Antimicrobial Susceptibility Pattern of Bacterial Isolates in Patients with Lower Respiratory Tract Infections

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Abstract: Lower respiratory tract infection (LRI) is one of the global public health problems accounting for millions of deaths annually. The present study aim is to determine bacterial pathogens’ bacteriological profile and antibiotic susceptibility pattern affecting the patients with LRI attending a tertiary care hospital. Two hundred and eight sputum samples were collected from patients with signs and symptoms of LRI. The samples were processed in Microbiology laboratory for bacterial pathogens. Bacterial isolates were identified using standard microbiological techniques and antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Klebsiella pneumoniae (39.6%), followed by Pseudomonas (32%), were the most predominant bacteria isolated in the present study. The other bacteria isolated were Escherichia coli (16.9%), Staphylococcus aureus (5.6%), Streptococcus species (1.8%), and Acinetobacter (3.7%). Gram-negative bacteria showed high drug resistance to ampicillin and cephalosporins and maximum susceptibility to piperacillin-tazobactam, amikacin, and gentamicin. Most of the isolates showed resistance to the Beta-lactam group of antibiotics. The isolated bacterial pathogens’ resistance profile indicates the need for the appropriate diagnosis and antibiotic susceptibility testing before managing the cases.

Keywords: Antibiotic susceptibility, bacteria, Lower respiratory tract infections, multi-drug resistance
1. INTRODUCTION

Antibiotic resistance is one of the major global issues on the rise in the past few decades. Irrational use of antibiotics is considered one of the major causes of the growing antibiotic resistance1. Lower respiratory tract infection (LRI) is one of the commonest infectious diseases affecting people of all age groups leading to high morbidity and mortality globally. LRI can affect the nasal pathway, bronchi, and lungs, causing signs and symptoms such as a sore throat, running nose, cough, and fever. Most people with respiratory tract infections have no or only mild symptoms. However, some people develop more serious symptoms such as chest pain and difficulty in breathing with complications like fatal pneumonia, pleural effusion, pneumothorax, chronic obstructive lung disease (COPD), etc. It has been reported that Europe, in particular the United Kingdom (UK) report high mortality from respiratory system diseases, particularly in the elderly. World Health Organisation (WHO) has reported that among the various infectious diseases, LRI accounts for 20% of mortality2. The causative agents of LRI were diverse, including bacteria, viruses, fungi, and parasites. Among the bacterial causative organisms of LRI, Gram-positive organisms like Streptococcus pneumoniae, Staphylococcus aureus and Gram-negative organisms like Hemophilus influenzae, Klebsiella pneumoniae, Pseudomonas, Acinetobacter are frequently implicated4. These bacteria invade the lungs and multiply, causing an infection. Antibiotics are used to treat infectious diseases. Antibiotic resistance refers to the ability of bacteria to survive when exposed to antibiotics. The overuse and misuse of antibiotics is one of the main causes of antibiotic resistance. LRIs caused by bacteria that are resistant to antibiotics are difficult to treat. These bacteria are referred to as antibiotic-resistant bacteria. The rising trend of multi-drug resistant (MDR) bacteria is alarming, especially among Gram-negative bacteria. Most investigators have recommended the judicious use of antibiotics in tackling the growing problem of antibiotic drug resistance. Clinicians, pharmacists, and others must follow proper antibiotic guidelines when handling infectious diseases.

Respiratory infections are considered an important health issue resulting in around 50 million deaths annually. It has been reported that respiratory infections account for 13.3% of Disability-adjusted life years (DALY)4. More information about the microbial agents and the antimicrobial profile among LRIs in our settings need to be available. Knowledge about the causative agents of LRIs and their antibiotic susceptibility pattern is of utmost importance in selecting antimicrobial agents in treating patients. The present study aims to determine the bacterial etiological agents causing the LRI in patients attending a tertiary care hospital in Chennai. The study’s objective is to isolate the bacterial pathogens from the sputum samples of patients with signs and symptoms of LRI using standard microbiological techniques and to study the antibiotic susceptibility pattern of the bacterial isolates to the commonly prescribed antibiotics.

The study’s results would help to formulate antibiotic policies and guidelines for the rational use of antimicrobial agents to combat the growing resistance among pathogens.

2. MATERIALS AND METHODS

The study was conducted in a tertiary care hospital in Chennai over 6 months from June 2022 to November 2022. The study has been approved by the institutional research and ethical committee (Ref. No.002/SBMC/IHEC/2022/1721). Written consent was obtained from all the patients included in the study. Clinical samples were collected from the patients attending the outpatient department and those admitted to wards during the study period with symptoms and signs of lower respiratory tract infections. The clinical samples were processed immediately in the Microbiology laboratory. Patients already on antibiotic therapy and those with a previous hospital admission in the last 14 days of data collection were excluded from the study.

2.1. Bacterial isolation and identification

Sputum samples were collected in a disposable, sterile, wide-mouthed container with a tight-fitting lid after the patients were given proper instructions. The sputum samples were transported to the Microbiology laboratory. As per the standard protocols, the samples were inspected macroscopically and microscopically. Samples contaminated with saliva were rejected. Suitable samples were cultured aerobically. The purulent portion of the sputum samples was inoculated on Nutrient agar, MacConkey agar, and Blood agar plates and incubated at 37°C aerobically for 24 to 48 hrs. After 24 hours, the plates were examined for growth, if any. The bacterial organisms were identified using standard biochemical and microbiological techniques. If there is no appreciable growth, the plates were further incubated for 48 hours before reporting no growth.

2.2. The antibiotic susceptibility testing

The antibiotic susceptibility of the bacterial isolates in the current study was performed using the Kirby Bauer disc diffusion technique as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The bacterial suspension was seeded as lawn culture on the Mueller-Hinton agar plate, and antibiotic discs were placed on it and incubated at 37°C overnight. The zone of inhibition was measured and interpreted as per CLSI guidelines. The following antibiotic discs were tested for this research: ciprofloxacin, gentamicin, amikacin, ampicillin, amoxycillin clavulanic acid, piperacillin tazobactam, Imipenem, cefazidime, cefotaxime, ceftriaxone, cefepime, cotrimoxazole, penicillin, erythromycin, clindamycin, linezolid, tetracycline, and cefoxitin. Standard reference strains (E. coli (ATCC-25922), S. aureus (ATCC-25923), P. aeruginosa (ATCC-27853)) were used as quality control during culture and antimicrobial susceptibility testing.

3. RESULTS

A total of 208 patients were included in the study. 56% of the study subjects enrolled in the study were males, and 44% of patients were females.

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20 years</td>
<td>16</td>
<td>7.6</td>
</tr>
<tr>
<td>20 – 40 years</td>
<td>68</td>
<td>32.6</td>
</tr>
<tr>
<td>40 – 60 years</td>
<td>86</td>
<td>41.3</td>
</tr>
<tr>
<td>60 – 80 years</td>
<td>38</td>
<td>18.3</td>
</tr>
</tbody>
</table>
The study population included patients of all ages under 80 with clinical evidence of lower respiratory tract infections (Table 1). Most of the patients enrolled in the study were 40-60 years (41.3%). The other major group of patients (32.6%) were in the middle age between 20 to 40 years. 18.3% of the study group were elderly (60-80 years). 7.6% of patients were less than 20 years old.

The microorganisms isolated in the study are shown in Table 2. The present study isolated Gram-negative organisms more frequently than Gram-positive microorganisms. The bacteria isolated from the clinical samples include Gram-negative organisms like Klebsiella pneumoniae (39.6%), Pseudomonas (32%), Escherichia coli (16.9%), Acinetobacter (3.7%), and Gram-positive organisms like Staphylococcus aureus (5.6%) and Streptococcus species (1.8%).

The various bacterial pathogens isolated in the present study have been depicted in Figure 1, which shows that among the microorganisms isolated in the current study, Klebsiella pneumoniae outnumbers the rest of the organisms (39%). The second most commonly isolated organism was Pseudomonas aeruginosa (32%). Klebsiella and the Pseudomonas account for more than 50% of the cases. The third common bacteria isolated was Escherichia coli (17%). The other bacteria, like Staphylococcus, Streptococcus, and Acinetobacter, account for less than 10% of the cases, as shown in Figure 1.

### Table 2: Incidence and distribution of the pathogens

<table>
<thead>
<tr>
<th>S.No</th>
<th>Microorganism</th>
<th>Number of Culture positive Samples (N=106)</th>
<th>Percentage of occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Klebsiella pneumoniae</td>
<td>42</td>
<td>39.6</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>Streptococcus</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>Staphylococcus</td>
<td>6</td>
<td>5.6</td>
</tr>
<tr>
<td>5</td>
<td>Escherichia coli</td>
<td>18</td>
<td>16.9</td>
</tr>
<tr>
<td>6</td>
<td>Acinetobacter</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td>Total isolates</td>
<td>106</td>
<td>100</td>
</tr>
</tbody>
</table>

(N – number of isolates tested)

![Distribution of Bacterial isolates](image)

**Fig 1:** Distribution of various bacterial pathogens of lower respiratory tract infections

The various bacterial pathogens isolated in the present study have been depicted in Figure 1, which shows that among the microorganisms isolated in the current study, Klebsiella pneumoniae outnumbers the rest of the organisms (39%). The second most commonly isolated organism was Pseudomonas aeruginosa (32%). Klebsiella and the Pseudomonas account for more than 50% of the cases. The third common bacteria isolated was Escherichia coli (17%). The other bacteria, like Staphylococcus, Streptococcus, and Acinetobacter, account for less than 10% of the cases, as shown in Figure 1.

### Table 3: Antimicrobial susceptibility pattern of Gram-positive isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Staphylococcus N = 6</th>
<th>Streptococcus N = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td>Penicillin</td>
<td>3(50)</td>
<td>3(50)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>4(67)</td>
<td>2(33)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>5(83)</td>
<td>1(17)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>3(50)</td>
<td>3(50)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>6(100)</td>
<td>0</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>3(50)</td>
<td>3(50)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2(33)</td>
<td>4(67)</td>
</tr>
<tr>
<td>Ciprofloxacine 5(83)</td>
<td>1(17)</td>
<td>NT</td>
</tr>
</tbody>
</table>

(N – number of isolates tested, NT - Not Tested)
The antibiotic susceptibility pattern of Gram-positive isolates is shown in Table- 3. All the Staphylococcus isolates were found to be susceptible to penicillin, clindamycin, tetracycline, and erythromycin (83%). The Staphylococcal isolates showed greater resistance to tetracycline (67%). The Streptococcal isolates were susceptible to linezolid. Staphylococcus isolates showed greater susceptibility to clindamycin (83%), ciprofloxacin (83%), and ampicillin (83%).

The antimicrobial susceptibility pattern of Gram-negative isolates is shown in Table 4. The Gram-negative isolates showed greater susceptibility to piperacillin–tazobactam, gentamicin, amikacin, cepfime, and imipenem and showed a variable susceptibility to the amoxycillin clavulanic acid, ciprofloxacin, cotrimoxazole, and other cephalosporins.

4. DISCUSSION

Lower respiratory tract infections are the most common cause of lung infections in adults, and the rise in antimicrobial resistance is a life-threatening public health problem. In this study, bacterial pathogens were isolated from 51% of the total sputum specimen of patients with clinical presentation of lower respiratory tract infections. The most common organism isolated was Klebsiella pneumoniae (39.6%), followed by Pseudomonas (32%). The other Gram-negative bacilli were Escherichia coli (16.9%) and Acinetobacter (3.7%). Among the Gram-positive cocci, Staphylococcus aureus accounts for 5.6% and Streptococcus pneumoniae for 1.8% of the total bacterial pathogens isolated in the current study. This is comparable to another study conducted by Luan et al., which showed Klebsiella pneumoniae to be 27.4%, Escherichia coli to be 17.9%, Pseudomonas aeruginosa 10.3%, Acinetobacter baumannii 5.1% and Streptococcus pneumoniae 9.4%. In contrast, a 5-year study conducted by Biagio Santella et al. showed Acinetobacter baumannii as the leading causative agent of LRI, followed by Pseudomonas aeruginosa and Klebsiella pneumoniae. Another study by El-sokkary et al. on community-acquired pneumonia among adult patients at an Egyptian university hospital showed higher rate of isolation of K. pneumoniae (10.37%) followed by Staphylococcus aureus (12.0%), S. pneumoniae and P. aeruginosa (7.78% each). The percentage of microbial causes of lower respiratory tract infections in our study was 50.9% which is comparable to various studies done in other parts of India, which is 75.6% in Shimla, 47.7% in Chandigarh, northern India 72% and other parts of the world including 62% in UK, 68% in Singapore and 56% in Philippines. On the contrary, a study conducted in southern India showed the culture positivity rate among community-acquired pneumonia cases to be 30%. The gender distribution in our study was 56% males and 44% females, similar to another study conducted by Chaithra Kanishan et al., which showed males to be 55.4% and females to be 44.5%. The increased prevalence in males may be attributed to associated risk factors such as smoking, alcoholism, and COPD. 41.3% of the subjects of LRI belonged to the age group of 40–60 in the current study, which shows that LRI is more common among aged individuals more than 40 years when compared to young subjects. This is consistent with other studies, which showed the mean age distribution between 54-58 years of age 16. Studies conducted among Western countries showed a greater incidence of LRI in older adults, with considerable morbidity and mortality among them. Gram-negative bacteria were commonly isolated in the present study. This shows a shift in the trend of pathogens. Among the Gram-negative isolates, Klebsiella pneumoniae was the predominant isolate in our study. Studies in Africa showed similar trends. Klebsiella pneumoniae showed high resistance to ampicillin (81%), cotrimoxazole (48%), ceftazidime, and cefotaxime (33%), followed by cefpime and ceftriaxone (29%). This result is in line with a previous study by Moini et al. Most of the Gram-negative isolates were susceptible to piperacillin tazobactam, amikacin, and gentamicin. Pseudomonas aeruginosa was the second commonest organism among Gram-negative bacteria isolated. In the case of P. aeruginosa, gentamycin and amikacin were the most effective antibiotics, contrary to ciprofloxacin, which was the least effective. These results are comparable to those of Biagio Santella et al. The Multidrug resistance pattern of microbes poses a great threat in treating infections. It is essential to monitor and regulate antibiotic usage through antibiotic stewardship programs. Many studies prove that combination therapy helps to prevent the emergence of novel wild-resistant strains. Treatment failures are usually found in patients who only receive a single antibiotic. Clinicians and microbiologists should collaborate to manage infections accurately as instituted by the Rational
Use of Medicine (RUM) Program. In our study, S. aureus was the majority among Gram-positive isolates, of which Methicillin Resistant Staphylococcus aureus (MRSA) was observed among 50% of them. None of the isolates showed resistance to linezolid. A study conducted by Global Initiative for Methicillin-Resistant Staphylococcus Aureus Pneumonia (GLIMP) recorded a very low prevalence of MRSA pneumonia in India (1.4%) in comparison to all over the world (3%)\(^ {25} \). In the US, the prevalence of MRSA is 2.4% among CAP cases\(^ {24} \). But it can manifest as severe necrotising pneumonia exhibiting high morbidity and mortality following influenza infection\(^ {27,28} \). A study by Eshwara VK et al. reported bacterial community-acquired pneumonia due to S.aureus infections exhibited fatal illness, similar to other studies from high-income countries that showed a similar outcome\(^ {29,30} \).

In a study done in South India on the antimicrobial resistance of bacterial agents of the upper respiratory tract, S. aureus (45.6%) was the most predominant bacterial isolate, followed by Streptococci species (22.8%), Klebsiella (14.9%) and Pseudomonas (8.33%)\(^ {31} \). Positive sputum culture with predominant Gram-negative bacteria and second-line antibiotic resistance were associated with delayed recovery and prolonged hospital stay. A recent review has shown that CAP due to multi-drug resistant organisms has become common and is related to considerable morbidity and mortality\(^ {32} \). Emphasis was made on the early identification of these pathogens and segregating patients based on the risk factor to start appropriate antibiotic treatment \(^ {32} \). Several factors like prolonged antibiotic therapy, chronic use of invasive devices, high-risk procedures, the immunocompromised status of the patient, insufficient use of standard and isolation precautions, etc., have been attributed to the growing antibiotic resistance\(^ {32,34} \). It is important to periodically monitor the prevalence as well as the antibiotic susceptibility pattern of the commonly isolated bacterial pathogens of lower respiratory tract infections before initiation of empirical therapy. The current study provides data about the antibiotic susceptibility pattern of the pathogens causing LRI, which would help the physicians to gain knowledge about local epidemiology and to choose appropriate antibiotics for managing Lower respiratory tract infections.

5. CONCLUSION

Gram-negative bacteria are found to be the predominantly isolated bacterial pathogens causing LRI in this study. Klebsiella pneumoniae, followed by Pseudomonas, are the most common pathogens in the current study. LRI was predominant in the age group of 40 – 60 years. The organisms were found to show greater susceptibility to piperacillin tazobactam, amikacin, and gentamicin drugs and were found to show greater resistance to ampicillin and cephalosporins. Future studies evaluating the influence of age, co-morbidity, and disease severity on the microbial and antibiotic susceptibility pattern of LRI are recommended. The emerging drug-resistant pathogens affect the patients and the healthcare setting. The rising MDR isolates from the respiratory tract are alarming. They must be managed with knowledge and treatment based on the culture and antibiotic susceptibility pattern of organisms causing lower respiratory tract infections.

6. AUTHORS CONTRIBUTION STATEMENT

Monisha R gathered the data with regard to the work and prepared the original draft. Dr. Lakshmi K and Dr. Sharanya K gave the necessary inputs towards finalising the manuscript, reviewed and edited the final manuscript. All authors have made a substantial, direct, and intellectual contribution to the manuscript and approved it for publication.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES


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