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Clinico-bacteriological profile of lower respiratory tract infections in a tertiary care hospital in Kolkata – A cross-sectional and observational study

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ABSTRACT

Background: Lower respiratory tract infection (LRTI) is the most common infection causing significant mortality with a 3–5% death rate in adults. The emerging nature of antimicrobial resistance to pathogens makes its treatment more challenging and complicated for physicians. **Aims and Objectives:** The study was designed to obtain a comprehensive insight into the microbial profile and resistance patterns of the isolated pathogens in LRTI in a tertiary care hospital in Kolkata. **Materials and Methods:** The cross-sectional observational study was carried out in the Microbiology Department of N.R.S Medical College from January to December 2021. Specimens collected from 782 clinically suspected LRTI cases were processed as per standard laboratory protocol following clinical and laboratory standard Institute guidelines. **Results:** Out of 782 respiratory samples, 47.83% showed significant bacterial growth. The major risk factor was found to be type II diabetes mellitus (47.44%) followed by chronic obstructive pulmonary disease (36.57%) and smoking (21.35%). 85.56% growth was monomicrobial and Gram-negative organisms (88.50%) predominated. *Klebsiella pneumoniae* (36.90%) was the most predominant Gram-negative bacilli followed by *Pseudomonas aeruginosa* (17.11%) and *Acinetobacter* species (13.90%). *Staphylococcus aureus* was the predominant Gram-positive isolate (7.49%). Gram-negative organisms showed the highest resistance to penicillin and cephalosporins, whereas the lowest resistance in carbapenems. Piperacillin/tazobactam and levofloxacin showed good susceptibility. Linezolid was the most susceptible antimicrobial followed by vancomycin in the case of Gram-positive organisms. Extended-spectrum β-lactamase was detected as the mechanism of resistance in 31.12% of cases and carbapenemase was detected in 19.64% of cases. **Conclusion:** Increasing antimicrobial resistance and varied bacterial etiology make it necessary to develop appropriate antibiotic policy and implement antimicrobial stewardship for effective and prompt therapy.

Key words: Lower respiratory tract infections; Risk factors; Anti-microbial resistance pattern; Respiratory pathogens

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INTRODUCTION

Lower respiratory tract infection (LRTI) encompasses inflammation from the trachea to the bronchus and includes bronchitis, bronchiolitis, bronchiectasis, emphysema, pleural effusion, lung abscess, and pneumonia.[1] It is the most common infection of humans accounting for around 6% of outpatient consultations and 4.4% of admissions in

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hospitals imposing great economic burden.[2] Pneumonia is the most common cause of hospital admission in developing countries, accounting for 3–5% of deaths in adults.[3] LRTI-associated outcome depends on several predisposing factors including age, sex, smoking, chronic obstructive pulmonary disease (COPD), drinking alcohol, appropriate antibiotic therapy, and status of antimicrobial resistance.[4] Pneumonia may be community-acquired and hospital-acquired. In hospital-acquired pneumonia (HAP), symptoms occur after 2 days of hospital infection, provided that the patient was not incubating the disease during admission, whereas in community-acquired pneumonia, the patient does not encounter to hospital or healthcare facilities within 14 days before the onset of symptoms.[5,6] Major etiological agents of LRTIs may vary geographically and over time among different types, epidemiology, clinical features, and outcome, but include both Gram-negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Haemophilus influenza,* and Gram-positive organisms such as *Staphylococcus aureus* and *Streptococcus pneumoniae*.[7] Although viruses are an important pathogen for a large proportion of LRTI, but indiscriminate use of empirical antimicrobial therapy has contributed to emerge antimicrobial resistance thus complicating the treatment and outcome of LRTI more complicated and challenging.[8]

Aims and objectives

The aim of the present study is to evaluate the prevalence of bacterial pathogens causing LRTIs and its antimicrobial resistance pattern in the current scenario along with associated risk factors to endorse and formulate a rational and competent antibiotic policy for appropriate treatment of the disease.

MATERIALS AND METHODS

Study design

An institution-based; prospective, cross-sectional study was conducted after Institutional Ethical Clearance in the Department of Microbiology of Nil Ratan Sircar Medical College and Hospital from January 2021 to December 2021.

Study population

A total of 782 clinically suspected LRTI cases were included in this study from both the outpatient department and inpatient department including intensive care units.

Exclusion criteria

Unwilling patients, patients of <15 years of age, and those who were reactive to HIV, were excluded from this study.

Data collection

A thorough history was taken regarding demographic and clinical parameters.

Specimens' collection and processing

Sputum, endotracheal secretions, tracheal aspirates, and bronchoalveolar lavage (BAL) were collected as respiratory specimens. Sputum samples were collected appropriately in a wide-mouth, sterile universal container following standard sample collection guidelines.[9] Quality of sputum samples were checked using the Murray and Washington grading system. Sputum with <10 epithelial cells and more than 25 polymorphonuclear leukocytes per low power field were graded as good quality sputum and included in this study.[10] For BAL fluid, organisms present in concentrations $>10^3$–10^4 colony forming units (CFU)/mL and specimens demonstrating intracellular presentation in more than 25% of inflammatory cells were selected. Growth of organisms $>10^5$ CFU/mL was selected as the standard for endotracheal aspirate.[11] The isolated found as commensals or contaminants were excluded from the study.

Specimens were processed in the bacteriology laboratory and inoculated into MacConkey agar, Chocolate agar, and 5% sheep blood agar and incubated overnight aerobically as well as in a candle jar for *S. pneumoniae* and *H. influenzae*. Isolated organisms were identified conventionally by biochemical testing as well as by automated method in VITEK2.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed conventionally by modified Kirby–Bauer disc diffusion method as well as by automated method in VITEK2 as per clinical and laboratory standard Institute (CLSI M100) guidelines.[12] The antibiotics tested were amoxicillin (10 μg), amoxicillinclavulanic acid (20/10 μg), amikacin (30 μg), ampicillin (10 μg), cefepime (30 μg), ceftazidime (30 μg), cefuroxime (30 μg), cefotaxime (30 μg), Cefoperazone (75 μg), co-trimoxazole (25 μg), ciprofloxacin (5 μg), cefixime (5 μg), vancomycin (30 μg), linezolid (30 μg), erythromycin (15 μg), azithromycin (15 μg), doxycycline (30 μg), gentamicin (10 μg), imipenem(10 μg), levofloxacin(5 μg), meropenem (10 μg), piperacillin + tazobactam (100 + 10 μg), polymyxin B (300 μg), and cefoperazone + sulbactam. ATCC *E. coli* 25922, ATCC *P. aeruginosa* 27853, and ATCC *S. aureus* 25923 were used as controls.

Detection of antimicrobial resistance

All Gram-negative pathogens resistant to third-generation cephalosporins were screened for extended-spectrum β-lactamases (ESBLs) phenotypically by conventional or automated method as per CLSI guidelines.[12] Carbapenemase production was detected from carbapenem-resistant Gram-negative isolates phenotypically by modified carbapenem inactivation methods (mCIM with/without eCIM) as per CLSI guidelines.[12]

All culture media, reagents, and chemicals were obtained from HI MEDIA Private Limited, Mumbai, Maharashtra India.

Statistical analysis

Microsoft Excel and Microsoft Word (version 10) were used to generate the tables and figures. All statistical analysis was done using the Chi-square test. The software used for the statistical analysis was GraphPad Prism 7.

RESULTS AND ANALYSIS

Out of 782 respiratory samples collected within the period of 1 year, significant bacterial growth was noted in 374 (47.83%) cases. The patient's profile showed male preponderance (565/72.25%) than female (217/27.75%) with a male: female ratio of 2.6:1 (Figure 1).

The most affected age group was 46–60 years of age (38.24%) followed by >60 years of age (29.54%) (Table 1).

The majority of suspected LRTI cases were suffering from single or multiple comorbidities or risk factors. The major risk factor was found to be Type II diabetes mellitus (371/47.44%) followed by COPD (286/36.57%), and smoking (167/21.35%) (Table 2).

The majority of patients (452/57.80%) were admitted to the hospital and from the Chest department (189/41.81%) (Figure 2).

The **m**ajority of the respiratory samples collected were sputum (514/65.73%) followed by BAL fluid (156/19.95%) (Figure 3).

Among the positive cultures, 320/85.56% growth was monomicrobial whereas, 54/14.44% growth was polymicrobial (Table 3).

Growth of Gram-negative organisms was noted in 331/88.50% cases, out of which 197/52.67% were from Enterobacteriales and 134/35.83% were non-fermenters. Among the Gram-negative isolates, *K. pneumoniae* (138/36.90%) was the most predominant species followed by *P. aeruginosa* (64/17.11%) and *Acinetobacter* species (52/13.90%). Among Gram-positive isolates, *S. aureus* (28/7.49%) was the major pathogen isolated (Table 4).

Gram-negative organisms showed the highest resistance to penicillin and cephalosporins, whereas the lowest resistance was noted in carbapenems. Piperacillin/tazobactam and levofloxacin showed good susceptibility (Figure 4a).

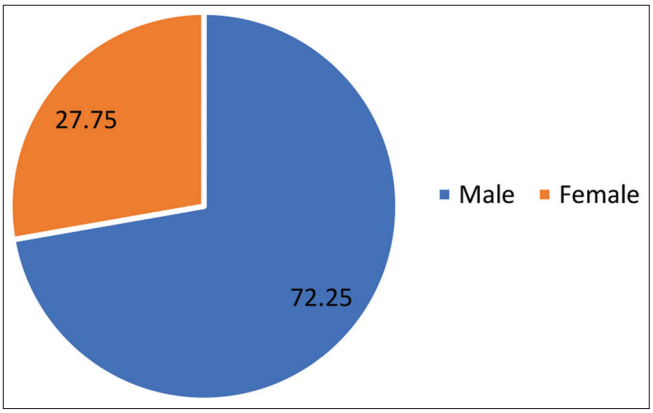

Figure 1: Sex-wise distribution of cases (n=782)

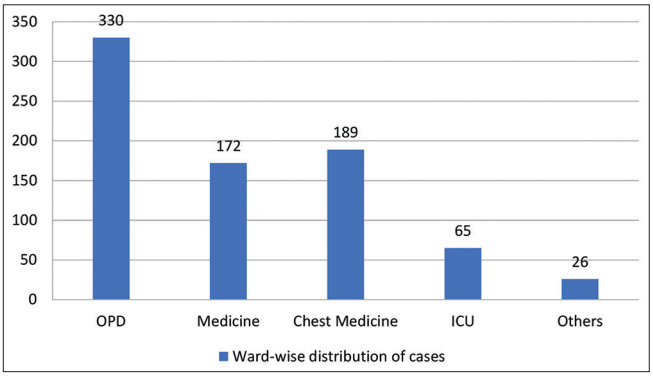

Figure 2: Ward-wise distribution of cases (n=782)

Table 1: Age-wise distribution of cases (n=782)

Age	Number	Percentage
15–30	84	10.74
31–45	168	21.48
46–60	299	38.24
>60	231	29.54
	782	100

Table 2: Various comorbidities or habit-wise distribution of cases (n=782)

Comorbidities/habit	Number	Percentage
Smoking	167	21.36
Alcohol	122	15.60
COPD	286	36.57
Bronchial asthma	145	18.54
T2DM	371	47.44

COPD: Chronic obstructive pulmonary disease, T2DM: Type II diabetes mellitus

Linezolid was the most susceptible antimicrobial followed by vancomycin in the case of Gram-positive organisms (Figure 4b).

ESBL was detected as the mechanism of resistance in 31.12% of cases and carbapenemase was detected in 19.64% of cases (Figure 5).

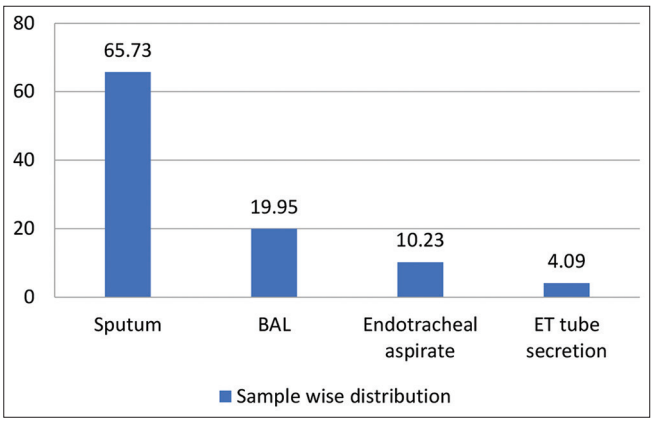

Figure 3: Sample-wise distribution of cases (n=782)

Table 3: Growth-wise distribution of cases (n=782)

	Number	Percentage
Bacterial growth	374	47.83
Single growth	320	85.56
Mixed growth	54	14.44
No growth/No pathogenic growth	408	52.17

Table 4: Organism-wise distribution of culture-positive cases (n=374)

Organisms isolated	Number	Percentage
Klebsiella pneumoniae	138	36.90
Pseudomonas aeruginosa	64	17.11
Pseudomonas species	15	4.01
Acinetobacter	52	13.90
Escherichia coli	38	10.16
Citrobacter species	13	3.48
Proteus mirabilis	5	1.34
Burkholderia cepacia	3	0.80
Serratia marcescens	3	0.80
Total Gram-negative	331	88.50
Staphylococcus aureus	28	7.49
Pneumococcus	6	1.60
Group A *Streptococcus*	9	2.41
Total Gram-positive	43	11.50
Total	374	100

DISCUSSION

In our study, significant bacterial growth was detected in 47.83% of cases, out of which 85.56% was monomicrobial. Similar isolation rates were also noticed by Mishra et al.,[13] (44%) and Khan et al.,[14] (49.3%). Ramana et al.,[15] and Regha and Sulekha[16] showed little lower isolation rates (39.4% and 26.34%, respectively). A higher prevalence rate was noted in Panda et al.[17] (83%) 86.67% monomicrobial growth was detected in Singh et al.[18] Similar findings were also described by Saxena et al.,[19] and Narayanagowda et al.[20] Higher isolation rate mainly depends upon appropriate specimen collection, rapid transport to the laboratory and proper processing. Similar to the present study, male predominance was detected in Singh et al.,[18] Panda et al.,[17] Saha et al.,[21] Akingbade et al.,[22] Shah et al.,[23] and Ahmed et al.[24] Adults and elderly male preponderance were seen mostly due to exposure to different population and associated various risk factors.[18] Age-related physiological and immunological alterations may also be responsible for such findings.[24] In the present study, 38.24% of cases belong to 46–60 years of age and 29.54% belong to >60 years of age. Similar findings were also noted in Singh et al.,[18] Shah et al.,[23] and Ahmed et al.[24] Most of the cases were admitted in our study (57.80%) and the maximum contribution was from the Chest Medicine Department (41.81%) and the Internal Medicine Department (38.05%) followed by ICUs (14.38%). Similar type of findings was noted in the study of Singh et al.,[18] and Ahmed et al.[24] This is probably due to the fact that people with LRTIs usually present to medicinal wings first.[24] Similar to the present study, diabetes mellitus, COPD, and smoking were found to be major comorbidities in the study of Saha et al.,[21] and Gaikwad et al.[25] Growth of Gram-negative organisms were noted in maximum cases (88.50%), out of which *K. pneumoniae* was the most common (36.90%) followed by *Pseudomonas* (21.12%) and *Acinetobacter* spp. (13.90%). Predominant growth of Gram-negative organisms was also seen in the study of Wajid et al.,[26] Gebre et al.,[5] Bajpai et al.,[27] and Singh et al.,[18] Regha and Sulekha,[16] 2018 (Kerala) showed *K. pneumoniae* (31.1%) as the predominant isolates followed by *Pseudomonas* spp. (30.2%), which was in concordance with the present study. A similar finding was also noted in Panda et al.,[17] Verma et al.,[28] Ratna,[29] Ritchie and Wedzicha.[30] *S. aureus* was found to be the predominant Gram-positive isolates in the present study (7.49%). Similar findings were also found in the study of Regha and Sulekha[16] (4.5%), Saha et al.,[21] (3%), and Gaikwad et al.[25] (4%).

In this study, monomicrobial growth was noted in 85.56% of cases, whereas 14.44% of cases showed polymicrobial or mixed growth. About 86.67% monomicrobial growth was detected in Singh et al.,[18] along with 13.33% mixed growth. A similar finding was also noted in the study of Saxena et al.,[19] 52.17% of cases showed poor growth or growth of commensal organisms. This may be due to inappropriate sample collection or prior antibiotic therapy. Singh et al.,[18] also found more than 50% growth of non-pathogenic commensal organisms. Only a 13.91% growth of commensal was noted in the study of Bajpai et al.[27]

In the present study, Gram-negative organisms showed higher resistance to penicillin and cephalosporins. *K. pneumoniae* showed 81.16% resistance to amoxicillin–clavulanic acid and 76.58% resistance to cefuroxime. 100% resistance was noted in the case of ampicillin, whereas *E. coli* showed 94.35% resistance. Regha and Sulekha[16] also found higher resistance to cephalosporin (70.7%), whereas Gaikwad et al.[25] showed 100% resistance to cefuroxime and

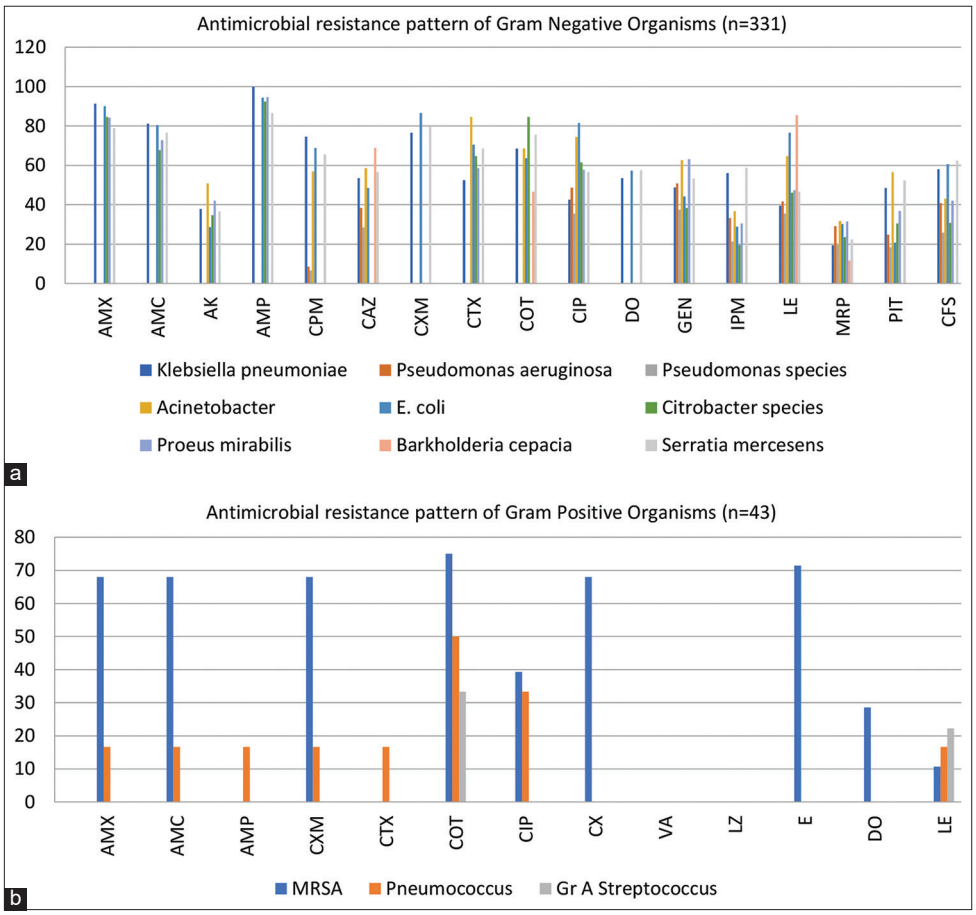

Figure 4: (a) Antimicrobial resistance pattern of Gram-negative organisms isolated (n=331). (AMX: Amoxicillin, AMC: Amoxicillin–clavulanic acid, AK: Amikacin, AMP: Ampicillin, CPM: Cefepime, CAZ: Ceftazidime, CXM: Cefuroxime, CTX: Cefotaxime, COT: Cotrimoxazole, CIP: Ciprofloxacin, DO: Doxycycline, GEN: Gentamycin, IPM: Imipenem, LE: Levofloxacin, MRP: Meropenem, PIT: Piperacillin-Tazobactam, CFS: Cefoperazone-sulbactam), (b) Antimicrobial resistance pattern of Gram-positive organisms isolated (n=43). (AMX: Amoxicillin, AMC: Amoxicillin–Clavulanic acid, AMP: Ampicillin, CXM: Cefuroxime, CTX: Cefotaxime, COT: Cotrimoxazole, CIP: Ciprofloxacin, CX: Cefoxitin, VA: Vancomycin, LZ: Linezolid, E: Erythromycin, DO: Doxycycline, LE: Levofloxacin)

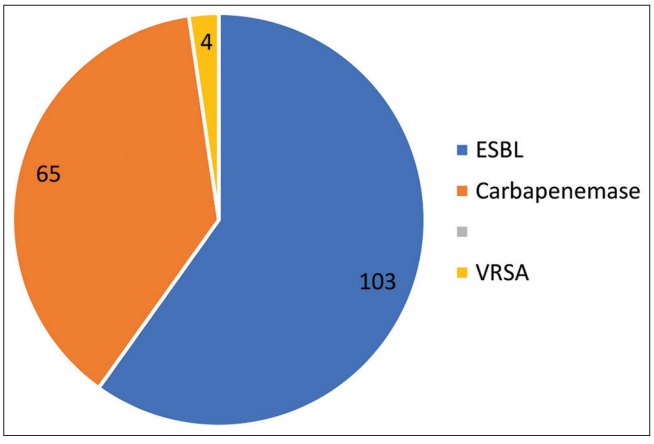

Figure 5: Resistance mechanism of the isolated organisms

97.4% resistance to ampicillin. Ceftazidime was found to be relatively better than cephalosporin in the present study, where *Pseudomonas* showed 38.46% resistance and *Klebsiella* showed 53.56% resistance. This finding was concordant with the study of Regha and Sulekha[16] (36.8% for *Pseudomonas*) and Gaikwad et al.,[25] (42.4% for *Pseudomonas*). Higher resistance to ceftazidime was noted in the study of Ahmed et al.,[24] (84.1% for *Klebsiella* and 57.1% for *Pseudomonas*). In the present study, Cefepime was found to be a better alternative for the treatment of LRTI with *Pseudomonas*, for which it showed lesser resistance (8.6%). Although Gaikwad et al.,[25] and Regha and Sulekha[16] showed 42.4% and 25.3% resistance, respectively. It showed 74.53% resistance for *Klebsiella*, 56.96% for *A. baumanii,* and 68.85% for *E. coli*. These findings are concordant with the study of Gaikwad et al.,[25] and Regha and Sulekha.[16]

The aminoglycoside resistance was found more in gentamycin than in amikacin. *Klebsiella* and *E. coli* showed 48.86% and 44.21% resistance to gentamycin, respectively, whereas for Amikacin resistance rate was 37.86% and 28.63%, respectively. Similar findings were also noted in the study of Regha and Sulekha[16] (Gen/AK 42.2/30%) and Bajpai et al.,[27] (Gen/AK 39.55/28.95%). *Acinetobacter* showed relatively higher resistance to aminoglycosides

(Gen/AK 62.7/50.9%). About 61.9% resistance to amikacin by *A. baumanii* was also reported by Ahmed et al.[24]

For beta-lactam and beta-lactam inhibitor combination drugs, although amoxicillin-clavulanic acid showed higher resistance (81.16% for *Klebsiella*), it was pretty lesser in the case of Piperacillin-Tazobactam (48.56% for *Klebsiella*, 20.7% for *E. coli*). This finding was similar to the study of Regha and Sulekha[16] (34.4% for *Klebsiella*, 27.3% for *E. coli*). Gaikwad et al.[25] showed higher resistance (87.1% for *Klebsiella*, 60% for *E. coli*), whereas Ahmed et al.[24] showed lower resistance to piperacillin–tazobactam (18.2% for *Klebsiella*, 3.6% for *E. coli*). *P. aeruginosa* showed 24.8% resistance in this study and *A. baumanii* showed 56.55%. Regha and Sulekha[16] showed 21.83% resistance for *P. aeruginosa* and 48.8% resistance for *A. baumanii*.

Among quinolones, Levofloxacin was found to be less resistant than Ciprofloxacin. Levofloxacin showed 39.56% resistance for *Klebsiella* and 76.58% resistance for *E. coli*. In the case of ciprofloxacin, the resistance rate for *Klebsiella* was 42.56% and for *E. coli* was 81.62%. Similar findings were noted in Ahmed et al.,[24] (CIP/LE 59.1/52.3 for *Klebsiella* and 81.8/68 for *E. coli*) and Regha and Sulekha[16] (to Ciprofloxacin 51.1% for *Klebsiella* and 54.5% for *E. coli*). Sarkar et al.,[31] showed little lesser resistance (CIP/LE 38.7/20.2 for *Klebsiella* and 71.4/42.9 for *E. coli*) whereas Gaikwad et al.,[25] showed higher resistance to quinolones (CIP/LE 92.9/89.3 for *Klebsiella* and 80/62.5 for *E. coli*). For non-fermenter, the resistance rate to ciprofloxacin was 48.69% for *Pseudomonas* and 74.5% for *Acinetobacter*, whereas it was 41.67% for *Pseudomonas* and 64.7% for *Acinetobacter*. It was almost similar to the study of Gaikwad et al.,[25] (CIP/LE 45.5/41.2 for *Pseudomonas* and 80/62.5 for *Acinetobacter*).

Carbapenems showed the highest effectivity against Gram-negative isolates. Meropenem showed 19.52% resistance for *Klebsiella*, 29.16% for *P. aeruginosa*, 31.78% for *Acinetobacter,* and 23.56% for *E. coli*. Nidhi Goel et al.,[32] showed 22.8% meropenem resistance for *P. aeruginosa* and 25.6% for *Acinetobacter* spp.

Among Gram-positive isolates, Linezolid was found to be the most susceptible antibiotic followed by Vancomycin. Linezolid was also found to be the most effective antimicrobial in the study of Singh et al.,[18] Bajpai et al.,[27] Gaikwad et al.,[25] and Regha and Sulekha.[16] In the present study Methicillin resistance was detected in 68% of cases. A similar finding was also noted in the study of Singh et al.,[18] (56.9%), Bajpai et al.,[27] (55.55%), Gaikwad et al.,[25] (83.3%) and Regha and Sulekha[16] (15.4%).

In the present study, ESBL was detected in 31.12% of cases, whereas carbapenemase was detected in 19.64% of cases. Bajpai et al.,[27] showed ESBL production in 36.62% of cases. 31.81% carbapenemase production was shown in the study of Majumdar et al.[33]

Limitations of the study

The present study could not differentiate between community and HAP among the cases. It could not isolate the pathogen for atypical pneumonia due to the use of routine culture media.

CONCLUSION

It was revealed in the study that Gram-negative pathogens have major contributions to the development of LRTIs, out of which *K. pneumoniae* and *P. aeruginosa* were found to be the major pathogen followed by *E. coli* and *A. baumanii*. Meropenem was found to be the most sensitive antibiotic. Piperacillin tazobactam, levofloxacin, and amikacin were found better alternatives for treatment. The alarming rise of resistance to antibiotics makes early laboratory diagnosis and judicious use of antibiotics necessary for prompt and effective treatment of LRTI.[18]

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SB- Definition of intellectual content, literature survey, coordination, and manuscript revision; **SJC-** Concept, design, and review manuscript**; BG-** Literature survey, data analysis and interpretation, and manuscript revision; **SS-** Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data analysis, manuscript preparation, and submission of article; **AR-** Concept, design, coordination, and manuscript revision; **AD-** Data collection, literature survey, and manuscript revision; **PJ-** Data collection, literature survey, and manuscript revision.

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