Int.J.Curr.Microbiol.App.Sci (2017) *6*(3): 631-639

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 3 (2017) pp. 631-639**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.603.073

Bacterial Profile of Lower Respiratory Tract Infections in Adults and their Antibiotic Susceptibility Pattern with Detection of MRSA, ESBLs and MBLs

C. Amutha[1]*, M. Suganthi[1], Radhika Katragadda[2], K.V. Leela[1], J. Jayachitra[1] and Padmanaban[3]

[1]Department of Microbiology, Govt. Kilpauk, Medical College and Hospital, Chennai- 600010, India
[2]Government Omandurar Medical College, Chennai, India
[3]NIIRH FU, ICMR, Govt. Kilpauk Medical College and Hospital, Chennai, India
**Corresponding author*

Keywords

Lower respiratory tract infection, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Extended spectrum beta lactamases, Metallobetalactamases, Methicillin resistant *Staphylococcus aureus.*

Article Info

Accepted:
10 February 2017
Available Online:
10 March 2017

A B S T R A C T

LRTIs are one of the most common infectious diseases of humans. They are associated with morbidity and mortality worldwide. This study aims to find out the bacterial profile of lower respiratory tract infections in adults and the antibiotic susceptibility pattern of the isolated pathogen including detection of MRSA, ESBLs and MBLs. This study was conducted for a period of 6 months from March to August 2015 at a tertiary care hospital, Chennai. A total of 830 samples were collected during the study. They were processed following standard laboratory protocol. Antibiogram was done using appropriate antibiotics by Kirby-Bauer disc diffusion method and the occurrence of MRSA, ESBLs and MBLs was seen. Out of the 830 samples, 480 (57.8%) were male and 350 (42.2%) were female. 426 (51%) samples showed growth of pathogenic bacteria. Patients in the age group 41-50 were predominantly affected. *Klebsiella pneumoniae* (57.5%) was found to be the commonest organism isolated followed by *Pseudomonas aeruginosa* (28.8%). 90% of *Klebsiella pneumoniae*, 95.6% of *Pseudomonas aeruginosa* were sensitive to Piperacillin-tazobactum. ESBL was detected to be 33%, MBL was detected to be 3.2%, MRSA was detected to be 25%.All the MRSA isolates were sensitive to vancomycin. All the ESBL isolates were sensitive to Imipenem. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the commonest bacteria causing lower respiratory tract infection in adults in this centre. Multidrug resistance among the isolates was common. Periodic analysis of Sputum culture and their antibiotic sensitivity report should be made to identify the changing trends in etiological and sensitivity patterns.

Introduction

Lower respiratory tract infection (LRTIs) includes a group of disease entities namely acute bronchitis, pneumonia and exacerbations of chronic lung disease (Woodhead *et al*., 2011). LRTIs are one of the most common infectious diseases of humans (Karen caroll, 2002). They are associated with morbidity and mortality worldwide Anuradha mokkapati and mainly affect older individuals and those with chronic diseases or immunodeficient patients (Bhattacharya, 2006). Age, gender and season can affect the occurrence of LRTIs (Salman *et al.*, 2015). Pneumonia in adults occurs in 4% of Indians with male to female ratio of 1.56:1.14 (WHO, South East Asia region, 2012) and an annual

incidence rate of (1.12–3.16 per 1000 people), which is increased as age advances (Sowmya *et al.*, 2016). For diagnosis of LRTIs, expectorated sputum is the important sample received in the laboratory. Sputum can be easily and non-invasively obtained from the patients (Anuradha mokkapati and Madhavi Yalamanchili, 2013). It is important to find out the bacterial profile of LRTIs and determine the antimicrobial resistance pattern of the etiological agents. This will guide the clinician in giving the antibiotic therapy and also to watch the change in trend of these infections (Salman khan *et al*., 2015). Good communication between the treating clinician and the clinical microbiologist helps in the effective treatment of the patient.

This study was designed to study the occurrence of bacterial pathogens causing lower respiratory tract infections in adults at a tertiary care hospital in Chennai and determine the antibiotic susceptibility pattern of the isolated pathogen including detection of MRSA, ESBLs and MBLs.

Materials and Methods

This prospective observational study was conducted for a period of 6 months from March to August 2015 at a tertiary care hospital, Chennai, after obtaining due approval from the Institutional ethics committee. A total of 830 samples were collected during the study.

Inclusion criteria

1. Patients clinically suspected for LRTIs.
2. Patients above 18 years of age(adults)

Exclusion criteria

1. Patients suffering from tuberculosis
2. Children suffering from LRTIs
3. Patients who had received antibiotics before sputum could be sent for culture and sensitivity

Informed consent was obtained from the patients and strict confidentiality about the patient details was maintained.

Sample collection

Sputum – expectorated or induced (Mackie, Sandeep Kumar *et al*., 2014)

Deeply coughed out or when the sputum is scanty it was induced with saline nebulisation and was collected in a disposable leak proof sterile, wide mouthed container with tight fitting lid after giving proper instruction to the patient. Spontaneous early morning sputum is preferred as it contains pooled overnight secretions (ICMR guidelines). The specimen should be sent to the laboratory as quickly as possible.

Sample Processing

Macroscopic examination[mackie]

The sputum was examined for colour (rusty, red currant jelly was noted), consistency, purulent/non purulent to distinguish it from saliva.

Direct microscopy

The Sputum specimens were subjected to microscopic examination using standard laboratory techniques. Gram staining was done and examined for the presence of relative number of polymorphonuclear cells and squamous epithelial cells.

Criteria for assessing the quality of respiratory samples (Koneman *et al*., 2006)

Bartlett's grading

Number of neutrophil (LPF)	Grade
<10	0
10 - 25	+1
>25	+2
Presence of mucus	+1

Number of epithelial cells	Grade
10 - 25	-1
>25	-2

Total number of polymorphonuclear cells and epithelial cells in 20-30 LPFs was calculated and the total score was seen. A final score of 0 or less indicated lack of active inflammation or contamination, and a score of 1 and above were considered an acceptable sample.

Sputum culture

Sputum samples were then plated into the following agar media: Nutrient agar, 5% Sheep blood agar, Chocolate agar and MacConkey agar. All cultures were incubated at 37°C under aerobic condition and addition to this blood agar and Chocolate agar plates were kept under 5-10% carbon dioxide atmosphere. Plates were evaluated for growth at 24 and 48hours. Bacterial isolates grown in culture were identified by means of Gram's staining and biochemical reactions by standard microbiological techniques.

Antibiotic Susceptibility Testing (Wayne, 2015)

Antibiotic sensitivity testing was done on Mueller Hinton agar using Kirby Bauer disk diffusion method. Interpretation of the results was done by measuring the sizes of the zone of inhibition according to CLSI guidelines 2015(M-100-S25). Quality control strains used are as follows: ATCC 25922 *Escherichia coli*, ATCC 27853 *Pseudomonas aeruginosa* and ATCC 25923 *Staphylococcus aureus*.

Tests to detect methicillin resistant *Staphylococcus aureus* (MRSA) (Amutha Chelliah *et al*., 2014)

Cefoxitin disc (30ug) diffusion test

The test is performed with 30 µg of cefoxitin per disc placed on 25ml Mueller Hinton agar plate. The zone of inhibition is determined after 24 hrs of incubation at 37˚C. The zone size is interpreted according to CLSI guidelines.

Susceptible ≥22mm
Resistant ≤ 21mm

Quality control used for MRSA detection

ATCC *S. aureus* 43300 (positive control)
ATCC *S. aureus* 25923 (negative control)

Detection of Extended Spectrum Beta Lactamases

All Enterobacteriaceae isolates were screened for betalactamases production by disk diffusion method (Veena *et al*., 2013) and confirmed by Phenotypic confirmatory disc diffusion test (Veena *et al*., 2013) (Maninder Kaur *et al*., 2013) (Manisha sahu *et al*., 2014)

Disk diffusion methods-screening for ESBL

Disk diffusion test was done for all Enterobacteriaceae isolates against Cefotaxime (30 µg), Ceftriaxone (30 µg), and Ceftazidime (30 µg) antibiotic disks for the screening of the isolates for potential ESBL production.

Overnight incubation was done at 37˚C after which the zone size was read as per CLSI recommendations for ESBL screening criteria in which the isolates showed resistant to two or more 3rd generation Cephalosporins.

Antibiotics	Zone of inhibition – interpretation
Cefotaxime (30µg)	≤27mm
Ceftriaxone(30µg)	≤25mm
Ceftazidime(30µg)	≤22mm

Quality controls were performed using
Klebsiella pneumoniae ATCC 700603 - Positive control

Phenotypic confirmatory disc diffusion test

This is done in the isolates positive in the screening test. Ceftazidime (30 µg) antibiotic discs with and without clavulanic acid (10 µg) were used. These discs were placed on a Mueller –Hinton agar plate inoculated with bacterial suspension equivalent to 0.5 McFarland standards. Overnight incubation was done at 37°C after which the result was interpreted as follows:

If the zone diameter of Ceftazidime with clavulanic acid was increased ≥ 5 mm when compared with Ceftazidime alone was taken as positive for ESBL production.

Phenotypic detection of MBL (manisha sahu *et al*., 2014)

Phenotypic detection of MBL was carried out using Imipenem (10mcg) and Imipenem (10mcg) +EDTA (750mcg) discs

Statistical Analysis

The test outcome was observed, recorded and analysed. The data, that were analysed and presented in the form of statistical tables if necessary in appropriate places. P values were calculated by Chi –Square test to compare the proportion between categorical variables. The significant findings was further discussed in detail and compared with other similar studies published in reputed scientific journals. The clinical application of these findings will be stressed for better patient care.

Results and Discussion

Out of the 855 samples, 25 were rejected due to oral contamination and rest 830 samples were collected for the growth in culture to study the common bacterial pathogens and its antibiotic sensitivity.

Klebsiella pneumoniae was the most common bacteria found to be causing lower respiratory tract infection. 245(57.5%) samples were found to show growth for Klebsiella. *Pseudomonas* spp were found in 123(28.8%) samples which was the second commonest and *Staphylococcus aureus* in 22(5%) samples. In this study, Growth of yeasts was present in 8 samples but they were present as a mixed growth along with Gram negative bacteria and so were reported to correlate it clinically.

Investigation as a part of diagnostic procedures done in the microbiological lab is needed in bringing out the antibiotic

sensitivity pattern. An accurate appreciation of the severity of illness is critical in making decisions regarding antibiotic prescription. According to WHO, antimicrobial resistance is one of the three greatest threats to human life (Gilbert *et al*., 2010). Broad spectrum antibiotics are used as initial empirical therapy in most hospital set up. Knowledge of the prevalence of pathogens in the local context can help to devise an antibiotic policy. This can reduce mortality and prevent development of complications (Kollef *et al*., 2008). Broad spectrum therapy can be narrowed down after the culture reports. Examination of expectorated sputum has been the primary means of determining the bacterial pathogens. Good sputum samples depend on thorough healthcare worker education and patient understanding (Fuseliar *et al*., 2002).

In this study Male contributed predominantly to about 57.8% (Table 1) and female for about 42.2% in this study. Age wise distribution studies were also done (Table 2) and the age group from 41-50 yrs was found to have the maximum number of cases followed by 51-60 yrs of age group.

In the present study, 426(51%) showed growth. This is almost similar to study by Salman khan *et al*., 2015 (49.3%), (Tamang *et al*., 2005 -50.4%) whereas study by Anuradha Mokkapati *et al.*, showed culture positivity of 61.66%, In our study, direct Gram stain results correlated with pathogens isolated in culture in 70% of cases.

Clinical importance of species level identification is important as they differ in antibiotic susceptibility. As per Table 3 and Table 4, *Klebsiella pneumoniae* is the major cause of all the LRTI accounting for about 57.5% of all cases. This is in concordance with Anuradha Mokkapati *et al*., 2013 and Shashidhar *et al*., 2013. The role of *Pseudomonas aeruginosa* is also considerable with about 28.8% of cases. This is similar to Shashidhar *et al*., 2013 in which *Pseudomonas aeruginosa* is the second common pathogen. *Staphylococcus aureus* isalso being implicated in LRTIs (5%).In study by Salman *et al*., 2015, *Pseudomonas aeruginosa* is the commonest organism. As per this study, 391(91.8%) samples were monomicrobial and 35(8.2%) showed mixed growth (polymicrobial). As per Salman khan *et al*., 2015, 80% were monomicrobial and 20% showed mixed growth.

As per table 5, amikacin is sensitive in 87% of *Klebsiella* and 70% of *Pseudomonas*. Piperacillin-tazobactumare beta-lactamase stable and are alternatives to the penicillins like ampicillin. About 90% of *Klebsiella pneumoniae* and 95.6% of *Pseudomonas aeruginosa* were sensitive to Piperacillin-tazobactum. About 67% of *Klebsiella pneumoniae* and 69.5% of *Pseudomonas aeruginosa* were sensitive to Ceftazidime.

Table.1 Gender distribution

Gender	Positive	Negative	Total
Male	254	226	480
Female	172	178	350
Total	426	404	830

chi square= 1.154 p=0.2841

Positivity with respect to gender distribution is not statistically significant

Age Distribution: The study covered people from adult age group

Table.2 Age distribution

Age Group	Positive	Negative	Total
19-20	6	5	11
21-30	47	44	91
31-40	67	58	125
41-50	104	102	206
51-60	94	90	184
61-70	74	72	146
71-80	32	29	61
81-90	2	4	6
Total	426	404	830

chi square =1.204 p=0.9908

The outcome positivity and negativity with respect t o different age is not statistically significant

Table.5 Sensitivity pattern of Gram negative bacilli

Antibiotics	***Klebsiellla pneumoniae* (n=245)**	***Pseudomonas aeruginosa* (n=123)**
Amoxycillin	12 (4.76%)	8 (6.66%)
Cephalexin	23 (9.52%)	8 (6.66%)
Ceftazidime	164(67%)	85(69.5%)
Piperacillin-tazobactum	220 (90%)	117 (95.6%)
Ciprofloxacin	115(47%)	71 (58%)
Gentamicin	159(65%)	80 (65%)
Amikacin	213 (87%)	86 (70%)
Imipenem	245 (100%)	119 (97%)

Table.6 Percentage of ESBL (n= 259 of Enterobacteriaceae)

Isolates	Combined disc test positive	ESBL %
259	85	33%

Table.7 MBL detection

Isolates	MBL +ve	MBL %
n=133	4	3.2%

Table.3 Gender wise occurrence of organisms

	Total Cases	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	Escherichia coli	*Staphylococcus aureus*	CONS	Acinetobacter	Enterococcus	Total
Male	480	155	69	6	12	4	6	2	254
Female	350	90	54	8	10	4	4	2	172
Total	830	245	123	14	22	8	10	4	426

chi square=4.318 p=0.6338

There is no statistical significance of the occurrence of different organisms with respect to gender

Table.4 Age wise distribution of organisms

Age Group	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	Escherichia coli	*Staphylococcus aureus*	CONS	Acinetobacter	Enterococcus	Total
19-20	5	1	0	0	0	0	0	6
21-30	6	15	1	1	4	0	0	27
31-40	39	14	3	6	1	4	0	67
41-50	68	34	2	5	1	3	1	114
51-60	66	26	2	6	1	3	0	104
61-70	42	23	3	3	1	0	2	74
71-80	17	10	3	1	0	0	1	32
81-90	2	0	0	0	0	0	0	2
Total	245	123	14	22	8	10	4	426

chi square=65.84 p=0.018

There exists a statistical significance in occurrence of different organisms with respect to different age group

Table.8 Sensitivity of *Staphylococcus aureus*

Antibiotics	*Staphylococcus aureus* (n=20)
Ampicillin	5
Gentamycin	12
Amikacin	16
Ciprofloxacin	12
Doxycycline	12
Erythromycin	28
Vancomycin	20

About 65% sensitivity is found in Klebsiella and *Pseudomonas* for Gentamicin. For Ciprofloxacin, *Klebsiella pneumoniae* shows 47% sensitivity and *Pseudomonas aeruginosa* showing 58%.100% of *Klebsiella* and 96.8% of *Pseudomonas* being sensitive to Imipenem. ESBL was detected to be 33% (Table 6) and MBL was 3.2% (Table 7). This is in contrast to Shashidhar Viswanath *et al*., 2013 which showed 65% of ESBL.

Staphylococcus aureus showed 80%sensitivity to Amikacin, 60% to Gentamicin and Doxycycline and 100% sensitivity to Vancomycin, MRSA being 25%.

In conclusion, the study revealed *Klebsiella pneumoniae* to be the most common etiological agent behind the LRTIs.

Imipenem is the most sensitive drug, next sensitive being Piperacillin-tazobactum and Amikacin against the bacterial pathogens causing LRTIs and should be used for the empirical therapy.

Amoxycillin and Cephalexin have shown very low sensitivity to most of the bacterial pathogens and should be avoided so as to prevent failure of treatment.

Periodic analysis of the sputum culture and their antibiotic sensitivity report should be made so that changing trends in the etiological and sensitivity patterns can be identified and therapy adjusted accordingly so that emergence of resistance will be prevented. Strict infection control measures should also be followed to contain hospital acquired infections.

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How to cite this article:

Amutha, C., M. Suganthi, K. Radhika, K.V. Leela, J. Jayachitra and Padmanaban. 2017. Bacterial Profile of Lower Respiratory Tract Infections in Adults and their Antibiotic Susceptibility Pattern with Detection of MRSA, ESBLs and MBLs. *Int.J.Curr.Microbiol.App.Sci.* 6(3): 631-639. doi: https://doi.org/10.20546/ijcmas.2017.603.073