ORIGINAL ARTICLE

Etiological Trends and Antibiotic Susceptibility of Bacterial Strains Causing Respiratory Tract Infections

Fareeha Adnan, Faisal Iqbal Afridi, Nazia Kurshid
Department of Microbiology, the Indus Hospital, Karachi, Pakistan.

ABSTRACT

Background: Lower respiratory tract infections (LTRIs) are emerging as the most common infectious diseases of humans. Antibiotic resistance has increased in all the major pathogens therefore, this project engrossed on defining the current drift of bacterial etiologies of respiratory tract infections among the patients and their antimicrobial susceptibility pattern.

Methods: This cross-sectional study with non-probability consecutive sampling was conducted in the microbiology laboratory of Ziauddin Hospital. Bacterial isolates (163) were recuperated from respiratory sputum specimens obtained from patients with lower respiratory tract infections. The pathogens collected for study were Streptococcus pneumonia, Haemophilus influenzae, and Moraxella catarrhalis. Frequencies and percentages were computed for categorical variables like microorganism, gender, age, duration of lower respiratory tract infections, etc. Mean and standard deviation were calculated for quantitative variables like age and infection duration. Furthermore, duration of disease was stratified by post stratification Chi Square with \( p \) value ≤ 0.05 was considered significant.

Results: Most commonly isolated pathogen is Moraxella catarrhalis 72.39% followed by Haemophilus influenza 14.72% and Streptococcus pneumonia 12.88%. For Streptococcus pneumoniae 47% sensitivity showed to Ampicillin, 52% Penicillin, 61.9% Erythromycin and 57% to Ceftriaxone. For Haemophilus influenzae 100%, sensitivity showed to Ceftriaxone, 100% Amoxicillin and 62.5% Co-trimoxazole. Similarly, for Moraxella catarrhalis 54% sensitivity was showed to Erythromycin, 100% Ceftriaxone and 27% with Levofloxacin.

Conclusion: Moraxella catarrhalis, Haemophilus influenzae and Streptococcus pneumoniae were the most common bacterial isolates recovered from LTRIs. We found M. catarrhalis resistant rate was elevated for Levofloxacin, Streptococcus pneumonia for Co-trimoxazole and Haemophilus influenzae to all β-lactams.

Keywords: Respiratory Tract Infection; Haemophilus influenzae; Moraxella catarrhalis; Streptococcus pneumonia.

Corresponding Author:
Dr. Fareeha Adnan
Department of Microbiology,
The Indus Hospital, Darussalam Society,
Sector 39, Korangi, Karachi, Pakistan.
Email: fareehaadnan1@gmail.com
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INTRODUCTION

Lower respiratory tract infection (LRTI) are recognized to be the most widespread and serious infections\(^1\). According to United Kingdom (UK) studies over all incidence of LRTI in adult population, in general practice is approximately 44 – 84 cases per 1000 population per year\(^2\). LRTI include Community Acquired Pneumonia (CAP) and Acute Exacerbation of Chronic Bronchitis (AECB)\(^3\). Etiology of LRTI varies with age, season and geographic region\(^3\). Bacterial pathogens responsible for typical CAP include Streptococcus pneumoniae (S. pneumoniae), Haemophilus influenzae (H. influenzae), Klebsiella pneumoniae (K. pneumoniae) and Moraxella catarrhalis (M. catarrhalis) \(^3^4\). These pathogens are responsible for 85% of CAP cases. Among these, the most common one is S. pneumoniae\(^5\).

The increase in antibiotic resistance is a serious
A vigilant and shrewd use of antibiotics will minimize the prevalence of atypical pathogens in different geographical location, it is crucial to frequently update the etiology, predisposing factors and advance laboratory diagnostic facilities to ascertain the infectious cause of LRTIs.

Due to emergence and dissemination of multidrug resistant pathogens, difference of etiological agent and prevalence of atypical pathogens in different geographical location, it is crucial to frequently update the etiology, predisposing factors and advance laboratory diagnostic facilities to ascertain the infectious cause of LRTIs.

Usually empirical treatment is adopted for CAP but because of increased frequency of antibiotic resistance, isolated respiratory pathogens has complicated the selection process of antimicrobial agents. In developing countries like Pakistan most LRTI are treated empirically, may be due to higher cost of laboratory services or non-availability of standardized laboratories. Ongoing surveillance is essential to update the consultants and general practitioners to choose the appropriate antibiotics. In reference to the infected organisms similarly vigilant and shrewd use of antibiotics will minimize the burden of multidrug resistance. The purpose of this study was to extract data of the most common bacterial pathogens responsible for LRTI and their antimicrobial susceptibility patterns in tertiary care hospitals setting in Pakistan.

**METHODS**

This descriptive study evaluated the frequency of isolation of S. pneumoniae, H. influenzae, M. catarrhalis, and their susceptibility profile. Study conducted from October 2014 to November 2015 at Department of microbiology, in Ziauddin university hospital (Ref.MIC-2013-201-144). To determine the frequency of bacterial pathogens of LRTI, sample size of patient was calculated with a 0.05 margin of error and 95% confidence level. Prevalence of bacterial pathogens in LRTI is 10.6% after putting the values in the formula n = \( \frac{Z_{.05}^2 \times P \times Q}{d^2} \) = 163. Specimens were obtained from patients with LRTI and bacterial isolates were recovered from sputum. Isolates from patient of 35-70 years of age were obtained after 48 hours. Patients with diagnosis of ventilator associated pneumonia and health care associated pneumonia were not considered. Clinical laboratory standard institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing for bacterial identification and antibiotic susceptibility testing was done. Exclusion criteria were strappingly fulfilled. Written approval was taken from the institutional ethical committee.

Consent was also taken from the patients.

Age, gender, source of specimen and visit type were collected and recorded. Samples were inoculated on “sheep blood agar (SBA), chocolate agar and MacConkey agar”. Streaking was performed with a sterile wire loop on these media plates following standard procedure. The culture plates were incubated at “35°C in ambient atmosphere” for 24-48 hours and noticed for growth by formation of colonies. Organism grown were identified and their antibiotic susceptibility testing was performed following standard procedures.

Colonies of H. influenzae were identified as “Gram negative rods, tiny, moist, and smooth gray colonies with absence of hemolysis, positive catalase and oxidas test although oxidase test may be variable. However, its oxidase test may be negative due to the presence of growth factors X and V, satellite growth around streaks of Staphylococcus aureus”.

Presence of M. catarrhalis was identified as “gram negative cocci on gram staining, colony morpholo, oxidase test, hockey puck sign, catalase test, and butyrate esterase production, and their inability to ferment sugars”. Identification features of S. pneumoniae are defined as “presence of tiny, round, flat, and transparent colonies, with central depression (checker piece and nail head colonies), a hemolysis, catalase negative and oxidase negative, absence of bile-esculin hydrolysis, lysis by bile-salts, susceptibility to optochin and other biochemical characters”.

The susceptibilities testing of isolated organisms were done by Kirby-Bauer disc diffusion method was used using Mueller Hinton agar plates. Twenty-four hours incubated isolated colonies were suspended in normal saline and a suspension of 0.5 McFarland turbidity was made. They were streaked using sterile swabs over the surface of Mueller Hinton agar plates. Antimicrobial discs of different strengths were placed on the surface of the MHA plates and gently pressed. The antimicrobial discs were obtained from Oxoid (UK) and Bioanalyse (Turkey). The tested Antimicrobials were “ampicillin (10 μg), co-amoxiclav (amoxicillin/clavulanic acid 20/10 μg), cefadroxil (30 μg), ceftriaxone (30 μg), meropenem (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), co-trimoxazole (trimethoprim/sulfamethoxazole 1.25/23.75μg), erythromycin (15 μg) and clindamycin (2 μg), Vancomycin (30 μg).” The plates were inverted and incubated at 37°C for 16 to 18 hours. The inhibition zone diameters were measured and recorded.

For data analysis, Statistical Package for Social Sciences (SPSS) version 17 was used. For variables like microorganism, gender and antibiotic susceptibility frequencies and percentages were calculated. For quantitative variables like age, duration of LRTI, etc., mean and standard deviation was considered. Effect modifiers like age, gender and duration were measured and recorded.
of disease stratified as post stratification Chi-square test applied and $p$ value $\leq 0.05$ was considered significant.

RESULTS

The average age of the patients ($n=163$) was 52.61±11.8 (95% CI: 50.78-54.45) years and mean duration of LRTI was 8.55±1.96 months. Histogram (Figure 1) shows the age distribution in different groups. Out of 163 patients, 85(52.15%) were male and 78(47.85%) were female patients.

Frequency of major pathogen of community acquired lower respiratory tract infection is presented as Moraxella catarrhalis organism was commonly observed i.e. 72.39% (118) followed by Haemophilus influenzae 14.72% (24) and Streptococcus pneumoniae was observed in 12.88% (21) patients. For all three bacteria there were no significant $p$-value found Streptococcus pneumonia (0.66), Haemophilus influenzae (0.76) and Moraxella catarrhalis, (0.92). Sensitivity pattern (Table 1) of these organisms (Streptococcus pneumonia, Haemophilus influenzae and Moraxella catarrhalis) were reported and showed that Streptococcus pneumonia was 85.7% to 100% sensitive to Levofloxacin, Clindamycin, Linezolid, and Vancomycin. Haemophilus influenzae was sensitive to Ampicillin, Ceftriaxone, Co-amoxiclav, Moxifloxacin, Cefixime and Meropenem. Moraxella catarrhalis was sensitive to Ceftriaxone and Co-amoxiclav.
Table 1: Susceptibility pattern of major bacterial pathogens of lower respiratory tract infections (LTRIs).

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Streptococcus pneumonia</th>
<th>Haemophilus influenza</th>
<th>Moraxella catarrhalis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=21)</td>
<td>(n=24)</td>
<td>(n=118)</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10[47.6%]</td>
<td>11[52.4%]</td>
<td>24[100%]</td>
</tr>
<tr>
<td>Penicillin</td>
<td>11[52.4%]</td>
<td>10[47.6%]</td>
<td>NT</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>13[61.9%]</td>
<td>8[38.1%]</td>
<td>NT</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>12[57.1%]</td>
<td>9[42.9%]</td>
<td>24[100%]</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>19[90.5%]</td>
<td>2[9.5%]</td>
<td>NT</td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>10[47.6%]</td>
<td>11[52.4%]</td>
<td>24[100%]</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>3[14.3%]</td>
<td>18[85.7%]</td>
<td>15[62.5%]</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>NT</td>
<td>NT</td>
<td>20[83.3%]</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cefixime</td>
<td>NT</td>
<td>NT</td>
<td>24[100%]</td>
</tr>
<tr>
<td>Meropenem</td>
<td>NT</td>
<td>NT</td>
<td>24[100%]</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>18[85.7%]</td>
<td>3[14.3%]</td>
<td>NT</td>
</tr>
<tr>
<td>Linezolid</td>
<td>21[100%]</td>
<td>0[0%]</td>
<td>NT</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>21[100%]</td>
<td>0[0%]</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT= Not Tested.

Table 2: Frequency of major pathogen of community acquired lower respiratory tract infection (LRTI) with respect to age groups, gender and duration of disease.

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Age Groups (Years)</th>
<th>Gender</th>
<th>Duration of Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤40 n=41</td>
<td>41-50 n=33</td>
<td>51-50 n=45</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>4 (9.8%)</td>
<td>4 (12%)</td>
<td>5 (11%)</td>
</tr>
<tr>
<td>Haemophilus influenza</td>
<td>5 (12%)</td>
<td>2 (6%)</td>
<td>8 (17.8%)</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>32 (78%)</td>
<td>27 (81.8%)</td>
<td>32 (71%)</td>
</tr>
</tbody>
</table>

Chi-Square test applied.

Stratification analysis (Table 2) was performed and Moraxella catarrhalis was observed highest (81.8%) among 41-50 age group and in females (76.9%). However, all three bacteria [Streptococcus pneumonia, Haemophilus influenza; Moraxella catarrhalis] showed no significant results among different age groups, gender and duration of disease.
DISCUSSION

Lower respiratory tract infection (LRTIs) is in the midst of the most common infectious diseases globally. Bacterial agents responsible for these LRTIs are different in different areas, so the susceptibility profile will also vary between geographical locations. Current information of the bacteria responsible for LRTIs and their sensitivity profile are therefore essential for the selection of appropriate therapy. This study was conducted to ascertain the antimicrobial resistance pattern among microorganisms isolated from patients with an innovative approach toward developing antibiotic policies in association with the clinicians.

Sputum Gram stains and culture are considered as a simple test for the diagnosis of LRTI. In our study, M. catarrhalis was the most predominant isolate recovered from patients with LRTIs. However, another study conducted in Pakistan reported S. aureus as the most dominant bacteria followed by S. pneumoniae. In another study conducted in Pakistan, the most common cause of LRTI was Streptococcus pneumoniae followed by H. influenza, whereas M. catarrhalis was on sixth position. In last 20 to 30 years; the M. catarrhalis has arisen as a pathogen and is now considered as a principal cause of upper respiratory tract infections in healthy kids and elderly people.

All the isolated of S. pneumoniae in our study displayed erratic sensitivity to linezolid and vancomycin. Similar results were gathered from various studies conducted in other parts of the world. In S. pneumoniae resistance to penicillin is of special concern. Guèye Ndiaye observed 1% S. pneumoniae resistance to penicillin. In the early 1990, a study conducted in Greece, evaluated the antimicrobial susceptibilities of S. pneumonia isolated from patients with CAP. That study showed 14% resistance to penicillin while we observed 47% resistance.

The antibiotic resistance patterns of Streptococcus pneumoniae isolates vary widely from one country to another. Data from the Sofia Maraki project stated ceftriaxone resistance levels in S. pneumoniae 0% in Greece to 30% in south India in 2013. Similarly, Ndiaye et al., in Dakar observed negligible resistance against Co-amoxiclav and ceftriaxone while we observed high antimicrobial resistance to ampicillin (47%), ceftriaxone (42%), and Co-amoxiclav (47%).

Within context to the present study, H. influenzae seems to be surprisingly susceptible to numerous classes of antibiotics, including the β-lactams and β-lactamase inhibitor combinations. The prevalence rate of co-amoxiclav, ceftriaxone, cefixime, and meropenem is 100% sensitive. The same picture is observed to other studies conducted in different parts of the world. Over the past ten to twenty years, M. catarrhalis has progressed to a well-recognized pathogen. Indeed, beta-lactam producing strains appear to be more prevalent, and this may play a vital role in the therapy of infections. British Thoracic Society and American Thoracic Society guidelines suggest empirical antibiotic treatment with amoxicillin or a macrolide for outpatients in case of first use of antibiotic. For in patients not requiring ICU, treatment recommended is a respiratory fluoroquinolone or a beta lactam plus macrolide. For patients requiring ICU, a beta lactam plus either clarithromycin or a respiratory fluoroquinolone is advised. In addition to this, it is important to collect information on the local resistance pattern, which could contribute, to some knowledge for selection of appropriate empirical antibiotic therapy. Although prevalence and antibiotic susceptibility profile of these bacteria vary from area to area, continuous surveillance at indigenous and national levels remains imperative to notice any auxiliary changes in frequency of pathogens and observe any fluctuations in their sensitivity profile. Therefore, this may help physicians and doctors to select appropriate antimicrobial options for the management of LRTIs. Furthermore, it may also curtail the antimicrobial resistance in the community as well as in health care setup.

CONCLUSION

The most prevalent bacterial agents of LRTIs are M. catarrhalis followed by Haemophilus influenzae and...
Streptococcus pneumoniae. Similarly, the isolates collected in our setup M. catarrhalis resistant rate was elevated for levofloxacin, Streptococcus pneumonia for Co-trimoxazole. However, Haemophilus Influenzae showed minimum resistant and remained susceptible to all β-lactams. Thus, vigilant use of antimicrobial agents will reduce the load of multidrug resistance and thereby enabling improved patient management and limiting the resultant morbidity and mortality arising from LRTI’s.

CONFLICT OF INTEREST

The authors would like to acknowledge the Ziauddin laboratory staff and the supervisor for their immense contribution.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ETHICS APPROVAL

Ethics review committee of Ziauddin University Pakistan approved this study (Ref.MIC-2013-201-144).

PATIENT CONSENT

Verbal and written signed consent were taken from patients.

AUTHORS’ CONTRIBUTION

FA analyzed and interpreted the patient data; FIA reviewed the manuscript while NK majorly contributed in the manuscript writing.

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