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The value of microbiology rapid on-site evaluation of sepsis caused by pulmonary infection

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Abstract. – OBJECTIVE: This study aims to evaluate the value of microbial rapid on-site evaluation (M-ROSE) of sepsis, and septic shock caused by pulmonary infection.

PATIENTS AND METHODS: Thirty-six patients with sepsis and septic shock due to hospital-acquired pneumonia were analyzed. Accuracy and time were compared with M-ROSE, traditional culture, and next-generation sequencing (NGS).

RESULTS: A total of 48 strains of bacteria and 8 strains of fungi were detected by bronchoscopy in 36 patients. The accuracy rate of bacteria and fungi was 95.8% and 100%, respectively. M-ROSE took an average of 0.34±0.01 hours, much faster than NGS (22h±0.01 h, $p<0.0001$) and traditional culture time (67.50±0.91 h, $p<0.0001$).

CONCLUSIONS: M-ROSE may quickly identify common bacteria and fungi, so it may be a useful method for the etiological diagnosis of sepsis and septic shock caused by pulmonary infection.

Key Words:
Pathogenic microorganism, Rapid on-site evaluation (ROSE), Sepsis, Pulmonary infection.

Introduction

Sepsis and septic shock are major healthcare problems, affecting millions of people worldwide each year, and it may lead to fatalities in as many as one in four cases, or even more [1-4]. Searching for infection focus and targeted anti-infection strategy is the key to curing sepsis patients. Sepsis guidelines[4,5] indicate that early antibiotics therapy is necessary, and that specimen culture is completed before antibiotics therapy. According to the sepsis guideline[6-10], initial anti-infection therapy is appropriate for empiric antibiotics. Failure to initiate appropriate empiric therapy in sepsis and septic shock patients is associated with a substantial increase in morbidity and mortality[11-13]. However, it has been shown[14] that treatment by empiric antibiotics is often unreliable, particularly in sepsis and septic shock caused by pulmonary infection. The lag in the detection of traditional pathogenic microorganisms reduces the reference significance. Currently, the next-generation sequencing (NGS) that is popular in China and abroad takes 16-24 hours to get the results. Rapid polymerase chain reaction (PCR) detection has become a hotspot of current research. But NGS and PCR still cannot meet the conditions of antibiotics application within 1 hour in the sepsis guidelines[4,5,15]. Using microbial rapid on-site evaluation (M-ROSE) to guide the clinical antibiotics therapy may be a useful choice to meet the guidelines.

By treating specimens with Diff staining, trained microbiologists can identify common bacteria and fungi in 10 minutes[16-18]. In consultation with a microbiologist, the clinician can recommend the best anti-infection strategy within the recommended period. According to the results of Diff staining and discussion, the use of antibiotics is of great significance for sepsis and septic shock patients caused by pulmonary infection.

Patients and Methods

Study Design and Subjects

We investigated 36 sepsis or septic shock patients caused by pulmonary infection treated in the China Aerospace Science & Industry Corporation 731 Hospital between November 2021 and June 2022. Hospital-acquired pneumonia (HAP), sepsis, and septic shock are diagnosed based on international guidelines[4,18]. Inclusion criteria

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Table I. The basic information characteristics of sepsis and septic shock patients.

Male	Age	Specimen pass rate1	Number of cultured bacteria	Agreement rage of M-ROSE with bacterial culture	Number of culture fungi	Agreement rage of M-ROSE with bacterial fungi
21	66.3±10.6	100%	48	95.80%	8	100%

1 squamous epithelium ≤5% and ciliated columnar epithelial cells ≤5%.

were age >18 years. According to the guidelines, HAP is defined as pneumonia not incubating at the time of hospital admission and occurring 48 hours or more after admission, new lung infiltrates and clinical evidence that the infiltrate is of an infectious origin, which includes the new onset of fever, purulent sputum, leukocytosis, and decline in oxygenation. Sepsis is defined as life-threatening organ dysfunction (SOFA score ≥2 points) caused by a dysregulated host response to infection. Septic shock is defined as a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain mean arterial pressure (MAP) ≥65 mm Hg and having a serum lactate level >2 mmol/L despite adequate volume resuscitation. Exclusion criteria: those who don't sign the informed consent form and don't agree to share information, those who refuse bronchoscopy examination, and those who refuse NGS testing. The basic information characteristics of sepsis and septic shock patients are listed in Table I.

Microbiological Analyses

Sputum samples were obtained by a skilled respiratory physician during the patient's hospitalization. The specimens were divided into three equal parts used for ROSE, traditional culture, and NGS respectively. Sputum specimens were tested by cytology rapid on-site evaluation (C-ROSE) and M-ROSE. Specimens were prepared for 1 minute (at least 3 smears), dried by natural air for 2 minutes, and about 90 seconds for Diff staining (20-30 s with solution A, 20-30 s with solution B).

Statistical Analysis

SPSS 26.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Normality test was performed, and measurement data that conformed to the normal distribution were expressed as mean±standard deviation (SD) and analyzed using *t*-tests. $p<0.05$ was considered statistically significant.

Results

The number of cells in the specimens was observed by C-ROSE. The qualified specimens were squamous epithelium ≤5% and ciliated columnar epithelial cells ≤5% at low magnification. The qualified rate of all 48 specimens was 100% (Table I).

Among the 36 samples, a total of 56 stains were cultured, and the NGS detected the corresponding strain sequences in all the cultured stains. Among 56 strains of dominant pathogeny microbiology, the accuracy of M-ROSE in predicting dominant bacteria was 95.8%, and that of fungi was 100%. Comparing the detection time for M-ROSE with that of NGS and traditional microbial culture, it was found that the detection time of M-ROSE had obvious advantages. The application time of M-ROSE was 0.35±0.01 h, 60 times faster than that of NGS (22±0.01 h, $p<0.0001$), and 200 times faster than that of traditional microbial culture (67.80±0.91 h, $p<0.0001$) (Figure 1).

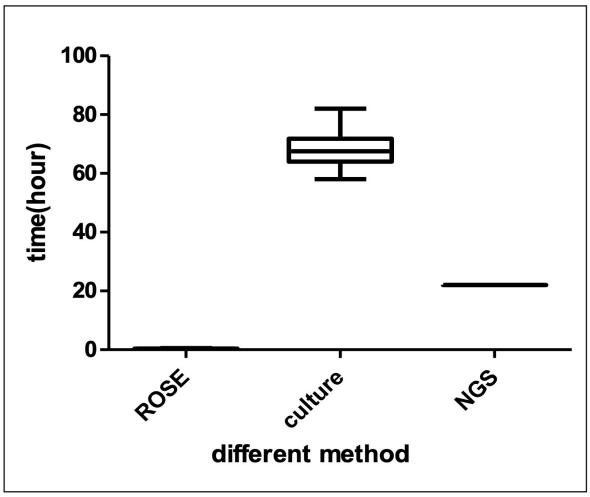

Figure 1. Comparison of the time taken by different methods.

Discussion

It is accurate and reliable to use C-ROSE to judge whether the specimen is qualified or not. The number of neutrophils, lymphocytes, eosinophils, and other cells in the specimen can be determined, which is particularly important as it assesses whether sepsis or septic shock is caused by pulmonary infection[19,20]. Squamous epithelium and ciliated columnar epithelial cells are the gold criteria for determining whether the specimen originated from the upper respiratory tract[21].

As early as 1982, T Tomita found that the movement of bacteria can promote the phagocytosis of neutrophils[22]. Recently, Liew et al[23] elaborated the movement of bacteria and phagocytosis of bacteria.

Specimen smear can find neutrophils or macrophages phagocytic bacteria phenomenon if a certain proportion (>5%, almost no phagocytic cells phagocytic microorganisms in the normal specimen), it indicates that pathogenic microorganisms may be involved in infection. On the contrary, if no pathogenic microorganisms such as bacteria and fungi are found in the specimen, and no inflammatory cells such as neutrophils are found, the possibility of non-infection is considered to be high. In our study, many neutrophilic phagocytic bacteria were found, and the phagocytic bacteria were clinically confirmed to be pathogenic bacteria (Figure 2-5).

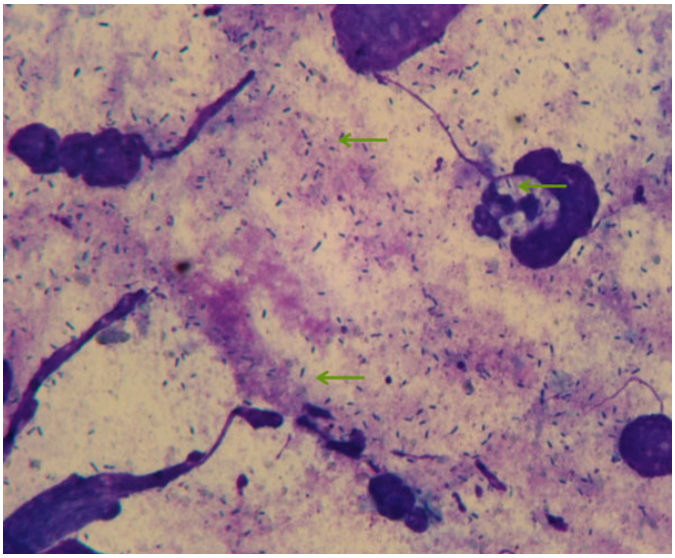

Figure 2. *Stenotrophomonas maltophilia*, Neutrophil phagocytic bacteria (x1,000).

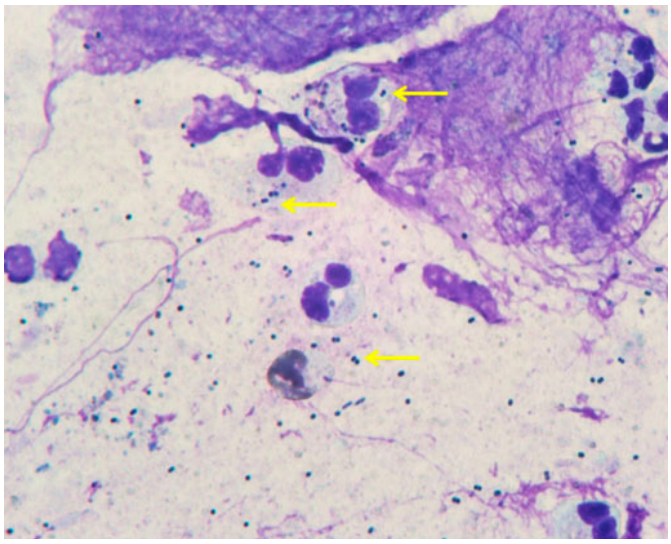

Figure 3. *Acinetobacter baumannii*, Neutrophil phagocytic bacteria (x1,000).

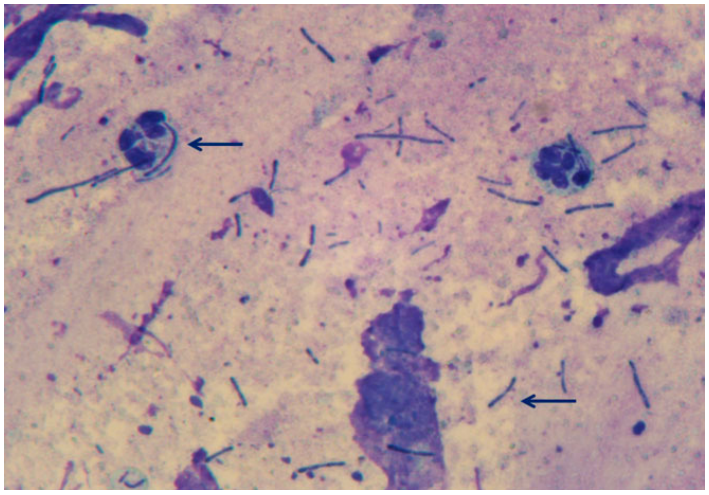

Figure 4. *Pseudomonas aeruginosa* (x1,000).

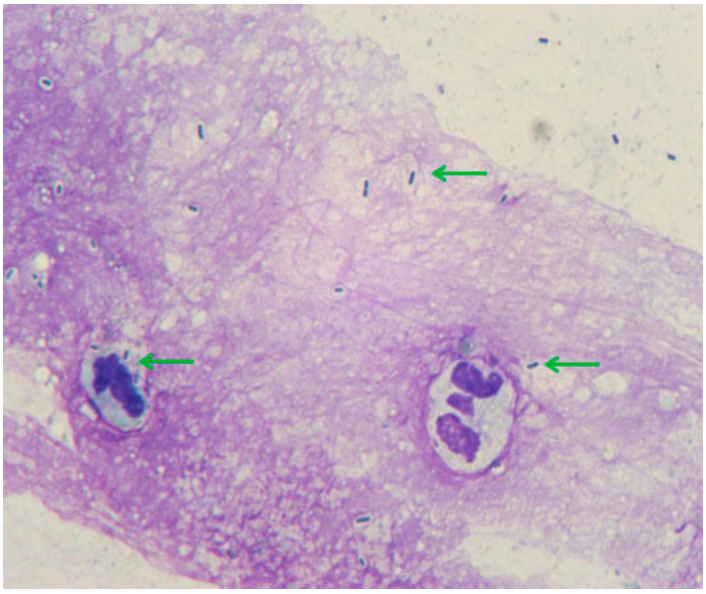

Figure 5. *Klebsiella pneumoniae* (x1,000).

Therefore, the C-ROSE can also rapidly assess if a specimen is qualified and the likelihood of infectious *vs.* noninfectious causes of acute illness[15].

The ability to accurately diagnose sepsis early in its course would allow physicians to appropriately shape antimicrobial regimens, thereby maximizing therapeutic effect and decreasing morbidity and the risk of propagating drug-resistant organisms. Gram staining takes more time to get results than ROSE, which takes about 40 minutes. Previous studies[24] have shown that Gram staining can identify common bacteria and fungi well, and its specificity can reach more than 90%. In our study, experienced microbiologists could identify common bacteria with 95.8% accuracy. The application of M-ROSE can quickly and accurately identify fungi because their characteristics are more evident[25]. In this study, experienced microbiologists could identify fungi with 100% accuracy. Compared with Gram staining, Diff staining takes less time and can quickly read cell morphology, so it can quickly judge whether the specimen is qualified or not, and also identify common bacteria, fungi, and so on. In our study, we could complete the C-ROSE and M-ROSE in 0.34 h, it met the conditions of antibiotics application within 1 hour considering the sepsis guidelines[4,5,15]. The maximum effect of rapid evaluation must be a combination of C-ROSE and M-ROSE.

In addition to a variety of rare bacteria, clinicians will gradually improve their identification ability and expand the range of identification after the increase of clinical reading specimens[26]. We can also dynamically evaluate the number of bacteria in each high field. If the number of bacteria in each high field decreases, it indicates that anti-infection treatment is effective; otherwise, it indicates that it is ineffective. Therefore, the M-ROSE is particularly important for the timely adjustment of anti-infection strategies during the treatment of sepsis, and septic shock caused by pulmonary infection.

M-ROSE can quickly know whether pathogenic bacteria exist in the specimen, which is particularly for differentiating infectious and non-infectious diseases[27,28]. To determine sepsis caused by infectious diseases, M-ROSE can also quickly determine the approximate number of pathogenic bacteria and preliminarily determine their genus, which has important reference significance for the timely selection of effective antibiotics[24,29]. At the same time, M-ROSE may be based on the neutrophil number, and whether the neutrophil phagocytic pathogen, judges the pathogen as pathogenic bacteria[30,31]. Dynamic observation of infected specimens in the same part of the same patient and comparison of the number and changes of cells and bacteria can be used to evaluate the effect of anti-infection therapy. If M-ROSE can be combined with other rapid diagnostic techniques, such as Polymerase Chain Reaction (PCR), gene probe, mass spectrometry, flow cytometry, and NGS, it can further improve the sensitivity and specificity of rapid evaluation of pathogens.

Conclusions

This study shows M-ROSE may quickly identify common bacteria and fungi. Although this study has some limitations, it also has good clinical application prospects and can save the problem of empiric anti-infection therapy, especially in patients with sepsis, severe sepsis, and septic shock caused by pulmonary infection.

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Ethics Approval
The study was approved by the Institutional Review Board (Research Review Committee and Ethical Review Committee) of the Ethics Committee of China Aerospace Science & Industry Corporation 731 Hospital; the ethics code is 2022-0302-01.

Informed Consent
All patients signed informed consent before the procedure.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Authors' Contributions
G-X. M, and L-X. X designed the study. L-C. S., X-L. W., and F. W. did the literature search. Q-Y. Y., K-F. W., and Y. T. collected the data. P. Y. and Q-X. H. analyzed and interpreted the data and wrote the manuscript.

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Data Availability
Not applicable.

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