

Bacterial Isolates and their Antimicrobial Susceptibility Pattern from Tracheal Secretions and Sputum of Admitted and Outdoor Patients

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Abstract

Objective: The purpose of study was to determine the frequent pathogens in tracheal secretions and sputum specimens along with the antimicrobial susceptibility pattern.

Methods: This descriptive study was conducted in the department of Pathology and Microbiology, Farooq Hospital Lahore, Pakistan and data included was taken from 1st August 2022 to 31st December 2022. The specimens were inoculated on blood agar and MacConkey agar and incubated for 24 hours at 37 °C. The Kirby Bauer disc diffusion method was employed to find out the antimicrobial susceptibility pattern. The SPSS was used to assess the data.

Results: From the total 102 positive culture growths, Klebsiella species (35.2%) was frequently isolated followed by Pseudomonas species (25.4%), Acinetobacter species (23.5%), *Escherichia coli* (9.8%), *Staphylococcus aureus* (3.9%), Citrobacter species (0.9%), and Proteus species (0.9%). The doxycycline and linezolid were completely effective (100%) against the gram positive cocci. The Klebsiella species, Pseudomonas species, Acinetobacter species, and *Escherichia coli*, showed maximum sensitivity to tigecycline.

Conclusion: The most commonly isolated gram negative rod was Klebsiella species and tigecycline was found to be the most effective antibiotic against it. Multidrug resistance among respiratory pathogens is the major issue so it is necessary to administer antibiotic therapy in a limited and objective manner.

Keywords: Antibiotic sensitivity pattern, gram negative rods, gram positive cocci, sputum specimens, tracheal secretions.

How to cite: Farooq O, Wali N, Yousaf Z, Rashid S, Mumtaz A, Aslam M. Bacterial Isolates and their Antimicrobial Susceptibility Pattern from Tracheal Secretions and Sputum of Admitted and Outdoor Patients. *Esculapio - JSIMS* 2023;19(02): 145-150

DOI: <https://doi.org/10.51273/esc23.251923>

Introduction

Tracheobronchial secretions are produced by the tracheobronchial tree's mucous glands and goblet cells. In addition to facilitating the interchange of heat and water during breathing, these secretions also serve to protect the respiratory tract. High morbidity and mortality rates are related to respiratory infections, parti-

cularly in critically ill patients.¹ An acute lung infection in a patient that develops outside of a hospital setting without recent hospitalization is referred to as "community-acquired pneumonia" (CAP). Since the advent of antibiotics, the etiology of CAP has undergone significant evolution. The CAP etiology and its clinical manifestations are complex and vary with age, region and current exposure.²

Hospital-acquired pneumonia is one of the most prevalent issue in health care associated infections. Previous literature reports that chronic respiratory diseases, multiple organ disorders, aspiration, intubation and mechanical ventilation are some of the risk factors.³ Ventilator-associated pneumonia (VAP) accounts for approximately 15% of all hospital-acquired infections with the highest morbidity and mortality, ranking second in frequency. A patient who develops pneumonia after 48 hours of

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Submission Date:	03-05-2023
1st Revision Date:	26-06-2023
Acceptance Date:	30-06-2023

intubation and mechanical ventilation is known as VAP.⁴⁵

VAP has decreased in high-income nations through a combination of surveillance, education, prevention and intervention.⁶ There is a lack of data on VAP rates, the most common associated pathogens, and their antibiotic susceptibility profiles in Asia. The orderly survey and meta-analysis⁷ of VAP occurrence, microbiological etiology, and cost sums up this information by country pay level, intervention guidelines and further research in the region.

One of the life-saving techniques for intensive care unit (ICU) admitted patients are mechanical ventilation, although this technique carries a greater risk of causing respiratory diseases. Multidrug-resistant infections in hospitals are always more likely to affect critically ill ICU patients. This occurs as a result of their prolonged hospitalization, immune-compromised profile, severe illness, and use of catheters and antibiotics.⁸ The incessant and unselective use of wide range of antibiotics without promoting microbiological cultures and awareness prompts the advancement of these multidrug safe pathogens in the realm of microbial science and complicates the treatment of ICU patients.⁹ The invasion of multidrug-resistant strains of microorganisms is increasing the morbidity of these patients. Pneumonia-associated pathogens include *Klebsiella* species, *Pseudomonas* species, *Staphylococcus aureus* (*S. aureus*), and *Acinetobacter* species.¹⁰

The diagnosis of VAP necessitates a high degree of clinical suspicion, physical and radiological examination along with microbiological investigation of respiratory secretions. The Centers for Disease Control and Prevention's criteria recommend bronchoalveolar lavage, lung biopsy, and tracheal aspirate as the three diagnostic options for VAP. The collection of tracheal aspirate specimens is the simplest and potentially the safest of the three; however, tracheal aspirate specimens have low diagnostic specificity for VAP and rarely differentiate between infection and colonization.¹¹

A significant information gap in the prevention and treatment of especially VAP is being caused by the absence of recent national studies, and therefore is resulting in suboptimal outcomes for such patients in the ICU. The objective of this study was to determine the common pathogens and their antimicrobial sensitivity and resistance pattern in tracheal secretions and sputum specimens which can provide guidelines to physicians for appropriate empirical antibiotic treatment.

Materials and Methods

It was a descriptive study carried out in the Department of Pathology and Microbiology, Farooq Hospital Lahore, Pakistan. The data was taken from 1st August 2022 to 31st December 2022. All ethical considerations were addressed and the study was conducted after approval from the ethical review committee (IRB No. 0198). This study comprised all patients with tracheal secretions and sputum specimens that were examined throughout the specified time. Patients with other respiratory tract infections and immuno-compromised patients were excluded. The convenient sampling technique was used to choose the total 126 specimens which were independent of age and gender.

These specimens were collected from the outdoors and admitted patients of the ICU, private rooms, dental ward, medical ward, and pediatric ward. The patient collected the sputum specimen in a sterilized container. The endotracheal tube and a suction catheter tip were used to obtain the tracheal secretions from the patients who were unable to provide the sputum. The tracheal secretions and sputum specimens were applied on blood and MacConkey agars, which were then incubated for 24 hours at 37°C. The cultures were checked for any positive or negative growth after 24 hours. The bacteria were primarily identified on the basis of their colonial morphology, fermenter or non-fermenter, and presence or absence of hemolysis on blood agar. Gram staining, bench and biochemical tests were additionally used to detect the gram positive cocci and gram negative rods.¹²

The Kirby Bauer disc diffusion method was used to evaluate the antimicrobial susceptibility testing on Mueller-Hinton agar and these agar plates were incubated at 37°C for 24 hours. The results were analyzed in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations. The gram negative rods were tested with amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), cefoperazone-sulbactam (105 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), fosfomycin (200 µg), gentamicin (10 µg), imipenem (10 µg), levofloxacin (5 µg), meropenem (10 µg), piperacillin-tazobactam (110 µg), tigecycline (15 µg), tobramycin (10 µg), and trimethoprim-sulphamethoxazole (25 µg) antibiotic discs. For gram positive bacteria, amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), azithromycin (15 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), doxycycline (30 µg), erythromycin (15 µg), gentamicin (10 µg),

levofloxacin (5 µg), linezolid (30 µg), penicillin (10 µg), trimethoprim-sulphamethoxazole (25µg), tigecycline (15 µg), and vancomycin (30 µg) antibiotic discs were used. Cefoxitin (30 µg) was used for the identification of methicillin-resistant Staphylococcus species.¹³ Statistical Package for the Social Sciences (SPSS) v.25.0 was used to assess the data. To determine the percentages and frequencies, descriptive statistics were applied.

Results

A total of 126 specimens including 60 (47.6%) tracheal secretions and 66 (52.4%) sputum specimens were taken from 69(54.7%) males and 57(45.3%) females. The mean age of study participants was 59.65±20.36. Three age groups were targeted in this study: children (<14 years), adults (15 years to 64 years), and elders (>65 years). Children constituted 11(8.7%) of the patient population whereas, 62(49.3%), and 53(42.0%), belonged to adults and elderly age groups, respectively. The outdoor patients were 06 (4.7%) while 120 (95.3%) patients

were admitted to the hospital. Of admitted patients, 80 (63.4%), 18 (14.2%), 11 (8.7%), 08 (6.3%), and 03 (2.3%) were in ICU, medical ward, private rooms, pediatrics ward, and dental ward, respectively.

From 126 specimens, no growth was observed in 46 (36.5%) specimens while positive growth was observed in 80(63.5%) specimens. The gram negative rods were responsible for the majority of the isolates. From total of 80 positive growth specimens, 22(27.5%) specimens have double growth of organism. Specimens 76 (95.0%) were gram negative rods while the remaining 04 (5.0%) were gram positive cocci.

From 102 positive growth cultures, Klebsiella species (n=36; 35.2%) was most prevalent subsequently followed by Pseudomonas species (n=26; 25.4%), Acinetobacter species (n= 24; 23.5%), Escherichia coli ([E. coli]; n = 10; 9.8%), S. aureus (n= 04; 3.9%), Citrobacter species (n= 01; 0.9%), and Proteus species (n= 01; 0.9%). All isolated S. aureus were resistant to the surrogate marker (cefoxitin) hence, identified as methicillin resistant S.

Table 1: : Bacterial isolation with respect to gender, age, category and departments of patients, and specimens

	No growth N (%)	Klebsiella species N (%)	Pseudomonas species N (%)	Acinetobacter species N (%)	E. coli N (%)	S. aureus N (%)	Citrobacter species N (%)	Proteus species N (%)
Genders								
Females	24/46 (52.1%)	16/36 (44.5%)	12/26 (46.2%)	11/24 (45.8%)	3/10 (30%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
Males	22/46 (47.9%)	20/36 (55.5%)	14/26 (53.8%)	13/24 (54.2%)	7/10(70.0%)	3/4 (75%)	1/1 (100%)	1/1(100%)
Age groups								
Children	3/46 (6.5%)	6/36 (16.7%)	6/26 (23.1%)	1/24 (4.1%)	0/10 (0%)	0/4 (0%)	0/1 (0%)	0/0 (0%)
Adults	25/46 (54.3%)	14/36 (38.8%)	15/26 (57.6%)	15/24 (62.5%)	4/10 (40%)	0/4 (0%)	0/1 (0%)	1/1(100%)
Elders	18/46 (39.2%)	16/36 (44.5%)	5/26 (19.3%)	8/24 (33.4%)	6/10 (60%)	4/4 (100%)	1/1 (100%)	0/0 (0%)
Category of patient								
Outdoor	4/46 (8.6%)	1/36 (2.7%)	1/26 (3.8%)	0/24 (0%)	0/10 (0%)	0/4 (0%)	0/1 (0%)	0/1 (0%)
Admitted	42/46 (91.4%)	35/36 (97.3%)	25/26 (96.2%)	24/24 (100%)	10/10(100%)	4/4 (100%)	1/1 (100%)	1/1(100%)
Admitted patient departments								
ICU	26/46 (56.5%)	25/36 (69.4%)	12/26 (46.1%)	19/24 (79.1%)	8/10(80.0%)	4/4 (100%)	1/1 (100%)	0/1 (0%)
Medical ward	7/46 (15.2%)	5/36 (13.8%)	5/26 (19.2%)	1/24 (4.1%)	2/10 (20%)	0/4 (0%)	0/1 (0%)	1/1 (100%)
Private rooms	7/46 (15.2%)	1/36 (2.7%)	2/26 (7.6%)	1/24 (4.1%)	0/10(0%)	0/4 (0%)	0/1 (0.0%)	0/1 (0%)
Pediatrics ward	4/46 (8.6%)	3/36 (8.3%)	5/26 (19.2%)	2/24 (8.3%)	0/10(0%)	0/4 (0%)	0/1 (0%)	0/1 (0%)
Dental ward	2/46 (4.3%)	2/36 (5.5%)	2/26 (7.6%)	1/24 (4.1%)	0/10(0%)	0/4 (0%)	0/1 (0%)	0/1 (0%)
Specimens								
Tracheal secretion	13/46 (28.2%)	24/36 (66.6%)	12/26 (46.1%)	21/24 (87.5%)	8/10 (80%)	2/4 (50%)	1/1 (100%)	1/1(100%)
Sputum	33/46 (71.8%)	12/36 (33.4%)	14/26 (53.9%)	3/24 (12.5%)	2/10 (20%)	2/4 (50%)	0/1 (0%)	0/1 (0%)

aureus (MRSA). Klebsiella species was the most prominent bacterial isolate while Proteus species was the least common (Figure 1).

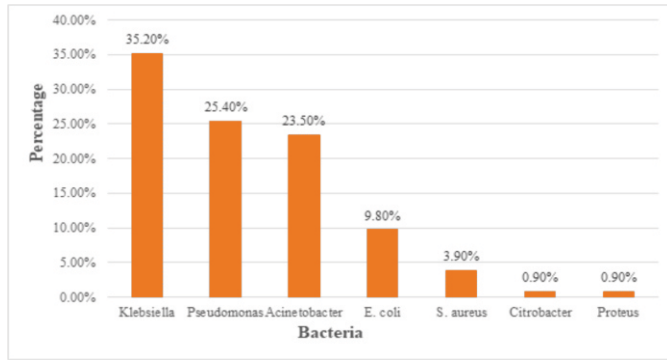


Figure 1: Bacterial growth in tracheal secretions and sputum

In females, Pseudomonas species (46.2%) was the most common isolate while in males Citrobacter species (100.0%), Proteus species (100.0%), and E. coli (70.0%) were the common isolates. In total, 23.1% of children developed an infection of Pseudomonas species which represented the commonest bacterial growth as compared to other bacterial growths. In the adult age group, Proteus species (100.0%), and Acinetobacter species (62.5%) were common while in the elder age group S. aureus (100.0%), Citrobacter species (100.0%), and

E. coli (60.0%) were common isolates. The majority of bacterial growths were observed in admitted patients and many of them were in the ICU department. The tracheal secretions had highest number of bacterial growth. The frequency and percentages of descriptive characteristics with respect to no growth and bacterial growth were also observed (Table 1).

The doxycycline and linezolid were 1(100%) sensitive to gram positive cocci. The Proteus 1(100%), E. coli 9(90%) and Klebsiella species 25(69.4%) showed maximum sensitivity to tigecycline. Similarly, the sensitivity of Proteus species was maximum for carbapenems 1(100%) and piperacillin-tazobactam 1(100%). In addition, the aminoglycosides performed better in the case of Acinetobacter species. However, a worrisome trend was observed for Citrobacter species which tested completely resistant to all applied antibiotics. In addition, the sensitivity of carbapenems was less for E. coli, Pseudomonas and Klebsiella species. The majority of the bacteria were completely resistant to ampicillin and amoxicillin-clavulanate except ampicillin 2(5.6%) was sensitive in the case of Klebsiella species. All antibiotic sensitivity results have been summarized in Figure 2.

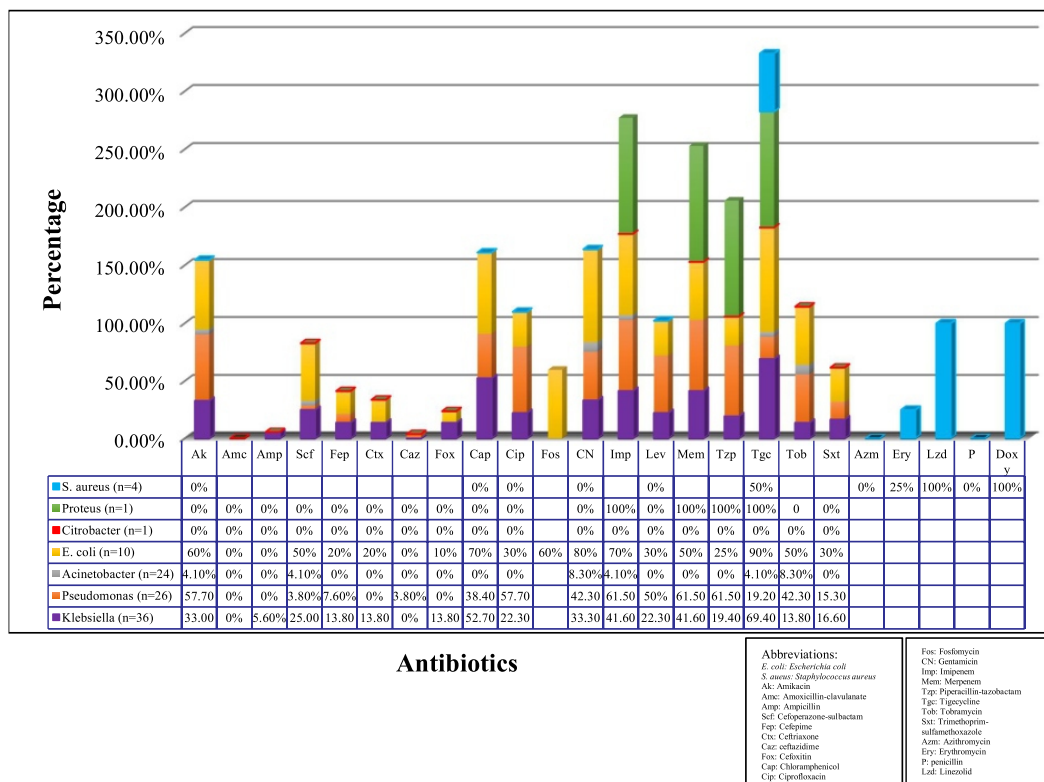


Figure 2: Antimicrobial sensitivity of gram negative rods and S. aureus from tracheal secretions and sputum specimens

Discussion

In clinical and non-clinical settings, bacteria are becoming more and more resistant to conventional antibiotics.¹⁴ Due to excessively invasive procedures, such as the use of artificial ventilator support, the rate of nosocomial infection in patients admitted to the ICU is also gradually rising.¹⁵ This emerging resistance is a serious issue, with the proper use of antibiotics and improvement in hospital settings can stop the bacteria from showing more resistance. In the present study, 63.5% of the samples showed positive bacterial growth. Gupta et al. conducted a study in which, the positive growth rate was 53%.¹⁶ Chandra et al. conducted another study, in which 72.3% of the samples were positive.¹⁷ Malik et al. conducted a research project in Pakistan; the percentage of positive cultures was 83%.¹⁸ The improved infection control measures in our hospital's ICU setup are to blame for the significant drop in our study. However, the decreased proportion of positive growth may be limited by our study's convenient sampling technique.

The gram negative rods were the frequent source of infection, outnumbering gram-positive cocci, which caused only 4% of all positive culture growths in our research study. This was in accordance with Chandra et al. findings. In Gupta et al. studies, gram negative rods accounted for 85% of the bacterial growth, where 86% of the specimens contained gram negative rods.^{16,17} The majority of the gram negative bacteria in the isolates of patients belonged to the Klebsiella, Acinetobacter, and Pseudomonas species according to Deepti et al. study.¹⁷ The bulk of gram negative infections are contracted in hospitals which are more tenacious and challenging to treat. Strenuous measures should be taken to prevent the spread of gram negative rods, particularly in the ICU.

The most prevalent isolate in the present study was a Klebsiella species (35.2%). A study conducted in Lahore, Pakistan revealed Klebsiella pneumoniae as the most frequently isolated bacterium from tracheal secretions (35.4%).¹⁸ Chandra et al. reported the most prevalent isolate was also Klebsiella (32.35%).¹³ In George et al. study, Acinetobacter (37.5%), Pseudomonas (21.8%), and Klebsiella (15.6%) were the most prevalent isolates.¹⁹ The considerable rise of multidrug-resistant isolates can be credited with the rise in Klebsiella species found in this study, particularly in the ICU department.

This study showed Klebsiella species was highly sensitive to tigecycline (73.5%) and chloramphenicol (54.3%).

Pseudomonas species exhibited 62.5% sensitivity to tigecycline. Klebsiella was 62% sensitive to sulzone, while Pseudomonas showed maximum sensitivity to piperacillin-tazobactam (73%).²⁰ The sensitivity to drugs of Klebsiella species and Pseudomonas species gradually decreased. Cross-infections and the improper use of antibiotics, may be blamed for this increasing prevalence of multidrug resistant gram-negative bacteria²¹. The remaining gram-negative rods were most resistant to fluoroquinolones, cephalosporins, and antibiotics like ceftazidime, ceftriaxone, ciprofloxacin, and cefepime. This was almost in agreement with the trend described by Gupta et al. and Malik et al. studies.^{16,20}

The time duration of the study was one of the limitations. Additionally, this research study was performed in a single tertiary care facility, making it impossible to apply the findings to the entire population.

Conclusion

Klebsiella species was the most frequently isolated species of gram-negative rods in tracheal secretions and sputum specimens. Moreover, this study concludes that Tigecycline is highly effective against gram negative rods in vitro. According to the new guidelines that have been updated in light of such research, it is necessary to administer antibiotic therapy in a limited and objective manner.

Conflict of Interest: *None*

Funding Source: *None*

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Authors Contribution

OF: Conceptualization of Project

SR: Data Collection

MA: Literature Search

ZY: Statistical Analysis

ZY, AM: Drafting, Revision

NW: Writing of Manuscript