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Etiologies, risk factors, and antibiotic pattern of lower respiratory tract infection in patients coming to Deccan College of Medical Sciences, Hyderabad, South India

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ABSTRACT

Background. Lower Respiratory Tract Infections (LRTI) impose a huge problem on society due to their tenacious and unescapable health problems, and they were the reasons for consultation and hospitalization. Patients with LRTI were present with a wide spectrum of diseases that range from life-threatening infections to minor self-limiting illnesses.

Materials and methods. Sputum samples from 310 patients with complaints of lower respiratory tract infections were collected and analyzed. After microscopic analysis, 30 were excluded from the study. Of the 280 that were included in the study, 171 were males and 109 were females. The age group between 56 and 65 years comprised the highest number of patients in the study. Different antibiotics were used to check antibiotic sensitivity patterns against isolated Gram-positive and negative bacteria.

Results. Of the 280 samples processed, 122 (43.5%) were culture-positive. Among the 122 bacterial isolates, the predominant organism was *Klebsiella pneumoniae* 55 (45%). The most common predisposing factor identified in 115 (37%) patients was smoking. Using the Double Disk Synergy Test, 11 (20%) out of a total of 55 *K. pneumoniae* isolates and 4 (30.7%) out of 13 *Pseudomonas* isolates showed Extended Spectrum Beta Lactamases (ESBL). The *Klebsiella* and *Pseudomonas* isolates showed resistance to 2nd and 3rd generation Cephalosporins.

Conclusion. Because of different geographical regions and conditions, the etiology and the antibiotic sensitivity patterns of LRTI vary, so the etiology, predisposing factors, and the antibiotic sensitivity pattern of LRTI need to be updated regularly. The diagnostic facilities for early and rapid identification of LRTI also need to be improved.

Keywords: AST, etiology, LRTI, Hyderabad, South India, ESBL

INTRODUCTION

Lower Respiratory Tract Infections are an obstinate and universal health problem that poses a massive problem to the world and society and are also considered a significant reason for clinical consultation and hospitalization.

Symptoms like cough, expectoration, dyspnoea, wheezing, and chest pain/ discomfort, usually for a period ranging from 1-3 weeks are all common in Lower Respiratory Tract Infection (LRTI). Bronchitis, bronchiolitis, influenza, community-acquired pneumonia (CAP) either with or without radiological evidence, acute exacerbations of chronic obstructive pulmonary disease (COPD), and acute exacerbations of bronchiectasis [1] are all acute manifestations of Lower Respiratory Tract Infection that may or may not involve lungs.

Community-acquired pneumonia (CAP) or pneumonia is defined as radiological evidence of new or increasing pulmonary infiltrates plus one or more of the following fever, hypothermia, cough, with or without sputum production, tachypnoea, dyspnoea, hemoptysis, wheezing, physical findings such as rales and hypoxemia [2]. Etiological agents for LRTIs can

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be a bacterium, an intracellular bacterial pathogen, virus, fungi, or parasite [2,3].

The causative agent of LRTIs depends on various factors, like the place of study, age, and other factors like hospitalization [4]. The other risk factors constituted are Smoking, COPD, and structural Lung disease, Diabetes Mellitus, Altered consciousness, Chronic alcoholism.

Microbiological investigation is required to identify the causative agent and for the management and treatment of lower respiratory tract infections (LRTI) as they cannot be identified clinically [5]. Therefore, microbiologists in the clinical labs have a key part to play in the early diagnosis and management of LRTI in the clinical setting [6,7].

Due to various biosafety reasons sputum culture to diagnose LRTI cannot be performed by many routine/small labs, this leads to empirical and presumptive antimicrobial therapy in LRTI cases. The unaccountable or irrational use of antibiotics is the main cause of the increased prevalence of drug resistance in LRTI bacteria as well as in other bacteria [8].

The main objective of this study is to note the prevalence of various bacterial pathogens in patients with LRTI in the metropolitan city of Hyderabad, using expectorated sputum samples and assessing the antimicrobial susceptibility pattern of the isolated bacterial pathogen. No viral cultures were performed due to a lack of facilities. Detection of the presence of Methicillin-Resistant Staphylococcus aureus (MRSA) and Extended Spectrum Beta Lactamase (ESBL) production was also done among the isolates.

MATERIAL AND METHODS

The study was conducted from January 2022 to November 2023 (22 Months) at the Department of Microbiology of Deccan College of Medical Sciences, Hyderabad, India.

Subjects and inclusion criteria: Samples were taken from patients 15 years to 65 years of age, from the Inpatient (IP) (non-intensive Care Unit) and Outpatient (OP) departments.

Ethical approval: The research was ethically cleared by the ethical board of Deccan College of Medical Sciences, Hyderabad. Written informed consent was taken from each participant after explaining the purpose and procedure of the study. The patients' results in this study were kept confidential.

Inclusion criteria: Patients having at least two of the following symptoms, like Fever above 37°C, Cough, production of purulent sputum, Breathing difficulty, in association with physical findings suggestive of consolidation, 2 Chest pain and Leucocytosis (W.B.C > 10000/cumm).

Exclusion criteria: 1. Pulmonary tuberculosis Patients 2. Patients with congestive heart failure 3. AIDS and those patients receiving Immunosuppressive Therapy, 4. Patients on antibiotic therapy.

A total of 310 sputum samples from patients with complaints suggestive of lower respiratory tract infections were analyzed, and after microscopy, 30 were excluded from the study as 17 were positive for Acid Fast Bacilli and 13 were positive for Candida species. Of these 280 subjects which were included in the study, 171 were males and 109 were females.

Sample collection: After giving instructions to the patient regarding sputum collection, 310 sputum samples were collected early morning into a sterile wide-mouthed container and transported to the laboratory according to standard protocol [9].

Macroscopy: Quality, color, and consistency (watery, mucoid, purulent, blood-tinged) of the sputum were noted. The most purulent of the sputum was subjected to further processing.

Microscopy: In order to separate Gram-positive from Gram-negative bacteria and to detect the predominant morphotypes patient sputum samples were collected and analyzed [10]. To identify sputum samples contaminated with saliva, microscopic analysis was done. Ziehl Neelsen-Stained sputum smears were examined to exclude sputum samples positive for AFB and samples showing Candida on microscopy were not subjected to culture [11].

Specimen culture and biochemical identification: Microscopically satisfactory samples were cultured on Blood, MacConkey, and Chocolate agar media [10,12,13]. After an incubation period of 24 hrs the plates were observed for size, shape, growth, elevation, odor, pigmentation, hemolysis, and swarming of the colonies. Using earlier studies as a reference, staining and biochemical tests were done for the bacterial species differentiation [13,14].

Antibiotic susceptibility testing methods: The antimicrobial susceptibility of Bacterial isolates was performed using the Kirby-Bauer Disk Diffusion Method on MHA plates and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020) [15]. Antibiotic discs and Mueller-Hinton agar media were procured from Himedia laboratories, Mumbai.

Antimicrobial discs used for gram positive isolates: Oxacillin (1 µg), Cefotaxime (30 µg), Amoxyclav (30 µg), Vancomycin (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Co-trimoxazole (25 µg), Clindamycin (2 µg), Doxycycline (30 µg), Linezolid (30 µg), Erythromycin (15 µg), Gentamycin (10 µg), Cefoxitin (30 µg).97 (12)

Gram negative disks: Imipenem (10 µg), Piperacillin-Tazobactam (100/10 µg), Amoxyclav (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Cotrimoxazole

(25 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Gentamicin (10 µg) [15].

Preparation of inoculum: 3-5 isolated colonies of similar morphology of the test organisms were sub-cultured into a test tube containing 4 ml of sterile peptone water/nutrient broth and were incubated for 2-4 hours at 37°C to produce a bacterial suspension of moderate cloudiness. To standardize the inoculum density for susceptibility tests, a barium sulphate turbidity standard equivalent to 0.5 Mc Farland's standard was used [15].

Detecting B-Lactam Resistance to Oxacillin 1µg discs among *S. pneumoniae* isolates (CLSI-2020)

Methodology: Disc Diffusion test was performed to predict the sensitivity of Beta-Lactam drugs. A direct colony suspension equivalent to a 0.5 McFarland standard was used as a standard. Colonies that were prepared from an overnight incubation (18-20 hours) on Sheep blood agar plates were inoculated on Mueller Hinton Agar with 1 µg Oxacillin discs and incubated at 35 ± 2° C using 5% CO_2.

Interpretation: Isolates of Pneumococci with Oxacillin 1 µg zone size of >20 mm are susceptible to penicillin.

Control: *Streptococcus pneumoniae* ATCC 49619 [15].

Detection of E.S.B.L producing organism

Gram-negative isolates showing resistance to 2nd and 3rd Generation Cephalosporins (Ceftriaxone >25, Ceftazidime- >17, cefotaxime >27) were selected for ESBL confirmatory test as per CLSI guidelines 2020. The ESBL phenotypic confirmatory test was done by Double Disc Synergy Test [15].

Detection of Methicillin Resistance among *S.aureus* isolates using Cefoxitin disc 30 mcg

Methodology: The test organism was subcultured in nutrient broth for 2-4 hours at 37° C. The turbidity was matched with 0.5 Mc Farland. The test strain was swabbed using a sterile cotton swab dipped into the inoculum over Mueller Hinton Agar and a Cefoxitin disc of 30 µg was placed on the plate. Interpretation- *S. aureus* was considered as Methicillin sensitive if the zone diameter was >22 mm = mec A Negative. The Methicillin-resistant strains, i.e., mec A positive if the zone diameter was < 21 mm, as per CLSI Guidelines 2020.

Control: *S. aureus* ATCC 43300 – mec A Positive (Zone <21 mm) [15].

RESULTS

A total of 310 sputum samples from patients with complaints suggestive of lower respiratory tract infections were analyzed, and after microscopy, 30 were excluded from the study as 17 were positive for Acid Fast Bacilli and 13 were positive for Candida species. Of the 280 that were included in the study, 171 were males and 109 were females. In the current study majority of the subjects were in the age group of 56-65, which was followed by 46-55 years. The age group of 15-25 years comprised the least number of subjects. The gender ratio was approximately 3:2. i.e. male-to-female respectively. Age and sex distribution are shown in Table 1.

TABLE 1. Age and gender wise distribution of patients with LRTI under study

Sl. No	Age Group (Years)	Male	Female	Total	Percentage
1.	15-25	21	11	32	11
2.	26-35	22	14	36	13
3.	36-45	23	18	41	15
4.	46-55	50	26	76	27
5.	56-65	55	40	95	34
TOTAL		**171**	**109**	**280**	**100**

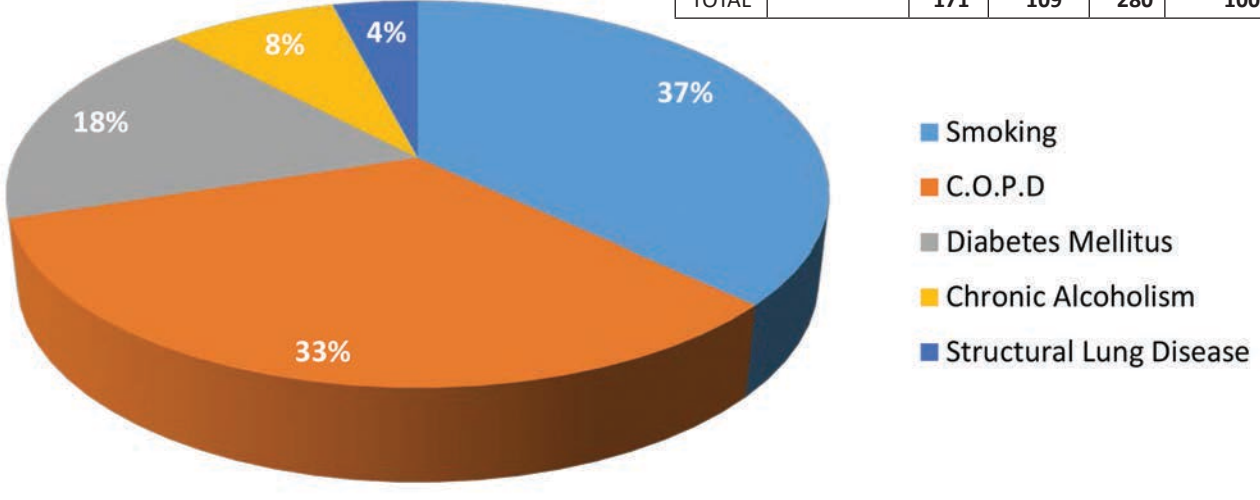

FIGURE 1. Distribution of various risk factors

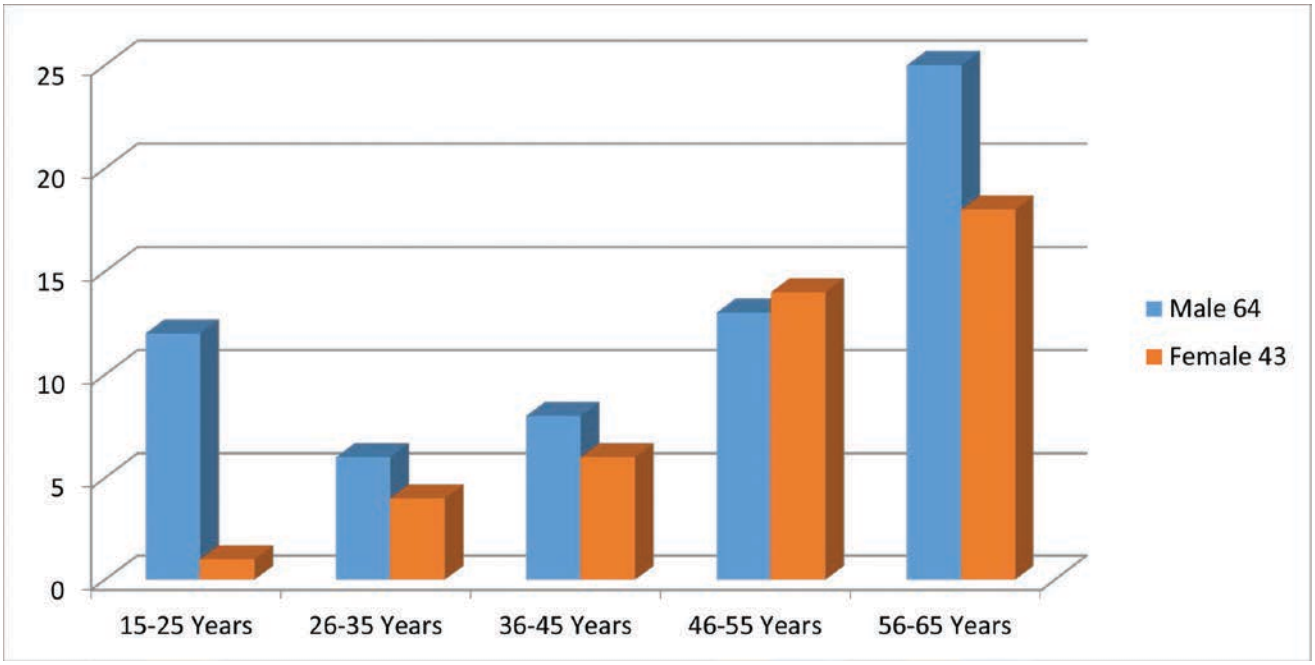

FIGURE 2. Age wise distribution of OP/IP patients enrolled in the study OP (107)

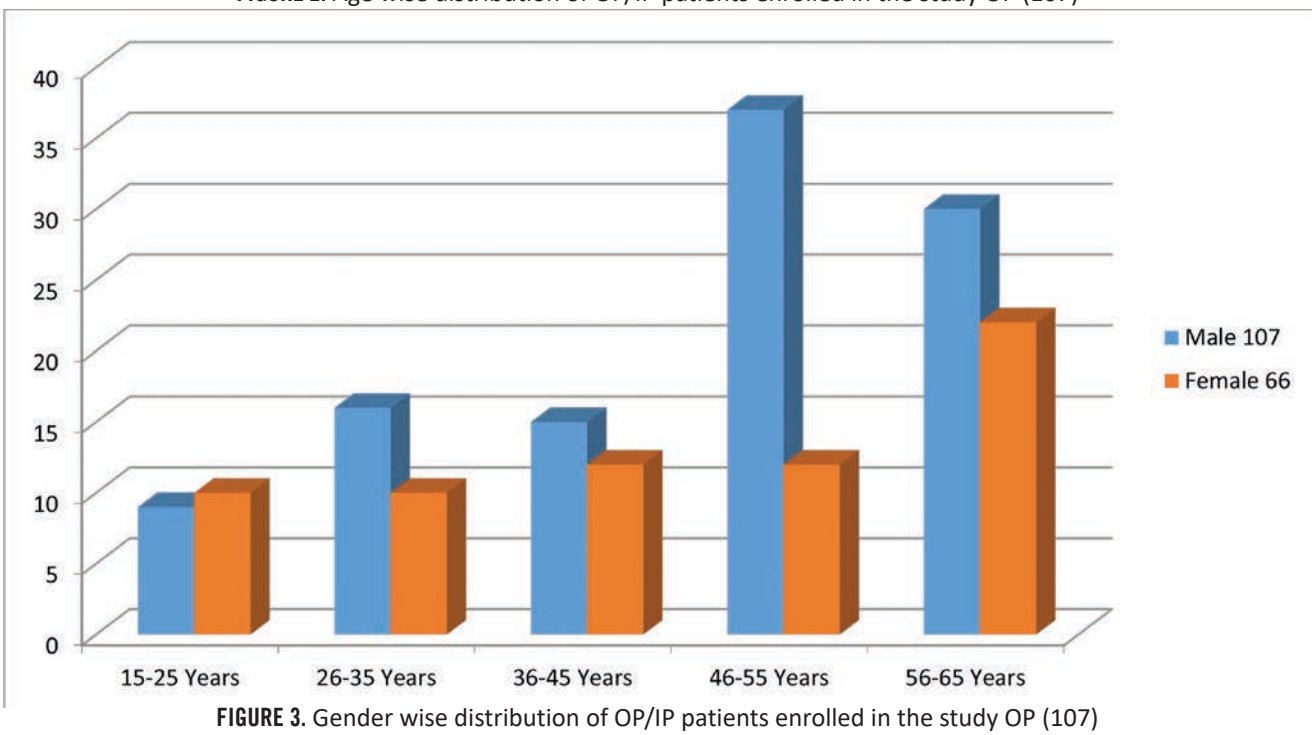

FIGURE 3. Gender wise distribution of OP/IP patients enrolled in the study OP (107)

In the current study, smoking was identified as the most common risk factor in 115 (37%) patients, followed by Chronic Obstructive Pulmonary Disease (C.O.P.D) in 102 (33%), Diabetes Mellitus (D.M) in 56 (18%), Chronic alcoholism in 25 (8%), Structural lung disease in 12 (4%) (Figure 1).

Of the 280 samples processed, 122 (43.5%) were culture positive. Among the 122 bacterial isolates, the predominant organism was *Klebsiella pneumoniae* 55 (45%) followed by *Streptococcus pneumoniae* 31 (25.4%), *Staphylococcus aureus* 16 (13.1%), *Pseudomonas aeruginosa* 13 (10.6%), *Acinetobacter baumanni* 5 (4%), *Moraxella catarrhalis* 2 (1.6%) The data is shown in Table 2 and in Figures 4-10.

TABLE 2. Distribution of various bacterial isolates obtained from LRTI patients in the present study

Sl. No	Bacterial isolates	OP (107)	IP (173)	Total
1.	Gram Positive Isolates (n=47)			
	a. *S. pneumoniae*	10	21	31 (25.4%)
	b. *S. aureus*	4	12	16 (13.1%)
2.	Gram Negative Isolates (n=75)			
	a. *K. pneumoniae*	22	33	55 (45%)
	b. *P. aeruginosa*	-	13	13 (10.6%)
	c. *A. baumanni*	-	5	5 (4%)
	d. *M. catarrhalis*	1	1	2 (1.6%)
TOTAL		**37 (34.6%)**	**85 (49.1%)**	**122**

FIGURE 4. Golden yellow colonies of *S. aureus* on Nutrient agar

FIGURE 5. Colonies of *S. aureus* on Blood agar

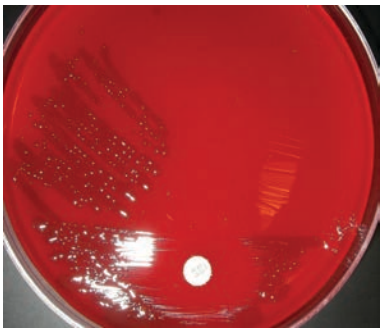

FIGURE 6. *S. pneumoniae* optochin sensitivity

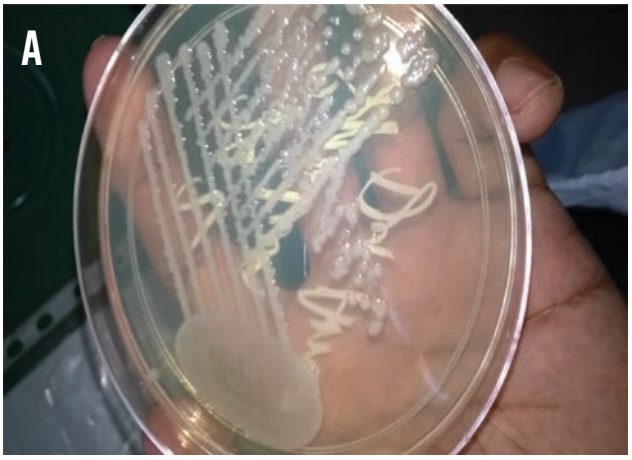

FIGURE 7 A-B. Mucoid colonies of *Klebsiella pneumoniae* on Nutrient Agar and McConkey Agar

FIGURE 8 A-B. Greenish pigment production on Nutrient Agar and Non-Lactose Fermenting colonies on McConkey agar of *Pseudomonas aeruginosa*

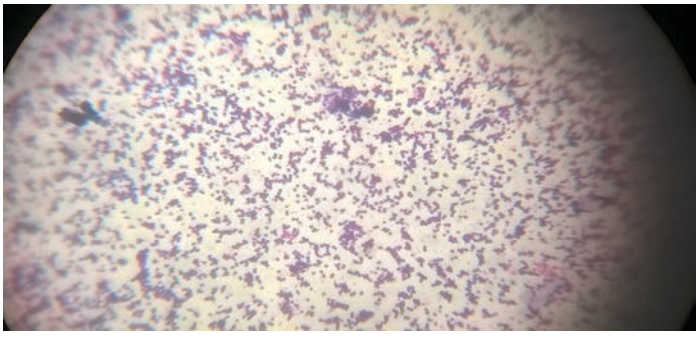

FIGURE 9. Gram stain showing Gram Positive Cocci in Clusters- *Staphylococcus aureus*

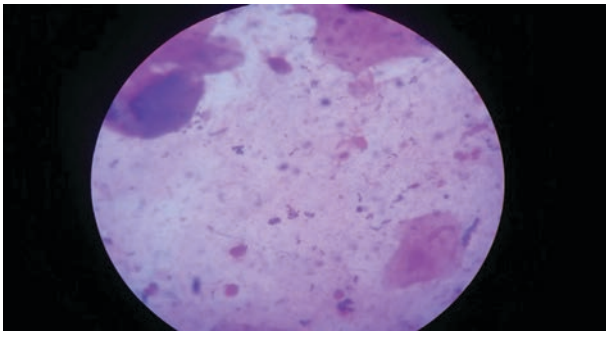

FIGURE 10. Gram Stain showing Gram Positive Diplococci *Streptococcus pneumoniae*

TABLE 3. Incidence of bacterial isolates with age distribution

Sl. No	Bacterial isolates	15-25 Yrs (n=32)	26-35 Yrs (n=36)	36-45 Yrs (n=41)	46-55 Yrs (n=76)	56-65 Yrs (n=95)	TOTAL
1.	*K.pneumoniae*	7 (22%)	6 (17%)	5 (12%)	17 (22%)	20 (21%)	55
2.	*S.pneumoniae*	3 (9.3%)	4 (11%)	5 (12%)	6 (8%)	13 (14%)	31
3.	*S.aureus*	2 (6.2%)	4 (11%)	-	3 (4%)	7 (7.3%)	16
4.	*P. aeruginosa*	2 (6.2%)	-	2 (5%)	3 (4%)	6 (6.3%)	13
5.	*A. baumanni*	-	-	-	2 (3%)	3 (3%)	5
6.	*M. catarrhalis*	-	-	1 (2.4%)	-	1 (1%)	2

TABLE 4. Cultures positive for polymicrobial growth

Sl. No	Mixture of organisms	Male	Female	Total
1.	*K. pneumoniae* + CONS	7	3	10
2.	*S. aureus* + non-albicans Candida	2	4	6
3.	*S. pneumoniae+ Candida albicans*	1	1	2
4.	*K. pneumoniae + Candida albicans*	2	2	4
Total		**12**	**10**	**22**

TABLE 5. Antibiotic sensitivity profile of the total gram-positive isolates

Sl. No	Antibiotic Discs	*S. pneumoniae* (n=31)	*S. aureus* (n=16)
1.	Amikacin (30 µg)	22 (70%)	12 (75%)
2.	Amoxyclav (30 µg)	23 (74%)	10 (63%)
3.	Cefotaxime (30 µg)	28 (90%)	7 (44%)
4.	Ciprofloxacin (5 µg)	23 (75%)	9 (56%)
5.	Cotrimoxazole (25 µg)	25 (80%)	11 (68%)
6.	Clindamycin (2 µg)	22 (70%)	10 (63%)
7.	Doxycycline (30 µg)	24 (79%)	9 (56%)
8.	Erythromycin (15 µg)	25 (80%)	11 (69%)
9.	Gentamycin (10 µg)	20 (65%)	9 (56%)
10.	Linezolid (30 µg)	31 (100%)	16 (100%)
11.	Oxacillin(1µg)[a]	26 (84%)	-
12.	Vancomycin (30 µg)	31 (100%)	13 (81%)
13.	Cefoxitin (30 µg)[b]	-	16 (100%)

[a]Detection of Penicillin Resistance among Pneumococcal isolates-5 (16%) isolates showed a zone size <20 mm – indicating resistance to the Penicillin group of drugs. Thus β-Lactam producing.
[b]None of the 16 S. aureus isolates showed resistance to cefoxitin 30 µg, indicating there were no MRSA strains isolated.
- Not Applicable

Gram Negative Bacterial Isolates showing resistance to 2nd and 3rd Generation Cephalosporins were subjected to ESBL phenotypic confirmation by Double Disk Synergy Test. 11 (20%) out of a total 55 *K. pneumoniae* isolates and 4 (30.7%) out of 13 *Pseudomonas* isolates were positive for ESBL production. The overall prevalence of ESBL production was 22% (Table 7, Figure 14).

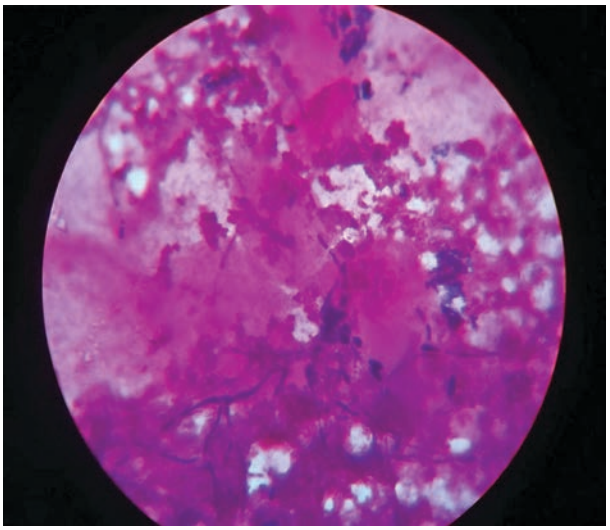

FIGURE 11. Direct Gram Stain showing *Candida* species with *Pseudohyphae*

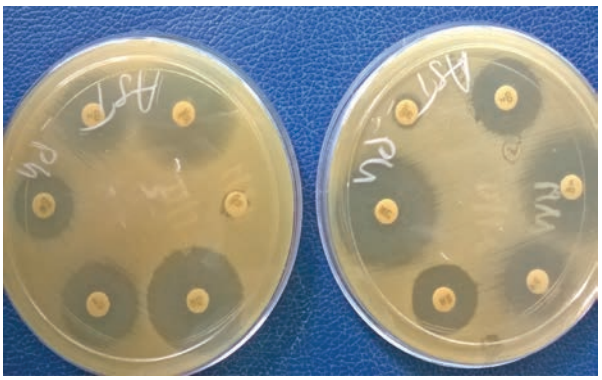

FIGURE 12. Mueller Hinton agar supplemented with 2-4% NaCl showing Antibiotic susceptibility testing and showing sensitivity towards Cefoxitin 30 µg streaked with *S. aureus*

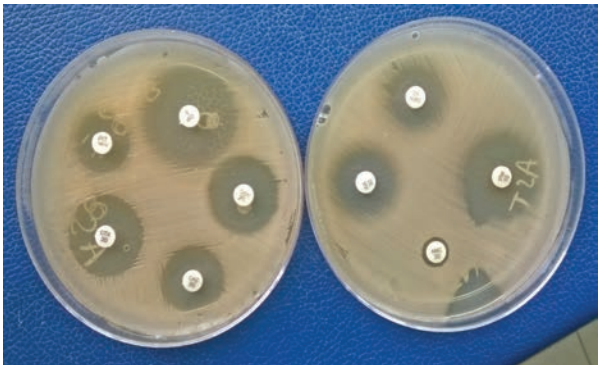

FIGURE 13. Antibiotic susceptibility testing for Gram-Negative Bacilli

TABLE 6. Antibiotic sensitivity profile of the total gram-negative isolates

No	Isolates	Antibiotic Discs								
		Amikacin 30 µg	Amoxyclav 30 µg	Cefotaxime 30 µg	Ceftazidime 30 µg	Cotrimoxazole 30 µg	Ciprofloxacin 5 µg	Gentamicin 10 µg	Imipenem 10 µg	Piperacillin Tazobactam 100/10 µg
1.	*K.pneumoniae* (n=55)	41 75%	25 45%	26 47%	20 36%	20 36%	31 56%	35 64%	55 100%	54 98%
2.	*P. aeruginosa* (n=13)	11 85%	3 23%	5 38%	4 31%	10 77%	7 54%	8 62%	11 92%	12 94%
3.	*A. baumannii* (n=5)	3 60%	3 60%	1 20%	1 20%	2 40%	1 20%	2 40%	4 80%	4 80%
4.	*M. catarrhalis* (n=2)	1 50%	1 50%	1 50%	1 50%	0 0%	2 100%	1 50%	2 100%	2 100%

TABLE 7. Table showing potential ESBL producers

Sl. No	Isolates	Total No: of Isolates	No: of isolates tested for ESBL production	No: of Positives for ESBL	Percentage
1.	*K. pneumoniae*	55	26	11	20.0
2.	*P. aeruginosa*	13	7	4	30.7

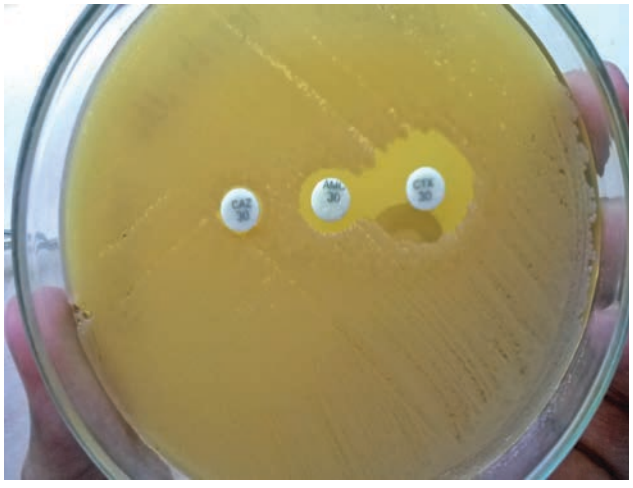

FIGURE 14. Showing E.S.B.L Confirmatory test by Double Disk Synergy Test

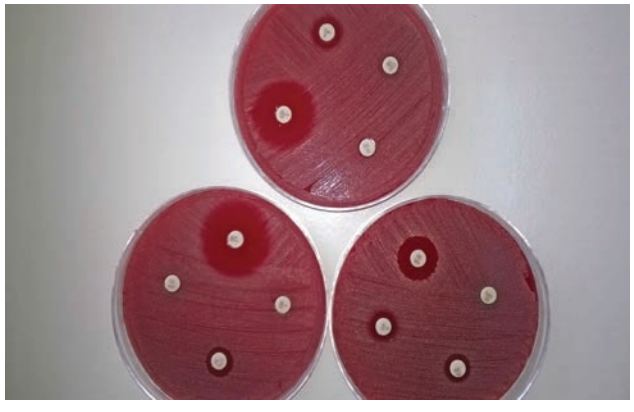

FIGURE 15. Antibiotic susceptibility using Sheep Blood Agar for S. pneumoniae and showing Penicillin resistance using Oxacillin 1µg disk

DISCUSSION

This study was conducted to determine the bacterial etiology of patients with LRTI and their sensitivity profile, as LRTI is one of the leading causes of morbidity and mortality in the world [16].

Clinically, etiologic agents of LRTI cannot be determined, as these agents vary from area to area, and so do their antibiotic susceptibility profiles. Some of the earlier studies done on LRTI reported some Gram-positive and negative bacteria like *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas spp., Acinetobacter spp.,* and *Klebsiella spp* respectively [17].

In the present study, 43.5% (122) of the bacterial isolates were recovered from 280 sputum samples that were included in the study. A similar percentage of occurrence of pathogens in sputum samples was reported by Mishra SK et al in 2012 [18].

In our study, S. pneumoniae was isolated from 68% of IP patients and 32% of OP patients as the commonest Gram-positive bacterial isolate. *S. pneumoniae* has been identified as a common Gram-positive bacterial isolate causing community-acquired pneumonia all over the world [19]. In the present study, 13 (14%) of the 31 Streptococcus isolates were obtained from patients with the age range 56-65 years requiring hospitalization. Our study finding is similar to one earlier study where the causative agent of community-acquired pneumonia was S. pneumoniae with an occurrence of 23.40% in the age group 51-60. 2013 [20] (Figures 4-10).

The isolated Pneumococcal cultures in our study were found to be susceptible to most of the antimicrobials tested. The 100% sensitivity was observed with Linezolid and Vancomycin. A similar level of sensitivity to Linezolid and Vancomycin was reported in earlier studies [21].

In our study, *Streptococcus pneumoniae* isolates were screened for Penicillin resistance using Oxacillin 1 µg disc as per CLSI 2020 Guidelines [15], and only 5 (16%) of 31 pneumococcal isolates showed resistance to Penicillin (Oxacillin 1 µg) indicating the low occurrence of multidrug resistance phenotype. Multidrug-resistant *S. pneumoniae* is defined as resistant to Penicillin and two or more non-β-Lactam agents such as Macrolides, Cotrimoxazole, or Tetracycline which are reported from many parts of the globe [22] (Figure 15).

Although Cefotaxime (90%) and Erythromycin (80%) are widely used drugs for the treatment of acute respiratory infections in our population, they have escaped resistance in the pneumococcal isolates obtained. Hence, they can still be used for empirical treatment.

In the present study, *Staphylococcus aureus* 16 (13.1%) was the third common bacterial isolate which is similar to another study carried out by R.K. Ramamurthy in Bangalore Medical College in 2013. [23]. Community-acquired *S. aureus* inhalation pneumonia is commonly a secondary complication of influenza and parainfluenza virus infection. *S. aureus* pneumonia can also be acquired by the hematogenous transmission of the organism from various niduses, including skin infection, septic phlebitis, and endocarditis vegetations. Staphylococci can progress rapidly to cavitation with 10% of patients with pneumonia developing pleural empyema [24].

In our study, 81% of *S. aureus* were found susceptible to Vancomycin, and a similar study done by K.V. Ramana reported 85% sensitivity [4]. In our study, no MRSA isolates were detected, in contrast to a study done from Andhra Pradesh, where 1 isolate of S. aureus was detected as Methicillin Resistant [4] (Figure 12).

Over the last 3 decades, there have been reports of greater Gram-negative bacteria incidence among culture-positive pneumonia cases [25,26]. In the present study, the majority of the bacteria isolated were Gram-negative, with *Klebsiella pneumoniae* sharing the major part, i.e. 45%.

In our study, *K. pneumoniae*, which is the dominant Gram-negative bacteria isolated, is more frequently found among the elderly age group >50 years. Our study finding is in coherence with an earlier study done by Supriya Panda et al., 2012 [5]. This susceptibility to Gram-negative bacteria may be because of weakening immunity, poor pulmonary defense mechanisms, underlying diseases with chronic conditions, and silent aspiration. Poor care of patients in hospital and health care settings can also make them more susceptible to Gram-negative pneumonia [5]. However, in our study, almost a similar percentage of *K. pneumoniae* isolates were recovered from both IP and OP patients. A study by Mishra S.K et al. in 2012 reported a higher percentage of *K. pneumoniae* isolates from IP compared to OP patients [18].

In the present study, *K. pneumoniae* isolates showed 100%, 98%, 75%, 64%, 56%, and 45% susceptibility to Imipenem, Piperacillin tazobactam, Amikacin, Gentamicin, ciprofloxacin, and Amoxyclav respectively. The findings of our study are in coherence with other studies done in India and other parts of the world [4,27,28] (Figure 13).

Klebsiella in our study showed a lower degree of sensitivity towards Cefotaxime and Ceftazidime, i.e., 47% and 36%, respectively. The emergence of drug resistance is alarming to the commonly used antibiotics in our country against LRTI. In the same way, Pseudomonas aeruginosa another Gram-negative isolate in our study, also showed lower sensitivity towards Cephalosporins namely, Cefotaxime and Ceftazidime. i.e., 38% and 31% respectively. These Gram-Negative bacterial isolates showing resistance to 2nd and 3rd generation Cephalosporins may be subjected to ESBL phenotypic confirmation (Figure 14).

Several previous studies have reported the incidence of bacterial resistance mediated by β-lactamase. Due to the ability of failing the treatment, the clinical relevance of the β-lactamase enzyme is enhanced. ESBLs were reported first in the year 1983, since its first report they have spread worldwide and are most common in certain genera of the *Enterobacteriaceae* family like *E. coli*, and *Klebsiella pneumoniae*, and it is also found in some other bacteria which are out of Enterobacteriaceae family like *Staphylococcus aureus, Haemophilus influenza,* and *Pseudomonas aeruginosa* [29].

In the present study, 20% (11) of 55 *K. pneumoniae* isolates were ESBL producers. The increased prevalence of ESBL producers in any hospital/healthcare setting depends on factors like the use of disinfection in the ICU, the usage of antibiotics, and the rate of carriage of ESBL-producing bacteria among hospital personnel [30].

The present study reported 30.7% ESBL production among *P. aeruginosa* isolates. The high isolation of ESBLs among *Pseudomonas* isolates in this study could be due to acquired resistance by plasmids which is a problem in *P. aeruginosa.* Plasmid-mediated resistance involving modifying enzymes is particularly associated with indiscriminate antibiotic use and with sites where high levels of antibiotics are achieved [31]. Pseudomonas are more versatile

than *Enterobacteriaceae* in acquiring drug resistance by various mechanisms. ESBL production is one of them [32].

Compared to other studies, the low isolation of ESBL-producing organisms in our study is due to the inclusion of only non-ICU patients, whereas other studies included ICU patients, hence their high isolation of ESBLs.

In this study, polymicrobial growth was (22) 18% and this was in concordance with a study done by Supriya Pandey et al. in 2012 who reported 21.7% of mixed infections [5]. The above percentage is consistent with the fact that the overall incidence of mixed infections does not usually exceed 30% [33] (Figure 11).

The most commonly identified risk factor in this study was smoking with 37%, and the findings of our study are similar to the study from Karnataka [34]. Bacterial colonization of the lower respiratory tract is more prevalent in smokers than in non-smokers, as there is a disruption of respiratory flora, mechanical clearance, and cellular defense [35].

In the present study, COPD was found to be the second most important comorbid condition with 33%. In another study, it was also the most common underlying comorbid condition among 40 cases (57%) [36]. Smoking is one of the risk factors for COPD, so quitting smoking can lower the risk of COPD, and lower mortality is observed among those patients who don't have COPD [37].

In the present study, 18% of LRTI cases were associated with diabetes, whereas a study done by Shah et al. reported 13% [38]. Diabetes is associated with higher mortality and was also found to be more frequent in patients with bacteremic pneumococcal pneumonia compared to those with either non-bacteremic pneumococcal pneumonia or CAP of other etiologies [39].

CONCLUSION

LRTIs are among the most common infectious diseases in humans worldwide. Among the acute manifestations of LRTI, which include acute bronchitis, bronchiolitis, influenza, and CAP with exacerbations of COPD, CAP remains an important public health problem. Demographic factors like age (Young/Middle/Old) and some predisposing factors like hospitalization can lead to a change in the causative agent of LRTI.

In the current study, LRTIs were more common in males than in females, with a higher incidence in the age group of 56-65 years among males (34%). In this study, Smoking, COPD and Diabetes mellitus were among the most important risk factors associated with increased prevalence of CAP.

The etiology of LRTI could be established in 43.5% of cases with 34.6% of OP and 49.1% of IP cases. Gram-negative organisms were the most common cause of LRTI in this study. *Klebsiella pneumoniae* (45%) was the most common Gram-negative isolate, while *Streptococcus pneumoniae* (25.4%) was the most common Gram-positive isolate among the CAP patients.

In this study, *K. pneumoniae* showed the highest susceptibility towards Imipenem (100%) and Piperacillin Tazobactam (98%) and the least sensitivity of around (36%) towards Cephalosporins and cotrimoxazole.

S. pneumoniae showed maximum sensitivity to Vancomycin and Linezolid while the least susceptibility was shown towards Gentamicin.

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REFERENCES

1. Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Ieven M, et al; Joint Taskforce of the European Respiratory Society and European Society for Clinical Microbiology and Infectious Diseases. Guidelines for the management of adult lower respiratory tract infections--full version. *Clin Microbiol Infect.* 2011 Nov;17 Suppl 6(Suppl 6):E1-59. doi: 10.1111/j.1469-0691.2011.03672.x.
2. Carroll KC. Laboratory diagnosis of lower respiratory tract infections: controversy and conundrums. *J Clin Microbiol.* 2002 Sep;40(9):3115-20. doi: 10.1128/JCM.40.9.3115-3120.2002.
3. Beck JM, Young VB, Huffnagle GB. The microbiome of the lung. *Transl Res.* 2012 Oct;160(4):258-66. doi: 10.1016/j.trsl.2012.02.005.
4. Ramana KV, Kalaskar A, Rao M, Rao SD. Aetiology and Antimicrobial Susceptibility Patterns of Lower Respiratory Tract Infections (LRTIs) in a Rural Tertiary Care Teaching Hospital at Karimnagar, South India. *Am J Infectious Diseases Microbiol.* 2013; 1(5):101-105. doi: 10.12691/ajidm-1-5-5.
5. Panda S, Nandini BP, Ramani TV. Lower respiratory tract infections-Bacteriological profile and antibiogram pattern. *Int J Cur Res Rev.* 2012; 4(21): 149-155.
6. Campbell S, Forbes BA. The Clinical Microbiology Laboratory in the Diagnosis of Lower Respiratory Tract Infections. *J Clin Microbiol.* 2011 Sep; 49(9 Suppl): S30–S33. doi: 10.1128/JCM.00789-11.
7. Reimer LG, Carroll KC. Role of the microbiology laboratory in the diagnosis of lower respiratory tract infections. *Clin Infect Dis.* 1998 Mar;26(3):742-8. doi: 10.1086/514583.
8. Guthrie R. Community-acquired lower respiratory tract infections: etiology and treatment. *Chest*. 2001 Dec;120(6):2021-34. doi: 10.1378/chest.120.6.2021.
9. Colle JG, Miles RS, Wan B. Tests for the identification of bacteria. In: Mackie and Mc Cartney Practical Medical Microbiology. Eds. Collee JG, Fraser AG, Marmion BP & Simmons A. 14th Ed. Edinburg: Churchill Livingstone, 1996. p. 131-50.

10. Tortora FC. Microbiology, An Introduction Benjamin. London: Cumming publishing Company, Inc.; 2004. p. 66 – 67.
11. Isenberg HD. Clinical Microbiology Procedures Handbooks. 2nd Ed. Washington DC: A.S.M press, 2004.
12. Till PM. Bailey and Scott's, Diagnostic Microbiology. 13th Ed. Elsevier publishers. 2014; Chapter 69, p. 882.
13. Ahmed KS, Tewelde K, Thomas PD, Fisehatsion S, Yitbarek G/ Her, Shano AK, Anijajo TT. Prevalence and drug susceptibility pattern of urinary tract infections among war disabled patients of Denden Hospital, Asmara, Eritrea. *Ethiopian Med J.* 2023;61(1):21-35.
14. Ahmed KS, Bhayal BK, Eman D, Tekeste Y, Tadelle M, Omaha F, et al. Identification of bacterial profile, common associated risk factors, and antimicrobial susceptibility patterns, of bacterial keratitis in community hospitals of Asmara, Eritrea. *Ethiopian Med J.* 2022; 60(3):245-56.
15. Performance Standards for Antimicrobial Susceptibility Testing. 30th Ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2020.
16. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet*. 1997 May 3;349(9061):1269-76. doi: 10.1016/S0140-6736(96)07493-4.
17. Ozyilmaz E, Akan OA, Gulhan M, Ahmed K, Nagatake T. Major bacteria of community-acquired respiratory tract infections in Turkey. *Jpn J Infect Dis.* 2005 Feb;58(1):50-2.
18. Mishra SK,Kattel HP, Acharya J, Shah NP, Shah AS, Sherchand JB, et al. Recent trend of bacterial aetiology of lower respiratory tract infections in a tertiary care centre of Nepal. *Int J Infect Microbiol.* 2012;1(1):3-8.
19. Howard LS, Sillis M, Pasteur MC, Kamath AV, Harrison BD. Microbiological profile of community-acquired pneumonia in adults over the last 20 years. *J Infect.* 2005 Feb;50(2):107-13. doi: 10.1016/j.jinf.2004.05.003.
20. Menon RU, George AP, Menon UK. Etiology and Anti-microbial Sensitivity of Organisms Causing Community Acquired Pneumonia: A Single Hospital Study. *J Family Med Prim Care*. 2013 Jul;2(3):244-9. doi: 10.4103/2249-4863.120728.
21. He R, Luo B, Hu C, Li Y, Niu R. Differences in distribution and drug sensitivity of pathogens in lower respiratory tract infections between general wards and RICU. *J Thorac Dis.* 2014 Oct;6(10):1403-10. doi: 10.3978/j.issn.2072-1439.2014.09.22.
22. Lalitha MK, Pai R, Manoharan A, Appelbaum PC; CMCH Pneumococcal Study Group. Multidrug-resistant Streptococcus pneumoniae from India. *Lancet*. 2002 Feb 2;359(9304):445. doi: 10.1016/s0140-6736(02)07578-5.
23. Ramamurthy RK, Ningappa R, Jayachandra. Changing Bacterial Organisms and Antimicrobial Resistance Pattern Among Hospitalised Community Acquired Pneumonia Patients in a Tertiary Care Center. *J Evol Medical Dental Sci.* 2013 Mar;2(12):83.
24. Carroll KC, Adams LL. Lower Respiratory Tract Infections. Microbiology Spectrum. 2016 Jul;4(4). doi: 10.1128/microbiolspec.dmih2-0029-2016.
25. Chawla K, Mukhopadhay C, Majumdar M, Bairy I. Bacteriological profile and their antibiogram from cases of acute exacerbations of chronic obstructive pulmonary disease: A hospital-based study. *J Clin Diag Res*.2008; 2:612–6.
26. Ailani RK, Agastya G, Ailani RK, Mukunda BN, Shekar R. Doxycycline is a cost-effective therapy for hospitalized patients with community-acquired pneumonia. *Arch Intern Med.* 1999 Feb 8;159(3):266-70. doi: 10.1001/archinte.159.3.266.
27. Akter S, Shamsuzzaman SM, Jahan F. Community acquired bacterial pneumonia: aetiology, laboratory detection and antibiotic susceptibility pattern. *Malays J Pathol.* 2014 Aug;36(2):97-103.
28. Agmy G, Mohamed S, Gad Y, Farghally E, Mohammedin H, Rashed H. Bacterial profile, antibiotic sensitivity and resistance of lower respiratory tract infections in upper egypt. *Mediterr J Hematol Infect Dis.* 2013 Sep 2;5(1):e2013056. doi: 10.4084/MJHID.2013.056.
29. Sarma JB, Ahmed GU. Prevalence and risk factors for colonisation with extended spectrum beta-lactamase producing enterobacteriacae vis-à-vis usage of antimicrobials. *Indian J Med Microbiol.* 2010 Jul-Sep;28(3):217-20. doi: 10.4103/0255-0857.66476.
30. Sarojamma V, Ramakrishna V. Prevalence of ESBL-Producing Klebsiella pneumoniae Isolates in Tertiary Care Hospital. *ISRN Microbiol.* 2011 Dec 1;2011:318348. doi: 10.5402/2011/318348.
31. Shahid M, Malik A, Sheeba. Multidrug-resistant Pseudomonas aeruginosa strains harbouring R-plasmids and AmpC beta-lactamases isolated from hospitalised burn patients in a tertiary care hospital of North India. *FEMS Microbiol Lett.* 2003 Nov 21;228(2):181-6. doi: 10.1016/S0378-1097(03)00756-0.
32. Aggarwal R, Chaudhary U, Bala K. Detection of extended-spectrum beta-lactamase in Pseudomonas aeruginosa. *Indian J Pathol Microbiol.* 2008 Apr-Jun;51(2):222-4. doi: 10.4103/0377-4929.41693.
33. de Roux A, Ewig S, García E, Marcos MA, Mensa J, Lode H, Torres A. Mixed community-acquired pneumonia in hospitalised patients. *Eur Respir J.* 2006 Apr;27(4):795-800. doi: 10.1183/ 09031936.06.00058605.
34. Madhulatha CK, Pratibha Malini J, Ravikumar KL, Rashmi KS. 2013. Bacteriological Profile and Antibiotic Sensitivity Pattern of Micro Organisms from Community Acquired Pneumonia. *RJPBCS*. 2013;4(3):1005-1011.
35. Abdullah BB, Zoheb M, Ashraf SM, Ali S, Nausheen N. A Study of Community-Acquired Pneumonias in Elderly Individuals in Bijapur, India. *ISRN Pulmonology.* 2012;1-10.
36. Bansal S, Kashyap S, Pal LS, Goel A. Clinical and bacteriological profile of community acquired pneumonia in Shimla, Himachal Pradesh. *Indian J Chest Dis Allied Sci.* 2004 Jan-Mar;46(1):17-22.
37. Plouffe JF, Martin DR. Pneumonia in the emergency department. *Emerg Med Clin North Am.* 2008 May;26(2):389-411, ix. doi: 10.1016/j.emc.2008.02.005.
38. Shah BA, Singh G, Naik MA, Dhobi GN. Bacteriological and clinical profile of Community acquired pneumonia in hospitalized patients. *Lung India.* 2010 Apr;27(2):54-7. doi: 10.4103/0970-2113.63606.
39. Marrie TJ. Bacteraemic pneumococcal pneumonia: a continuously evolving disease. *J Infect.* 1992 May;24(3):247-55. doi: 10.1016/s0163-4453(05)80029-5. PMID: 1602145.