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Research Article

Lower respiratory tract pathogens and their antimicrobial susceptibility pattern in a medical hospital of central Nepal

Sony Shrestha*[1], Anju Acharya[2], Hari Prasad Nepal[3], Rajendra Gautam[4], Shamshul Ansari[5], Goma Upadhyay[6] and Avisekh Gautam[7]

[1,5]Lecturer, [2]Associate professor, [3,4]Assistant Professor, [6]Assistant Lecturer, Department of Microbiology, Chitwan Medical College (CMC), Chitwan, Nepal.

[7]Lecturer, Department of Microbiology, St. Xavier's College, Kathmandu, Nepal.

***Correspondence Info:**
Sony Shrestha
Department of Microbiology,
Chitwan Medical College (CMC), Chitwan, Nepal.
E.mail: friendlysony@gmail.com

Abstract

Background: Lower respiratory tract infection (LRTI) is one of the major causes of morbidity and mortality in young children and elderly people. It is among top ten diseases of Nepal and accounts for deaths of 2.6 million people per year.

Objectives: To identify causative agents of lower respiratory tract infection and to determine their susceptibility to antimicrobial agents, with special interest to multidrug resistance (MDR).

Materials and Methods: The study was carried out in 240 sputum specimens received in the Microbiology Laboratory of Chitwan Medical College Teaching Hospital within a period of 6 months (April- September, 2010).

Results: Total positive result was observed in 73 specimens (30.42%). Infection rate in males (58%) was higher than in females (42%). LRTI was mostly seen in age group of 61-75 years. Altogether 9 different species of bacteria were identified, majority of which were Gram-negative bacteria (73%). On performing antimicrobial susceptibility testing, Gram-positive organisms exhibited maximum sensitivity to gentamicin (100%) while Gram-negative bacteria showed variable response towards different antimicrobials. Prevalence of MDR was higher in *Enterobacter* spp (100%) followed by *Escherichia coli* (90%).

Conclusion: Varieties of pathogens are responsible for LRTI and antimicrobial resistance has become significant public health problem in central Nepal.

Keywords: Antimicrobial susceptibility, bacterial isolates, LRTI, MDR, Nepal

1. Introduction

Lower respiratory tract infections include infection of trachea, bronchi and lungs. It usually occurs when infecting organisms reach the airway of pulmonary parenchyma by bypassing the mechanical and other nonspecific barriers of the upper respiratory tract. Infection may result from inhalation of infectious aerosols, aspiration of oral or gastric contents or by heterogeneous spread[1]. Most common lower respiratory infections are acute tracheobronchitis, acute exacerbations of chronic bronchitis, and pneumonia[2] and common causative agents are *Streptococcus pneumoniae, Haemophilus influenzae, Enterococcus* spp, *Klebsiella pneumoniae* etc.

Infection of the respiratory tract is the most common human ailment. While they are a source of discomfort, disability and loss of time for adults, they are a substantial cause of morbidity and mortality in young children and the elderly people. Every year acute respiratory tract infection (ARI) in young children is responsible for an estimated 3.9

million deaths worldwide. It is estimated that Bangladesh, India, Indonesia and Nepal together account for 40% of the global ARI mortality. About 90% of the ARI deaths are due to pneumonia, which is usually bacterial origin[3].

Among 20 most frequent causes of death, ischemic heart disease and cerebrovascular disease are the leading causes of death, followed by lower respiratory infections (including pneumonia), chronic obstructive pulmonary disease and diarrhoeal diseases. It is estimated that 4.2 million deaths occurs each year due to LRTI, 17% death is due to diarrheal diseases and the same proportion to LRTI[4].

Several studies[5,6,7] have been conducted throughout the world to derive information about etiological agents of LRTI and their antimicrobial susceptibility pattern. No study can speculate exactly the situation of LRTI among the different people of different parts of world. Although there has been few literatures on documentation of LRTI in Nepal[5,8,9], causative organism and their antimicrobial susceptibility pattern is subject to change which occurs with time, place and human activities. Therefore, this study was conducted with the aim of studying the etiological agents causing LRTI in patients of all age groups and their antimicrobial susceptibility pattern with special interest to MDR in Chitwan Medical College Teaching Hospital, situated in central Nepal.

2. Materials and Methods

This was a hospital based cross sectional study conducted on 240 sputum specimens received in the Laboratory of Microbiology Department, Chitwan Medical College Teaching Hospital within a period of 6 months (April 2010-September 2010). Sputum specimen for bacteriological culture was subjected to Gram-staining and examined microscopically. In microscopic examination sputum smear containing less than 10 squamous epithelial cells and more than 25 leucocytes or pus cells per low power field confirmed the reliability of the specimen indicating that it was not contaminated with saliva. Specimens were inoculated onto MacConkey agar, blood agar and chocolate agar media[2]. *In vitro* antimicrobial susceptibility tests for the isolates were performed by modified Kirby-Bauer Disk Diffusion Technique[10] following CLSI guidelines[11]. All the plates were incubated at 37^0C and read after 24 hours of incubation. Defining criterion for MDR in our study was resistance to more than two antimicrobial agents of different chemical structure[12].

3. Results

Of the 240 sputum specimens submitted to Microbiology Laboratory of Chitwan Medical College Teaching Hospital for bacterial culture and sensitivity, 73 specimens showed the bacterial growth, of which 62 were Gram negative bacilli (GNB) and 11 were Gram positive cocci (GPC). Among 240 patients, 49 male patients and 35 female patients were infected.

Among 62 Gram negative bacilli, *Pseudomonas* spp (20) was the most common isolates obtained followed by *Klebsiella pneumoniae* (16), *Acinetobacter* spp (14), *E. coli* (10), and *Enterobacter* spp (2)

while in case of Gram positive cocci (11 in total), *Staphylococcus aureus* (5) was the most common organism followed by *Enterococcus* spp (3) and *Streptococcus pneumoniae* (3).

The highest number of culture positive cases was seen in age group of 61-75 year (table 1).

Table 1: Age wise distribution of Isolates obtained from LRTI

Age group (Year)	Bacterial isolates								Total
	Gram Negative Bacilli					Gram Positive Cocci			
	Pseudomonas spp	*Klebsiella pneumoniae*	*Acinetobacter* spp	*E. Coli*	*Enterobacter* spp	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Enterococcus* spp	
Upto 15	2	0	0	1	0	0	0	0	3
16-30	0	0	1	0	0	0	1	0	5
31-45	4	4	1	0	0	1	0	1	14
46-60	2	0	0	2	0	1	1	1	8
61-75	7	5	5	4	2	2	1	0	29
>75	5	7	7	3	0	1	0	1	26
Total	20	16	14	10	2	5	3	3	85

Antimicrobial susceptibility pattern of the isolates is presented in table 2 and table 3.

Table 2: Antimicrobial susceptibility profiles of Gram positive isolates

Antibiotic disc used	Pathogens					
	***Staphylococcus aureus* (n=5)**		***Streptococcus pneumoniae* (n=3)**		***Enterococcus species* (n=3)**	
	Sensitive (%)	**Resistant (%)**	**Sensitive (%)**	**Resistant (%)**	**Sensitive (%)**	**Resistant (%)**
Amikacin	100	0	100	0	66.66	33.33
Ampicillin	Not tested		Not tested		100	0
Ceftriaxone	Not tested		100	0	Not tested	
Cephalexin	20	80	Not tested		66.66	33.33
Cefotaxime	40	60	0	100	Not tested	
Ciprofloxacin	50	50	100	0	66.66	33.33
Erythromycin	20	80	66.66	33.33	100	0
Cotrimoxazole	0	100	0	100	Not tested	
Gentamicin	100	0	100	0	100	0
Ofloxacin	50	50	100	0	66.66	33.33
Penicillin	0	100	0	100	Not tested	
Tetracycline	80	20	100	0	100	0
Abbreviations: n- number of isolates						

Table 3: Antimicrobial susceptibility profiles of Gram negative bacilli

Antibiotics used	Pathogens														
	***Pseudomonas species* (n=20)**			***Klebsiella pneumoniae* (n=16)**			***Acinetobacter* species (n=14)**			***E. coli* (n=10)**			***Enterobacter* species (n=2)**		
	S %	I %	R %	S %	I %	R %	S %	I %	R %	S %	I %	R %	S	I	R %
AK	89	0	11	94	0	16	57	0	43	70	10	20	0	0	100
AMP	Not tested			0	0	100	25	0	75	0	0	100	0	0	100
CB	100	0	0	Not tested			Not tested			Not tested			Not tested		
CAZ	50	10	40	14	0	86	0	0	100	0	0	100	0	0	100
CTR	10	1	2	27	13	60	36	0	64	10	0	90	0	0	100
CTX	100	0	0	0	0	100	38	8	54	25	0	75	0	0	100
C	Not tested			91	0	19	70	20	10	86	0	14	0	0	100
CIP	100	0	0	63	0	37	63	0	37	0	0	100	0	0	100
COT	Not tested			29	0	71	60	0	40	17	0	83	0	0	100
G	90	0	10	60	7	33	50	0	50	60	10	30	0	0	100
OF	94	0	16	69	0	31	54	0	46	17	0	83	0	0	100
PI	29	24	47	Not tested			Not tested			Not tested			Not tested		
TOB	88	0	12	Not tested			Not tested			Not tested			Not tested		

Abbreviations: n – total number of isolates, S-sensitive, I – intermediate, R – resistant, AK- Amikacin, AMP- Ampicillin, CB- Carbenicillin, CAZ- Ceftaxidime, CTR- Ceftriaxone, CTX- Cephotaxime, C- Chloramphenicol, CIP- Ciprofloxacin, COT- Cotrimoxazole, G- Gentamicin, OF- Ofloxacin, PI- Piperacillin, TOB- Tobramycin

Prevalence of MDR was 100% in *Enterobacter* species, 90% in *E.coli*, 80% in *Staphylococcus aureus*, 69% in *Klebsiella pneumoniae*, 50% in *Acinetobacter* species, 33% in *Streptococcus pneumoniae* and 30% in *Pseudomonas* species (table 4).

Table 4: Percentage of MDR among total isolates

S. No.	Pathogens	Total isolates	No (%) of MDR
1.	*Pseudomonas* spp	20	6 (30)
2.	*Klebsiella pneumoniae*	16	11(69)
3.	*Acinetobacter* spp	14	7 (50)
4.	*E.coli*	10	9 (90)
5.	*Enterobacter* spp	2	2 (100)
6.	*Staphylococcus aureus*	5	4 (80)
7.	*Streptococcus pneumoniae*	3	1 (33)
8.	*Enterococcus* spp	3	0
Total		73	40

3. Discussion

In our study, 30.42% of total specimens showed growth on culture. The isolation rate is close to the observation of Gauchan *et al*[5] (41.4%), Sharma *et al*[14] (39.46%) and Okesola *et al*[15] (27%) while Dawadi *et al*[13] (48.43%) and Egbagbe *et al*[7] (47.2%) reported slightly higher isolation rate. Lower yield in the present study may be attributed to various factors. For example, viruses like adenovirus, respiratory syncytial virus, parainfluenza virus and rhino virus, which are significant contributors of LRTI, were not looked for in our study due to limitation of resources. Likewise, common pulmonary pathogens such as *Mycobacterium tuberculosis, Mycoplasma, Chlamydia, Pneumocystis,* Fungi, *Legionella*, and anaerobes could not be cultured by routine methods.

Among 73 bacterial isolates, altogether 8 different types of bacteria were identified. Greater number of the isolates was GNB, i.e. 62 (73%), and 11 (13%) were GPC. Of 62 gram negative bacilli in total, *Pseudomonas* spp (34%) was the most common isolate. Infection with *P. aeruginosa* is a serious problem affecting hospitalized patients, particularly those who are critically ill and immunocompromised, such as patients with cystic fibrosis[16]. However, there were intermixing of hospital acquired and community acquired LRTI cases in our study. Some studies have also pointed to the predominance of other gram negative bacilli in lower respiratory tract infection. For example, Gauchan et al[5] and Sharma et al[14] isolated *Hamemophilus influenzae* as the most common isolate while Okesola et al[15] and Egbagge et al[7] did *Klebsiella pneumoniae*. Though *H. influenzae* is regarded as a common pathogen incriminated in lower respiratory tract infection, we did not encounter any isolates of *H. influenzae* in this study probably due to different environmental conditions and inadvertent use of antibiotics.

We have observed the infection rate in males (58%) was higher than females (42%). Females enrolled in the study comprised largely of housewives. Since they were less mobile, they must have experienced less exposure to respiratory risk factors. Vulnerability of males may also be attributed to predisposing factors like smoking and alcoholism[17].

In this study majority of infected patients belonged to age group of 61-75 years, the observation being close to the findings of Serchan *et al*[5] who noticed that LRTI was found most prevalent in the age group of 50-59 year (21.3%) while Sharma *et al*[14] reported it in the age group of 51-60 year. An increasing incidence of LRTI as they get older may be due to less effective immune system in older patients owing to either malnutrition or underlying degenerative diseases such as diabetes mellitus, emphysema, uraemia etc[18,19].

The increasing resistance to antibiotics by respiratory pathogens has complicated the use of empirical treatment with traditional agents and a definitive bacteriological diagnosis and susceptibility testing would, therefore, be required for effective management of LRTI.[20]

Antimicrobial susceptibility test performed for 73 bacterial isolates in the current study showed that gentamicin

(100%) was the most effective (100% sensitivity) antibiotic against Gram-positive bacteria and penicillin the least effective one (100% resistance). For Gram- negative bacteria, susceptibility pattern was variable. In case of *Pseudomonas* spp carbenicillin (100%) was the most effective (100% sensitivity) antibiotic and piperacillin the least effective (29% sensitivity) one. *Klebsiella pneumoniae* was most sensitive to amikacin (94% sensitivity) while *Acinetobacter* spp was to chloramphenicol (70%). Acinetobacter spp exhibited 100% resistance to ceftazidine. Seventy percent of *E. coli* were sensitive to amikacin (70%) but all of them showed resistance to ampicillin. *Enterobacter* spp exhibited 100% resistance against the entire antibiotics tested. In the similar study conducted by Gauchan *et al*[5] antibiotic susceptibility test of the isolates showed that 100% Gram-negative bacteria were sensitive to chloramphenicol but only 20.6% to co-trimoxazole. Similarly, for Gram-positive bacteria, ciprofloxacin was found to be the most effective (79.2% sensitivity) antibiotic and the least was co-trimoxazole.

Emergence of serious infections due to multidrug resistant organisms poses a therapeutic challenge[21]. The prevalence of MDR in this study was 100% in *Enterobacter* spp, 90% in *E.coli*, 80% in *Staphylococcus aureus*, 69% in *Klebsiella pneumoniae*, 50% in A*cinetobacter* spp, 33% in *Streptococcus pneumoniae* and 30% in *Pseudomonas* spp. Pokhrel *et al*[8] observed that 70% of *Klebsiella pneumoniae* and 71.4% of *Pseudomonas aeruginosa* among the respiratory pathogens were having multidrug resistance. On the other hand, Sharma *et al*[14] reported 100% MDR in *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* and 80% MDR in *Klebsiella pneumoniae*.

4. Conclusion

From this study it becomes clear that varieties of pathogens are responsible for LRTI and antimicrobial resistance has become significant public health problem. Thus longitudinal surveillance program, institution of infection control practices and rational use of antibiotics are highly recommended to reduce the infection rate and limit the spread of resistance.

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