

## Bacteriological profile and antibiotic susceptibility patterns of lower respiratory tract infections in a tertiary care hospital, Central Kerala

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### Abstract

**Background and Objective:** Lower respiratory tract infections (LRTIs) is one of the leading human diseases causing high morbidity and mortality worldwide. The prevalent etiological agents and their antimicrobial resistance patterns differs, both geographically and over time. With emerging resistance of respiratory bacterial pathogens to commonly used antibiotics, it is imperative to study their recent trends for effective management of these cases.

**Materials and Methods:** A retrospective, record based study was conducted on culture and sensitivity reports of lower respiratory samples obtained in the microbiology lab during 1<sup>st</sup> January 2015- 31st December 2017. The samples were processed by standard methods for isolation and identification followed by antimicrobial sensitivity testing using Kirby Bauer disc diffusion method.

**Results:** 288 (26.34%) of total 1093 samples were positive for bacterial culture. 244 (84.7%) were gram negative bacilli (GNB) and 44 (15.3%) were gram positive cocci. The predominant pathogen isolated was *K.pneumoniae* (31.1%) followed by *P.aeruginosa* (30.2%). The overall susceptibility of GNB was highest towards Imipenem followed by Amikacin and Piperacillin tazobactam with resistance rates of 11.5%, 26.2% and 31.6% respectively. Gram positive organisms exhibited highest susceptibility towards Vancomycin and Linezolid. 15.4 % of *Staphylococcus aureus* were Methicillin resistant (MRSA).

**Conclusion:** Imipenem is the most sensitive antibiotic followed by Amikacin and Piperacillin tazobactam which can be used for empirical therapy for LRTI. The antibiotic therapy should be modified as per the culture and sensitivity report. Regular determinations of the type of bacterial pathogens and updation of antibiogram must be followed in every institution to aid in better patient management by helping the clinician in the judicious use of antibiotics.

**Keywords:** Antibiotic resistance patterns, Lower respiratory tract infections.

### Introduction

Lower respiratory tract infection (LRTI) is defined as the inflammation of the respiratory tract starting from trachea to the alveoli with subsequent multiplication of an infectious agent.<sup>1</sup> It includes bronchitis, bronchiectasis, bronchiolitis, emphysema, lung abscess, pleural effusion and pneumonia.<sup>2</sup> Acute lower respiratory tract (ALRI) infections are among the most common infectious diseases affecting humans worldwide causing significant morbidity and mortality for all age groups.<sup>1,3</sup> It is responsible for 4.4% of all hospital admissions and 6% of general practitioner consultations.<sup>4</sup> It accounts for 3 to 5% of deaths in adults. Globally, about 4.2 million ALRI deaths are estimated to occur among all age groups.<sup>1</sup> The problem is much greater in developing countries where pneumonia is the most common cause of hospital attendance in adults.<sup>5</sup>

Each type of LRTI vary in the epidemiology, pathogenesis, clinical presentation, and outcome.<sup>2,5</sup> The major respiratory pathogens are Gram negative bacilli (GNB) like *Klebsiella pneumoniae* (*K.pneumoniae*), *Escherichia coli* (*Esch.coli*), *Pseudomonas aeruginosa* (*P.aeruginosa*), *Acinetobacter* species, other Non-Fermentative Gram-Negative Bacilli (NFGNB) and gram positive organisms like *Streptococcus pneumoniae* (*Str.pneumoniae*), *Staphylococcus aureus* (*S.aureus*) etc.<sup>2</sup>

However, results from various surveillance studies show wide variations in the prevalent etiological agents and their antimicrobial resistance patterns, both geographically and over time. The dramatic rise in the antimicrobial resistance among the respiratory pathogens, presumably due to the prophylactic administration of antibacterial therapy even before the availability of the culture reports, is a matter of potential concern worldwide. Failure to de-escalate the therapy after getting the culture and sensitivity report is another important reason for the drug resistance. In this context, management of these infections has become a challenge to the physicians.<sup>6</sup> Hence the present study was conducted to investigate the bacterial aetiology of LRTIs in our institution and to update the clinicians on the current antibiotic susceptibility pattern of these pathogens.

### Materials and Methods

A retrospective, record based study was conducted on all culture and sensitivity (C/S) reports of lower respiratory samples (sputum, endotracheal aspirate and bronchoalveolar lavage) obtained in the microbiology lab of a tertiary care hospital, Central Kerala during three consecutive years (1<sup>st</sup> January 2015-31st December 2017).

**Inclusion criteria:** All predominant bacterial isolates from sputum, endotracheal aspirate and bronchoalveolar lavage (BAL) during the study period

**Exclusion criteria:**

1. Bacterial isolates from repeat culture of previously recruited patients
2. Bacterial isolates identified as commensals or contaminants
3. Mixed bacterial growth

**Method**

Sputum, endotracheal aspirate and BAL were collected in a sterile wide mouth container. The quality of sputum and endotracheal tube samples were assessed based on criteria laid by American Society for Microbiology (ASM).<sup>6</sup> According to this, a reliable specimen after gram staining 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. The undiluted sputum samples were inoculated on the culture medium using a Nichrome wire loop. The culture media used for inoculation were blood agar, chocolate agar and MacConkey's agar. The inoculated plates were incubated at 37°C for 18-24 hours. The predominant bacterial growth obtained from sputum samples were recorded.

Endotracheal secretions were vortexed for 1 minute, centrifuged at 3000 rpm for 10 minutes and semi-quantitative culture was performed by the calibrated loop method using a wire loop of capacity 0.001 mL. The bacterial colonies were counted. Colony counts of  $\geq 10^5$ /mL suggest potential pathogen.<sup>2</sup>

BAL were inoculated on to blood agar, chocolate agar and MacConkey's agar and brain heart infusion broth and all were incubated at 37°C for 18-24 hours. From broth it was subcultured on to blood agar and MacConkey's agar and a further 24 hours of incubation was done.

Identification of the isolates were performed by standard microbiological procedures such as study of colony morphology, Gram stain reactions and a battery of standard biochemical tests.<sup>7</sup> Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and on Blood agar for fastidious organisms. After incubation at 37°C for 18-24 hours, the results were read and interpreted as per CLSI guidelines.<sup>8</sup> The antibiotic discs used were Ampicillin (10µg), Amoxicillin-clavulanate (20/10 µg), Piperacillin (100 µg), Piperacillin-Tazobactam (100/10 µg), Gentamicin (10 µg), Amikacin (30 µg), Cefazolin (30 µg), Ceftriaxone (30 µg), Cefuroxime (30 µg), Cefoxitin (30 µg), Cefepime (30 µg), Ceftazidime (30 µg), Cotrimoxazole (1.25/23.75 µg), Tetracycline (30 µg), Ciprofloxacin (5), Imipenem (10), Penicillin (10U), Erythromycin (15 µg), Clindamycin (2 µg), Linezolid (30 µg), and Vancomycin (30 µg). Methicillin resistant Staphylococcus aureus (MRSA) were detected using Cefoxitin 30 µg disc. Strains with zone size  $\leq 21$  mm

were considered as MRSA.<sup>8</sup> Penicillin sensitivity of Str. pneumoniae was detected using Oxacillin 1 µg disc and a zone size  $\geq 20$ mm were considered as sensitive.

For quality control of disc diffusion tests ATCC control strains of Esch.coli ATCC 25922, S.aureus ATCC 25923 and P.aeruginosa 27853 were used.

**Statistical analysis**

Descriptive statistics was used for analysis. The collected data was entered in MS-Excel and statistical analysis was done using SPSS 17 software and were expressed as percentages.

**Results**

Out of 1093 lower respiratory samples, 288 (26.34%) were positive for bacterial culture. Remaining samples yielded normal pharyngeal flora/ mixed flora/no growth/fungal growth. Among the bacterial isolates 244 (84.7%) were GNB and remaining 44 (15.3%) were gram positive cocci. The predominant pathogen isolated was K.pneumoniae (31.1%) followed by P.aeruginosa (30.2%). Among gram positive bacteria, S.aureus (4.5%) and Strp.pyogenes (4.5%) were predominant organisms followed by Enterococci (4.2%). The rate of isolation of different bacterial isolates is shown in Table 1.

The antibiotic resistance pattern of GNB is presented in Table 2. The overall resistance of GNB was lowest towards Imipenem followed by Amikacin and Piperacillin-tazobactam. Resistance to first, second and third generations of cephalosporins were significantly high. Ciprofloxacin and Cefepime exhibited comparatively good activity against GNB. Among antipseudomonal cephalosporins tested, Cefepime showed the highest rate of sensitivity followed by Ceftazidime. NFGNB demonstrated higher rate of resistance to almost all groups of antibiotics including Imipenem.

Gram positive organisms showed highest sensitivity towards Vancomycin followed by Linezolid. (Table 3). 84.6 % of S.aureus were resistant to penicillin. 15.4% of S.aureus isolates were Methicillin resistant (MRSA). 100 % of Strp.pyogenes and Strp.pneumoniae were sensitive to Penicillin.

**Discussion**

The etiological agents of LRTIs and their susceptibility patterns vary from area to area. Hospital antibiograms are mandatory to guide empirical antimicrobial therapy and are an important component of detecting and monitoring trends in antimicrobial resistance. Reliable statistics on antibiotic resistance are mandatory to control resistant pathogens. The present study provides an insight on the prevalence and the antibiogram of respiratory pathogens in Central Kerala.

In our study, the bacterial etiology for LRTI was noticed in 26.34% of samples. The isolation rates by Mishra et al,<sup>9</sup> Salman Khan et al<sup>10</sup> and Ramana et al<sup>11</sup> were 44%, 49.3% and 39.4% respectively. Higher

prevalence was shown by some other authors.<sup>6,12,13</sup> Lower bacterial isolation rate may be due to improper sample collection, delay in transportation or prior antimicrobial therapy before sample collection.

In this study Gram negative bacilli were more frequently isolated than gram positive bacteria. Many other studies also found out considerable predominance of GNB among respiratory pathogens.<sup>2,3,5,13-16</sup> The gram negative predominance might partly be due to the unequal distribution of patients with community acquired and hospital acquired infections and also due to the spread of antibiotics resistance in hospital settings. The predominant pathogen isolated was *K.pneumoniae* (31.1%). This is in concordance with Ratna S,<sup>5</sup> Verma D et al,<sup>17</sup> Madhavi et al<sup>18</sup> and Mokkapati A et al.<sup>19</sup> But in some other studies the predominant pathogen was *P.aeruginosa* followed by *K.pneumoniae*.<sup>2,10</sup> *P.aeruginosa* was the second most common isolate in the present study as shown by Viswanath S et al.<sup>20</sup>

Among gram positive bacteria, *S.aureus* (4.5%) and *Str.pyogenes* (4.5%) were most frequently isolated. *S.aureus* was isolated as the predominant gram positive pathogen in studies by Amutha C et al,<sup>13</sup> Egbe et al<sup>3</sup> and Anvari MS et al<sup>15</sup> with isolation rates of 5%, 15.41% and 20.8% respectively. But frequency of isolation of *Str.pyogenes* was relatively low in their studies.<sup>3,13,1</sup>

Against GNB, the most active antibiotics were Imipenem followed by Amikacin and Piperacillin tazobactam. *K.pneumoniae* and *P.aeruginosa* showed comparatively good susceptibility rate towards these three antibiotics. Some studies found out good activity against them but some authors pointed out higher resistance rates (Table 4). According to our findings, Imipenem followed by Amikacin and Piperacillin tazobactam are the most suitable drugs for empirical therapy for LRTI in our settings.

46% of GNB isolates were resistant to Ciprofloxacin. Recent studies from various parts of India demonstrated

high resistance rates of *K.pneumoniae* towards Ciprofloxacin, but the antibiotic showed comparatively good activity against *P.aeruginosa*(Table 4). The GNB isolates in our hospital showed high resistance to all generations of Cephalosporins. 76.4 % and 70.7% of GNB were resistant to first and second generation cephalosporins respectively. They showed 65% resistance to third generation cephalosporins. The overall resistance to Cefepime was 42.2% which is lower when compared to other generations. 53.3% of *K.pneumoniae* exhibited resistance to Cefepime. Some studies showed higher resistance rate to Cefepime in the range of 65-70%.<sup>6,16</sup> But some contemporary studies found out good activity against this antibiotic.<sup>5,6,16</sup> Among antipseudomonal cephalosporins, Cefepime showed the best activity against the organism as noted by some other investigators.<sup>2,6,13</sup> Higher resistance of *P.aeruginosa* in a range of 57-65% against Cefepime were recorded by other authors also.<sup>3,15,16</sup> Thus, in our settings, cephalosporins and fluoroquinolones were found to be ineffective for empirical therapy for LRTI. They can be tried in those isolates with proven sensitivity by susceptibility testing

Gram positive organisms showed highest sensitivity towards Vancomycin followed by Linezolid. 15.4% of *S.aureus* isolates were Methicillin resistant (MRSA), which is lower when compared to other studies from various states of India. In a study from Chennai it was 25%, from Jharkhand it was 23.29%, from Manipur it was 62.06%, 82.7% in Iran and 55.6% in Nagpur.<sup>5,13-16</sup> *Str.pyogenes* showed 23.1% resistance to Erythromycin and only 7.7% resistance to clindamycin and no resistance to Penicillin was detected. This is in concordance with a recent study by Ratna S.<sup>5</sup> *Str.pneumoniae* was 100% sensitive to Penicillin as demonstrated by Kombade et al.<sup>16</sup>

**Table 1: Distribution of bacterial isolates from respiratory specimens**

Organism	Number of isolates (N)	Percentage of isolation
<i>Klebsiella pneumoniae</i>	90	31.1
<i>Pseudomonas aeruginosa</i>	87	30.2
Other Non -fermenting GNB	43	14.93
<i>Staphylococcus aureus</i>	13	4.5
<i>Streptococcus pyogenes</i>	13	4.5
Enterococci	12	4.2
<i>Esch.coli</i>	11	3.8
<i>Streptococcus pneumoniae</i>	6	2.1
Citrobacter species	4	1.4
Proteus species	3	1.1
<i>Moraxella catarhalis</i>	3	1.1
<i>Burkholderia cepacia</i>	3	1.1
Total	288	

**Table 2: Antibiotic resistance patterns of Gram negative bacteria**

Antimicrobial agent	K.pneumoniae N(%)	P.aeruginosa N(%)	NFGNB N(%)	Esch.coli N(%)	Citrobacter sp. N(%)	Proteus sp. N(%)	M.catarhalis N(%)	B.cepacia N(%)	Total (%)
Ampicillin	90(100)	NT	32(74.4)	8(72.7)	4(100)	2(66.7)	0 (0)	0 (0)	136 (86.6)
Amoxycillin-clavulanate	85(94.4)	NT	31(72.1)	7(63.6)	2(50)	1(33.3)	0 (0)	0 (0)	126 (80.3)
Piperacillin	76(84.4)	27(31)	27(62.8)	7(63.6)	4(100)	0(0)	0 (0)	0 (0)	141 (57.8)
Piperacillin-Tazobactam	31(34.4)	19(21.83)	21(48.8)	3(27.3)	3(75)	0(0)	0 (0)	0 (0)	77 (31.6)
Gentamicin	38(42.2)	43(49.4)	20(46.5)	4(36.4)	3(75)	0(0)	0 (0)	0 (0)	108 (44.3)
Amikacin	27(30)	18(20.7)	17(39.5)	1(9.1)	1(25)	0(0)	0 (0)	0 (0)	64 (26.2)
Cephazolin	76(84.4)	NT	34(79.1)	5(45.5)	4(100)	1(33.3)	0 (0)	0 (0)	120 (76.4)
Ceftriaxone	59(65.6)	NT	34(79.1)	4(36.4)	4(100)	1(33.3)	0 (0)	0 (0)	102 (65)
Cefuroxime	71(78.9)	NT	31(72.1)	4(36.4)	4(100)	1(33.3)	0 (0)	0 (0)	111 (70.7)
Cefepime	48(53.3)	22(25.3)	24(55.8)	4(36.4)	4(100)	1(33.3)	0 (0)	0 (0)	103 (42.2)
Cefoxitin	47(52.2)	NT	25(58.1)	3(27.3)	2(50)	1(33.3)	0 (0)	0 (0)	78 (49.7)
Ceftazidime	NT	32(36.8)	NT	NT	NT	NT	0 (0)	NT	32 (35.6)
Cefaperazone	NT	33(37.9)	NT	NT	NT	NT	0 (0)	NT	33 (36.7)
Ciprofloxacin	46(51.1)	32(36.8)	25(58.1)	6(54.5)	2(50)	0(0)	0 (0)	1(33.3)	112 (46%)
Cotrimoxazole	55(61.1)	NT	16(37.2)	4(36.4)	4(100)	4(100)	1(33.3)	0 (0)	84 (53.5)
Tetracycline	56(62.2)	NT	29(67.4)	7(63.6)	0(0)	0(0)	1(33.3)	0 (0)	93 (59.2)
Imipenem	8(8.9)	3(3.4)	17(39.5)	0(0)	0(0)	0(0)	0(0)	0 (0)	28 (11.5)

NT: not tested

**Table 3: Antibiotics resistance patterns of Gram positive cocci**

Antimicrobial agents	S.aureus N(%)	Str.pyogenes N(%)	Enterococci N(%)	Str.pneumococci N(%)	Total (%)
Penicillin	11(84.6)	0 (0)	8 (66.7)	0 (0)	19 (43%)
Erythromycin	7(53.8)	3(23.1)	NT	2(33.3)	12 (37.5)
Clindamycin	2(15.4)	1(7.7)	NT	0(0)	3 (9.4)
Cotrimoxazole	0(0)	NT	NT	0(0)	0 (0)
Cefoxitin	2(15.4)	NT	NT	NT	2 (15.4)
Tetracycline	2(15.4)	NT	NT	0(0)	2 (10.5)
Rifampin	0(0)	NT	NT	NT	0 (0)
Linezolid	0(0)	0(0)	3(25)	0(0)	3 (6.8)
Vancomycin	NT	0(0)	1(8.3)	0(0)	1 (3.2)

NT: not tested

**Table 4: Resistance of K.pneumoniae and P.aeruginosa to Imipenem, Amikacin, Piperacillin tazobactam & Ciprofloxacin in various studies**

Studies	K.pneumoniae (resistance %)				P.aeruginosa (resistance %)			
	Imipenem	Piperacillin tazobactam	Amikacin	Ciprofloxacin	Imipenem	Piperacillin tazobactam	Amikacin	Ciprofloxacin
Present study	8.9	34.4	30	51.1	3.4	21.8	20.7	36.8
Amutha C et al <sup>13</sup>	0	10	13	53	3	4.4	30	42
Elumalai et al <sup>6</sup>	2.6	9.3	44.2	54.3	0	0	10.8	37.5
Ratna S <sup>5</sup>	5	42.62	NT	18.03	7.14	42.85	NT	14.28
Thomas et al <sup>2</sup>	40	70	70	80	33.33	41.7	25	33.3

### Limitations

A distinction between community-acquired and hospital-acquired infections could not be made.

### Conclusion

The study revealed GNB as major pathogens causing LRTIs. *K.pneumoniae* was the predominant respiratory pathogen followed by *P.aeruginosa*. Imipenem was the most sensitive drug, next being Amikacin and Piperacillin tazobactam and should be used for empirical therapy for LRTI. The treatment should be modified as per the culture and sensitivity report from the microbiology lab.

Antibiotic resistance among respiratory bacterial pathogens is alarming. Strict implementation of the concept of 'antibiotic stewardship' has become necessary to conserve the already available antibiotics. Hospitals should have an 'antibiotic policy' and facilities for proper monitoring of antibiotic usage along with effective infection control practices to check the issue of antibiotic resistance worldwide. Periodic analysis of types of respiratory pathogens and regular updation of their antibiograms should be done in every institutions, so that changing trends can be identified and therapy adjusted accordingly.

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