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Spectrum of Lower Respiratory Bacterial Pathogens and their Antimicrobial Susceptibility in a Tertiary Care Hospital at Rawalpindi

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ABSTRACT

Objective: To study the spectrum of bacterial etiological agents causing lower respiratory tract infections and their antimicrobial susceptibility pattern in a tertiary care hospital at Rawalpindi.

Methodology: A descriptive, cross-sectional study was conducted in the department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from June to December 2021. Total 130 patients with lower respiratory tract infections (LRTIs) irrespective of age and gender were included in the study while patients of pulmonary tuberculosis, fungal diseases and patients having prior antibiotic therapy before sample collection were excluded. Non quantitative cultures were performed for sputum, non- directed bronchial lavage (NBL), pleural fluid and quantitative cultures for bronchoalveolar lavage (BAL) and endobronchial (EB) washings specimens. Antibiotic susceptibility was performed using Clinical and Laboratory Standards Institute (CLSI) 2021 guidelines.

Results: Pseudomonas aeruginosa (26.9%) was found to be the most prevalent bacterial pathogen followed by Klebsiella pneumoniae (23%). Both Gram negative and Gram-positive bacteria showed highest antimicrobial resistance to flouroquinolones. Gram negative bacteria were more susceptible to aminoglycosides and carbapenems. Gram positive bacteria were most susceptible to linezolid and vancomycin. Staphylococcus aureus was isolated from 8 samples, of which 5(62.5%) were Methicillin-resistant Staphylococcus aureus (MRSA).

Conclusion: This study concluded that the most frequent bacterial pathogen causing lower respiratory tract infections was Pseudomonas aeruginosa and both Gram negative and Gram-positive bacteria showed highest antimicrobial resistance to flouroquinolones.

Key words: Antimicrobial susceptibility pattern, bacterial etiological agents, lower respiratory tract infections.

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Introduction

Lower respiratory tract infections (LRTIs) are one of the most common infectious diseases worldwide and a major cause of death and disability in all age groups.[1] These infections account for a large proportion of outpatient and inpatient work load, imposing a huge economic burden on our society. Antimicrobial therapy for LRTIs is frequently empirical and presumptive often without the evidence of culture results, hence this practice is presumably playing a significant role in the emergence of antimicrobial resistance among respiratory pathogens.[2,3]

Etiology of LRTIs cannot be clinically differentiated and significant variations exist among geographical regions. The etiologies also vary with age, gender, immune status,

co-morbid conditions and antimicrobial usage. Therefore, determining the local antimicrobial susceptibility profile is important for decision making regarding empirical antibiotics, need for hospitalization and measures for isolation.[2,4] We are living in an era of multi and extensively drug resistant pathogens. In order to conserve the available treatment options judicious use of antibiotics with continuous surveillance of microbial etiology and susceptibility should be our priority.[5,6]This way we can guide clinicians regarding antimicrobial stewardship, control the rise of antimicrobial resistance and improve health care as a whole.[5,6,7]

Majority of bacterial pathogens causing LRTIs are Gram negative bacilli.[2,7,8]A study conducted in Nepal showed *Pseudomonas aeruginosa* (35.32%) to be the most prevalent Gram negative bacterial pathogen followed by *Haemophilus influenzae* (33.83%) and *Klebsiella pneumoniae* (17.19%).Among Gram positive bacteria, *Streptococcus pneumoniae* (51.7%) was most predominant followed by *Staphylococcus aureus* (48.3%).[6]Another study conducted in Lahore General Hospital also showed *Pseudomonas aeruginosa* (35.74%) to be the most prevalent bacterial pathogen causing LRTIs followed by *Klebsiella pneumoniae* (33.11%). Among Gram positive bacterial pathogens *Staphylococcus aureus* was found significant followed by *Streptococcus pneumoniae* exhibiting high susceptibility to linezolid and vancomycin.[7] Bacterial isolates exhibit high resistance to Beta-lactam antibiotics (penicillins, cephalosporins) and co-trimoxazole while lower resistance to combination drugs(piperacillin/tazobactum and cefoperazone-sulbactam). Gram negative bacterial pathogens causing LRTIs exhibit low resistance to aminoglycosides and carbapenems and are considered good therapeutic options.[2,7]

Limited data regarding etiological factors and susceptibility pattern of LRTIs was available from Rawalpindi region. The aim of this study was to determine the antimicrobial profiles of bacterial pathogens causing lower respiratory tract infection.

Methodology

The study was carried out in the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from 1st June 2021 to 1st December 2021 after taking permission from ethical review board. Specimens were received from Combined Military Hospital (CMH) Rawalpindi using non- probability, consecutive sampling technique. A sample size of 130 specimens was calculated using WHO sample size calculator with 95% confidence level, 0.1719 anticipated population proportion: and 0.065 absolute precision required. Non-duplicate specimens from outdoor and indoor patients irrespective of age and gender were included in the study of patients who had at least two of the following symptoms - fever, severe cough with phlegm, pleuritic chest pain, shortness of breath along with TLC >11000cm^3 and/or radiological evidence. Patients taking antimicrobial therapy before sample collection or who were diagnosed with pulmonary tuberculosis and on ATT (anti tuberculosis therapy) were excluded from the study. Specimens that turn out positive for fungal growth were also excluded from our data. Permission was obtained from Institutional Ethical Committee. Consent was taken from all patients included in the study.

Demographic information including age, gender, address was recorded. The samples were processed as per standard microbiological protocol. Non quantitative cultures were performed for sputum, NBL, EB washings and quantitative cultures for BAL specimen. The threshold for positive BAL cultures is taken as 10^4 in quantitative culture. The samples were inoculated on Chocolate agar (CHA), 5% Sheep Blood agar (BA) and MacConkey agar (MA) (Oxoid, UK) plates. The CHA plates were incubated in Carbon dioxide incubator (10% CO2) at 35 +/- 2°C, while BA and MA plates were incubated at 37°C under aerobic conditions. The plates were observed for 48 hours for growth before reporting as negative. All isolates were identified on the basis of Gram stain, colony morphology and biochemical characteristics following standard procedures. Respective API test strips were used for definitive identification to species level.[9] Antibiotic susceptibility were performed in compliance with CLSI 2021 guidelines on Mueller Hinton agar (Oxoid, UK) by Modified Kirby Bauer disc diffusion method. Vancomycin was tested by E test method for *Staphyloccocus aureus* as guided by CLSI 2021.[10]

Data obtained was entered in SPSS version 24. For Qualitative variables like gender, hospital setting (Indoor/Outdoor), bacterial isolate, antibiotic susceptibility and antibiotic resistance was measured in percentages and frequencies were calculated. For Quantitative variables like age, mean±SD was calculated. Effect modifiers like age and gender were stratified by post stratification chi-square test and P value of ≤0.05 was considered significant.

Results

Participants of all ages were included in this study with mean age of 54.67 ±13.49 years. Majority of the patients were between 46 to 75 years of age.

Out of 130 patients, 52 (40%) were male and 78 (60%) were females. Distribution of patients according to samples is shown in Table I. Distribution of patients according to bacterial isolates is shown in Table II.

Antimicrobial Susceptibility profile of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, Heamophilus *influenzae* to antibiotics is shown in Table III. Antimicrobial Susceptibility profile of *Streptococcus pneumoniae* and *Staphylococcus aureus* to antibiotics is shown in Table IV.

Table I: Distribution of patients according to sample (n=130).

Samples	No. of Patients	Percentage
Sputum	20	15.4
BAL	68	52.3
EB Washing	37	28.5
Pleural Fluid	5	3.8
Total	130	100.0

Table II: Distribution of patients according to bacterial isolate. (n=130)

Isolates	No. of Patients	%
Pseudomonas aeruginosa	35	26.9
Klebsiella pneumoniae	30	23
Heamophilus Influenzae	5	3.8
Streptococcus pneumoniae	5	3.8
Staphylococcus aureus	8	6.2
No growth	47	36.1
Total	130	100.0

Table IV: *Antimicrobial Susceptibility profile of Streptococcus pneumoniae and Staphylococcus aureus.*

Antibiotics	Bacterial Isolate			
	Streptococcus pneumoniae		*Staphylococcus aureus*	
	Sensitive	Resistant	Sensitive	Resistant
Penicillin	100	0	2	98
Tetracycline	80	20	75	25
Doxycycline	80	20	75	25
Erythromycin	80	20	50	50
Azithromycin	80	20	NT	NT
Clindamycin	80	20	75	25
Vancomycin	100	0	100	0
Linezolid	100	0	100	0
Levofloxacin	80	20	NT	NT
Gentamycin	NT	NT	75	25
Chloramphenicol	NT	NT	75	25
Ciprofloxacin	NT	NT	25	75
Rifampicin	NT	NT	100	0
Cefoxitin	NT	NT	75	25
Co-trimoxazole	80	20	50	50

Stratification of bacterial isolates with respect to age and gender done using chi-square test was found to have p-value of 0.319 and 0.001 respectively. There was no role of stratification of age and gender for antimicrobial susceptibility testing.

Discussion

In our study, LRTI was more common in patients between the ages of 46-75 years and higher prevalence was found in males (60%) than in females (40%).

LRTI due to bacterial etiology was found in 63.84% of cases. The isolation rates by Regha IR *et al*, Mishra *et al*, Salman Khan et *al* and Ramana *et al* were 26.34%, 44%,

Table III: Antimicrobial Susceptibility profile of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Heamophilus influenzae.*

Antibiotics	Bacterial Isolate					
	Pseudomonas aeruginosa		***Klebsiella pneumoniae***		***Heamophilus influenzae***	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Cefepime	60	40	40	60	NT	NT
Ceftazidime	60	40	30	70	100	0
Piperacillin-tazobactam	80	20	30	70		
Imipenem	80	20	30	70	100	0
Meropenem	80	20	30	70	100	0
Ciprofloxacin	40	60	20	80	80	20
Levofloxacin	40	60	20	80	80	20
Gentamycin	80	20	30	70	NT	NT
Amikacin	80	20	30	70	NT	NT
Aztreonam	60	40	NT	NT	100	0
Ceftriaxone	NT	NT	30	70	100	0
Amoxicillin-clauvulanate	NT	NT	20	80	100	0
Doxycycline	NT	NT	20	80	NT	NT
Minocycline	NT	NT	20	80	NT	NT
Cotrimoxazole	NT	NT	30	70	80	20
Azithromycin	NT	NT	NT	NT	100	0
Tetracycline	NT	NT	NT	NT	80	20

49.3% and 39.4% respectively.[4,5,6,11] Lower bacterial isolation rates in these settings maybe due to lesser burden of disease, improper sample collection, delay in transportation or prior antimicrobial therapy before sample collection. Higher isolation rates found in our study highlights the burden of disease and failure of empirical therapy in our setting.

In this study Gram negative bacilli (GNB) were more frequently isolated than Gram positive bacteria. Many other studies also found out considerable predominance of GNB among respiratory pathogens.[9,11-13] In our study Gram negative predominance might be partly due to the unequal distribution of indoor and outdoor patients as higher number of samples are received from indoor settings and there is a predominance of Gram-negative bacteria in hospital care settings than in the community.

The findings of our study isolated *Pseudomonas aeruginosa* (26.9%) as the predominant pathogen. This is in concordance with Salman K *et al*, Malik M *et al* and Thomas AM *et al*.[6,7,9] In some other studies the predominant pathogen was *Klebsiella pneumoniae* followed by *Pseudomonas aeruginosa*.[14,15] *Pseudomonas aeruginosa* was found to have more than 70% susceptibility to amikacin, piperacillin–tazobactam, gentamicin, imipenem, and meropenem. However, it was less than 50% susceptible to ciprofloxacin and levofloxacin. *Klebsiella pneumoniae* was more resistant than *Pseudomonas aeurginosa* in its antimicrobial profile and showed less than 50% susceptibility to all tested antibiotics. Out of which it was least susceptible to ciprofloxacin and levofloxacin with only 15% susceptibility. Multiple studies from different geographical areas have shown high prevalence of multidrug resistant *Klebsiella pneumoniae* and its management has become a major challenge for clinicians.[16-17]

In the current investigation, *Hemophilus influenzae* was isolated in 3.85% cases. Our results are comparable to studies done by Chen J et al, however slightly higher percentage (6.5%) was isolated in their study.[18] The first line of treatment is ampicillin, but steady rise in ampicillin resistance is being observed. In our study, the isolate was 100% susceptible to all the tested beta-lactam antibiotics (penicillin, cephalosporin, carbapenem, aztreonam). Resistance was found against co-trimoxazole (15%), ciprofloxacin (20%) and tetracyclines (20%). However, a study conducted in Poland with a sample size of 1481 collected from 2005 to 2019 showed 12.6% ampicillin resistance. Higher resistance was noted for co-trimoxazole (24.7%) while resistance to ciprofloxacin was found to be only 0.1%.[19]

Out of 130 samples, Gram positive organisms were isolated from 13(10%) samples. *Staphylococcus aureus* was isolated from 8 (6.2%) and *Streptococcus pneumoniae* from 5 (3.8%). Similarly, *Staphylococcus aureus* was isolated as the predominant Gram positive pathogen in studies by Egbe *et al*, Amutha C *et al* and Anvari MS *et al* with isolation rates of 15.41%, 5% and 20.8% respectively.[1,20] The current study revealed that *S. aureus* was highly sensitive to vancomycin (100%), linezolid (100%), rifampicin (90%). However, isolates of *S. aureus* were resistant to ciprofloxacin (80%), co-trimoxazole (40%) and penicillin (98%). Cefoxitin, which was used as a surrogate for methicillin which was found to be 75% susceptible, therefore 25% of *S.aureus* isolates were found to be Methicillin resistant Staphylococcus aureus (MRSA). However, our results differ from a study conducted in southern Ethiopia which reported *S. aureus* to be highly sensitive to cefoxitin 90.6% and ciprofloxacin 71.4% with resistance to tetracycline 61.9%, co-trimoxazole 47.6% and penicillin 45%.[21] *Streptococcus pneumoniae* showed 25% resistance to Erythromycin and only 20% resistance to clindamycin and no resistance to Penicillin, vancomycin or linezolid was detected.

In our study, the isolated GNB were most susceptible to carbapenems including imipenem and meropenem followed by amikacin and gentamicin. Therefore, carbapenems followed by aminoglycosides are the most suitable drugs for empirical therapy for LRTI in our setting. The GNB isolates in our study showed high resistance to all generations of cephalosporins and flouroquinolones. Thus, cephalosporins and flouroquinolones were found to be ineffective for empirical therapy for LRTI in our setting.

The limitations found in this study were that distinction between community-acquired and hospital-acquired infections could not be made. Patient comorbidities and previous illnesses were not taken into consideration. Equal number of indoor and outdoor patients were not taken. Indoor patients were not segregated into medical wards and ICUs. Patients on ventilator were not distinguished from other indoor patients.

Conclusion

This study concluded that the most frequent lower respiratory tract bacterial pathogen causing lower

respiratory tract infections was *Pseudomonas aeruginosa (26.9%)* followed by *Klebsiella pneumoniae* (23%), *Staphylococcus aureus* (6.2%), *Streptococcus pneumoniae* (3.8%) and *Heamophilus influenzae* (3.8%). Overall, both groups of bacteria (Gram negative, Gram positive) showed highest resistance to quinolone antimicrobials. Carbapenems and Aminoglycosides could be used as empirical therapy when suspecting Gram negative bacterial etiology. The treatment should be modified as per the culture and sensitivity report from the microbiology lab.

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