

Recent trend of bacterial aetiology of lower respiratory tract infections in a tertiary care centre of Nepal

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ABSTRACT

INTRODUCTION: Lower respiratory tract infections (LRTIs) are among the most common infectious diseases of humans. This study focused on determining the recent trend of bacterial aetiology of LRTIs among the patients attending Tribhuvan University Teaching Hospital (TUTH) in Kathmandu.

MATERIALS AND METHODS: This was a prospective study conducted over a period of six months in the bacteriology laboratory of TUTH. A total of 1120 specimens representing lower respiratory tract were received from patients with suspected LRTIs. The specimens were collected and processed according to standard methodology.

RESULTS: Respiratory pathogens were recovered from 44.4% cases (n=497). Gram-negative bacteria were recovered in 84.1% (n=488). Bacteria were more commonly recovered from endotracheal secretion (41/61, 67.2%) than in sputum (454/1039, 43.7%) and bronchial washing (2/20, 10%). Ninety-one percent (n=454) growth was monomicrobial while the rest accounted for mixed growth. Among the organisms isolated, *Haemophilus influenzae* (112, 21%) was the most predominant pathogen followed by *Klebsiella pneumoniae* (102, 19.1%), Pseudomonads (91, 17.1%), *Acinetobacter baumannii calcoaceticus* complex (60, 10.9%), *Streptococcus pneumoniae* (46, 8.6%), *Escherichia coli* (37, 6.9%).

CONCLUSIONS: *H. influenzae* and *S. pneumoniae* were the most common Gram-negative and Gram-positive bacterial isolates recovered, respectively from LRTIs urging for monitoring and surveillance of these pathogens.

KEY WORDS: Bacterial infection, Lower Respiratory tract, Respiratory aspirates

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INTRODUCTION

Lower Respiratory Tract Infection (LRTI) is one of the leading causes of the morbidity and mortality in the world.¹ LRTI is not a single disease but a group of specific infection each with a different epidemiology, pathogenesis, clinical presentation and outcome. The etiology and symptomatology of respiratory diseases vary with age, gender, season, the type of population at risk and other factors. These are frequently the first infection to occur after birth and pneumonia is too often the final illness to occur before death.² Out of total 3,941,000 deaths in the world, respiratory infection account for 34.60% deaths in the South-East Region.³ Talking in Nepal's context, LRTI is one of the commonest domiciliary and nosocomial infections.

The aetiological agents of LRTIs cannot be determined clinically. These agents vary from area to area, so do their antibiotic susceptibility profile.⁴ Gram-positive bacteria like, *Staphylococcus aureus*, *Streptococcus pneumoniae* etc and Gram-negative bacteria like, *Haemophilus influenzae*, *Pseudomonas* spp., *Acinetobacter* spp., *Klebsiella* spp. have been recovered from LRTIs.^{4,5} However, not much is known about the prevalence of microbial agents causing LRTIs in Nepal. Age, gender, and season are factors that have been implicated to affect the prevalence of LRTIs.⁵ Therefore, current knowledge of the organisms that cause LRTIs are necessary for the management of patients. This information is obtained only after microbiological investigation in the laboratory. Hence, this study was conducted to determine the microbial agents of LRTIs among the patients attending Tribhuvan University Teaching Hospital (TUTH).

MATERIALS AND METHODS

This was a prospective study conducted over a period of six months in bacteriology laboratory of TUTH among the patients suspected of LRTI. A total of 1120 specimens were processed from patients ranging from 6 to 96 years. Lower respiratory tract (LRT) representing specimens, viz., sputum, endotracheal (ET) secretion and bronchial washings received for culture and sensitivity which met the criteria as recommended by American Society for Microbiology (ASM) were included in the study.⁶ Information regarding duration of hospital stay and antibiotic history was also taken into consideration whenever possible during the processing of specimens. These data were noted in the clinical and microbiological profile sheet of patients. After receiving the sample at the sample collection site, it

was immediately transported to the bacteriology laboratory and was processed further.

Specimens which were very thick and mucoid were first homogenized with commercially available sputasol containing 0.01% dithiotheritol. Briefly, to homogenize, 5 ml of sterile distilled water was added to the given vial containing 1 gm sputalysin, dithiotheritol powder and mixed gently to dissolve the powder completely. The content was then added with aseptic precaution to 95 ml of sterile distilled water which makes 1:20 dilution. Then equal volume of diluted sputasol was added to the sputum sample aseptically, then mixed gently and was incubated at 37°C for 30 minutes for complete homogenization of sputum.

The quality of the sputum and ET secretion specimens were evaluated according to the criteria given by ASM. According to this, a reliable specimen would have more than 25 leucocytes and fewer than 10 epithelial cells per low power field of microscope.⁶

Culture of the specimen: The digested sputum samples were cultured on Chocolate agar (CHA), 5% Sheep Blood agar (BA) and MacConkey agar (MA) (Oxoid, UK) plates. On the CHA, bacitracin disk (10 Unit) and optochin disk (5 µg) (Oxoid, UK) were placed at primary and secondary inoculation to screen *H. influenzae* and *S. pneumoniae* respectively. The CHA plates were incubated in CO₂ incubator (10% CO₂) at 37°C for 24 hours while BA and MA plates were incubated at 37°C for 24 hours in aerobic atmosphere.

Identification of isolated organisms: Identification of significant isolates were done following standard microbiological techniques which involved morphological study of the colonies, Gram's staining reactions, and a battery of biochemical tests as required. A colony count of $\geq 10^4$ CFU/ml was considered to be significant for bronchial washing⁷ while for other specimens, $\geq 10^5$ CFU/ml was suggestive for infection⁸.

This study was approved by Institutional Review Board of Institute of Medicine. Data were analyzed using Microsoft Excel 2007.

RESULTS

Number of specimens and result pattern

A total of 1162 specimens from lower LRT were processed according to the standard microbiological

Table 1. Pattern of bacterial isolation in different Lower respiratory tract specimens

Specimen	Significant growth	Insignificant growth	No growth
	No. (%)	No. (%)	No. (%)
Sputum (N=1039)	454 (43.7)	574 (55.3)	11 (1.0)
ET secretion (N=61)	41 (62.2)	0	20 (32.8)
Bronchial washing (N=20)	2 (10)	0	18 (90)

methods. Specimens processed in this study were sputum (n=1081), ET secretion (n=61) and bronchial washing (n=20). Out of total 1081 sputum specimens, only 1039 specimens were further processed while the remaining 42 specimens were rejected as they implied oral contamination. Among the total processed specimens (n=1120), only 497 showed significant growth (44.4%). Of the different specimens, ET secretion showed the highest microbial isolation (67.2%) (Table 1).

Trend of microbial isolates

Out of total 497 microbial growth, there was significant polymicrobial growth (growth of two different microbes) in 43 specimens (8.7%) while predominant monomicrobial growth was seen in 454 cases (91.3%). Among the bacterial isolates, 84.1% were Gram-negative and 15.9% were Gram-positive (Table 2).

Distribution of bacterial isolates

Among the 533 bacterial isolates, *H. influenzae* was found to be the most predominant organism followed by *Klebsiella pneumoniae* sub spp. *pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii calcoaceticus* complex (ABC complex). *S. pneumoniae* was the most common isolate among Gram-positive bacteria (54.1%) (Table 2).

DISCUSSION

This study was conducted among the patients attending TUTH, Kathmandu to find out the current trend of the microbial spectrum causing LRTIs. Pathogens were recovered from 44.4% of the total specimens. Similarly, 43.7%, 67.2% and 10% of pathogen were recovered from sputum, ET secretion and bronchial washings respectively. Previous studies in the same hospital in 1994 and 2004 had shown the growth in 34.6% and 30.5% cases respectively.^{9,10} This shows that there has been an increment in the prevalence of bacterial LRTIs.

Table 2. Distribution of bacterial isolates among indoor and outdoor patients

Bacteria (n=533)	Inpatients n=267	Outpatients n=266
	n (%)	n (%)
Gram-positives (n=85)		
<i>S. pneumoniae</i>	6 (13)	40 (87)
<i>S. aureus</i>	25 (75.8)	8 (24.2)
<i>Enterococcus</i> spp.	5 (100)	0 (0)
<i>S. pyogenes</i>	1 (100)	0 (0)
Gram-negatives (n=448)		
<i>H. influenzae</i>	3 (2.7)	109 (97.3)
<i>K. pneumoniae</i>	65 (63.7)	37 (36.3)
<i>P. aeruginosa</i>	56 (81.2)	13 (18.8)
ABC complex	51 (85)	9 (15)
<i>E. coli</i>	24 (64.9)	13 (35.1)
<i>M. catarrhalis</i>	9 (40.9)	13 (59.1)
<i>Pseudomonas</i> spp.	8 (36.4)	14 (63.6)
<i>C. freundii</i>	6 (50)	6 (50)
<i>C. koseri</i>	2 (66.7)	1 (33.3)
<i>M. morgani</i>	1 (33.3)	2 (66.7)
<i>E. aerogenes</i>	1 (50)	1 (50)
<i>S. marcescens</i>	2 (100)	0 (0)
<i>A. lwoffii</i>	2 (100)	0 (0)

A study conducted in Western Nepal recovered respiratory pathogens in 50.4% samples.¹¹ Likewise, prevalence studies done in China, Turkey and Iran showed there was significant growth in 53.1%, 59.4% and 40.0% of cases respectively.^{4,12,13} The lower yield of pathogens in this study as compared to other studies might be attributable to various factors. Natural history of infectious disease in the patient will have already been modified by the use of different type of antibiotics by health professionals at different levels before the patient lands into this tertiary care centre. The use of antibiotics might have played a significant role in culture negativity. Likewise, there is no prudent use of antibiotics in the country and the self-prescribing practice by patients could be another contributing

factor. Low prevalence of pathogen isolation was also noted in 40 to 60% of cases elsewhere.¹⁴

The observation that more than one causative pathogen can be identified in a patient has been demonstrated in several studies. The exact rate of polymicrobial infection, which depends on the number of the pathogens tested for and the laboratory techniques used, has been reported to vary from 3 to 40%, and *Chlamydia pneumoniae* seems to be the most common organism of coinfection.¹⁵ In this study too, monomicrobial growth was found in 91.3% of cases while 8.7% were polymicrobial. The mixed pathogens were also seen in 11.2% samples in other study.⁴ Polymicrobial growth in 13.3% and 19.0% respectively were also recovered.^{15,16} The slightly lower number of mixed infections in our setting could be due to the reason that microbes like *C. pneumoniae*, *Mycoplasma pneumoniae*, were not looked for in this study.

In this study, Gram-negative bacteria accounted for 84.1% of all bacteria isolated. The most common isolate was *H. influenzae* not only among the Gram-negative bacteria (25%), but also among the total isolates (21%). *H. influenzae* was also common in other studies in Nepal (39.3%, 26.9%) and outside.^{4,9,11,17} Low prevalence of *H. influenzae* in this study could be due to biofilms formation in *in-vivo* which may yield negative cultures.^{18,19} Also, several lines of evidence indicate that *H. influenzae* is viable inside host cells, including macrophages and respiratory epithelial cells.²⁰⁻²² It is tempting to speculate that *H. influenzae* may modulate its form of growth under different conditions in the human respiratory tract, accounting for negative sputum cultures. It has been found that 34 to 47% of sputum cultures are negative with proven *H. influenzae* pneumonia.^{23,24}

Non-typeable *H. influenzae* colonizes the respiratory tract of adults with chronic obstructive pulmonary disease (COPD) and causes intermittent exacerbations.²⁵ Fifty three percent of the patients with acute exacerbation of COPD showed the growth of *H. influenzae* in this study which complies with the finding (>50.0%) in Turkey.⁴ Out of total 112 isolates of *H. influenzae*, 45 (40.2%) were seen in patients of age >50 years (data not shown); this result is consistent with the study done in Nepal which also found 39.4% of *H. influenzae* among older patients.⁹ The high incidence of the isolate in this study coincides with that of Marfarlane.²⁶ A higher number of *H. influenzae* was isolated from outpatients (97.3% vs. 2.7%). On reviewing 15

published studies from North America, over 3 decades, it was found that *S. pneumoniae* was the most common cause of community-acquired pneumonia (up to 60% cases), followed by Gram-negative bacteria including *H. influenzae*, *K. pneumoniae* and *P. aeruginosa*. *S. aureus*, *Legionella* spp., *M. pneumoniae*, *C. pneumoniae*, and viruses were among the minors.²⁷ Similarly, a study in England and Wales observed a high rate of *S. pneumoniae* while *H. influenzae* was the second common isolate.²⁶ Buccheri GF, in Italy, also showed that *S. pneumoniae* was the most frequent isolate followed by *H. influenzae*.²⁸ However, in this study *H. influenzae* was isolated more frequently than *S. pneumoniae*. Reason behind the low number of *S. pneumoniae* could be due to the hospital-based study, where there is higher recovery of Gram-negative bacilli in hospital-acquired LRTIs. Moreover, even in patients with bacteremic *S. pneumoniae* pneumonia, it has been estimated that the usual laboratory methods can not detect the pathogen in 45 to 50% of cases even when large numbers of organisms have been noted on Gram stain.²⁹ Nevertheless, the growth of *S. pneumoniae* in this study is comparable with that of earlier studies in the similar set up (10.7%) and also with a multicentric study conducted in China (10.3%).^{9,12}

Out of 46 *S. pneumoniae* isolates, 40 (87%) were isolated from outpatients and it was 15% of the total bacterial isolates from the outpatients; this complies with the finding in Thailand where 13.3% of the isolates were *S. pneumoniae*.¹⁵ According to South Asian Pneumococcal Alliance (SAPNA) Project, *S. pneumoniae* is also more common in Nepal.³⁰

Among 85 Gram-positive isolates, besides *S. pneumoniae*, *S. aureus* (38.8%), *Enterococcus* spp. (5.9%) and *S. pyogenes* (1.2%) were recovered. Regarding the isolation of *S. aureus*, 75.8% of them were from inpatients whereas 24.2% were from outpatients. Similar result was found by in China.¹² *S. aureus* has emerged as a secondary opportunist in lungs of patients with opportunistic diseases and prior viral respiratory disease predisposes patient to primary staphylococcal pneumonia and a considerable number of *S. aureus*-in this study-were found as mixed pathogens.³¹ *Enterococci* can cause pneumonia in elderly, debilitated patients with multiple chronic problems and here also similar result was found as all *Enterococci* were isolated from inpatients of age >50 years (data not shown).³² A total of 102 *K. pneumoniae* (19.1%) were isolated which constituted the second major Gram-negative bacteria. The majority of the isolates (55.9%) were

diagnosed in patients >50 years age group. Similar incidence of *Klebsiella* spp. (19.4%) was found from sputum samples in 2004.¹⁰ Among the total *Klebsiella* isolates, 63.7% were from inpatients which accounted for 24.3% of the total bacteria from inpatients. In earlier studies at TUTH, 17.9% and 24.3% *Klebsiella* spp. were encountered among inpatients respectively.^{9,33}

Non-fermentative bacterial isolates like *Pseudomonads* and *Acinetobacter* spp. were the third and fourth major bacteria recovered. *Pseudomonads* (17.1%) comprised *P. aeruginosa* (12.9%), and other *Pseudomonas* spp. (4.1%). Similarly, *Acinetobacter* spp. constituted ABC complex (10.9%) and *A. lwoffii* (0.4%). Out of total 91 *Pseudomonads* isolates, 70.3% were from hospitalized patients. It should be noted that *P. aeruginosa* is the epitome of an opportunistic pathogen of human and is notorious for nosocomial infections.

Prevalence study done a decade back at TUTH had showed the growth of *Pseudomonas* spp. and *Acinetobacter* spp. in 11.9% and 1.19% of cases respectively.⁹ Another study in 2004 at the same setting, showed the increment in growth of these bacteria by more than twofold which was *P. aeruginosa* (28.2%) and *A. calcoaceticus* (2.9%).³⁴ This shows that though the prevalence of *Pseudomonas* spp. has decreased in 2008 as compared to 2004 at TUTH, there is a small increment of *Acinetobacter* spp. associated LRTIs. This data is again higher than that of a study carried out in Manipal Teaching Hospital in which *P. aeruginosa* and *Acinetobacter* spp. accounted for 7.5% and 3.9% of the LRTI cases respectively.¹¹

E. coli is an uncommon cause of acute LRTI and in this study, they comprised of 6.9% of the total cases. This finding corroborates with that of Pokhrel et al (5.8%).¹⁰ Likewise, other enteric pathogens along with *E. coli* constituted 11.07% of the total isolates. Among these, 64.9% were from inpatients and 54.24% were found in elderly patients (data not shown).

Another emergent pathogen was *M. catarrhalis* which accounted for 4.1% of the total isolates. Different studies have shown its prevalence ranging from 1 to 12%.^{4,11,12,17}

CONCLUSIONS

H. influenza and *S. pneumoniae* were the common Gram-negative and Gram-positive bacteria,

respectively, causing LRTIs in our setting. Since bacterial aetiology may vary in different geographical regions and even over time in the same location and population, routine surveillance of microbial aetiology of LRTI is important.

CONFLICT OF INTEREST: None to declare.

FINANCIAL INTEREST: None to declare.

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