ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 03 | March - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

BACTERIAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF LOWER RESPIRATORY TRACT INFECTION AMONG PATIENTS ATTENDING DARBHANGA MEDICAL COLLEGE AND HOSPITAL, BIHAR.

Microbiology

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ABSTRACT

Lower respiratory tract infections are the commonest health problem demanding frequent consultation and hospitalization. Moreover, in recent years, there has been dramatic rise in antibiotic resistance among respiratory pathogens. **Objective:** To determine bacteriologically confirmed burden, profile and antibiotic susceptibility pattern of bacteria causing lower respiratory tract infections. **Materials and Methods:** This study was conducted over a time period of one and a half years in the department of Microbiology, DMC, Darbhanga. Sputum samples were collected by using convenient sampling technique and inoculated on to MacConkey agar, Chocolate agar and blood agar and then incubated for 24 hrs at 37oc. Biochemical tests were performed and antimicrobial susceptibility testing was done using disk diffusion method. **Results:** From a total of 240 samples processed 77 (32.1%) showed growth of various species of bacteria. Among the isolates, *K. pneumoniae* 28 (36.4%) was the most isolated organism, *S. pneumoniae 15* (19.5%) was next, followed by *E. coli* 10 (12.9 %).Gram-negative bacilli were highly sensitive to Meropenem (98%), Tobramycin (93 %), Amikacin (90%), ceftazidime (90%) and Ceforoxime (90%) while highly resistance to Ampicilin (83%), Tetracyclin (53%), Ciprofloxacilin (34%) and Trimethoprim-sulfamethoxazole (31%). **Conclusion:** Even though, the results obtained in this study indicated that some of the antibiotics used to treat respiratory tract infections in this community are effective, but still there is danger of drug resistance which need to be tackled. Hence regular monitoring of LRTI etiologies and antimicrobial drug resistance testing is essential to maximize the shelf life of effective drugs.

KEYWORDS

Lower Respiratory Tract Infection, Bacterial Agents, Antimicrobial susceptibility patterns.

INTRODUCTION :

Respiratory tract is the part of the human system that plays a vital role in breathing processes. The respiratory system can be subdivided into an Upper respiratory tract and a Lower respiratory tract based on anatomical features. The respiratory tract is constantly exposed to microbes due to the extensive surface area. Respiratory tract infections are the most frequently reported of all human infections [1]. Respiratory tract infection is one of the most important infectious diseases worldwide. This infection is the leading cause of morbidity and mortality in critically ill patients especially in developing countries. Respiratory tract infections are usually contracted through air and by direct contact [2].

Lower respiratory tract infection is a broad description of a group of disease entities, encompassing acute bronchitis, pneumonia and exacerbations of chronic lung disease, and it may be defined as those infections presenting with symptoms including cough, expectoration, dyspnoea, wheeze and /or chest pain/discomfort usually for a period ranging from 1-3 weeks. Acute manifestations of lower respiratory tract infections that may or may not involve lungs include acute bronchitis, bronchiolitis, influenza, community –acquired pneumonia either with or without radiological evidence, acute exacerbation of chronic obstructive pulmonary disease (COPD) and acute exacerbation of broncheictasis [3].

The most common bacteria that causes lower respiratory tract infection are *Streptococcus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Enterobacter* spp, *Acinetobacter* spp and *H. influenza* [7].

Use of antibacterial drugs has become widespread over several decades (although equitable access to antibacterial drugs is far from being available worldwide), and these drugs have been extensively misused in humans in ways that favour the selection and spread of resistant bacteria. Consequently, antibacterial drugs have become less effective or even ineffective, resulting in an accelerating global health security emergency that is rapidly outpacing available treatment options [8-12].

DATA ANALYSIS:

Data's were entered and analyzed using SPSS version 20. Simple frequencies were applied to see the distribution of the socio demographic variables. Proportions of categorical variables were compared by Chi square. P values< 0.05 was considered statistically significant and results were presented by words and tables.

Ethical Consideration:

Ethical clearance was obtained from the Ethical Committee of this Institute.

MATERIALS AND METHODS:

Sputum samples of Two hundred and fourty patients patients presenting with symptoms of LRTI (productive cough and dyspnea) were collected in wide - mouthed sterile containers by standard collection procedures after informing them to rinse their mouth with water. Quality of expectorated sputum samples were assessed by Bartlett's scoring method and checked for pulmonary tuberculosis by using the Ziehl-Neelsen (ZN) staining technique in the Department of Microbiology. Then only suitable and pulmonary tuberculosis negative sputum samples were transported immediately to Bacteriology Laboratory of EPHI by using triple package transportation system and inoculated on to MacConkey agar and incubated at 37°C for 18-24 hours, Blood agar (BAP) and Chocolate agar (CAP) incubated in a humid, 5% CO2 atmosphere for 18–24 hours at 35°C

37°C.All the plates were initially examined for growth after 24 hrs, the plates without the growth were further incubated for up to 48 hrs. Bacterial colonies differing in size, shape and color were selected from the different plates and further sub cultured. After obtaining pure colonies, further identification was done by using the standard microbiological technique like gram stain, colony morphology and biochemical tests. Preliminary identification of bacteria were based on Gram reaction, colony characteristics of the bacteria like hemolysis on blood agar and enzyme activities of the bacteria on different substrates. Isolation of Bacteria was done by biochemical test and antimicrobial susceptibility testing.

RESULTS :

Of 240 samples processed , 77 sputum samples had bacterial growth of different species. Making a burden of 32.1%. Out of 112 males enrolled in this study, 50 (44.6%) were positive for Pathogenic

bacteria. The result shows that LRTI were more prevalent in males than in females. Among 36 smokers 21 (58.3) were positive for bacterial pathogens of LRTI.

Among the isolates, *K. pneumoniae* 28(36.4%) was the most isolated organism, *S. pneumoniae 15*(19.5%) was next, followed by *E. coli* 10 (12.9%) and *Citrobacter* 7(9.1%) (2).

The most effective antibiotic for *K. pneumoniae* was Meropenim with 100 % sensitivity (28/28), followed by Amikacin 27 (96%), Ceftazidime 26(93%) and Tobramicin 26 (93%), while high resistance rate was recorded for Ampicillin with resistance patterns of 86 % (24 isolates). Results are given in Table 1 and 2 Respectively.

DISCUSSION :

In this study, 50 (44.6%) of 112 males were positive for Bacterial pathogens of LRTI, while 27 (21.1%) of 128 females were positive for bacterial pathogens of LRTI. our finding also agrees with study conducted in India [2] and Tribhuvan University, Nepal [10] that states the possible reasons for having high prevalence of LRTI in males than In females is due to different habits of males like cigarette smoking (P. value = 0.00) and also could be females enrolled in the study comprised largely of housewives, who being less exposed to respiratory tract risk factors.

Out of the 77 bacterial isolates, gram-negative organisms were the highest of the isolates, accounting for 59 (77%), while gram-positive were 18 (23%).

In this study, 28 (36.4%) *K. pneumoniae* were isolated, which is comparable with findings in India that showed as prevalence of *K. pneumoniae* was 31.5% - 42% [3, 9, 8,7].

The second dominant isolate in this study is *S. pneumoniae* which accounts 15(19.5%). This is comparable with studies, in India (20%) [9], Nigeria (17.5%) [10] and Ethiopia (11.8%) [10].

The third dominant isolate in this study is *E.coli* and it accounts 10 (12.9%). This is comparable with other studies, in India (8.3% - 11.5%) [9, 10], Nigeria (7.1% - 7.5%) [12, 10].

But lower than the findings in India (25%) [9], (17%) [10] and (13.3%) [10].

Three (3.9%) *S.aureus* were isolated from this study, which is comparable with studies in India who reported as prevalence of *S.aureus* in lower respiratory tract infection is 5% and 2.6% [3, 5].

Two (2.6%) of *P.vulgaris* were isolated and this is almost similar with the finding in Ethiopia (0.6%) [7].Similarly one (1.3%) *Acinetobacter* and one (1.3%) *Seratia* spp were isolated, this is comparable with others works done in India 2.6% *Acinetobacter* [3] and Nigeria 4.28% *Acinetobacter* and 2.85% *Seratia* spp[10] .

S. pneumoniae and *S.aureus* were the two gram positive isolates observed on this work which contrast to the study from Nigeria [12] who reported as *S. pneumoniae* was the only Gram positive isolate.

The most predominant isolate in our study is *K. pneumoniae* followed by *S.pneumoniae*Mixed infection was observed in 4(5.2%) participants from these 3 (75%) were mixed infection of *K. pneumoniae* and *E.coli* and 1(25%) were *K. pneumoniae* and *P. aeruginosa*. Similar study from Ethiopia by the same outer in 2015 on Antimicrobial susceptibility pattern of bacterial isolates from community-acquired pneumonia patients in Jimma University Specialized Hospital, Jimma, Ethiopia, reported a mixed infection of 4.5% [12].

The susceptibility patterns of gram negative isolates were performed for antibiotics like Tobramycine (TOB) 10µg, Trimethoprim-sulfamethoxazole (SXT) 1.25/ 23.75 µg ,Cefazidime (CAZ) 30 µg,Tetracycline(TTC) 30 µg, Amoxicillin-clavulanate (AMC) 20/10 µg, Cefuroxime (CXM) 30 µg,Ceftraxone (CTX) 30 µg, Norfloxacilin (Nor)10 µg, Gentamycin (CN) 10 µg, Ciprofloxacin(CRO) 5 µg, Cefoxitin (FOX) 30 µg, Cefepime (CFP) 30 µg, Amikacin (AK)30 µg, Meropenem (Mem) 10 µg and Ampicilin (Amp)10 µg. Similarly gram positive isolates were tested for Tobramycine (TOB) 10µg, Trimethoprim-sulfamethoxazole (SXT) 1.25/ 23.75 µg, Gentamycin (CN) 10 µg, Ciprofloxacin(CRO) 5 µg,Clindamycin (CLM) 2 µg, Erytromicin 2 µg Tetracyclin (TTC)30 µg,Penicilin (pen) 10 units, Chloraphinicole (CAF) 30 µg, Oxacilin (OXA) 1 µg and Vancomycine (VAN)30 µg according to CLSI 2018 guideline

The result of the sensitivity test indicated as gram-negative bacilli shows high sensitive to Meropenem (98%), Tobramycin (93 %), Amikacin (90%), ceftazidime (90%) and Ceforoxime (90%) while high resistance level was observed to Ampicilin (83%), Ciprofloxacilin (34%), Tetracyclin (53%) and Trimethoprim-sulfamethoxazole (31%). Similarly study from India revealed as Amikacin was the most effective antibiotics for Gram negative bacilli causing LRTI [14] and also study from the same country indicates as all the gram negative isolates were resistance to antibiotics such as ampicillin, amoxycillin-clavulinic acid and trimethoprim-sulphamethoxazole [12].

The most common causative agent among gram-negative bacilli, *K.pneumonia,* was showed high sensitivity to Meropenem (100%) and Amikacin (96%), And high resistance to Ampiciline (86%), Tetracycline (46%) and ciprofloxacilin(39%), which is comparable with the study conducted in India that sowed as susceptibility pattern of *K. pneumoniae* was 94% and 90.5%, to Meropenem and Amikacin respectively [12]

In this study, 90% *E.coli,* that showed susceptibility to Tobramycin, Amoxicillin-clavulanate, Cefrotoxin, Cefpime and Meropenem was also 100% susceptible to Amikacin and 80% resistant to Ampiciline, 30% resistant to Trimethoprim-sulfamethoxazole and 40% resistant to Gentamicin, similarly study from India showed 100% sensitivity to Amikacin, Ciprofloxacin, Ceftazidime, Cefepime and Ceftriaxone [2]. P.*arogenesa*, which was 83% resistant to Ampiciline and Amoxicillin-clavulanate, 67% to Trimethoprim-sulfamethoxazole, 50% to ceftriaxozone, Gentamycine and Norfloxaciline, was 100% sensitive to Meropenem and 83 % to Tobramycine, Cefuroxime and cefpime. Comparably Study from Nigeria indicates susceptibility pattern of P.*arogenesa* 100% to Cefuroxime and Ceftazidime. Although, citrobacter showed 100% sensitivity to Amikacine and Tobramycine, 86% sensitivity to Norfloxacine and Cefroxime; it was the second isolates that showed high resistance to two or more antibiotics (86% to Ampiciline, 43% to Trimethoprim-sulfamethoxazole, Tetracycline, Ceftraxozone, Amoxicillin-clavulanate and gentamycine) next to P.*arogenesa*. Similarly study from India on Multidrug resistant Gram-negative bacilli in lower respiratory tract infections

In present study gram positive organisms showed 95% susceptibility to Oxaciline and 93% to vancomycin while high resistance was to Tetracycline (56%), Trimethoprim-sulfamethoxazole (56%) and Peniciline (50%). Similar study from India reported as 100% of gram positive organism was susceptible to Vancomycin [3]. *S. pneumoniae,* which was the second dominant isolate in this study, showed 100% susceptible to oxacilin and 40 % to trimethoprim-sulfamethoxazole; while highly resistance to peniciline 53%, Tetracycline 47% and Eretromycine 40%.

In this study, 67% *S.aureus,* that showed susceptibility to Tobramycin, trimethoprim-sulfamethoxazole, Gentamycine, Ciprofloxacin, Clindamycine and Peniciline, also showed 100 % resistance to Tetracycline and Cloramphinicole; and 60 % resistance to Erytromycine.The majority 61(83.6%) of the pathogens showed multi-drug resistant (MDR) pattern to most of the antibiotics used. This finding is comparable with study conducted in Ethiopia , that showed as multi-drug resistance pattern was 60.3% on 2014 and 62.7% on 2015[8,12].

The difference in susceptibility pattern of isolates to different antibiotics is may be due to different antibiotic usage habit of study participant and different antibiotic using policy of each country.

CONCLUSION :

In the present study the most predominant organism isolated from patients with LRTI was *Klebsiella pneumonia* (36.4%) both as single pathogen & in mixed infection. The most effective antibiotics for gram negative bacilli causing LRTI was Amikacin and Meropenim while for gram positive cocci was Vancomycine and Oxaciline. Even though, the results obtained in this study indicated that some of the antibiotics used to treat respiratory tract infections in this community are still effective, but still there is danger of drug (especially for Ampicilin and Tetracycline) resistance which need to be tackled.

Table.1. Frequency of bacteria isolates among patients attending OPD.

Bacterial Isolates	Frequency (n)	Percent (%)
K. pneumoniae	28	36.4
S. pneumoniae	15	19.5
E.coli	10	12.9
Citrobacter spp	7	9.1
P. aeruginosa	6	7.8
K. pneumoniae and E.coli	3	3.9
S. aureus	3	3.9
P. vulgaris	2	2.6
Acinetobacter spp	1	1.3
K. pneumoniae and P. aeruginosa	1	1.3
Serratia spp	1	1.3
Total	77	100

Table 2. Antibiotics Susceptibility patterns of gram negative Isolates of LRTI among patients.

Isolates	N o		Antibiotic pattern (%)													
			TOB (%)	SXT (%)	CAZ (%)	TTC (%)	CXM (%)	CTX (%)	AMC (%)	Nor (%)	CN (%)	CRO (%)	Fox (%)	CFP (%)	Ak (%)	Mem (%)	Amp (%)
K.pneumoniae	28	S	26(93)	23(82)	26(93)	18(64)	24(86)	11(79)	10(80)	11(79)	23(82)	17(61)	25(89)	26(93)	27(96)	28(100)	4(4)
		R	2 (7)	5(18)	2(7)	13(46)	4(14)	6(21)	5(18)	7(25)	5(18)	11(39)	3(11)	2(7)	1(4)	0	24(86)
E.coli	10	S	9(90)	7(70)	7(70)	4(40)	7(70)	8(80)	9(90)	7(70)	6(60)	7(70)	9(90)	9(90)	10(100)	9(90)	2(20)
		R	1(10)	3(30)	3(30)	6(60)	3(30)	2(20)	1(10)	3(30)	4(40)	3(30)	1(10)	1(10)	0	1(10)	8(80)
P.aeruginosa	6	S	5(83)	2(33)	4(67)	2(33)	3(50)	4(67)	1(17)	3(50)	3(50)	4(67)	5(83)	5(83)	4(67)	6(100)	1(17)
		R	1(17)	4(67)	2(33)	4(67)	3(50)	2(33)	5(83)	3(50)	3(50)	2(33)	1(17)	1(17)	2(33)	0	5(83)
Citrobacter	7	S	7(100)	4(57)	6(71)	4(57)	6(71)	4(57)	4(57)	6(86)	4(57)	6(71)	6(86)	6(71)	7(100)	7(100)	1(14)
		R	0	3(43)	2(29)	3(43)	2(29)	3(43)	3(43)	1(14)	3(43)	2(29)	1(14)	2(29)	0	0	6(86)
P.vulgaris	2	S	2(100)	1(50)	2(100)	1(50)	1(50)	1(50)	2(100)	2(100)	1(50)	1(50)	2(100)	2(100)	2(100)	2(100)	1(50)
		R	0	1(50)	0	1(50)	1(50)	1(50)	0	0	1(50)	1(50)	0	0	0	0	1(50)
Acinetobacter	1	S	1(100)	1(100)	1(100)	0	1(100)	1(100)	0	1(100)	1(100)	0	1(100)	1(100)	1(100)	1(100)	0
		R	0	0	0	1(100)	0	0	1(100)	0	0	1(100)	0	0	0	0	1(100)
Serratia spp	1	S	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0
		R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1(100)
Over all		S	58(93)	41(69)	50(88)	28(47)	46(78)	40(79)	44(79)	40(79)	43(75)	39(66)	51(90)	53(90)	53(96)	58(98)	10(17)
		R	4(7)	18(31)	9(14)	31(53)	13(22)	14(24)	16(28)	14(24)	16(27)	20(34)	6(10)	6(10)	6(10)	1(2)	49(83)

Key: TOB-Tobramycin, SXT-Trimethoprim-sulfamethoxazole, CAZ-Ceftazidime, TTC-Tetracycline, AMC-Amoxicillin-clavulanate, CXM-Cefuroxime, CTX - Cefotaxime, Nor - Norfloxacillin, CN - Gentamycin, CRO - Ciprofloxacin, FOX - Cefoxitin, CFP -Cefepime, AK- Amikacin, Mem - Meropenem and Amp- Ampicilin.

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