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Isolation of Respiratory Pathogens and Antibiotic Sensitivity Study in Clinical Samples of Patients Attending Tertiary Care Hospital, Tirupati

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Abstract: *Lower Respiratory Tract Infection (LRTI) is one of the leading causes of morbidity and mortality globally. Present study was undertaken to isolate etiological agents and study their antibiotic sensitivity pattern. The study was carried out to isolate and identify the common bacteria causing lower respiratory tract infections among patients attending to S.V.R.G.G. Hospital, Tirupati from January to December, 2014. Specimens coming to the laboratory were processed by standard laboratory procedures. From a total number of 548 sputum samples processed, culture negative for pathogenic organisms 170 in number, no bacterial growth seen in 22 and Candida species isolated from 50 samples were excluded from our study. Out of total 548 samples, the pathogens identified were 306 (55.8%). Males were found to be more in number, 176 (57.5%) than females, 130 (42.5%). Pathogenic organisms isolated were Streptococcus pneumoniae 124 (40.5%), Klebsiella pnuemoniae 98 (32%), Staphylococcus aureus 34 (11.1%), Esch. coli 17 (5.6%), Pseudomonas aeruginosa 17 (5.6%), Moraxella species 10 (3.3%), Acinetobacter species 5 (1.6%) and Proteus mirabilis 1 (0.3%). Antibiotic sensitivity testing was done for all 306 pathogenic isolates. Antibiotic discs used were amikacin, cotrimoxazole, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, levofloxacin and piperacillin/tazobactam. Carbenicillin was used for Pseudomonas species, ampicillin and amoxicillin were added for Gram positive organisms, vancomycin and imipenem for resistant isolates.*

As per present study in lower respiratory tract infections, the predominant species isolated was Streptococcus pneumoniae followed by Klbesiella pneumoniae. In view of emergence of drug resistant organisms, judicious use of antibiotics is to be advocated and followed up to bring down the morbidity and mortality specially due to lower respiratory tract infections being the commonest and an alert is given for the dire necessity of antibiotic policy.

Key words: *Antibiogram, Bacterial isolates, Lower respiratory tract infections (LRTIs).*

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I. Introduction

Lower respiratory tract infections are the commonest of all the infections and are responsible for increasing morbidity and mortality globally [1]. Lower respiratory tract infections place a considerable strain on the health budget and are generally more serious than upper respiratory tract infections. Since 1993 there has been a slight reduction in the total number of deaths from lower respiratory tract infection. However, in 2002, they were still the leading cause of deaths among all infectious diseases and they accounted for 3.9 million deaths worldwide and 6.9% of all deaths that year (WHO, 2004) [2]. Microorganisms gain access to the lower respiratory tract in several ways. The most common is by aspiration from oropharynx. Many pathogens are inhaled as contaminated droplets [3]. Acute lower respiratory infections cause most of the respiratory disease-associated deaths worldwide and pneumonia kills significantly more children than any other illness [4]. Over the past decade, there has been an increase in Hospital acquired pneumonia caused by multidrug-resistant pathogens such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii* and *methicillin resistant Staphylococcus aureus* (MRSA) [5]. Specimens should be delivered to the laboratory promptly and processed without delay (within 1 hour of collection). If delays are unavoidable, the specimen should be refrigerated. *Streptococcus pneumoniae*, the most common aetiology of pneumonia is very susceptible to the conditions outside the body and may be missed by the culture when the sample is not plated immediately. There are many ways to assess the quality of respiratory tract specimens. A simple screening method involves assessment of

squamous epithelial cells only which are found in the oropharynx but not in the lower respiratory tract. Increased numbers (>10/10x objective microscopic field) indicate gross contamination with oro-pharyngeal contents, which includes usual members of the oral bacterial microbiota Detection of a potential pathogen in a grossly contaminated specimen may represent contamination with an oro-pharyngeal microbiota [6]. Infections in the lower respiratory tract usually occur when infecting organisms reach the lower airways or pulmonary parenchyma by bypassing the mechanical and other nonspecific barriers of the upper respiratory tract. Infections may result from inhalation of infectious aerosols, aspiration of oral gastric contents, or by haematogenous spread [7]. When infection develops through the respiratory tract some compromise of the upper air-way mechanisms for filtering or clearing inhaled infectious agents usually occurs. The most common compromises are those that impair the epiglottic and cough reflexes, such as drugs, anaesthesia, stroke and alcohol abuse. Toxic inhalations and cigarette smoking may also interfere with the normal mucociliary action of the trachea-bronchial tree [8]. LRTIs have been attributed to account for almost 20% mortality among the infectious disease deaths in India as reported by World Health Organization (WHO) [9]. Since Tirumala Tirupati is a pilgrim centre there is every possibility of spreading the infection fast among the other people. Hence needed the present study among the patients coming to Sri Venkateswara Ramnarayana Ruia Government General Hospital which is a tertiary care hospital situated at the foothill of Tiruamala Tirupati.

II. Material and Methods

548 clinical samples of sputum collected during the period between January to December, 2014 from S.V.R.R.G.G. Hospital, Tirupati were isolated and characterized by the standard methods by doing direct Gram staining, culturing on nutrient agar, MacConkey agar and blood agar. The isolated strains were first identified by colony morphology, Gram staining, catalase test, oxidase test, sugar fermentation tests, IMViC reactions, urease test, nitrate reduction test and TSI agar tests. All the isolated pathogenic organisms were subjected to antibiogram by Kirby-Bauer's method of disc diffusion as per the NCCLS guidelines [10]. Various antibiotics used for testing were amikacin (30µg), cotrimoxazole (25µg), ceftriaxone (30µg), ceftazidime (30µg), ciprofloxacin (5µg), Piperacillin/Tazobactam (100µg), Levofloxacin (5µm), gentamycin (10µm). Carbenecillin (100µg) is used for Pseudomonas species. Ampicillin and amoxycillin and were added for Gram positive organisms. Vancomycin (30µg) and imipenem (10µg) were used for resistant isolates.

III. Results

Out of 548 samples, culture negative for pathogenic organisms 170 in number, no bacterial growth seen in 22 and Candida species isolated from 50 samples were excluded from our study. Detailed study and antibiotic sensitivity testing was done for the remaining 306 significant pathogens. Males were found to be more in number (176) (57.5%) than females (130) (42.5%). (Table No.1) Out of 306 isolates, 158 isolates were Gram-positive (51.6%) and 148 were Gram-negative (48.4%). (Table No. 2)

Table No. 1 Sex-wise distribution of the samples tested

Sex	No. of samples	Percentage (%)
Males	176	57.5
Females	130	42.5
Total	306	100.0

Table No. 2 Gram's reaction-wise distribution of the samples tested

Gram's reaction	No. of pathogens	Percentage (%)
Gram-positive	158	51.6
Gram-negative	148	48.4
Total	306	100.0

Table No. 3 Occurrence of bacterial pathogens from patients in relation to age

Age group (in years)	No. of pathogens	Percentage %
Less than 10	4	1.3
11-20	13	4.3
21-30	24	7.8
31-40	72	23.5
41-50	61	19.9
51-60	64	20.9
61 and above	68	22.2
Total	306	100.0

More no. of cases (72) (23.5%) belonged to 31-40 age group followed by that of above 60 (68) (22.2%).

Table No. 4 Bacterial pathogens isolated from the lower respiratory tract of the patients

S. No.	Name of the isolate	No. of the isolates	Percentage (%)
1	*Streptococcus pneumoniae*	124	40.5
2	*Klebsiella pneumonia*	98	32.0
3	*Staphylococcus aureus*	34	11.1
4	*Pseudomonas aeruginosa*	17	5.6
5	*Escherichia coli*	17	5.6
6	*Moraxella species*	10	3.3
7	*Acinetobacter species*	5	1.6
8	*Proteus mirabilis*	1	0.3
	Total	306	100.0

Streptococcus pneumoniae was found to be highest in percentage (40.5%) followed by *Klebsiella pneumoniae* (38%) and *Staphylococcus aureus* (11.1%).

Table No. 5 Antibiotic susceptibility pattern of the isolates to commonly used antibiotics

Antibiotics	*Streptococcus pneumoniae* (124)	*Staphylococcus aureus* (34)	*Klebsiella pneumoniae* (98)	*Pseudomonas aeruginosa* (17)	*Esch. coli* (17)	*Moraxella spp.* (10)	*Acinetobacter spp.* (5)	*Proteus mirabilis* (1)
Amikacin (30 µm)	120 (96.8%)	16 (47.1%)	52 (53.1%)	15 (88.2%)	9 (52.9%)	8 (80%)	4 (80%)	1 (100%)
Amoxycillin (30µm)	64 (51.6%)	7 (20.6%)	10 (10.2%)	1 (5.9%)	6 (35.3%)	4 (40%)	2 (40%)	0
Ampicillin (10 µm)	30 (24.2%)	7 (20.6%)	10 (10.2%)	1 (5.9%)	0	3 (30%)	2 (40%)	0
Ceftriaxone (30 µm)	98 (79%)	17 (50%)	51 (52%)	6 (35.3%)	6 (35.3%)	4 (40%)	3 (60%)	0
Ceftazidime (30 µm)	101 (81.5%)	18 (52.9%)	51 (52%)	5 (29.4%)	11 (64.7%)	4 (40%)	3 (60%)	1 (100%)
Ciprofloxacin (5 µm)	40 (32.3%)	11 (32.4%)	42 (42.9%)	4 (23.5%)	7 (41.2%)	2 (20%)	3 (60%)	0
Cotrimoxazole (25 µm)	51 (41.1%)	8 (23.5%)	42 (42.9%)	4 (23.5%)	3 (17.6%)	4 (40%)	1 (20%)	0
Piperacillin/ Tazobactam (100/10 µm)	123 (99.2%)	32 (94.1%)	96 (98%)	15 (88.2%)	14 (82.4%)	8 (80%)	5 (100%)	1 (100%)
Levofloxacin (5 µm)	120 (96.8%)	30 (88.2%)	80 (81.6%)	13 (76.5%)	11 (64.7%)	7 (70%)	4 (80%)	1 (100%)
Imipenem (10 µm)	124 (100%)	34 (100%)	98 (100%)	15 (88.2%)	14 (82.4%)	10 (100%)	5 (100%)	1 (100%)
Vancomycin (30µm)	124 (100%)	34 (100%)	–	–	–	–	–	–
Gentamycin (10 µm)	32 (25.8%)	10 (29.4%)	50 (51%)	4 (23.5%)	5 (29.4%)	4 (40%)	1 (20%)	0
Carbenicillin (100 µm)	–	–	–	17 (100%)	–	–	–	–

More than 50% sensitivity was observed for the antibiotics amikacin, ceftriaxone, ceftazidime, piperacillin/tazobactam, imipenem and vancomycin (for Gram-positive isolates).

IV. Discussion

LRTIs are one of the commonest causes of morbidity and mortality among the population showing multidrug-resistance. Attention has now been focused on them because of their changing patterns of resistance. In this study 306 pathogenic isolates were identified as cause for lower respiratory tract infections. Males were more in number 176 (57.5%) than females 130 (42.5%). This could be because of more prevalent associated predisposing risk factors of pneumonia such as cigarette smoking and chronic alcoholism in males. Similar findings were observed in other studies of Preeti Srivastava and Pappu Kumar et al [11], Nihan Ziyade [12] and Christopher et al [13].

More number of cases belonging to 31-40 year age-group, 72 (23.5%) might be due to exposure to the risk factors such as drugs, anaesthesia, toxic inhalations, stroke and alcohol abuse, followed by above 60 year age-group, 68 (22.2%) due to waxing and waning of immunity. Least percentage was seen in less than 10 year age-group followed by 11-20 year age-group. Similar prevalence of infection was noticed in other studies like

Preeti Srivastava and Pappu Kumar et al [11] and Christopher et al [13] might be due to decreased immunity and compromised immune system in that age group. (Table No. 3).

In our study, out of 306 isolates the commonest pathogens isolated were *Streptococcus pneumonia*e, 124 (40.5%) followed by *Klebsiella pnuemoniae*, 98 (32%). Other species isolated were *Staphylococcus aureus* 34 (11.1%), *Escherichia coli* 17 (5.6%), *Pseudomonas aeruginosa* 17 (5.6%), *Moraxella* species 10 (3.3%), *Acinetobacter* species 5 (1.6%) and *Proteus mirabilis* 1 (0.3%). (Table No. 4). Similar results were observed in a study done by Dr. D.W. Taura and Dr. A. Hassan et al [14] from Nigeria. They observed *Streptococcus pneumoniae* 11 (25.6%), *Klebsiella pneumoniae* 9 (20.9%), *Staphylococcus aureus* 7 (16.3%), *Proteus* species 2 (4.7%), *Psedomonas aeruginosa* 2 (4.7%) and *Serratia* species 1 (2.3%). In another study conducted by C. Manikandan and A. Amsath [15] it is observed that out of 337 isolates studied, *Streptococcus pneumoniae* were 121 (36%), *Klebsiella pneumoniae* 96 (28.4%), *Staph. aureus* 81 (24%), *Psedomonas aeruginosa* 37 (11%) and *Escherichia coli* 2 (0.6%).

In our present study of the antibiotic susceptibility testing of the clinical isolates to routinely used antibiotic discs, Gram-positive organisms showed 100% susceptibility to Vancomycin and imipenem. For *Streptococcus pneumoniae,* susceptibility was to amikacin (96.8%), ceftazidime (81.5%), ceftriaxone (79%), piperacillin/tazobactam (99.2%) and levofloxacin (96.8%). Less than 50% sensitivity was seen with gentamicin, cotrimoxazole, ciprofloxacin and ampicillin. C. Manikandan and Amsath et al [15] showed similar sensitivity pattern for *Streptococcus pneumoniae*. *Klebsiella pneumoniae* showed 100% sensitivity to imipenem in our study followed by piperacillin/tazobactam (97.9%), levofloxacin (81.6%), amikacin (53.1%), ceftriaxone (52%) and ceftazidime (52%). Least sensitivity was seen with ampicillin, amoxycilln, ciprofloxacin and cotrimoxazole. (Table No. 5). Similar type of sensitivity pattern was observed in the study of Preethi Srivastava et al [11].

In our study *Staphylococcs aureus* showed 100% sensitivity to vancomycin and imipenem, more than 50% susceptibility to ceftazidime (52%), piperacillin/tazobctam (94.1%), levofloxacin (88.2%), ceftriaxone (50%) and ceftazidime (52%). Least sensitivity was to amoxycillin, ampicillin, gentamicin, cotrimoxazole, ciprofloxancin showing multi drug resistance (MDR). [Table No. 5]. This might be due to hospital acquired infections. Similar type of MDR was seen in the study observed by Dr. D.W. Taura and Hassan et al of Nigeria [14].

In our study *Pseudomonas aeruginosa* showed 100% sensitivity to carbenicillin followed by amikacin, imipenem, piperacillin/tazobactam (88.2% each), levofloxazone (76.5%), ceftazidime (38%) and ceftriaxone (36%). Gentamycin showed 23.5%, ciprofloxacin 23.5%, cotrimoxazole 23.5%, amoxycillin 2% and ampicillin 2% sensitivity. It was showing MDR pattern giving an alarm to stringent implementation of antibiotic policy in the tertiary care hospital. (Table No. 5). This was compared to the similar study observed by Dr. D.W. Taura and Hassan et al of Nigeria [14] and Preeti Srivastava and Pappu Kumar et al too [11].

Esch. coli showed sensitivity pattern of 82.4% to piperacillin/tazobactam and imipenem, 64.7% to Levofloxacin and ceftazidime, 52.9% to amikacin. Less than 50% sensitivity was seen for other antibiotics. *Moraxella* species showed more than 50% sensitivity to piperacillin/tazobactam, amikacin and levofloxacin, 100% to imipenem. (Table No. 5). This type of study correlated with other studies by SK. Mishra, HP Kattel et al, Nepal [16]. He showed *Esch. coli* 6.9% and *Moraxella catarralis* 4.1% of the total isolates. Only 5 isolates of *Acinetobacter* species and a single isolate of Proteus mirabilis were accounted for in our study.

In the present study, most of the isolates shown in Table No. 5 were sensitive to imipenem, levofloxacin, piperacillin/tazobactam, amikacin, ceftriaxone and ceftazidime. Resistant pattern was for amoxycillin, ampicillin, cotrimoxazole and ciprofloxacin.

V. Conclusion

As per the present study of lower respiratory tract infections the predominant species isolated was *Streptococcus pneumoniae* followed by *Klbesiella pneumoniae*. In view of emergence of drug resistant organisms, judicious use of antibiotics is to be advocated and followed up in the tertiary care hospital to bring down the mortality and morbidity especially due to lower respiratory tract infections which are the commonest and an alert is given for the dire necessity of antibiotic policy. Health sectors should be encouraged and educated to go for appropriate antibiotic sensitivity testing before going for treatment proper.

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