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Bacteria Etiological Agents Causing Lower Respiratory Tract Infections in the Western Part of Nepal

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Abstract

Objectives: Lower respiratory tract infections (LRTIs) are among the most common infectious disease. We determined the bacterial etiology of LRTIs among patients attending Nepalgunj Medical College teaching hospital in Banke, western Nepal. **Materials and Methods:** 426 specimens from patients with suspected LRTIs attending out-patients and in-patients departments were studied. The specimens were collected and processed according to standard methodology in the Central Laboratory of Microbiology at Nepalgunj Medical College and Teaching Hospital, Banke, Nepal during the period of January to December 2012. **Results:** Respiratory pathogens were recovered from 210 cases (49.3%). Bacteria were more commonly recovered from endotracheal secretions (86 cases; 41%) than in sputum (82 cases; 39%) and bronchial washing (42 cases, 20%). In168 cases, growth was monomicrobial while the rest was mixed growth. Gram-negative bacteria were isolated in 246 cases (80.9%) and Gram-positive bacteria in 58 cases (19.1%). Among the Gram-positive organisms isolated, *Streptococcus pneumoniae* (30, 51.7%) was the most predominant pathogen followed by *Staphylococcus aureus* (28, 48.3%) and in Gram-negative organisms isolated, *Pseudomonas aeruginosa* (116, 47.2%) was most predominant pathogen followed by *Haemophilus influenzae* (68, 27.6%), *Klebsiella pneumoniae* (36, 14.6%) and *Escherichia coli* (26, 10.6%). **Conclusions**: Respectively, *Pseudomonas aeruginosa and Streptococcus pnemoniae* were the most common Gram negative and Gram-positive bacterial isolates recovered from LRTIs. Close monitoring and surveillance of these pathogens is urged.

Key words: Bacteria etiological agent, Lower Respiratory tract infections, Respiratory aspirates, Nepal

Introduction

Respiratory tract infections are common and perhaps the

most frequently reported of all human infections. They are traditionally divided into two: upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs). Some of these infections are mostly mild, transient lasting and sometimes self-limiting. Due to this, many infected persons tend to disregard them (1). Respiratory infection account for 34.6% of the deaths in the south-east region out of the total 3,941,000 deaths worldwide (2). LRTIs is one of the leading causes of the morbidity and mortality in the world (3). In developing countries, the situation is more complicated and management is often difficult due to the problem associated with the identification of the etiological agents and administration of appropriate treatment in cases requiring antibiotic therapy (4). 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 disease 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 (5). The etiological agents of LRTIs cannot be determined clinically. These agents vary from area to area and so does their antibiotic susceptibility profile (6). Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* and Gram-negative bacteria like *Hemophilus influenzae*, *Pseudomonas spp., Acinetobacter spp*., and *Klebsiella spp*. have been recovered from LRTIs (6, 7). However, knowledge about the prevalence of microbial agents causing LRTIs in Nepal is sparse. Age, gender and season are factors that are implicated to affect the prevalence of LRTIs (7). Therefore, current knowledge of the organisms that cause LRTIs are necessary for the management of patients. Such information can only be obtained from microbiological investigation in the laboratory. Hence, this study was conducted to examine the spectrum of microbial agents isolated in LRTIs specimens collected from patients attending a teaching Hospital in Banke, Nepal.

Materials and Methods

Settings

This was a prospective study conducted on 426 patients of various chronic lung disease attending out-patients and in-patients departments of Nepalgunj Medical College and teaching Hospital, Banke, Nepal, during a full calendar year (January to December 2012). Specimens were processed at the central Laboratory of Microbiology from patients of LRTIs representing specimens. These included sputum, endotracheal secretion, bronchial washings that were received for culture and sensitivity and met the criteria recommended by American Society for Microbiology (8). Data regarding duration of hospital stay and antibiotic history were recorded whenever possible during the processing of specimens. These data were noted from the clinical and microbiological profile sheet of patients.

Cultures

After receiving the sample at sample collection site, it was immediately transported to the Microbiology laboratory and was processed further. The digested sputum samples were cultured on Chocolate agar (CHA), 5% of sheep blood agar (BA) and Maconkey agar (MA) (Oxoid, Basingstoke, UK) plates. On the CHA, bacitracin disk (10 Unit) and optochin disk (5μg) (Oxoid, Basingstoke,UK) were placed at primary and secondary inoculation to screen *Hemophilus influenzae* and *S. pnemoniae* respectively. The CHA plates were incubated in CO_2 incubator (5-10% CO_2) at 37°C for 24-48 hours while BA and MA were incubated at 37°C for 24 hours in aerobic atmosphere. Suspicious colonies were then subculture on a suitable solid culture media for purification and thereafter preserved on appropriate agar slants and stored in the refrigerator (4°C) for subsequent analysis.

Identification of organisms

Identification of significant isolates were done following standard microbiological techniques which involved morphological study of 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 (9) while for other specimens, $\geq 10^5$ CFU/ml was suggestive for infection (10). The API20E kit (Biomerieux, Marcy l'Etoile, France) was then used for the final confirmation of the isolates following the instruction by the manufactures.

Results

A total of 426 specimens from patients with LRT were processed according to the standard microbiological methods. Specimens processed in this study included sputum (n=144), ET secretion (n=8) and bronchial washing (n=42). Out of total 144 sputum specimens, only 82 specimens were further processed while the remaining 62 specimens were rejected as they implied oral contamination. 154 specimens showed no growth/sterile samples. Among the total processed specimens (n=426), 210 (49.3%) showed significant growth of the different specimens. Endotracheal

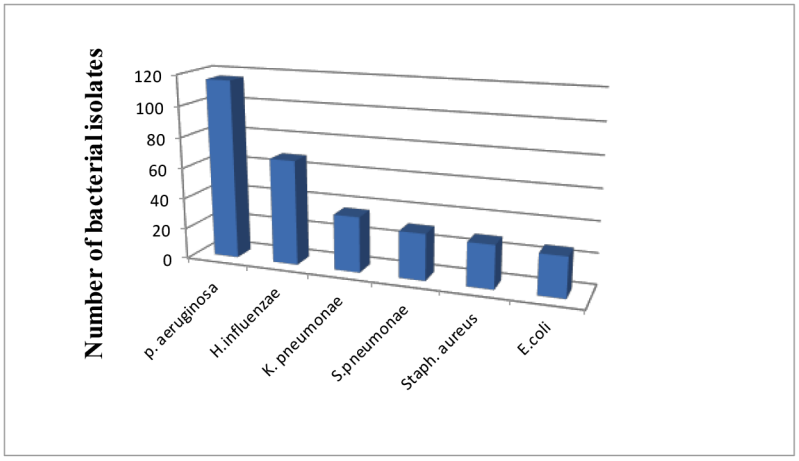

Figure 1. Distribution of various bacterial isolates.

Table 1. Distribution of total bacterial isolates according to Gram negative & Gram positive Bacteria

Gram-Negative Bacteria (n=246)			Gram-Positive Bacteria (n=58)		
Name of bacteria	Number of isolates	% of isolates	Name of bacteria	Number of isolates	% of isolates
P.aeruginosa	116	47.2	*S.pneumonae*	30	51.7
H.influenzae	68	27.6	*S. aureus*	28	48.3
K. pneumonae	36	14.6			
E.coli	26	10.6			

secretion specimens showed the highest microbial isolation rates at 41%. 80% of the growth was monomicrobial while the rest showed mixed growth. Gram-negative bacteria were in 80.9% (n=246) and Gram-positive bacteria were 19.08% (n=58). Among the total 304 bacterial isolates, *P. aeruginosa* (38.2%) was found to be the most predominant organism followed by *H. influenzae, K. pnemoniae, S. pnemoniae, S. aureus* and E. coli (Figure 2). Among the Gram-positive organisms isolated, S. pnemoniae (30; 51.7%) was the most predominant pathogen followed by *S. aureus* (28; 48.3%) and in Gram-negative organisms isolated, *P. aeruginosa* (47.2%) was the predominant pathogen followed by *H. influenzae* (27.6%) *K. pnemoniae* (14.6%) *E. coli* (10.6%) (Table 1).

Discussion

In the present study, we set to find out the bacterial etiological agents causing lower respiratory tract infections at the western part of Nepal. This is a very clinically relevant question as such knowledge may help guide the clinical choice of antimicrobial agents in day to day clinical practice. Pathogens were recovered from just under half of the total specimens. The best yield came from of pathogen ET secretions (41%) followed by sputum (39%) and bronchial washings (20%). Previous studies in the same part of Nepal (11), recovered respiratory pathogens in slightly greater proportion of samples (50.4%). There may have been a small decrease in the prevalence of bacterial LRTIs but the difference is very small. Another study from a different region of Nepal (Kathmandu) supported the

current trend of the microbial spectrum causing LRTIs (12). In the latter study, pathogens were recovered from 44.4% of the total specimens. The yield kept the same trend i.e. 67.2%, 43.7% and 10% of pathogens were recovered from ET secretion, sputum, and bronchial washings respectively (12). Previous studies by workers from same institution in Kathmandu, showed growth rates of 34.6% and 30.5% in 1994 and 2004 respectively (13,14). Likewise, prevalence studies done in China, Turkey and Iran showed matching growth rates of 53.1%, 59.4% and 40.0% of cases respectively (6,15,16).

The lower yield of pathogens in the present study compared to others might be attributed to several potential factors. The natural history of infectious disease in patients may have already been modified by the use of antibiotics by health professionals at different levels of care before patients reach a teaching hospital. Prior use of antibiotics might have played a significant role in culture negatively. Improper use of antibiotics by clinicians and self-prescribing practices by patients could be another contributing factor. Low prevalence of pathogen isolation was also noted in 40-60% of cases elsewhere (17).

The observation that more than a single 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 *Chlamydohila pnemoniae* seems to be the most common organism of co-infection (18). In the same study, monomicrobial growth was found in 80% of cases while 20% were polymicrobial. Monomicrobial growth was found in 91.3% of cases while polymicrobial growth was evident in the remaining 8.7% in another study (12). Mixed pathogens were also seen in 11.2% samples in other studies (6). Polymicrobial growth in 13.3% and 19.0% respectively were also recovered (18,19). In this study, Gram-negative bacteria accounted for 80.9% of all bacteria isolated. The most common isolate was *P. aeruginosa* not only among the Gram-negative bacteria (47.2%), but also among the total isolates (38.2%). It should noted that *P. aeruginosa* is the epitome of an opportunistic pathogen of human and is notorious for nosocomial infections. In the present study, *P aerogenosa* was greater is higher than that of a study carried out in Manipal teaching hospital in which *P. aeruginosa* accounted for 7.5 % of LRTI cases (11). In this study, *H. influenzae* was 27.6% and it secondary highest isolate among the Gram negative bacteria. *H. influenzae* was also common in other studies in Nepal (39.3%) and elsewhere (6,11,13,20). Low prevalence of *H. influenzae* in this study could be due to biofilms formation in vivo which may yield negative cultures (21,22). Also, several lines of evidence indicate that H. influenzae is viable inside host cells, including macrophages and respiratory epithelial cells (23-25). 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 34-47% of sputum cultures are negative with proven *H. influenzae* pneumonia (26,27).

Non-typeable *H. influenzae* colonies the respiratory tract of adults with chronic obstructive pulmonary disease (COPD) and causes intermittent exacerbations (28). Fifty five 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 (6). In this study, Gram-positive bacteria accounted for 19.08% of all bacteria isolated. The most common isolate was *S. pneumoniae*. In Gram positive bacteria *S. pneumoniae* was 51.7%. This was the highest percentage not only among the Gram-positive but also among the Gram-negative isolate. 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., *Mycoplasma pneumoniae*, C. pneumoniae, and viruses were among minors (29). Similarly, a study in England and Wales observed a high rate of *S. pneumoniae* while *H. influenzae* was the second common isolate (30). Buccheri et al. from Italy also showed that *S. pneumoniae* was the most frequent isolate followed by *H. influenzae* (31). Furthermore, even in patients with bacteremic *S. pneumoniae* pneumonia, it has been estimated that the conventional laboratory methods cannot detect the pathogen in 45 to 50% of cases even when large numbers of organisms were evident on Gram stain (32). According to the South Asian Pneumococcal Alliance (SAPNA) project, *S. pneumoniae* is more common in Nepal (33). Among 58 Gram-positive isolates, besides *S. pneumoniae*, 28 (48.3%) isolates of *S. aureus* were also recovered. Similar results were also reported from China (15). *S. aureus* has emerged as a secondary opportunistic diseases and prior viral respiratory disease predisposes the patient to primary staphylococcal pneumonia and a considerable number of *S. aureus* in this study were as mixed pathogens. A total of 36

K. pneumoniae (14.6%) were isolated which constituted the third major Gram-negative bacteria. Similar incidence of *Klebsiella spp*. (19.4%) was found from sputum samples in the 2004 study in Kathmandu (14). *E. coli* is an uncommon cause of acute LRTI and in this study, they comprised of 8.6% of total cases. The total 26 isolates were found E.coli which was 10.6% in among Garm-negative bacteria. These findings corroborate well with those of Pokhrel *et al*. and Mishra *et al* who reported rates of 5.8% and 6.9% respectively (14,12).

In conclusion, the spectrum of bacteria causing lower respiratory infection is still wide in Nepal. In the present study, *P. aeruginosa* and *S. pneumoniae* were the most common Gram-negative and Gram-positive bacteria isolated. Bacterial etiology may vary in different geographical regions and even over time in the same location and population. Hence, routine surveillance of microbial etiology of LRTI is important.

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