Comparison of the results of sputum culture and bronchoscopic lavage fluid culture:an analysis report of large sample size data from the Clinical Microbiology Laboratory

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Research

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Abstract

Background Sputum specimens were the most common for Clinical Microbiology Laboratory in China. For the results of sputum culture, it was difficult for clinicians to evaluate the significance, and even more difficult for laboratory physicians. At present, most Clinical Microbiology Laboratories in China executed quality assessment of sputum specimens. But how to evaluate the results of sputum culture was still very confusing. To solve this problem, we conducted a series of retrospective studies.

Methods Based on the culture results of bronchoscopic lavage fluid (BALF), the differences of sputum culture results before (2013-2015) and after (2016-2018) quality control of sputum samples in our hospital were compared.

Results Pseudomonas aeruginosa , Acinetobacter baumannii , Klebsiella pneumoniae and Staphylococcus aureus were the four most common pathogens in sputum and BALF culture, both in 2013-2015 years and 2016-2018 years. Antimicrobial susceptibility test from 2013-2015 and 2016-2018 both showed for P. aeruginosa and K. pneumoniae , the susceptibility rates of BALF isolates to all commonly used antibiotics were higher than those from sputum. For A. baumannii and S. aureus , the sensitivity rates of BALF isolates to most antibiotics were higher than those from sputum. After quality control of sputum samples, there was still a difference between the results of sputum culture and those of BALF.

Conclusions Even though the quality control of sputum specimens had been carried out, the results of culture and antimicrobial resistance of pathogens from qualified sputum samples were still different from those of BALF.

Background

Lower respiratory tract infections (LRIs) were the most common infectious disease of respiratory tract.[1–2] Pneumonia was the second most common infection in hospitalized patients, and was highly correlated with morbidity and mortality.[3] Irrational use of antibiotics would delay the patient's condition and cause serious bacterial resistance.[1–2] According to the data from China Antimicrobial Resistance Surveillance System (CARSS) in 2015, the major specimens type from inpatients in respiratory departments in China were sputum (81.6%, 41,131/50,417).[1–2] Due to the convenience of specimens collection, sputum had always been the most common type of specimens in clinical microbiology laboratories in China. But easily confused by oral colonization flora, it was difficult to judge the results of sputum culture as infectious, colonized or contaminated bacteria. For the results of sputum culture, it was difficult for clinicians to evaluate the significance, and even more difficult for laboratory physicians.

It was well known that unqualified sputum specimens, such as saliva, have no significance in bacterial culture. And the industry had reached a consensus that sputum culture without microscopic examination was of no value.[4] As early as the 1970s, the quality control of sputum specimens had been studied. Generally, sputum specimens were judged to be qualified by the number of inflammatory cells (primary

polymorphonuclear leukocytes) and epithelial cells examined by a stained smear of the specimen. According to six different interpretation criteria from Barrlett, Murray and Washington, Geckler et al, Van Scoy, Barry, Heineman and Radano, the results were significantly different from the six methods.[5] In this study, the Chinese national standard was used to determine whether the sputum specimens were qualified.[6]

Tongji Hospital was one of the largest teaching hospitals in China. Department of laboratory medicine was one of the first laboratories in China to pass both International Organization for Standardization (ISO) 15189 and College of American Pathologists (CAP) certification in the United States. Since 2016, quality control of sputum specimens had been carried out in the laboratory. Were the culture results of qualified sputum specimens necessarily the real pathogen of lower respiratory tract infection? To answer this question, we conducted a series of retrospective studies. Based on the culture results of BALF, the differences between the culture results of BALF and that of sputum samples in pre-control period from 2013 to 2015 and post-control period from 2016 to 2018 were compared and evaluated.

Materials And Methods

Study design and procedures

A retrospective analysis was made of the differences between sputum culture results and BALF culture results during the period of no quality control of sputum specimens from 2013 to 2015 and the period of quality control of sputum specimens from 2016 to 2018 in our hospital. For the main pathogenic bacteria, the difference of antimicrobial sensitivity between sputum specimens and BALF was analyzed.

Source of specimens

All specimens (Including sputum specimens and BALF specimens) were taken from the clinical departments of Tongji Hospital and sent to department of laboratory medicine.

Interpretation criteria for qualified sputum specimens

The qualified interpretation of sputum specimens in this study is based on the Chinese standard.[6] Since 2016, smear microscopy has been required for each specimen requiring sputum culture. Sputum specimens satisfying the following three conditions will be treated as qualified specimens. First, a specimen with ≥25 white blood cells (WBC) per average low-power field (LPF) and squamous epithelial cells (EPI) <25 per LPF. Second, the ratio of WBC to EPI was more than 10:1, and the single-form bacteria were predominant. Third, EPI <10 per LPF, and alveolar macrophages and columnar epithelial cells existed. In addition to the above three cases, when EPI> 10 per LPF, they were considered to be unqualified sputum specimens.

Identification of strains and antimicrobial susceptibility test

For isolates from the same position of the same patient, only the first isolate was included in the analysis according to CLSI M39.[7] The identification of strains was carried out by biochemical experiments, automatic identification system (Vitek–2-compact, BioMerier Products) and/or IVD-MALDI Biotyper (Bruker, Germany). Antimicrobial susceptibility test was carried out and explained according to CLSI 2018 by disk diffusion method and E test method. [8] ATCC 25922, 25923, 27853, 49247,49619, 90028, 35218, 700603, 29213 were used for quality control of indoor antimicrobial sensitivity tests, which were performed weekly.

Statistical analysis

All patient and strain information were stored in the WHONET software. WHONET 5.6 software was used to analyze antimicrobial susceptibility data.

Results

Pathogenic spectrum of respiratory tract infections

The number of bacteria isolated from sputum and BALF in 2013–2015 was 12957 and 848, respectively, compared with 6740 and 2239 in 2016–2018. During the period of no quality control for sputum specimens from 2013 to 2015, the main pathogens in sputum specimen culture were *S. aureus* (24%), *A. baumannii* (21%), *P. aeruginosa (*14%) and *K. pneumoniae* (13%). The detection rates of *Haemophilus influenzae* and *Streptococcus pneumoniae* were also in the top ten, 6% and 4% respectively. The top ten pathogens in sputum culture from 2013 to 2015 were listed in fig1 A. From 2013 to 2015, the culture results of BALF showed that the top four pathogenic bacteria were *P. aeruginosa (*22%), *A. baumannii* (21%), *K. pneumoniae* (16%) and *S. aureus* (14%). The detection rates of *H. influenzae* and *S. pneumoniae* were in the top 10, 8% and 4% respectively. The detection rate of *Candida albicans* ranked sixth, accounting for 5%. The top ten pathogenic bacteria in BALF were detailed in fig1 B. In 2016–2018, the top four pathogens in sputum culture were *A. baumannii (*27%), *S. aureus* (20%), *P. aeruginosa (*17%) and *K. pneumoniae* (13%). The detection rates of *H. influenzae, S. pneumoniae* and *Moraxella catarrhalis* ranked in the top ten, with 8%, 4% and 3% respectively. In fungi, the detection rate of *Aspergillus fumigatus* was the tenth, accounting for 2%. A list of other common pathogens was given in fig1 C. The culture results of BALF showed that *K. pneumoniae* (21%), *P. aeruginosa* (21%), *A. baumannii (*17%) and *S. aureus* (13%) were the most common pathogens. *H. influenzae* and *S. pneumoniae* ranked the top ten, with detection rates of 9% and 3%. Among fungi, *A. fumigatus* and *A. flavus* ranked the top ten, with detection rates of 4% and 3% respectively. The list of pathogenic bacteria cultured in BALF was detailed in fig1 D.

Antimicrobial susceptibility of major pathogens

The susceptibility rates of *P. aeruginosa* in sputum and BALF to commonly used antibiotics were compared in fig2 A (2013–2015) and fig2 B (2016–2018), respectively. The sensitivity of *P. aeruginosa* in BALF to commonly used antibiotics was higher than that of sputum isolates, whether in 2013–2015 or 2016–2018. The susceptibility rate of A. baumannii to commonly used antibiotics showed that the susceptibility rate of isolates from BALF in 2016–2018 was higher than that of sputum isolates, except minocycline, tegacycline and piperacillin. However, data from 2013 to 2015 showed that the susceptibility rates of sputum isolates to cefoperazone sulbactam, ampicillin sulbactam, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin and tigacycline were higher than those of BALF isolates. The susceptibility of *A. baumannii* to commonly used antibiotics is detailed in fig3 A and B. The sensitivity analysis of *K. pneumoniae* to commonly used antibiotics showed that the sensitivity of BALF isolates was higher than that of sputum isolates, whether in 2013–2015 or 2016–2018. Moreover, the difference in sensitivity between BALF isolates and sputum isolates was significantly higher in 2016–2018 than in 2013–2015, as shown in fig4 A and B. The susceptibility rate of *S. aureus* to commonly used antibiotics showed that the susceptibility rate of BALF isolates was higher than that of sputum isolates in 2016–2018. However, in 2013–2015, except erythromycin, clindamycin and trimethoprim/sulfamethoxazole, the sensitivity of BALF isolates was higher than that of sputum isolates. Specific sensitivity comparisons could be found in fig5 A and B.

Discussion

How to evaluate the significance of sputum culture had always been a puzzling problem. In this study, we evaluated the significance of sputum culture from a new perspective. This study compared the difference of pathogen spectrum and antimicrobial sensitivity between sputum samples and BALF, and compared the difference before and after quality control of sputum samples. Studies had shown that, even for qualified sputum specimens, the results of culture and antimicrobial sensitivity were still quite different from those of BALF. BALF was obtained through fiberoptic bronchoscope and could represent lower respiratory tract infection. However, sputum specimens were easily contaminated by colonies in the upper respiratory tract. Therefore, clinicians needed to be very careful in diagnosing and treating lower respiratory tract infections based on the results of sputum culture.

Sputum specimens were not a good type of specimens, from the point of view of diagnosis of LRIs. Doctors obtained biopsy specimens through fiberoptic bronchoscopy, which could represent LRIs. But after all, it belonged to the invasive operation and was not suitable for every patient. At present, qualified sputum specimens, together with some invasive surgical specimens (transtracheal aspiration, bronchoalveolar lavage, protected brush samples, etc) were acceptable in the global LRIs surveillance project. [3] In the face of LRIs, what kind of specimen to send was another difficult problem. The American Association of Pediatric Infectious Diseases told us that blood cultures should be sent for moderate to severe community-acquired pneumonia in children, especially complex pneumonia.[9] But at present, the rate of blood culture in Chinese patients with LRIs was not high. A multicenter study from China showed that blood culture isolates accounted for only 5.3% of all specimen types. [1–2] For LRIs, should we do blood culture or sputum culture? The Lancet, an authoritative medical journal, gave us the

answer. Different strategies should be adopted for different types of patients. For outpatient blood culture and sputum culture was not needed routinely. For inpatients with low severity, only sputum culture was needed. For inpatient with moderate severity and no ICU sputum culture, blood culture, legionella urinary antigen and pneumococcal urinary antigen should be adopted routinely. For inpatient in ICU with high severity, invasive sampling should also be performed in addition to all the above tests. [10]

Squamous epithelial cells only came from the upper respiratory tract, so the presence of squamous epithelial cells suggested that sputum specimens were contaminated by oral colonization. The presence of polymorphonuclear cells suggested infection. On the other hand, the presence of alveolar macrophages and columnar epithelial cells suggested that the specimen was from the lower respiratory tract. But bacterial engulfment in Gram-stained sputum did not indicate respiratory infections. Studies from Masafumi Shimoda, et al had shown that there was no significant difference in bacterial engulfment between infected and non-infected groups, but there were differences among different strains of bacteria. For example, *S. pneumoniae* appeared less engulfment, while *H. influenzae, Catarrhal Branhamella* and MSSA appeared more. [11] There were many criteria for evaluating the qualifications of sputum specimens, and there were great differences in the interpretation results of different criteria. [5] The study was carried out by industry standard of China and the standard combined the advantages of many different standards.[6]

There were several limitations in the studies. First, in this study, no distinction was made between natural expectoration, induced sputum and sputum aspiration. Second, whether BALF was a qualified sample had not been judged in this study. We hoped that in future studies, BALF would be interpreted as qualified as sputum specimens.

Conclusion

Even though sputum samples were strictly controlled, the results of qualified sputum culture were still different from those of BALF culture. If we only rely on the results of sputum culture to diagnose and treat lower respiratory tract infection, we should be cautious.

Abbreviations

LRIs: lower respiratory tract infections, CARSS, China Antimicrobial Resistance Surveillance System; ISO, International Organization for Standardization; CAP, College of American Pathologists; WBC, white blood cells; LPF, low-power field; EPI, squamous epithelial cells; BALF, bronchoscopic lavage fluid

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Tongji Hospital ethics committee for research in health. The Tongji Hospital ethics committee also approved the waiver of informed consent to participate in this study due to its retrospective design. All patient data were anonymous prior to the analysis.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Author Contributions

Ziyong Sun designed the study. Lei Tian analyzed the data and wrote the article. Zhen Zhang and Feng He revised the manuscript. All authors reviewed the manuscript prior to submission.

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Figures

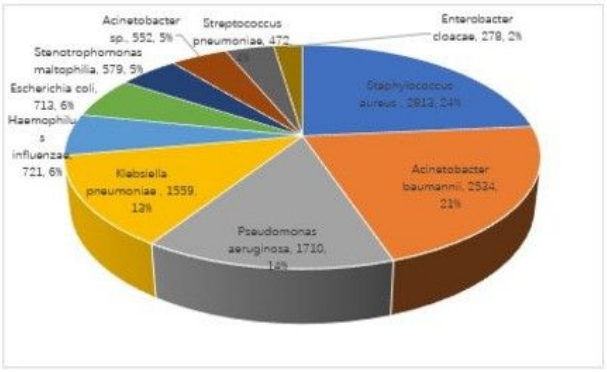

Fig 1A

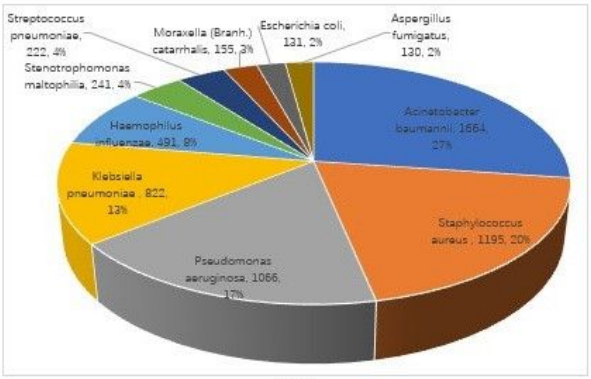

Fig 1C

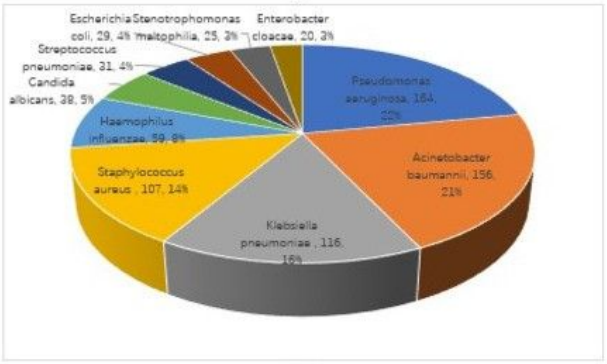

Fig 1B

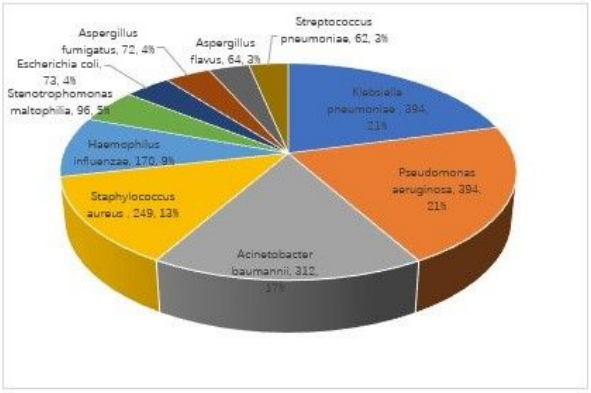

Fig 1D

Figure 1

Composition of main pathogens isolated from sputum culture during the period of non-quality control of sputum specimens from 2013 to 2015 (fig 1A). Composition of main pathogens isolated from bronchoalveolar lavage fluid culture during the period of non-quality control of sputum specimens from 2013 to 2015 (fig 1B). Composition of main pathogenic bacteria isolated from sputum culture during quality control of sputum specimens from 2016 to 2018 (fig 1C). Composition of main pathogenic bacteria isolated from bronchoalveolar lavage fluid culture during quality control of sputum specimens from 2016 to 2018 (fig 1D).

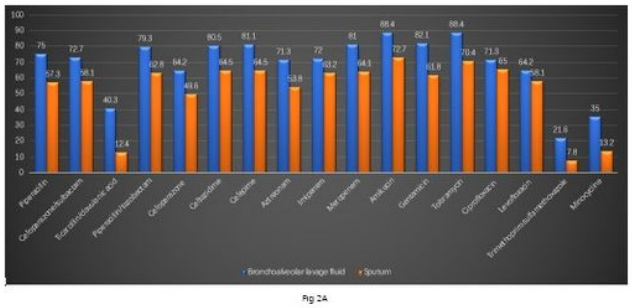

Fig 2A

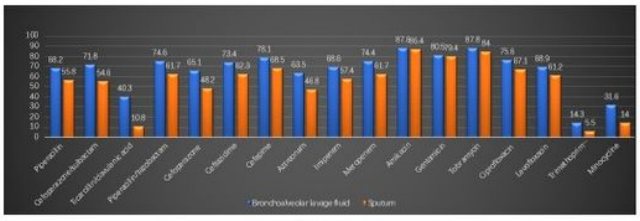

Fig 2B

Fig 2. Sensitivity (%) of Pseudomonas aeruginosa to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2013 to 2015 (fig 2A). Sensitivity (%) of Pseudomonas aeruginosa to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2016 to 2018 (fig 2B).

Figure 2

Sensitivity (%) of Pseudomonas aeruginosa to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2013 to 2015 (fig 2A). Sensitivity (%) of Pseudomonas aeruginosa to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2016 to 2018 (fig 2B).

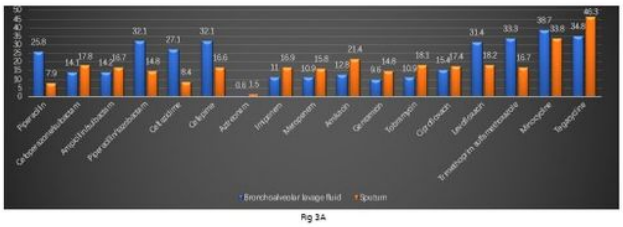

Fig 3A

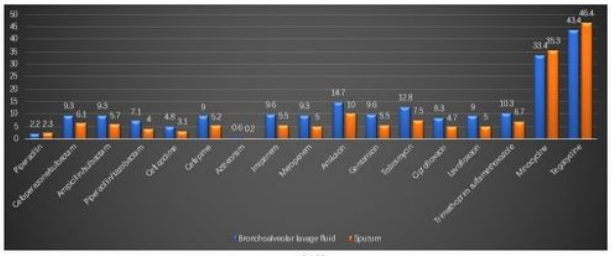

Fig 3B

Fig 3. Sensitivity (%) of Acinetobacter baumannii to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2013 to 2015 (fig 3A). Sensitivity (%) of Acinetobacter baumannii to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2016 to 2018 (fig 3B).

Figure 3

Sensitivity (%) of Acinetobacter baumannii to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2013 to 2015 (fig 3A). Sensitivity (%) of Acinetobacter baumannii to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2016 to 2018 (fig 3B).

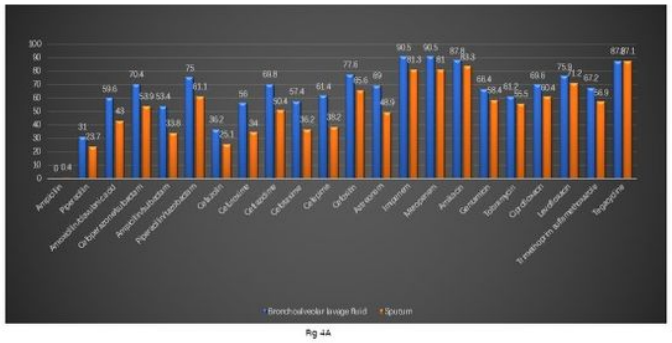

Fig 4A

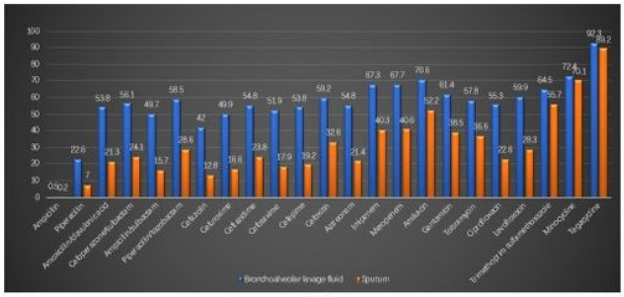

Fig 4B

Fig4. Sensitivity rate(%) of Klebsiella pneumoniae from sputum specimens and bronchoalveolar lavage fluid specimens to commonly used antibiotics from 2013 to 2015 (fig 4A). Sensitivity rate(%) of Klebsiella pneumoniae from sputum specimens and bronchoalveolar lavage fluid specimens to commonly used antibiotics from 2016 to 2018 (fig 4B).

Figure 4

. Sensitivity rate(%) of Klebsiella pneumoniae from sputum specimens and bronchoalveolar lavage fluid specimens to commonly used antibiotics from 2013 to 2015 (fig 4A). Sensitivity rate(%) of Klebsiella pneumoniae from sputum specimens and bronchoalveolar lavage fluid specimens to commonly used antibiotics from 2016 to 2018 (fig 4B).

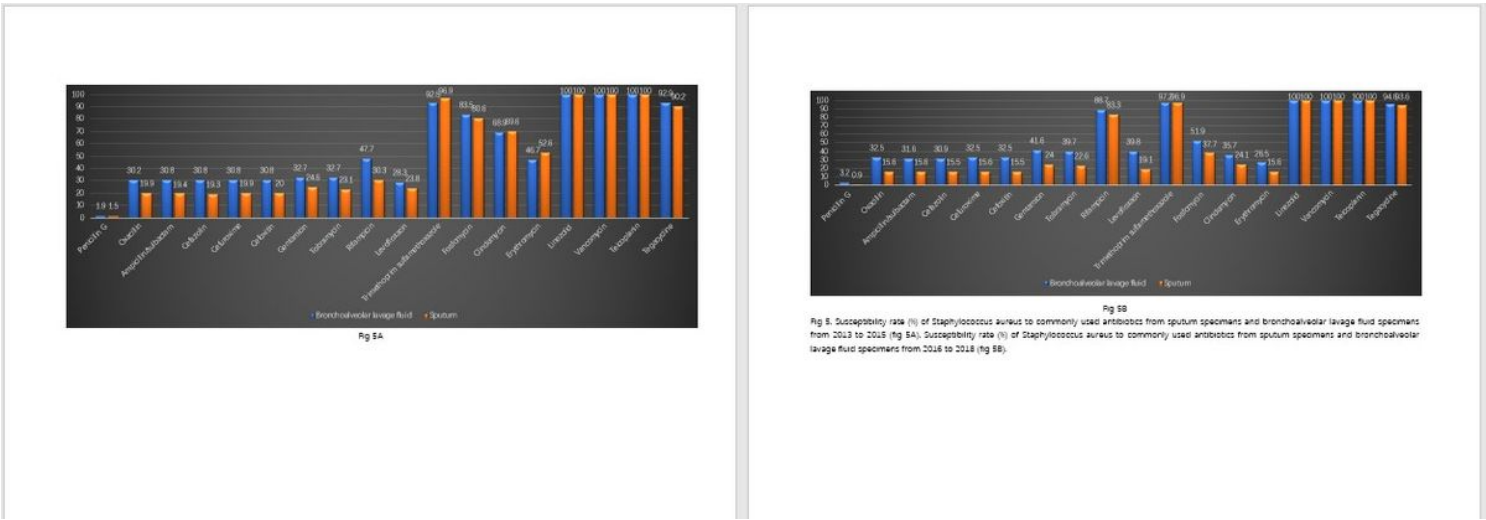

Fig 5A

Fig 5B

Fig 5. Susceptibility rate (%) of Staphylococcus aureus to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2013 to 2015 (fig 5A). Susceptibility rate (%) of Staphylococcus aureus to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2016 to 2018 (fig 5B).

Figure 5

Susceptibility rate (%) of Staphylococcus aureus to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2013 to 2015 (fig 5A). Susceptibility rate (%) of Staphylococcus aureus to commonly used antibiotics from sputum specimens and bronchoalveolar lavage fluid specimens from 2016 to 2018 (fig 5B).