

Antibiotic Resistance Patterns of Bacterial Pathogens Associated with Lower Respiratory Tract Infections

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ABSTRACT

Introduction: Respiratory tract infections are one of the most common and serious health problems in developing countries. Increasing incidences of multidrug-resistant isolates from cases of lower respiratory tract infections have been reported. This study was conducted to determine the bacterial pathogens associated with lower respiratory tract infections with their antibiotic resistance pattern in a tertiary care hospital in Western Nepal.

Methods: This hospital-based cross-sectional study was conducted among patients of Manipal Teaching Hospital, Pokhara, Nepal, from August 2022 to October 2022. A total of 376 specimens from the lower respiratory tract were included in this study. Processing of samples, isolation, identification and antibiotic sensitivity test of the bacterial pathogens was performed by standard methods.

Results: Respiratory pathogens were recovered in 68 samples. Gram-negative bacteria were isolated in 55 cases and Gram-positive bacteria in 13 cases. *Acinetobacter* species (42.6%), *Staphylococcus aureus* (16.9%) and *Pseudomonas aeruginosa* (13.9%) were the three most common pathogens isolated. The majority of the Gram-negative isolates were susceptible to tigecycline and colistin. Among *S. aureus* isolates, 54.5% (6/11) were methicillin-resistant *Staphylococcus aureus*.

Conclusion: Gram-negative bacteria were predominantly associated with lower respiratory tract infections with *Acinetobacter* species as the most common pathogen. Increasing antibiotic resistance was recorded among the pathogens and is alarming. Regular surveillance of pathogens and antibiotic resistance patterns is needed for better management of the patients.

Keywords: Drug Resistance; LRTI; Gram Negative Bacteria; MDR

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INTRODUCTION

Respiratory tract infections are the most common infections reported among humans. Lower respiratory tract infections (LRTI) are significant cause of morbidity and mortality in developing countries.[1,2] Lower respiratory tract infections occur when infectious agents reach the airway of pulmonary parenchyma by crossing the barriers of the upper respiratory tract. Respiratory infections are mostly of community origin but increasing cases of hospital-acquired infections have been reported.[3]

Respiratory infections are heterogeneous and complex group of diseases caused by wide range of infectious agents like viruses, bacteria, fungi or parasites. Viral agents are responsible for a large proportion of LRTI. Due to a lack of differential diagnosis, antibiotics are often unnecessarily prescribed and can contribute to the emergence of drug resistance.[4,5] Gram-negative bacteria commonly associated with LRTI include *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Pseudomonas* species, *Acinetobacter* species, *Enterobacter* species, *Citrobacter* species and others.[6] Gram-positive bacteria reported from cases of LRTI include *Streptococcus pneumoniae* and *Staphylococcus aureus*. The burden of antimicrobial resistance is increasing in developing countries which makes the treatment challenging. Increasing trends of multidrug resistance (MDR) among Gram-negative bacterial isolates have limited the therapeutic options among patients with LRTI. The reported incidence of LRTI in developing countries varies from 20–30% as compared to 3–4% in developed countries.[7] Infections of the lower respiratory tract cause more than 4 million deaths per year and with more cases from middle and low-income nations.[8] Respiratory tract infection accounts for 34.6% of total deaths in the South-East Asian Region.[9]

Etiological agents and antimicrobial resistance profiles of the isolates vary greatly from hospital to hospital within and outside the country.[10] Management of infections of the respiratory tract is more complex in developing countries due to the lack of proper diagnostic facilities. Therefore, this study was designed to determine the common bacterial pathogens associated with LRTI along with their antibiotic resistance pattern in a tertiary care hospital. The findings of the study would be useful in formulating guidelines for empirical antibiotic therapy among patients with LRTI.

METHODS

This hospital-based cross-sectional study was conducted among outpatients and admitted patients of Manipal Teaching Hospital, Pokhara, Nepal. Permission was taken from the Institutional Ethical Committee (MCOMS/IRC/532) of Manipal Teaching Hospital (MTH), Pokhara, before the commencement of the study. The study was conducted over three months (August 2022 to October 2022). The sample size was calculated using the following formula: $N = Z^2 p(1-p) / E^2$.

Isolation and identification of bacterial isolates

A total of 376 specimens from the lower respiratory tract (sputum, suction tip and bronchoalveolar lavage) received in the clinical Microbiology laboratory of Manipal Teaching hospital were included in this study. All the specimens were subjected to microscopy (Gram stain) and culture. Specimens were cultured on 5% Sheep Blood agar and Chocolate agar medium (Hi media, Mumbai, India). Inoculated culture plates were incubated at 37°C in a candle jar. Isolates were identified by colony morphology, microscopy and standard biochemical tests for Gram-positive bacteria (Catalase test, Coagulase test, Optochin sensitivity test etc.) and Gram-negative bacteria (Sugar fermentation test, Motility, Indole,

Methyl red, Voges-Proskauer, Citrate utilization, Urea hydrolysis etc.[11]

Antibiotic susceptibility test

Antibiotic susceptibility test of the bacterial isolates was performed on Mueller Hinton agar (HI media, Mumbai, India) by the Kirby Bauer disc diffusion method.[12] Following antibiotic discs were used: amikacin, ciprofloxacin, clindamycin, cefepime, colistin, cefoperazone sulbactam, erythromycin, gentamicin, imipenem, piperacillin-tazobactam, tigecycline, teicoplanin and vancomycin. Isolates showing resistance to at least one agent in three or more antimicrobial categories were labelled as multidrug-resistant (MDR).[13]

RESULTS

Out of 376 samples processed, bacterial pathogens were isolated in 68 (18.1%) cases, normal oropharyngeal flora was detected in 302 (80.3%) cases and no growth was seen in 6 (1.5%) cases as shown in figure 1. Among the bacterial pathogens isolated, 80.9% (55/68) were Gram-negative bacilli and 19.1% (13/68) were Gram-positive cocci. *Acinetobacter* species (29.4%) was the most common pathogen followed by *Klebsiella pneumoniae*

(20.5%), *Staphylococcus aureus* (16.1%), *Pseudomonas aeruginosa* (16.1%) and others. Samples received from Intensive Care Unit (ICU) and Critical Care Unit (CCU) yielded the highest number of *Acinetobacter* species. Among Gram-positive bacteria, *S. aureus* was the commonest pathogen. Details of bacterial pathogens isolated are depicted in Table 1.

Gender-wise distributions of organisms revealed that the majority of the bacterial pathogens 77.9% (53/68) were isolated from male patients. Age-wise distribution of the pathogens showed that 72% (49/68) of isolates were from patients more than sixty years of age. In the present study, 83.8% (57/68) of pathogens were cultured from patients admitted to various wards and remaining from the outpatient department.

Antibiotic resistance patterns of isolates revealed that a high percentage of *Acinetobacter* species were MDR (40%). The majority of the MDR isolates were cultured from ICU and CCU. The majority of *Acinetobacter* species were resistant to ciprofloxacin (70%). The most effective drugs in the case of *Acinetobacter* species were

Table 1: Bacterial pathogens isolated from lower respiratory tract specimen

S N	Organism	Number	Percentage
1	<i>Acinetobacter</i> species	20	29.4
2	<i>Klebsiella pneumoniae</i>	14	20.5
3	<i>Pseudomonas aeruginosa</i>	11	16.1
4	<i>Staphylococcus aureus</i>	11	16.1
5	<i>Escherichia coli</i>	05	7.3
6	<i>Haemophilus influenzae</i>	02	2.9
7	<i>Streptococcus pneumoniae</i>	02	2.9
8	<i>Enterobacter</i> species	01	1.4
9	<i>Citrobacter</i> species	01	1.4
10	<i>Moraxella catarrhalis</i>	01	1.4
Total		68	

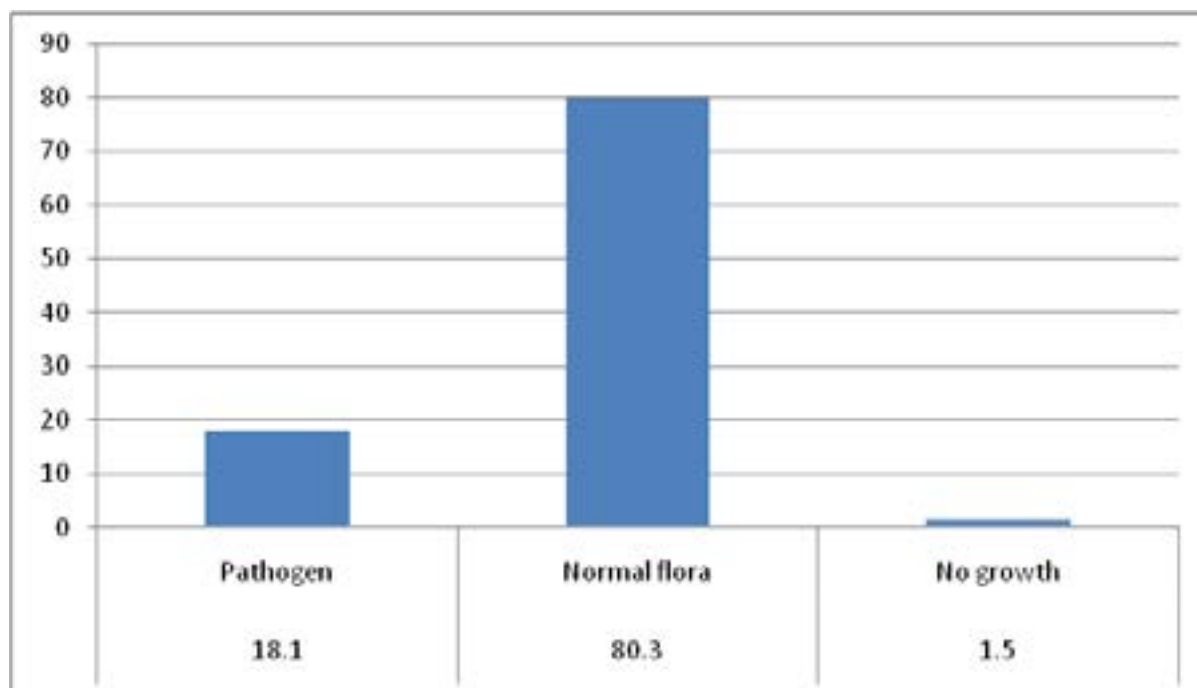


Figure 1: Patterns of bacterial growth (%) in respiratory samples

colistin and tigecycline with a lower percentage of resistance (10%). Colistin and tigecycline were equally effective in case of other Gram negative bacteria (*E. coli* and *Klebsiella*

species). Among *S. aureus* isolates (n=11), 6 (54.5%) were MRSA. Details of antibiotic resistance patterns of bacterial pathogens isolated are depicted in Table 2.

Table 2: Antibiotic resistance patterns of common bacterial pathogens

Antibiotic	<i>Acinetobacter</i> species n= 20 (%)	<i>K. pneumoniae</i> n= 14 (%)	<i>P. aeruginosa</i> n=11 (%)	<i>E. coli</i> n= 05 (%)	<i>S. aureus</i> n=11 (%)
Amikacin	7 (35%)	4 (28.5%)	4 (36.3%)	1 (20%)	2 (18.1%)
Ciprofloxacin	15 (75%)	5 (35.7%)	5 (45.4%)	2 (40%)	6 (54.5%)
Cefepime	--	8 (57.1%)	--	2 (40%)	--
Cefoperazone sulbactam	8 (40%)	4 (28.5%)	3 (27.2%)	1 (20%)	--
Colistin	2 (10%)	1 (7.1%)	00	00	--
Imipenem	7 (35%)	2 (14.2%)	2 (18.1%)	00	--
Piperacillin tazobactam	8 (40%)	4 (28.5%)	2 (18.1%)	00	--
Tigecycline	2 (10%)	1 (7.1%)	3 (27.2%)	00	--
Gentamicin	9 (45%)	5 (35.7%)	5 (45.4%)	1 (20%)	3 (27.2%)
Clindamycin	--	--	--	--	6 (54.5%)
Erythromycin	--	--	--	--	8 (72.7%)
Cotrimoxazole	--	--	--	--	5 (45.4%)
Cefoxitin	--	--	--	--	6 (54.5%)
Vancomycin	--	--	--	--	00

DISCUSSION

Lower respiratory tract infections of bacterial origin are one of the most serious clinical conditions among patients admitted to the hospital, especially in critical care units. The clinical outcome remains poor among patients with underlying comorbidities, diabetes mellitus, cystic fibrosis, chronic obstructive pulmonary disease, patients admitted to ICU, asthma and other conditions associated with immunodeficiency. The correct microbiological diagnosis, appropriate application of clinical and laboratory findings and early antibiotic treatment are crucial for the management of cases. This study aimed to determine the prevalence and antibiotic resistance patterns of bacterial pathogens causing LRTI among the patients of Manipal Teaching Hospital, Pokhara, Nepal.

Present research findings highlight the etiological agents associated with LRTI along with their antibiotic resistance patterns. In our study, bacterial pathogens were isolated in 18.1% of cases. Similar studies conducted in other parts of Nepal reported higher isolation rates as compared to our study findings. [14,15] A lower isolation rate in our study could be due to a higher percentage of admitted patients using broad-spectrum antibiotic therapy before sample collection. The findings of our study are comparable with a study by Ahmed et al. from India with an isolation rate of 17.03%. [16] The isolation rate from the clinical samples varies depending upon various factors such as the quality of the samples, transportation, preservation, processing methods, antibiotic therapy and many other factors. Isolation of bacterial pathogens was significantly higher in males as compared to females (p-value <0.001). This may be due to more associated risk factors of respiratory tract infection such as smoking, alcohol consumption etc. Similar findings are reported by Ahmed et al. and Shah et al. [16,17]

Etiological diagnosis is crucial in the management of LRTI, especially among patients with additional risk factors. In our study, association of Gram-negative bacteria was significantly higher 80.9% (55/68) as compared to Gram-positive bacteria 19.1% (13/68). Similar findings are reported by researchers from other parts of Nepal and neighbouring countries. [14-18] *Acinetobacter* species were the most common bacteria isolated followed by *Klebsiella* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In other studies from Nepal, *Klebsiella pneumoniae* was reported as the most common bacterial pathogen. [15,19] This could be due to a high number of the samples in our study were from admitted patients. *Acinetobacter* species are considered as one of the most important nosocomial pathogens with their ability to resist a wide range of broad-spectrum antibiotics. Isolation of a high number of *Acinetobacter* species is a big threat to the clinician due to the possibility of treatment failure. Isolation of bacterial pathogens was significantly higher among admitted patients as compared to outpatients (p value <0.001). Isolation of other Gram-negative respiratory pathogens like *Streptococcus pneumoniae* (2.9%), *Haemophilus influenzae* (2.9%) was less which is probably due to the routine immunization program introduced in Nepal. These findings are comparable with findings by Subedi et al. with an isolation rate of 2.8%. [20]

Increasing antibiotic resistance with an increasing percentage of MDR pathogens is a global burden, particularly in hospitals in developing countries. Antimicrobial resistance patterns of bacterial pathogens vary from hospital to hospital within and outside the country. As per our study findings, tigecycline and colistin were the most effective drugs against Gram-negative isolates. However, the emergence of colistin and tigecycline-resistant isolates is alarming due to limited treatment options. Colistin and tigecycline-resistant isolates of *Acinetobacter* species were reported

previously among ICU patients.[21] High resistance was detected against gentamicin, ciprofloxacin, cefepime and cefoperazone sulbactam. Among *Acinetobacter* species, 40% (8/20) were MDR isolates. The majority of these MDR isolates were cultured from patients with age >60 years. Among *S. aureus* isolates, 54.5% were MRSA. Isolation of a high percentage of MRSA increases the risk of systemic infections and is also associated with high mortality rates. All the isolates of *S. aureus* were susceptible to vancomycin.

CONCLUSION

Gram-negative bacilli were predominantly associated bacterial pathogens in LRTI and *Acinetobacter* species was the most common. *S. aureus* was common among the Gram-positive bacteria. A high percentage of MDR isolates were detected among Gram-negative bacilli. Colistin and tigecycline were the most effective treatment options for the MDR pathogens.

CONFLICT OF INTEREST

None

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None

REFERENCES

- Prajapati B, Talsania N, Lala M, Sonalia K. Epidemiological profile of acute respiratory infections (ARI) in under five age group of children in urban and rural communities of Ahmedabad district, Gujarat. *Int J Med Sci Public Health*. 2012;1(2):52. <http://dx.doi.org/10.5455/ijmsph.2012.1.52-58>
- Jacobs E, Dalhoff A, Korfmann G. Susceptibility patterns of bacterial isolates from hospitalised patients with respiratory tract infections. *Int J Antimicrob Agents*. 2009;33(1):52–7. <http://dx.doi.org/10.1016/j.ijantimicag.2008.07.017>
- Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, et al. Multistate point-prevalence survey of health care-associated infections. *N Engl J Med*. 2014; 370:1198-1208. <https://doi.org/10.1056/NEJMoa1306801>
- Pavia AT. “Viral infections of the lower respiratory tract: Old viruses, new viruses, and the role of diagnosis,” *Clinical Infectious Diseases*. 2011;52 (4):284–289. <https://doi.org/10.1093/cid/cir043>
- Ouedraogo AS, Pierre HJ, Banuls AL, Ouedraogo R, Godreuil S. “Emergence and spread of antibiotic resistance in West Africa: contributing factors and threat assessment”. *Medecine et Sante Tropicales*. 2017;27(2):147–154. <https://doi.org/10.1684/mst.2017.0678>.
- Ozyilmaz E, Akan OA, Gulhan M. Major bacteria of community-acquired respiratory tract infections in Turkey. *Jpn J Infect Dis*. 2005;58:50-52.
- Regional situation on health statistics reporting. Health Situation in the South-East Asia Region 1994-1997. New Delhi: EHI/WHO-SEARO. September 2007.
- World Health organization. Leading causes of deaths in the SEA Region and the World. Health Situation in the South East Asia Region 1998-2000. New Delhi. India: WHO, 2002.
- Alter SJ, Vidwan NK, Sobande PO, Omoloja A, Bennett JS. Common childhood bacterial infections. *Curr Probl Pediatr Adolesc Health Care*. 2011;41(10):256–83. <http://dx.doi.org/10.1016/j.cppeds.2011.06.001>
- Tchatchouang S, Nzouankeu A, Kenmoe S, et al. Bacterial aetiologies of lower respiratory tract infections among adults in Yaoundé, Cameroon. *Biomed Res Int*. 2019;2019:4834396. <http://dx.doi.org/10.1155/2019/4834396>
- Cheesbrough M. District laboratory practice in tropical countries. Cambridge University Press; 2006.
- Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing, Twenty- Second Informational Supplement,

- CLSI, Document M100- S27, CLSI, Wayne, PA, USA, 2017.
13. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske G, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18:268–81. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>
 14. Khan S, Singh P, Ansari M, Gurung K. Bacteria Etiological Agents Causing Lower Respiratory Tract Infections in the Western Part of Nepal. *Ibnosina J Med BS*. 2014;6(1):3-8. <https://doi.org/10.4103/1947-489X.210353>
 15. Mishra SK, Kattel HP, Acharya J, et al. Recent trend of bacterial aetiology of lower respiratory tract infections in a tertiary care centre of Nepal. *Int J Infect Microbiol*. 2012;1(1):3-8. <http://dx.doi.org/10.3126/ijim.v1i1.6639>.
 16. Ahmed SM, Jakribettu RP, Meletath SK, Arya B, Shakir VPA. Lower Respiratory Tract Infections (LRTIs): An Insight into the Prevalence and the Antibigram of the Gram Negative, Respiratory, Bacterial Agents. *Journal of Clinical and Diagnostic Research*. 2013;7(2):253-256. <https://doi.org/10.7860/JCDR/2013/5308.2740>.
 17. Shah BA, Singh G, Naik, MA, Dhobi, GN. Bacteriological and clinical profile of Community acquired pneumonia in hospitalized patients. *Lung India*. 2010;27:54-57. <https://doi.org/10.4103/0970-2113.63606>.
 18. Ullah B, Ahmed S, Shahariar M, Yesmine S. Current trend of antibiotic resistance in lower respiratory tract infections (LRTIs): An experience in a teaching hospital in Bangladesh. *Banglad Pharm J*. 2016;19(1):85–91. <http://dx.doi.org/10.3329/bpj.v19i1.29243>
 19. Nepal R, Shrestha B, Joshi DM, Joshi RD, Shrestha S, Singh A. Antibiotic susceptibility pattern of Gram-negative isolates of lower respiratory tract infection. *J Nepal Health Res Counc*. 2018;16(1):22–6. <http://dx.doi.org/10.3126/jnhrc.v16i1.19358>
 20. Subedi D, Devkota SP, Bhatta DR, et al. Antibiotic Susceptibility Profile of Respiratory Pathogens Obtained at Tertiary Care Hospital from Western Nepal. *Nepal Journal of Biotechnology*. 2019;7(1): 1-7. <https://doi.org/10.3126/njb.v7i1.26944>
 21. Bhatta DR, Hamal D, Shrestha R, et al. Burden of multidrug resistant respiratory pathogens in intensive care units of tertiary care hospital. *Asian J Med Sci*. 2019;10(2):14-19. <https://doi.org/10.3126/ajms.v10i2.21098>.