



## Study of Gram Negative Bacterial Isolates From Lower Respiratory Tract Infections (LRTI) and Their Antibigram Pattern in A Tertiary Care Hospital in South India

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### Abstract

**Introduction:** Lower respiratory tract infections (LRTI) are common human diseases, the morbidity and mortality of which varies depending on the underlying etiological agent and its virulence. With emerging drug resistance of organisms to commonly used antibiotics, it is imperative to study their recent trends, for effective management of these cases.

**Materials and Methods:** The study was done in the respiratory samples received at the microbiology department, from patients with LRTIs attending outpatient clinic at a tertiary care centre, Puducherry. The samples were processed by standard methods for isolations and identification followed by antimicrobial sensitivity testing using Kirby Bauer disc diffusion method.

**Results:** Nearly 109 respiratory samples were processed during the study period from July to October, 2014. Of the samples tested significant pathogens were isolated in 71(65.14%) cases. Among the gram negative bacteria grown i.e 42.56%, the predominant pathogen isolated was *Klebsiella pneumoniae* (52%), followed by *Pseudomonas aeruginosa* (25%), *Escherichia coli* (18%) and *Acinetobacter* species (5%). The antimicrobial resistance pattern showed high resistance rates to third generation cephalosporins and aminoglycosides.

**Conclusions:** In this study it was observed that significant number of gram negative isolates from the community acquired LTRI were showing resistance to third generation Cephalosporins. Hence it is advised to undertake routine testing in the clinical microbiology laboratory for screening of multidrug resistant isolates like ESBL and MBL producing organisms to give necessary information to the clinicians for accurate management of these infections.

**Keywords:** Lower respiratory tract infection, *Klebsiella* species, Gram negative bacterial isolates.

### Introduction

Lower respiratory tract infections (LRTI) are common human diseases, the morbidity and mortality of which varies depending on the

underlying etiological agent and its virulence. With emerging drug resistance of organisms to commonly used antibiotics, it is imperative to study their recent trends, for effective

management of these cases. Each year approximately 7 million people die as a direct consequence of acute and chronic respiratory infections<sup>(18)</sup>. The aetiological agents of LRTIs vary from area to area, so the susceptibility profile will also differ between geographical locations. Management of these infections has become a challenge to the physicians due to the rising resistance of these organisms to antibiotics.. Hence this study was done with the aim of studying the bacterial profile of lower respiratory tract infections and their antibiotic sensitivity pattern .

### Materials & Methods

This Prospective study was conducted in the department of Microbiology for a period of 6 months from December 2014 to May 2015. Nearly 109 patients attending Medicine, Pulmonology clinics and those admitted in Intensive Care Unit (ICU), who were provisionally diagnosed as pneumonia were enrolled in the study.

Appropriate samples representing the lower respiratory tract i.e sputum were collected. After taking the consent, patients were instructed to cough into the given sterile wide mouth container. The quality of sputum and endotracheal tube samples were assessed based on criteria laid by American Society for Microbiology (ASM). According to this, a reliable specimen would have more than 25 leucocytes and fewer than 10 epithelial cells per low power field of microscope. Samples not fulfilling these criteria were rejected for repeat specimen.

Samples were subjected to microscopy and then culture was done on Blood agar, and MacConkey's agar and incubated at 37° C for 24 hours in aerobic atmosphere. Significant pathogens were identified based on colony morphology, Gram's staining and battery of biochemical tests. The antibiotic sensitivity pattern of the isolates was performed by Kirby –

Bauer's disc diffusion method. Extended spectrum beta lactamase (ESBL) production among the gram negative bacilli was detected using CLSI approved standard disc diffusion method. Two antibiotic discs ceftazidime (30µg) and ceftazidime (30µg)+ clavulanic acid (10µg) were placed on the lawn culture of the test organism , at a distance of about 10mm. After an overnight incubation at 37° C, an increase in the zone size of at least 5 mm around ceftazidime (30µg) + clavulanic acid (10µg) disc is read as a positive result<sup>(1)</sup>. The study was approved by the institute ethical committee.

### Results

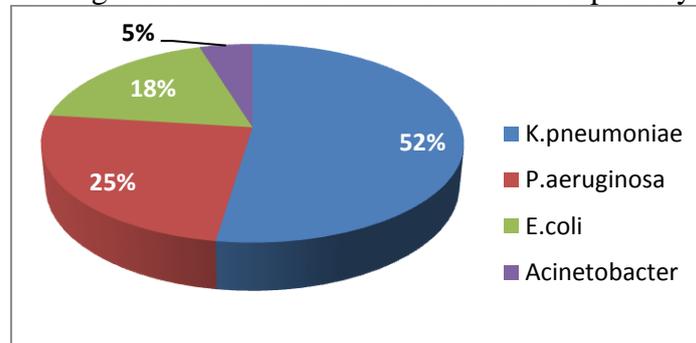
About 109 samples were processed based on the standard microbiological methods .The results of the study revealed that of the 109 samples tested significant pathogens were isolated from 71 (65.14%) samples. Of these 53 (74.6%) samples were from males and 18(25.4%) samples from females. The highest isolation rates was observed in the age group of 40-50 years. Among the gram negative isolates 38 (53.52%), the predominant pathogen isolated was *Klebsiella pneumoniae* (52%), followed by *Pseudomonas aeruginosa* (25%), *Escherichia coli* (18%) and *Acinetobacter species* (5%) (Table1., Chart.1) .

Most of the gram negative bacterial isolates showed resistance to commonly used cephalosporins. *Klebsiella* showed resistance to most of the drugs like Amoxyclav (87.8%), cefuroxime(83.3%),Ceftazidime(81.1%),ceftriaxone (70.5%). *Pseudomonas* showed relatively lesser resistance pattern , Amoxyclav(89%), Ceftazidime (37.5%) (Table 2). *E.coli* showed resistance to most of the cephalosporins, flouroquinolones and Amoxyclav (63.5%). *Acinetobacter* showed sensitivity only to higher level antibiotics like Imipenam (4.8%) and Piperacillin/Tazobactam (16.7%)

**Table 1.** Gram Negative bacterial isolates from lower respiratory tract infections

Organism	No of isolates	Percentage (%)
Klebsiella pneumoniae	20	52
Pseudomonas aeruginosa	9	25
Escherichia coli	7	18
Acinetobacter species	2	5

**Chart 1.** Distribution of Gram negative bacterial isolates from lower respiratory tract infections



**Table 2.** Resistance rates of the gram negative bacterial isolates from sputum samples

Antibiotic	K. pneumoniae	P. aeruginosa	E. coli	Acinetobacter
Amoxyclav	87.8	89	63.5	100
Cotrimoxazole	64.5	ND	88.2	89.2
Cefepime	74.2	ND	64	ND
Cefuroxime	83.3	ND	88	98.3
Ceftriaxone	70.5	ND	74	94.4
Ceftazidime	81.1	37.5	43.7	100
Ciprofloxacin	54.3	24	80.1	87.5
Ofloxacin	69	27	78.1	82.3
Amikacin	44.2	10.8	13.5	61.9
Aztreonam	76	0	56.3	90.9
Imipenem	2.6	0	0	4.8
Cefoperazone/Sulbactam	13.4	8.6	2.1	ND
Piperacillin/Tazobactam	9.3	0	5.6	16.7

**Discussion**

Management of LRTIs has become challenging to the clinicians in recent years due to several reasons. The first being emergence of drug resistance among the bacterial pathogens. Due to the inappropriate and indiscriminate use of antibiotics and self medication by the patients themselves, there has been a significant emergence of drug resistance among these organisms, more so among the gram negative bacteria. Moreover the natural course of the disease process is altered by the time the patients comes to a tertiary care center because of the previous visits to multiple clinics and the medications prescribed there. Hence the study was undertaken to assess the current trend of antibiotic drug resistance among the gram negative bacteria

in lower respiratory tract infections from patients attending our hospital.

In our study among the 109 sputum samples processed bacterial etiology was isolated in 71 (61.54%) samples. This is similar to the findings of Mishra et al <sup>(4)</sup> and Ramana et al <sup>(5)</sup> in which the isolation rates were 44% and 39.4% respectively. Similar higher prevalence rates of about 57.4% was shown in a study done by Thomas et al <sup>(7)</sup> in South India .The remaining 38 (38.46%) sputum samples which yielded no growth in culture could be due to viral etiology.

Of the 71 (65.14%) samples which showed growth, 53 (74.6%) were from males and 18 (25.4%) samples from females .Higher rates of isolation among males indicates increased susceptibility of male sex to lower respiratory

tract infections than female sex. This is similar to the findings of various similar studies done by which were done by Shah, et al. <sup>(1)</sup>, V.O Lugbue et al <sup>(8)</sup>, and Akingbade OA <sup>(9)</sup>. The highest isolation rates was observed in the age group of 40-50 years which was on par with the findings of studies done by Shah, et al <sup>(1)</sup>, and Mandell et al <sup>(10)</sup>.

Among the gram negative bacterial isolates 38 (53.52%), the predominant pathogen isolated was *Klebsiella pneumoniae*, 20 (52%) followed by *Pseudomonas aeruginosa* 9 (20%), *Escherichia coli* 7 (18%) and *Acinetobacter species* 2(5%) . Our findings are in concordance with studies done by Purti et al <sup>(11)</sup>, Ahmed et al <sup>(12)</sup>, Ramana et al <sup>(5)</sup> and Egbe et a <sup>(13)</sup> in which *K. pneumoniae* was the predominant isolate .

Multiple drug resistance (MDR) among the respiratory pathogens impose a major problem to the clinicians, as they are non responsive to treatment. In our study the major MDR isolates detected were *Klebsiella* (56.2%) ,*Acinetobacter* (75%) and *E.coli*(50.5%). Vishwanath et al (14) conducted a detailed study on MDR Gram negative bacilli causing lower respiratory infections. They had *K. pneumoniae* and *Acinetobacter* spp. as the commonest MDR isolates. Faimow and Nahra <sup>(15)</sup> state that the highest rates of MDR bacteria are found in the ICUs and selective pressures from intense antimicrobial exposure contributes to the emergence of MDR bacteria. De-escalation after receiving the culture and sensitivity reports is also not done in many ICUs, thus compounding the problem. *Pseudomonas* spp showed lesser resistance rates to various antibiotics i.e Amoxycillin Clavulanic acid (89%), Ceftazidime (37.5%), Ciprofloxacin (24%), ofloxacin (27%).

It was observed in our study that most of the Gram negative bacterial isolates showed high resistant rates to cephalosporins. This is similar to the findings of VO Lugbue et al <sup>(2)</sup> which showed 100% resistance to ceftazidime and the least resistance to ceftriaxone (76.5%).

Amikacin, Carbapenams and Piperacillin tazobactam were the antibiotics which showed

high in vitro efficacy against most of the gram negative bacterial isolates in our study. But these are the third line drugs, resistance to which is already on the rise as reported in studies done by Morrill et al (16). Cotrimoxazole showed high resistance rates to to most isolates in our study i.e *Klebsiella* (64.5%), *E.coli* (88.2%) and *Acinetobacter* (89.2%). Syed et al <sup>(17)</sup> showed similar results with resistance rates of 67.9% (*Klebsiella*), 91.3% (*E.coli*) and 87% (*Acinetobacter*).

### Conclusion

The current study has thrown light on the emergence of drug resistant strains among the gram negative isolates. Hence it is imperative to study the resistance pattern of these organisms on regular basis and review the management guidelines for the best management of the patients. It is also time to control and restrict the irrational use of antibiotics by formulating antibiotic policy guidelines based on the local resistance pattern. With strategies such as ‘antibiotic cycling’ and ‘antibiotic stewardship’, it is also necessary to conserve the already available antibiotics.

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