accuracy in identifying Gram-negative bacterial strains and conducting drug susceptibility testing, further reductions in error rates are still feasible.

In conclusion, the utilization of rapid extraction filtration technology offers an effective remedy to the time delay inherent in traditional inspection processes. Its exceptional identification success rate and consistency in drug susceptibility testing render it a potent tool in clinical diagnostics. By promoting early diagnosis and the timely commencement of appropriate antibiotic therapy, rapid diagnostic methods hold the promise of significantly ameliorating patient outcomes and curbing healthcare costs associated with

sepsis management. Hence, further research and implementation of rapid diagnostic technologies are warranted to optimize sepsis care and fortify medical delivery capabilities.

This study has two limitations. Firstly, due to the lower positivity rate of Gram-positive bacteria, Gram-positive samples were not included in this study. This limitation may result in an insufficient evaluation of the applicability and effectiveness of rapid identification methods for these bacteria. Future research could address this limitation by expanding the sample scope to comprehensively assess the method's performance across different types of bacteria. Secondly, this study lacks clinical application results. Therefore, further long-term observation and validation are necessary to fully understand the effectiveness and feasibility of the method in real-world clinical settings. Future studies could focus on broader clinical trials to further ascertain the benefits and reliability of the method.

Conflicts of Interest The authors declare no conflicts of interest regarding the publication of this paper.

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血流感染的新診斷方法：革蘭氏陰性菌的快速提取與過濾技術

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摘要

敗血症在醫療保健領域中是一個重大挑戰，全球的發病率呈逐年增加之勢。本研究旨在評估一種快速抽取和過濾法對血液樣本中革蘭氏陰性細菌進行鑑定的效力，以應對對及時診斷和干預的迫切需求。本研究於 2023 年 7 月 1 日至 2024 年 3 月 20 日在台北國泰綜合醫院進行，分析了 191 個陽性血液瓶樣本。實驗組採用了革蘭氏染色後進行快速抽取和過濾，而對照組則進行了傳統的亞培法。結果顯示，實驗組與對照組之間的鑑定一致率為 94%（177/188）。此外，藥物敏感性測試表明，基本一致性（EA）和類別一致性（CA）均達到了 98%（2694/2738）。儘管快速方法符合了接受標準並顯示出較低的錯誤率，但仍有改進的空間。本研究強調了快速診斷方法在改善敗血症管理方面的潛力，對提高患者護理和醫療效率具有重要意義。未來的研究應該聚焦於更廣泛的臨床試驗，進一步驗證和優化這些診斷方法。