

Susceptibility Profiles of Isolated Bacteria from Pneumonia Patients in Medical ICU

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⁴Critical revision of the manuscript for important intellectual content.

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Funding Source: None

Conflict of Interest: None

Received: November 14, 2023

Accepted: May 23, 2024

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ABSTRACT

Objective: To determine the susceptibility profiles of isolated bacteria from pneumonia patients in medical ICU.

Methodology: This cross-section observational study was conducted in the Medical ICU of Pakistan Ordinance Factories (POF) Hospital, Wah Cantt, Pakistan, between February and July 2023. The study included 100 patients aged ≥ 18 years, presented with severe symptoms of lower respiratory tract and exhibited bacterial growth. The patients' samples were collected from sputum, blood, tracheal secretions, bronchoalveolar lavage, and sent to hospital laboratory for sensitivity and culture analysis. Following overnight incubation, the agar plates were examined for bacterial growth and colonial morphology.

Results: Among 100 patients, 48% were female and 52% male. The mean age of the patients was 58.6 ± 14.5 years. 75% of patients were discharged from ICU, while 25% died. Sputum specimens were collected from 60% of patients, blood specimens from 21% of patients, tracheal secretions from 10% of patients, and bronchoalveolar lavage from 9% of patients. 30% tested positive for *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. *Pseudomonas aeruginosa* was detected in 20% of patients, while *E. coli* was found in 7% of patients. Among gram-positive bacteria, 6% of patients tested positive for *Staphylococcus aureus*, 3% for MRCONS, 2% for MRSA, and 2% for coagulase-negative staphylococcal organisms.

Conclusion: Significant levels of antibiotic resistance observed in isolates of gram-negative and gram-positive bacteria. The presence of increased resistance to multiple antibiotics indicates a high incidence of multidrug-resistant gram-positive and gram-negative organisms.

Keywords: Antibiosis; Bacteria; Pneumonia; Microbial sensitivity tests.

Cite this article as: Yasmin R, Hussain H, Hussain H, Naseer MA, Kazmi TH, Abidi STF. Susceptibility Profiles of Isolated Bacteria from Pneumonia Patients in Medical ICU. *Ann Pak Inst Med Sci*. 2024; 20(3):337-341. doi. 10.48036/apims.v20i3.1126

Introduction

Modern medical treatments have evolved significantly over the years, particularly with the advent of antibiotics. This evolution has led to improved healthcare, higher patient survival rates, and effective management of illnesses that were once potentially and often fatal.¹ ICU patients with severe illnesses often encounter multidrug-resistant bacteria, leading to limited therapeutic options

that effectively cover the spectrum of ICU pathogens.² Identifying the pathogen and its antibiotic pattern of sensitivity has essential for selecting appropriate antibiotics.³ The emergence of bacteria resistant to multiple antibiotics has been increasingly observed over the past two decades, coinciding with the advancement of broad-spectrum and extended-spectrum antibiotics.⁴

Staphylococcus aureus is a frequently encountered organism and is also found in anterior nares and normal

skin flora, thus serving as a primary origin of contamination in respiratory tract infection.⁵ Identifying coagulase in staphylococci is the basis for organisms' identification. Organisms are categorized as either coagulase-positive or coagulase-negative staphylococci based on the presence or absence of coagulase. *Staphylococcus aureus* is coagulase-positive organism, while *Staphylococcus epidermidis* is coagulase-negative gram-positive organism. Coagulase-negative *Staphylococcus* is primarily found on implanted devices, particularly in patients of advanced age or with compromised immune systems.⁶

β -Lactam, including carbapenem, monobactam, cephalosporin, and penicillin account for 60% of all antibiotics utilized. The primary cause for the heightened utilization of these medications is their high degree of safety and broad spectrum of bacteria activity, which leads to high efficacy. The extensive use of these antibiotics has led to a significant setback due to the emergence of resistance.⁷ Since the early 2000s, Methicillin-Resistant *Staphylococcus Aureus* (MRSA) has been recognized as the initial multi-drug resistant organism. However, in recently, gram-negative bacteria are documented to exhibit multi-drug resistance.⁸ Isolate organisms have demonstrated resistance to extended-spectrum beta-lactamases (ESBL), carbapenems, and even colistin. Carbapenems have demonstrated effectiveness against nosocomial infections and community-acquired diseases that are resistant to other antibiotics.⁹

The study objective was to determine the susceptibility profiles of isolated bacteria from pneumonia patients in medical ICU. This information aims to guide effective antibiotic selection and treatment strategies for these patients.

Methodology

This cross-section observational study was conducted after obtaining approval from the ethics committee at the Medical ICU of Pakistan Ordnance Factories (POF) Hospital, Wah Cantt, Pakistan from February to July 2023. 100 patients in total, of both genders, aged ≥ 18 years, who presented severe symptoms of the lower respiratory tract and exhibited bacterial proliferation, were included through consecutive sampling in the study. WHO calculator of sample size was used with the following parameters; a 95% confidence interval, a 5% significance level, and a 7% precision rate.² Patients with negative bacterial proliferation on culture reports and who had already received antibiotic treatment were excluded.

Informed consent was obtained from each patient before they were included in the study.

The samples were collected from the patients' sputum, blood, tracheal secretions, bronchoalveolar lavage, and sent to hospital laboratory for sensitivity and culture analysis. A blood sample of 1-3 ml was collected for this purpose. The samples were placed onto appropriate culture media like chocolate, blood, and MacConkey agars, and then incubated for 24hrs at 35-37°C in aerobic conditions. Following incubation overnight, the agar plates were inspected for bacterial proliferation and colony morphology. Gram-negative rods were detected using oxidase test, catalase test, and Gram staining by assessing motility. Gram-negative *Microbact 24E* detection kits were utilized to confirm the isolates. For the identification of Gram-positive cocci, coagulase, the DNAase, and catalase tests were conducted.

Isolate bacterial suspension was adjusted to a turbidity equal to 0.5 standard of McFarland was inoculated onto agar of Mueller-Hinton. The susceptibility test of antimicrobe was conducted using altered disc of Kirby-Bauer diffusion method. The outcomes of susceptibility were interpreted as sensitive, intermediate, or resistant based on the guidelines of the Clinical Laboratory Standards Institute. The department of microbiology reported the culture results in 5 days.

The data was analyzed using SPSS v 23. The association between antibiotic sensitivity patterns and types of organisms was determined by chi square. The significant p-value was set at ≤ 0.05 .

Results

The study included 100 patients in the medical ICU diagnosed with pneumonia. The patient's mean age was 58.6 ± 14.5 years, with 48% being female and 52% male. 75% of patients were discharged from the ICU, while the remaining 25% died. Sputum specimens were collected from 60% of patients, blood specimens from 21% of patients, tracheal secretions from 10% of patients, and bronchoalveolar lavage from 9% of patients. Community-acquired pneumonia and nosocomial pneumonia cases each accounted for 30%, 28% were classified as ventilator-associated pneumonia, and the remaining 12% were aspiration pneumonia. 30% tested positive for *Klebsiella pneumoniae*, and 30% tested positive for *Acinetobacter baumannii*. *Pseudomonas aeruginosa* was detected in 20% of patients, while *E. coli* was found in 7% of patients. Among gram-positive bacteria, 6% of patients tested positive for *Staphylococcus aureus*, 3% for MRCONS, 2%

for MRSA, and 2% for coagulase-negative staphylococcal organisms (Table I).

Table I: All patient's characteristics. (n=100)			
Variables		N	%
Age (years)	Mean \pm SD	58.6	14.5
Gender	Male	52	52.0
	Female	48	48.0
Mortality	Discharged	75	75.0
	Died	25	25.0
Specimen type	Sputum	60	60.0
	Blood	21	21.0
	Tracheal secretion	10	10.0
	Bronchoalveolar lavage	9	9.0
Pneumonia diagnosis	Community-acquired	30	30.0
	Nosocomial	30	30.0
	Ventilator-associated	28	28.0
	Aspiration	12	12.0
Isolated organism	E. coli	7	7.0
	Klebsiella	30	30.0
	Pseudomonas aeruginosa	20	20.0
	Acinetobacter baumannii	30	30.0
	MRSA	2	2.0
	Coagulase -ve Staph.	2	2.0
	Gram +ve S. aureus	6	6.0
	MRCONS	3	3.0

Table II: Antibiotic sensitivity and resistance of gram -ve organisms.			
Antibiotic	N	Sensitivity	Resistance
Amikacin	80	30	50
Ampicillin	35	5	30
Cefoperzone/Sulbactam	80	30	50
Cefoperazone	80	25	55
Cefotaxime	80	14	66
Ceftazidime	80	20	60
Ceftriaxone	80	5	75
Ciprofloxacin	80	10	70
Co-amoxiclav	70	7	63
Colistin	80	25	55
Cotrimoxazole	65	4	61
Gentamicin	80	10	70
Imipenem	80	30	50
Levofloxacin	80	8	72
Meropenem	80	35	45
Moxifloxacin	80	30	50
Piperacillin/Tazobactam	80	30	50
Tetracycline	70	4	66

Table III: Antibiotic sensitivity and resistance of gram +ve organisms.			
Antibiotic	N	Sensitivity	Resistance
Azithromycin	20	6	14
Clindamycin	20	5	15
Erythromycin	20	3	17
Linezolid	20	5	15
Penicillin	20	4	14
Teicoplanin	20	5	15
Vancomycin	20	6	14

Ampicillin sensitivity was observed in 5/35 patients, cotrimoxazole in 5/70 patients, and co-amoxiclav in 7/70 patients. Ciprofloxacin and gentamicin sensitivity was found equally in 10/80, and amikacin in 30/80 patients. The sensitivity of Cefotaxime was observed in 14/80, ceftriaxone in 5/80, piperacillin/tazobactam and cefoperazone/sulbactam was equally found in 30/80 patients. Similarly, high sensitivity was observed with imipenem (30/80) and meropenem (35/80). Whereas, low sensitivity was found for tetracycline (4/70), ceftazidime (20/80), cefoperazone (25/80), and levofloxacin (8/80).

The high sensitivity was found for moxifloxacin in 30/80 and colistin in 25/80 patients. For gram-positive organisms, sensitivity was recorded as follow: penicillin in 2/20, erythromycin in 3/20, clindamycin in 5/20, vancomycin in 6/20 patients, linezolid in 5/20, azithromycin in 6/20, and teicoplanin in 5/20 patients (Table II & III).

The frequency of antibiotic sensitivity for gram-positive organisms indicated that *Staphylococcus aureus* was sensitive to all tested antibiotics, including azithromycin, clindamycin, erythromycin, linezolid, penicillin, teicoplanin, tigecycline, and vancomycin (Table 5). MRSA demonstrated high resistance to azithromycin and penicillin, but demonstrated moderate sensitivity to clindamycin, erythromycin, linezolid, teicoplanin, and vancomycin (43%). CONS (coagulase-negative *Staphylococcus*) exhibited high resistance to erythromycin, linezolid, penicillin, and teicoplanin (100%). Whereas, azithromycin demonstrated enhanced sensitivity (100%). MRCONS were high resistant to azithromycin (86%), clindamycin (100%), and erythromycin (100%). Drugs such as penicillin demonstrated a 50% sensitivity, while linezolid, teicoplanin, and vancomycin each showed a sensitivity of 33%.

Discussion

In this study, 30% patients each tested positive for *Klebsiella pneumoniae* and *Acinetobacter baumannii*, followed by *Pseudomonas aeruginosa* in 20% patients, and 7% patients tested positive for *E. coli*. A study conducted in Islamabad, Pakistan reported that the most common organism among gram-negative patients was *E. coli* (15%), followed by *Pseudomonas aeruginosa* (13%) and *Klebsiella pneumoniae* (10%). However, MRSA was the most prevalent among gram-positive patients (6%). *Pseudomonas* showed sensitivity to colistin (93%), while *Klebsiella pneumoniae* demonstrated sensitivity to

Table IV: Frequency of antibiotic sensitivity and resistance of gram -ve organisms.

Antibiotic	Klebsiella (R/S)	E. coli (R/S)	Pseudomonas aeruginosa (R/S)	Acinetobacter baumannii (R/S)	p-value
Amikacin	18/8	4/3	13/7	15/5	.019
Ampicillin	-	6/0	-	-	.032
Cefoperzone/Sulbactam	22/14	6/0	16/4	13/7	.153
Cefoperazone	25/10	6/0	12/8	12/7	.243
Cefotaxime	30/3	4/3	13/5	19/3	.092
Ceftazidime	28/7	5/2	12/8	15/3	.295
Ceftriaxone	30/3	5/1	20/0	15/1	.181
Ciprofloxacin	30/5	6/0	20/2	14/3	.578
Co-amoxiclav	28/4	7/0	-	-	.751
Colistin	25/9	5/2	15/4	10/10	.722
Cotrimoxazole	30/2	6/0	-	25/3	.742
Gentamicin	31/4	4/1	18/3	17/2	.079
Imipenem	20/10	5/2	15/6	10/12	.797
Levofloxacin	32/2	6/0	14/4	20/2	.243
Meropenem	20/14	6/0	15/6	14/5	.176
Moxifloxacin	25/12	4/3	15/5	6/10	.678
Piperacillin/Tazobactam	25/10	5/2	7/13	13/5	.001
Tetracycline	30/3	6/0	-	30/1	.798

Table V: Frequency of antibiotic sensitivity and resistance of gram +ve organisms.

Antibiotic	S. aureus (R/S)	MRSA (R/S)	CONS (R/S)	MRCONS (R/S)	p-value
Azithromycin	1/0	6/2	1/2	6/2	.053
Clindamycin	1/0	4/3	3/1	7/1	.644
Erythromycin	1/0	4/3	5/0	7/0	.191
Linezolid	1/0	4/3	4/0	6/2	.452
Penicillin	1/0	7/0	4/0	4/4	.120
Teicoplanin	1/0	4/3	4/0	6/2	.452
Vancomycin	1/0	4/3	3/1	6/2	.785

tigecycline (100%) and minocycline (84%). The ICU had an overall mortality rate of 31%.¹⁰ In this study, the ICU had mortality rate of 25%.

Ghazal et al reported that the most common organism was *E. coli* (47%), followed by *Pseudomonas aeruginosa* (17%) and *Acinetobacter baumannii* (13%).¹¹ In this study, the sensitivity of *Klebsiella pneumoniae* was demonstrated as follows: amikacin (52%), meropenem (46%), cefoperazone-sulbactam (40%), imipenem (37%), moxifloxacin (34%), colistin (31%), cefoperazone (28%), and piperacillin-tazobactam (28%). Bhat et al demonstrated that *Klebsiella pneumoniae* positive patients, 53% were resistant to meropenem and imipenem. Similarly, for *Proteus mirabilis* positive patients, 50% resistance was found against meropenem and imipenem.¹²

CONS (coagulase-negative *Staphylococcus*) was found to be high resistant to erythromycin, linezolid, penicillin, and teicoplanin (100%), in this study. Azithromycin demonstrated enhanced sensitivity (100%). MRCONS exhibited high resistance to azithromycin (86%), clindamycin (100%), and erythromycin (100%). Drugs such as penicillin demonstrated a 50% sensitivity, while

linezolid, teicoplanin, and vancomycin each showed a sensitivity of 33%. A study conducted in Egypt on CONS revealed that amikacin, ciprofloxacin, and vancomycin were effective against CONS. CONS exhibited high resistance to commonly utilized first-line antimicrobe agents.¹³ Tehseen et al demonstrating that gram-negative bacteria were sensitive to vancomycin, while gram-positive bacteria were sensitive to amikacin.¹⁴ Li et al studied the sensitivity of organisms to meropenem and found that gram-negative were frequently resistance (54%) to meropenem, while gram-positive organisms showed lower resistance (39%) to meropenem.¹⁵ A study conducted in Egypt found that *Klebsiella pneumoniae* was frequent isolated organism in gram-negative bacteria (41%), followed by *Acinetobacter baumannii* (19%), *Pseudomonas aeruginosa* (17%), *E. coli* (15%), *Enterobacter aerogenes* (5%), and *Proteus mirabilis* (2%). The sensitivity antibiotics indicated carbapenem resistant was up to 36%, and colistin showed a sensitivity of 87%.¹⁶

Conclusion

Significant levels of antibiotic resistance observed in isolates of gram-negative and gram-positive bacteria. The

presence of increased resistance to multiple antibiotics indicates a high incidence of multidrug-resistant gram-positive and gram-negative organisms. Therefore, accurate identification of the organism is recommended to ensure the administration of empirical drugs that are mainly effective against the isolated organism, especially in severe cases.

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