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Original Research Article

Bacteriological Profile and Antibiotic Susceptibility Patterns in Lower Respiratory Tract Infection at a Tertiary Care Teaching Hospital

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ABSTRACT

Aim: To study the bacterial profile, along with antibiotic susceptibility pattern of isolates from suspected cases of LRTIs attending the tertiary care study area.

Materials and Methods: Respiratory samples like sputum, BAL & ET secretions from patients clinically suspected with LRTIs received from both in patient and OPD of a tertiary care teaching hospital, were processed as per standard protocol in Department of Microbiology. The bacterial isolates were identified using Gram's stain, motility and sets of biochemical tests. Antimicrobial susceptibility testing was performed and interpreted as per CLSI guidelines.

Results: This was a retrospective study based on the evaluation of data from January 2023 to June 2023. A total of 1943 respiratory samples (1709 sputum, 211 ET secretions and 23 BAL) were received during the study period. 669 sputum samples (39.14%), 102 ET secretion (48.34%) and 2 BAL fluid (8.69%) were positive for bacterial isolates. Out of these 773 culture positive samples, 855 microorganisms were isolated. *Klebsiella spp*. was the most common isolates being 39.5% (338 isolates) followed by *E. coli* 23.97% (205 isolates), *Pseudomonas spp*. 16.9% (145 isolates), *Acinetobacter spp*. 14.61% (125 isolates), *S. aureus* 3.04% (26 isolates) and *Enterococcus spp*. 1.87% (16 isolates).

Conclusion: Culture and susceptibility test is vital for proper diagnosis and management of patients with LRTIs. 39.78% culture positivity was observed in all the samples received with predominance of Gram-negative isolates.

Keywords: ICU, Klebsiella spp., LRTI, MDR isolates, Sputum

INTRODUCTION

One of the worst infectious diseases impacting people globally, lower respiratory tract infections (LRTI) significantly increase morbidity and mortality across all age groups.[1,2] They are considered as 3rd leading cause of death around the world, after ischemic heart and cerebrovascular diseases.[2,3] As its presentation is nonspecific, they are often misdiagnosed, mistreated, and underestimated. The problem is more grievous in developing world.[1-4] The spectrum of LRTI depends on the geographical area, its climatic condition, socioeconomic conditions, age group affected, associated risk factors as well as the antibiotics prescription pattern in community.[2,3,5]

LRTI is not a single disease but a broad category of specific infections, each with a different epidemiology, pathogenesis, clinical presentation and outcome.[3,5] These are frequently the first infection to occur after birth in neonates and often the final illness to occur before death in elderly. The underlying etiopathogenesis in Lower respiratory tract Infection can vary from bacteria, virus, fungus or protozoa. Sputum is generally the sample of choice for the diagnosis of lower respiratory tract infections, as it is easy to collect and non-invasive method. Though role of the sputum culture has been debatable and is limited by the fact that quality of the sample may not be up to the mark and may have to be repeated. The Gram stain remains a

major diagnostic method as it gives presumptive identification of suspected pathogen.[6,7]

With emerging drug resistance of the organisms to the commonly used antibiotics, management of these infections has become a challenge to the physicians.[8] The patterns of microorganisms causing infection and their antibiotic resistance pattern may vary from one country to other country and from hospital to hospital.[6] As per the primary guidelines developed by the British Thoracic Society (BTS) and the Infectious Disease Society of America (IDSA) for the diagnosis of LRTIs, it is crucial to identify the infectious organisms for appropriate management of cases.[9,10] Hence this study was done with the aim of studying the bacterial profile with antibiotic susceptibility pattern in cases of LRTI for better patient management.

MATERIALS AND METHODS

Study area
The study was carried out in the Department of Microbiology, of a tertiary care teaching hospital of Central Gujarat with almost 1100 beds from January 2023 to June 2023.

Sample size
A total of 1943 respiratory samples, which included 1709 sputum, 211 ET secretions & 23 BAL from patients clinically suspected with LRTIs received from in patients and OPD of a tertiary care hospital, were processed by conventional manual techniques as per the standard procedures in Department of Microbiology.[11]

Inclusion criteria
All the samples like sputum, BAL, ET secretions received in the department with adequate volume and proper labeling of patient demographic details were included in the study. The Sputum samples with Bartlett' score of 1 and more in Gram's stain were included in this study.

Sample Processing and isolate identification
Sample processing was done as per standard microbiological techniques including gross and microscopic examination of clinical samples. Gram's stain and KOH mount were performed for all the specimens received in the laboratory. For KOH mount 10% KOH was used and presence of fungal elements was evaluated. All the samples received in the laboratory were cultured on Mac Conkey agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India.), Blood agar and Chocolate agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India. with added blood at required temperature) and incubated at 37°C for 24 hours. Blood agar and Chocolate agar plates were incubated in CO2 incubator for isolation of fastidious organisms.

Identification of isolate was done using by manual techniques like Gram stain of colony, motility by hanging drop method and various biochemical tests like Catalase test, Oxidase test, IMViC, TSI, Urease, PPA and sugar fermentation test.[11] Antimicrobial susceptibility testing was done on Muller Hinton Agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India.). Antibiotics like Ampicillin (10µg), Ceftriaxone (30µg), Cefepime (30µg), Piperacillin-tazobactam (100+10µg), Doxycycline (30µg), Meropenem (10µg), Amikacin (30µg), Gentamycin (10µg), and Levofloxacin (5µg) were tested for Gram negative isolates. For *Pseudomonas spp.,* Ceftazidime (30µg), Imipenem (10µg), Cefepime (30µg), Piperacillin-tazobactam (100+10µg), Doxycycline (30µg), Amikacin (30µg), Gentamycin (10µg), and Levofloxacin (5µg) were tested. ESBL production was detected by ceftazidime and ceftazidime clavulanic acid discs. Zone size was interpreted as per CLSI guidelines.[12] For Gram positive isolates, Penicillin (10U), Cefoxitin (30µg), Amoxicillin clavulanic acid (30µg), Erythromycin (15µg), Clindamycin (2µg), Linezolid (30µg), Gentamycin (10µg) and Vancomycin E-strip were kept for susceptibility testing. MRSA was detected by Cefoxitin disc diffusion method. Antibiotic discs were procured from Hi-media Pvt. Ltd. Results were interpreted as Susceptible, Resistant and Intermediate according to the CLSI guidelines.[12]

Patients' demographic data, place of admission & the laboratory findings were entered in Microsoft excel for frequency distribution analysis. Only clinical samples received by the laboratory for routine analysis were included in the study with no direct patient involvement. All the data collected were from patients' requisition forms received along with the samples and LIS software of the hospital. Because of the descriptive nature of the study with no patient involvement, institutional ethics committee was not approached. Chi square test was applied for categorical data and statistical significance was studied using Statulator, an online calculator for analysis and interpretation of result.[13]

RESULTS

During the study period from, a total of 1943 respiratory samples were received from patients attending the OPDs or admitted in various clinical wards of the Hospital having suspected lower respiratory tract infection. Out of this, 1709 were sputum samples, 211 were ET secretions and 23 were BAL samples. Of these samples processed, 773 samples (39.7%) were found culture positive. Of the 773 samples positive, 550 (71.1%) were from male patients and 223 (28.9%) from female patients. M:F ratio was found as 2.5:1 in the study. When statistical analysis for sex of patients was calculated using Statulator, p-value of < 0.001 was found which is statistically significant in present study. In age group wise distribution, it was found that maximum, i.e. 37.5% patients belonged to 46-60 years, 23.9% in 60-75 years, 3% in 31-45 years,18.2% in 16-30 years, 5.8% in above 75 years and 4% in less than 15 years of age group.

Location wise distribution of culture positive samples is shown in Figure-1.

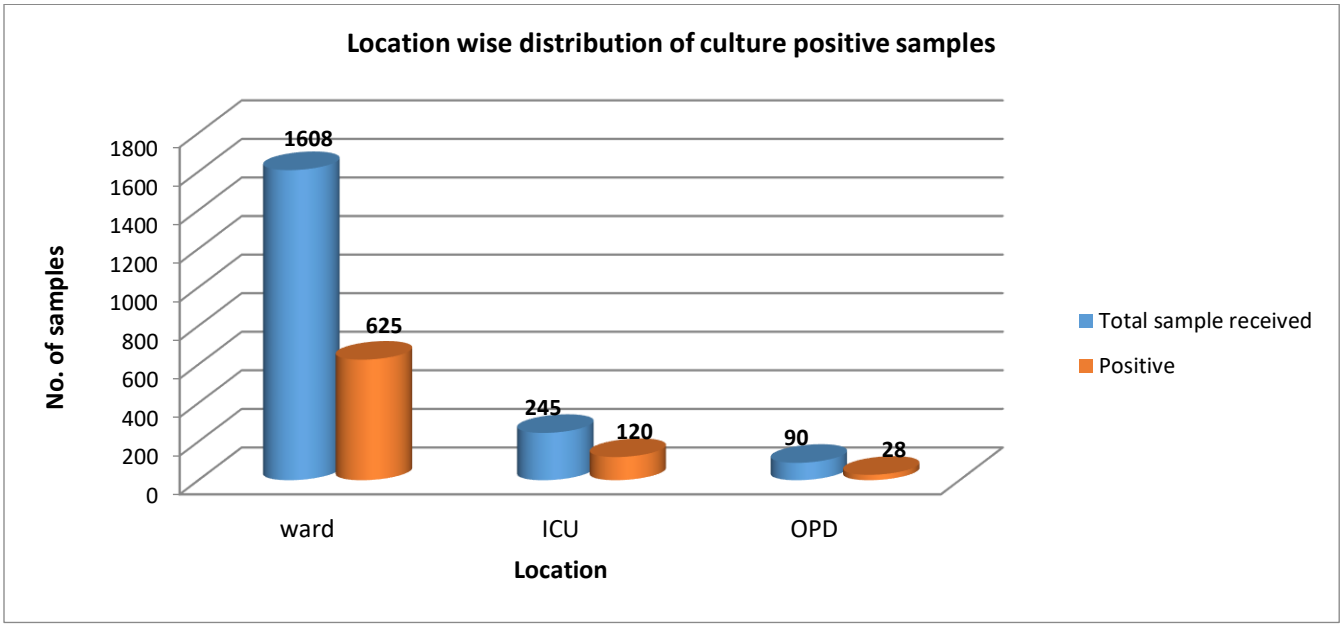

Figure-1: Location wise distribution of culture positive samples

Highest culture positivity of 48.9% was found in ICUs samples followed by 38.8% from wards and 31.1% from OPD samples. When culture positivity in LRTIs was compared in ICUs and ward using an online analysis tool, Statulator, p value of < 0.001 was found, which is statistically significant. Only bacterial pathogens were studied in the present study and their distribution in different respiratory samples is shown below in Figure-2.

Klebsiella spp. was the commonest isolate in all clinical samples being 39.5% (338 isolates) followed by *E. coli* 23.97% (205 isolates), *Pseudomonas spp.* 16.9% (145 isolates), *Acinetobacter spp.* 14.61% (125 isolates), *S. aureus* 3.04% (26 isolates) and *Enterococcus spp.* 1.87% (16 isolates) as shown in the Figure-3.

Antimicrobial susceptibility was recorded for all the isolates and interpreted as per current CLSI guidelines.[12] *Klebsiella spp.* was the most common isolate from sputum samples followed by *E. coli, Pseudomonas spp., Acinetobacter spp.* and Gram-positive isolates. 56% *Klebsiella spp.* were susceptible to Meropenem followed by 45% to Amikacin and only 31% to Piperacillin+Tazobactam. High resistance was observed to Fluroquinolones and Cephalosporins. Carbapenems resistance was reported in 34% isolates. 4% resistance to Colistin was observed in *Klebsiella spp.* isolated from sputum sample. *Acinetobacter spp.* was found to be highly resistant with only 45% isolates being susceptible to Amikacin. Figure-4 shows antibiotic susceptibility pattern of Gram-negative isolates in sputum samples. Amongst Gram positive isolates, 100% resistance to Penicillin was reported in *S. aureus* isolates. 33% isolates were MRSA. Vancomycin and Linezolid were 100% susceptible.

Klebsiella spp. was most common isolates in ET secretion followed by *Acinetobacter spp.* and *Pseudomonas spp.* Antibiogram of ET secretion isolates revealed high resistance in *Acinetobacter spp.,* almost 80% resistance to carbapenems. Meropenem and Amikacin was susceptible in 46% and 38% isolates of Klebsiella spp. Figure-5 shows antimicrobial susceptibility pattern of ET secretion isolates.

DISCUSSION

The aim of the present study was to determine the bacterial pathogens and their antibiotic susceptibility pattern in LRTIs patients in the study area. In the present study, 39.7% culture positivity was observed, while that was 43.3 % in Singh S *et al*[1] and 48% in Kumar M *et al*.[4] In other studies from Ethiopia, Gebre Ab et al[2] has reported positivity of 35% and Nurahmed N *et al*[3] has 32%. A study from Nepal by Khan S *et al*[5] has reported 49.3% culture positivity. Difference in culture positivity may be because of different sample size, geographical area, clinical presentation, use of antibiotics by health professionals at different levels of care before patients reach a teaching hospital and self-prescribing practices. LRTIs were common in male patients as compared to female patients

(71.1% v/s 28.9%) in present study which is comparable with the other Indian studies like, Singh S *et al*[1], Kumar M *et al*[4] and Dhivya G *et al*.[7] In other studies, from different part of the globe by Hassanzadeh S *et al*[14], Nguyen-Van T *et al*[15], Miriti DM *et al*[16] and Santella B *et al*[17] have also reported male preponderance in LRTIs. The prevalence of lower respiratory tract infections (LRTIs) in men may be related to risk factors for the infection, including alcoholism, smoking, more outdoor exposure and COPD.[1,17]

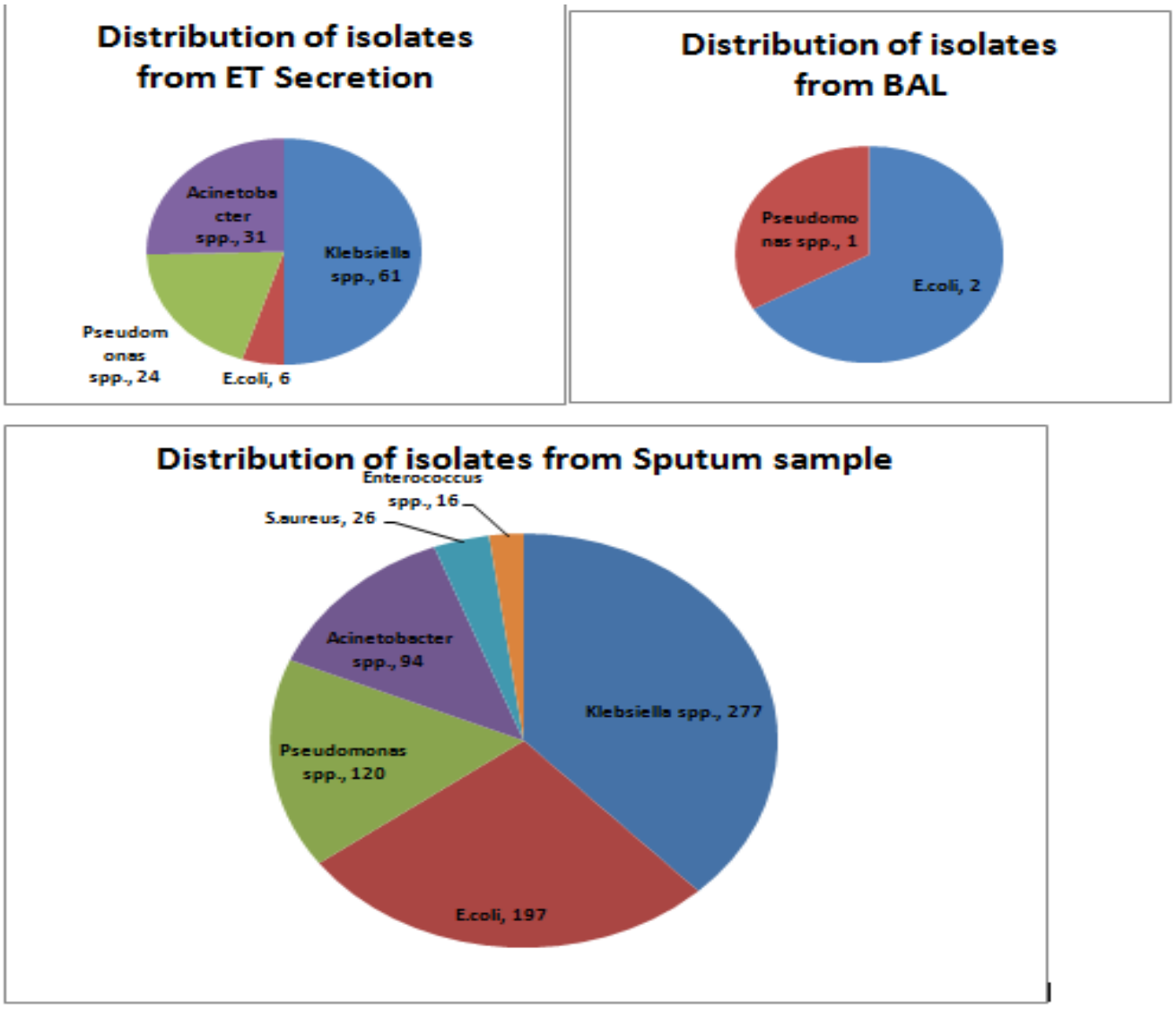

Figure-2: Organism wise distribution of clinical isolates in different samples

When the study population was analyzed for age group distribution, it was found that maximum patients, i.e. 37.5% of age group belonged to 46-60 years, 23.9% to 60-75 years, 21.3% to 31-45 years, 18.2% to 16-30 years, 5.8% to above 75 years and 4% to less than 15 years of age group. Indian study by Singh S *et al*[1] has shown maximum culture positivity of 35% in > 61 years of age followed by 30 % in 41-60 years. Miriti DM *et al*[16] has reported 30.3% culture positivity in 25-34 years of age group followed by 20% in 35-44 years of age group in Kenya population. Yulia R *et al*[18] has reported 54% patients with > 55 years of age followed by 44.5% in 26-55 years of age group in their Indonesian study. In present study, out of the culture positive samples, 48.9% were from ICUs followed by 38.8% from ward and 31.1% from OPD samples. Singh S *et al*[1] has reported highest positivity in ward samples (51.7%) followed by ICU (28.3%) and OPD (20%) samples.

Kumar M *et al*[4] has also reported 60% of culture positive samples from inpatient department, but not specified ICU and ward separately. Dhivya G *et al*[7] has reported 88.8% positivity in inpatient department.

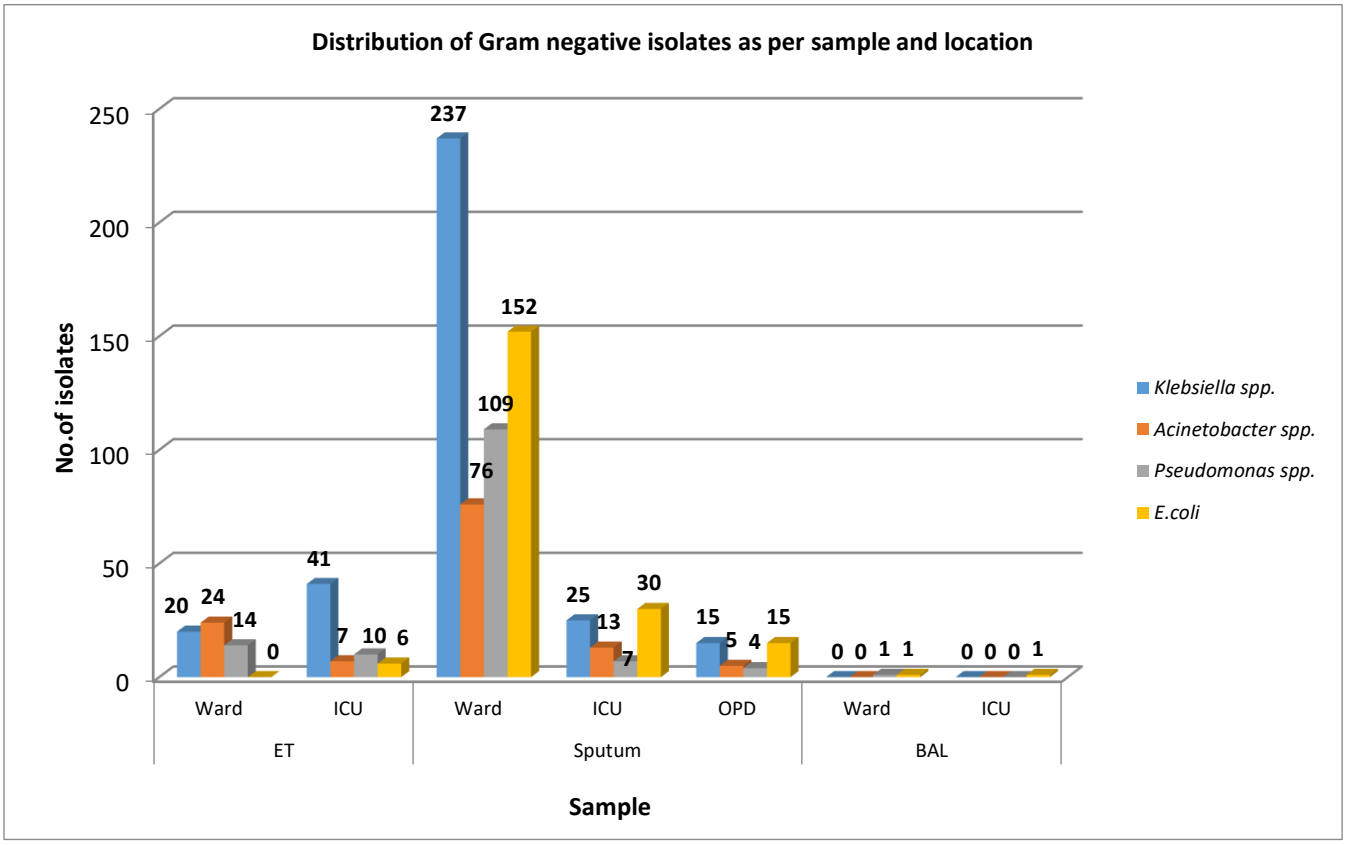

Figure-3: Distribution of Gram-negative isolates as per sample and location

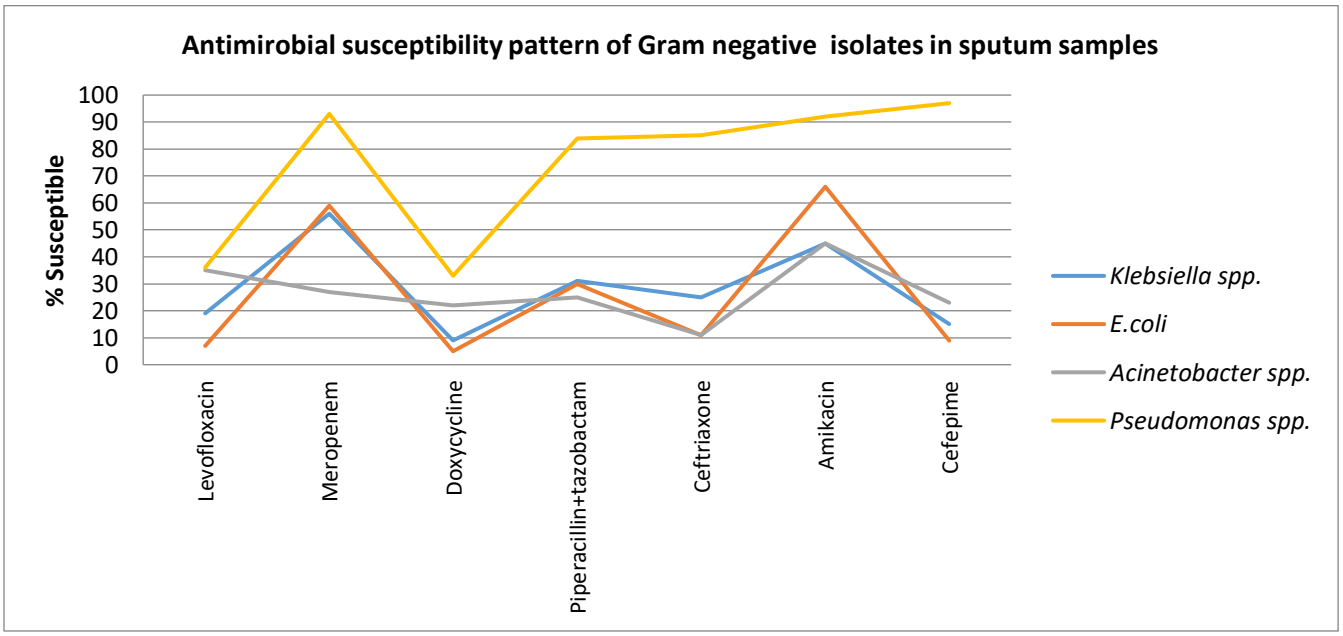

Figure-4: Antibiotic susceptibility pattern of Gram-negative isolates in sputum samples

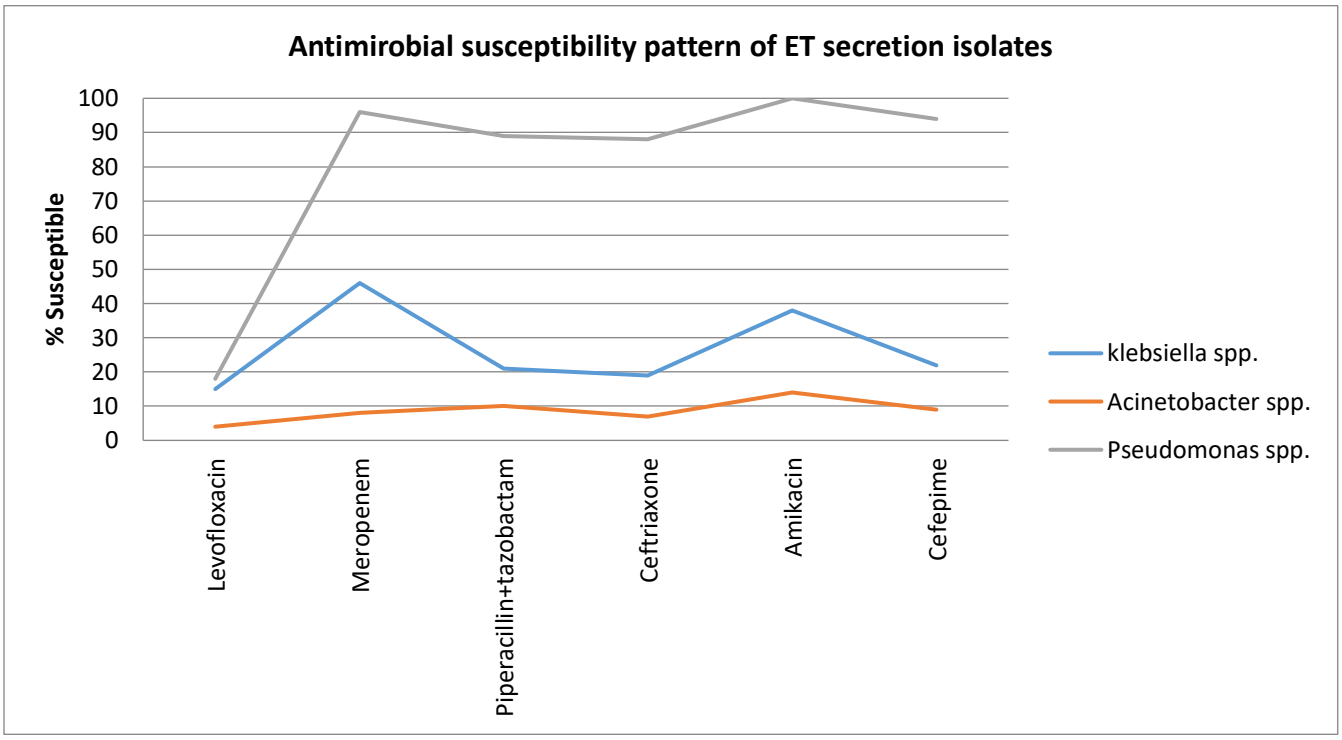

Figure-5: Antimicrobial susceptibility pattern of ET secretion isolates

Klebsiella spp. was most common isolate overall followed by *E. coli, Pseudomonas spp.* and *Acinetobacter spp*. in present study. A study from Rajasthan by Singh S *et al*[1] has reported *Pseudomonas spp.* as most common isolate followed by *Klebsiella spp.* and *Acinetobacter spp*, while study in Puducherry by Dhivya G *et al*[7] has shown *Pseudomonas spp.* as most common isolate followed by *Klebsiella spp.* and *E. coli.* A study by Debnath S *et al*[19] from Tripura has also shown *Klebsiella spp.* (52.1%) as the leading cause of LRTIs followed by *Acinetobacter spp.* (1.34%) and *Pseudomonas spp.* (13.2%). Ethiopian studies by Gebre AB *et al*[2] and Nurahmed N *et al*[3] have also reported *Klebsiella spp.* as the predominant isolates from LRTIs samples. Kenyan study by Miriti DM *et al*[16] and Italian study by Santella B *et al*[17] also have reported Gram negative organisms as a predominant cause of LRTIs in their study area. While Hassanzadeh S *et al*[14] in Iran and Nguyen-Van T *et al*[15] in Vietnam have reported *S. pneumoniae,* as the predominant cause of LRTI. This comparison justifies that though Gram-negative organisms are more common cause of LRTIs, causative organism may very geographically.

In present study, over all GNB isolates were 65% susceptible to Amikacin, 60% to Meropenem and 45% to Piperacillin+tazobactam. Levofloxacin was susceptible in 25% isolates only. In a study by Raghubanshi BR *et al*[9], 71% susceptibility to Amikacin and 65% to Meropenem has been reported. While Gupta E *et al*[10] has shown Imipenem susceptibility in 92% and Piperacillin – tazobactam in 89% and Amikacin 84% *Klebsiella spp.* isolates. In present study, Vancomycin and Linezolid susceptibility was 100% which is similar with other studies like Raghubanshi BR *et al*[9] and Gupta E *et al*.[10] Gebre AB *et al*[2] has reported 10% MRSA, while that in present study is 33%. Singh S *et al*[1] has reported 56.9% MRSA in their study area. Antibiotic susceptibility pattern varies in different organisms and different study area, thus making it mandatory to observe it keenly through. Regular monitoring of resistant isolates would be important for infection control in critical units for better patient management.

The present study has not evaluated viral and fungal etiology, risk factors and patient outcome in terms of total hospital stay, antibiotics total usage, mortality etc. in LRTIs.

CONCLUSION

This study reveals that a variety of pathogens are responsible for lower respiratory tract infections and increased antibiotics resistance has become a great public health issue. Gram negative organisms, *Klebsiella spp.* is the most common pathogens isolated from LRTIs and showed increased resistance to routinely used antibiotics. Higher level antibiotics have started showing resistance and treatment failure in many cases. Males are predominantly affected. Inpatient department has higher culture positivity rate in present study. Amikacin, Meropenem in Gram

negative and Vancomycin, Linezolid in Gram positive are effective antibiotics in study area. The present study recommends periodic analysis of types of respiratory pathogens and regular revision of their antibiotic susceptibility pattern to monitor the trend in local area and treatment policy formulations.

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