Original Article

Cross – sectional study of respiratory pathogens and their antibiotic susceptibility pattern in Tribhuvan University Teaching Hospital

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Background: Lower respiratory tract infection (LRTI) comprises an array of diseases ranging from bronchitis to pneumonia; its asperity can sway from non-pneumonic LRTI to pneumonia or chronic obstructive pulmonary disease (COPD). The HMG/WHO review in 1994 estimated the overall acute respiratory infections in Nepal to be 2.1%.

Methods: In brief, all total 132 sputum samples of patients suspected of LRTI those visiting Tribhuvan University Teaching Hospital during March to May 2005 were investigated with the use of standard microbiological methods and the bacterial isolates were subjected for antibiotic sensitivity test.

Results: Of the total, 31.0% sputum samples showed significant positive. Among them 75.6% were gram-negative bacteria (GNB) and 24.40% gram-positive bacteria (GPB). Eight different types of bacteria were isolated. *Haemophilus influenzae* (19.51%), *Klebsiella pnemoniae* (19.51%), *Pseudomonas aeruginosa*(19.51%), *were* the most common isolates followed by *Streptococcus pneumoniae* (12.19%), *Staphylococcus aureus* (12.19%), *Escherichia coli* (9.75 %), *Citrobacter fruendii* (4.87%), *Acinetobacter calcoaceticus* (2.43%). Antibiotic susceptibility test showed that, more than 50% of GPB were resistant to Erythromycin, Cephalexin and Ciprofloxacin whereas majority of GNB were resistant to Ciprofloxacin, Gentamycin, Ampicillin.

Conclusion: This study needs to be conducted round the year to acquire more information regarding seasonal variation.

Key words: LRTI, Antibiotic susceptibility pattern, Sputum, GPB, GNB

Introduction

According to the survey carried out in TUTH by Sayami and Shrestha in 1994 (1) respiratory diseases represent the number one hierarchy according to the frequency representing the 30.7% of the total medical admission.

Lower respiratory tract infections are among the most infectious diseases of human worldwide. An expanded variety of emerging pathogens likewise provides challenges for the microbiology laboratory. Over treatment of acute uncomplicated bronchitis, which is largely due to viruses, has led to incomparable level of multidrug resistance among invasive pathogens (2). There is growing need for the better understanding of the pathogens that causes lower respiratory tract infections, which should allow a logical approach to treatment(3). The treatment should be done with proper antibiotic and reporting of such antibiotic susceptibility pattern should be continued to improve the misuse of antibiotic as well as to impede multi-drug resistance strains and to guide prophylaxis.

Material and Methods

The study was hospital based cross-sectional study for which sputum specimens were collected from patients visiting TUTH; all the processing of the sputum and microbial study was conducted at bacteriology Laboratory, TUTH, IOM, Maharajgunj. All together 132 patients complaining about the respiratory illness visiting TUTH between March to May 2005 were involved. Sputum was collected in leak proof container. After microscopy, sputum were rejected or accepted on the basis of inclusion criteria (epithelial cells <10/Low power field (LPF) and pus cells>25/LPF). Accepted sputum was homogenized with 0.01% dithiotheritol. Culture was done in Blood agar, Chocolate agar (with Bacitracin and Optochin) and MacConkey agar plates. After 24 hours incubation at 37°C, the pattern of microbial growth was observed. In Blood agar α-haemolysis and β-haemolysis were checked whereas in chocolate agar bacitracin resistant watery colony and optochin sensitive organisms were looked. Any lactose fermenting and non-lactose fermenting colonies were observed in MacConkey agar. After studying the result of Gram Stain, gram positive and gram negative bacteria were further processed. For the further confirmation array of biochemical tests such as Catalase, Oxidase, IMViC (Indole, Methyl red, Voges Proskauer and Citrate), Motility, TSI (Triple sugar iron) tests were done for gram-negative bacteria except *Haemophilus influenzae.* For *H influenzae*, Bacitracin resistant watery colony was cultured on Muller Hinton Agar with X (hemin), V (NAD) and XV discs (combination of hemin and NAD). Growth around XV disc was identified as *H influenzae*. Cocci were identified following Bergy's Manual of Systematic Bacteriology, which included the study of colony morphology, gram staining, and biochemical properties, Catalase, oxidase, coagulase test.

Antibiotic sensitivity test was done by Kirby Bauer's method. The zone of inhibition was observed after 24 hours incubation at 37°C. The zone of inhibition was interpreted with the help of standard interpretive chart.

Results

Table 1 shows pattern of bacterial isolates from sputum sample. Among gram-negative bacteria *Haemophilus influenzae* (19.51%), *Klebsiella pnemoniae* (19.51%), *and Pseudomonas aeruginosa* (19.51%), were found to be equally common, followed by *E coli* (9.76%) *Citrobacter fruendii* (4.88%) and *Acinetobacter calcoaceticus* (2.43%). *Staphylococcus aureus* (12.19%) and *Streptococcus pneumoniae* (12.19%).

Table 1: Pattern of organisms isolated from lower respiratory tract

Gram negative bacteria	Number	%
Haemophilus influenzae	8	19.51
Klebsiella pnemoniae	8	19.51
Pseudomonas aeroginosa	8	19.51
Streptococcus pneumoniae	5	12.19
Staphylococcus aureus	5	12.19
E. coli	4	9.76
Citrobacter fruendii	2	4.88
Acinetobacter calcoaceticus	1	2.43
Total	41	100

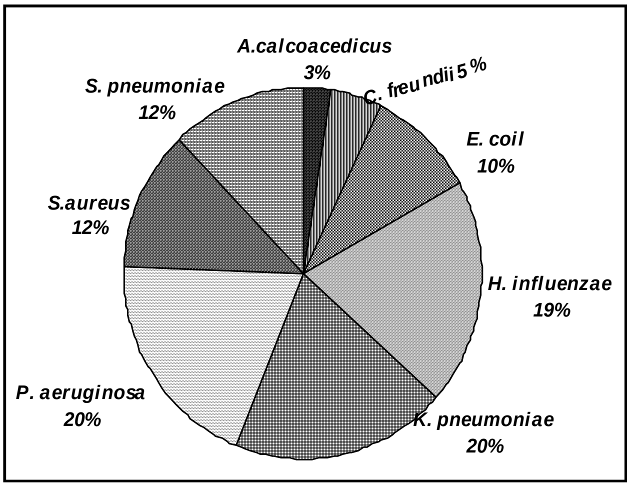

Fig. 1: Total organisms isolated from lower respiratory tract infection

It was found that gram-negative bacteria were higher in number from admitted patients and out patients whereas gram-positive bacteria were fewer in both cases. It was found that greater number of male outpatients and in-patients are infected than those of female outpatients and in-patients. The percent of infected in-patients were also found to be greater than that of infected outpatients. The number of bacterial isolates was greater in case of male in-patients and outpatients. *Haemophilus influenzae* was found in case of outpatients only whereas *Citrobacter fruendii*, *Acinetobacter calcoaceticus* was found only in in-patients. It was found that LRTI occurred most frequently between age 50-60 (30.43%) followed by 60-70 (20.58%), and in these groups male patients were higher in number than female patients *(Table 2).*

Table 2: Distribution of organisms in In-patients and Outpatient

Types of organisms	In-patients number Male	Female	Out-patients number Male	Female
Haemophilus influenzae	-	-	6	2
Klebsiella pneumoniae	3	1	3	1
Pseudomonas aeroginosa	3	3	1	1
Staphylococcus aureus	2	1	1	1
Streptococcus pneumoniae	2	-	1	2
Escherichia coli	3	1	-	-
Citrobacter fruendii	2	-	-	-
Acinetobacter calcoaceticus	1	-	-	-
Total	16	6	12	7

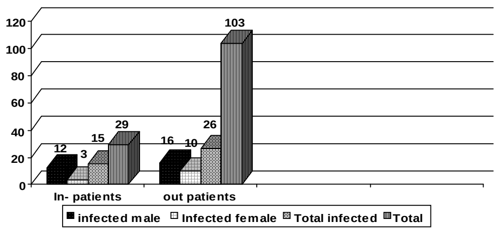

Fig. 2: Pattern of infection in in-patients and outpatients.

The pattern of antibiotic sensitivity in GNB except *Haemophilus influenzae* shown in table 3 reveals that 86.95 % GNB were sensitive towards Amikacin whereas 73.92 % GNB showed resistivity towards Cephalexin.

Table 3: Antibiotic sensitivity pattern of Gram Negative Bacteria except *Haemophilus influenzae.*

Antibiotic	Sensitive Number	(Percentage)	Resistant Number	(Percentage)
Amikacin	20	86.95	3	13.05
Ceftazidime	12	52.18	11	47.82
Ciprofloxacin	11	47.82	12	52.18
Gentamycin	10	43.47	13	56.53
Ampicillin	7	30.43	16	69.57
Cephalexin	6	26.08	17	73.92

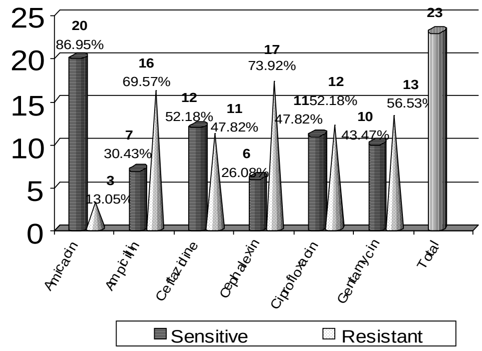

Fig. 3 Antibiotic sensitivity pattern of GNB except *Haemophilus influenzae*

Antibiotic sensitivity pattern of *Haemophilus influenzae* is shown in table 4. Of the total 87.5% of *Haemophilus influenzae* was susceptible to Chloramphenicol, followed by Doxycycline (75%), Ampicillin (75%), Ceftazidine (75%), and Ciprofloxacin (75%).

Table 4: Antibiotic sensitivity pattern of *Haemophilus influenzae*

Antibiotic	Sensitive Number	(Percentage)	Resistant Number	(Percentage)
Chloramphenicol	7	87.5	1	12.5
Ceftazidime	6	75	2	25
Ciprofloxacin	6	75	2	25
Doxycycline	6	75	2	25
Ampicillin	6	75	2	25
Cephalexin	3	37.5	5	62.5

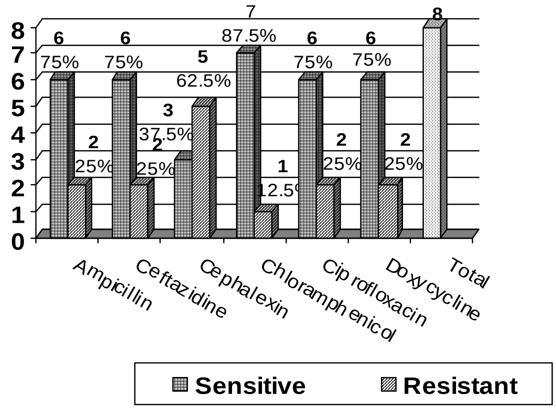

Fig. 4: Antibiotic sensitivity pattern of *Haemophilus influenzae*

In case of *Streptococcus pneumoniae* 80% of the organisms showed susceptibility towards Ampicillin followed by Erythromycin (60%) and Cloxacillin (60%) while, the least effective was Cephalexin. 80% *Streptococcus pneumoniae* showed resistance towards Cephalexin (Table 5).

Table 5: Antibiotic sensitivity pattern of *Streptococcus pneumoniae*

Antibiotic	Sensitive Number	(Percentage)	Resistant Number	(Percentage)
Ampicillin	4	80	1	20
Erythromycin	3	60	2	40
Cloxacillin	3	60	2	40
Ciprofloxacin	2	40	3	60
Cephalexin	1	20	4	80

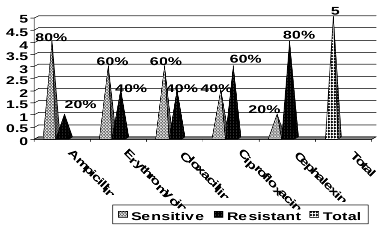

Fig. 5: Antibiotic sensitivity pattern of *Streptococcus pneumoniae*

Antibiotic sensitivity pattern of *Staphylococcus aureus showed* that Cloxacillin is the most effective drug. More than 50 % of the organisms showed resistance towards Ampicillin Ciprofloxacin and Erythromycin. The results are shown in table 6 and figure 6.

Table 6: Antibiotic sensitivity pattern of *Staphylococcus aureus*

Antibiotic	Sensitive Number	(Percentage)	Resistant Number	(Percentage)
Cloxacillin	4	80	1	20
Cephalexin	3	60	2	40
Ampicillin	2	40	3	60
Ciprofloxacin	1	20	4	80
Erythromycin	1	20	4	80

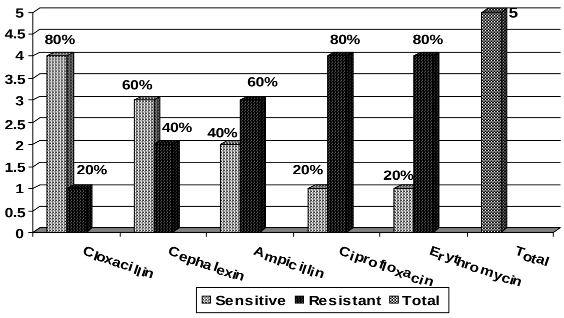

Fig. 6: Antibiotic sensitivity pattern of *Staphylococcus aureus*

Discussion

During this study, 132 sputum samples were examined and cultured, among which total 41(31.06 %) samples showed significant microbial growth. Out of total positive cases, 8 different types of bacteria were isolated. In a survey carried out in Tribhuvan University Teaching Hospital (TUTH) in the year 2004, out of the total 181 patients enrolled, 41.4% were infected with lower respiratory tract infection (4). In our case, the large percentage of negative results was obtained either due to the patients suffering from other infections such as *Chlamydia*, *Mycoplasma* ,Pneumocystis or viral infections which we did not look for.

Potential respiratory pathogens were isolated from 41 (31.06 %) patients. Out of which 75.61 % were gram negative and 24.39 % were gram-positive bacteria. The infection due to GNB is three times greater than GPB in our case. This incident correlated with the findings of Sayami and Shrestha (2)

Among GNB, *Haemophilus influenzae* (19.51%), *Klebsiella pnemoniae* (19.51) *and Pseudomonas aeroginosa* accounted 19.51% of the total infected cases. Mac Farlane *et al.*, 1993 (3) also reported in their study that the most common pathogen of LRTI was *Streptococcus pneumoniae* followed by *Haemophilus influenzae*. However, our results differ from these results. In our case, we isolated *Haemophilus influenzae* more frequent than *Streptococcus pneumoniae* which is similar to the study carried out by Shrestha B, Sharma A and Pokhrel B.M in 1997(6).

Sources of organisms such as *Pseudomonas aeruginosa, Escherichia coli, Citrobacter fruendii, and Acinetobacter calcoaceticus* could be endogenous. In our study it was found that greater numbers of in-patients (51.7%) were infected than those of out-patients (25.24%).

In our study, antibiotic sensitivity pattern of *Haemophilus influenzae* showed that Chloramphenicol was most effective (87.50%), this trend was followed by Doxycycline (75%). According to Thomsberry and Holley, 1999 (5) *Haemophilus influenzae* was generally sensitive to quinolones but there had been case reports of Ciprofloxacin resistance also. In our study only 25% of the total *Haemophilus* infected cases showed resistant towards Ciprofloxacin. Among GNB except *Haemophilus influenzae* the most effective drug of choice was Amikacin and the least effective was Cephalexin. As per in-vitro study effective antibiotic for *Staphylococcus aureus* was Cloxacillin and the least effective were ciprofloxacin and erythromycin. This result is well correlated with the findings of Shrestha B, Sharma A and Pokhrel B.M in 1995(6). Eighty percent *Streptococcus pneumoniae* were sensitive to Ampicillin and the least effective percent of drug was Cephalexin. According to Mac Farlane 1993 (3), Ampicillin was drug of choice in 80% cases of *Streptococcus pneumoniae*.

Conclusion

Our study showed that the most common organisms causing LRTI were *Haemophilus influenzae, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. *Streptococcus pneumoniae* and *Staphylococcus aureus* were found to be as second common pathogens. Antibiotic pattern of *Klebsiella pneumoniae* showed that the least effective drug was Ampicillin and most effective one was Amikacin. *Pseudomonas aeruginosa* was sensitive to Amikacin. Chloramphenicol was most effective and Cephalexin was least effective antimicrobial for *Haemophilus influenzae*. Cloxacillin was most effective while Erythromycin and Ciprofloxacin were least effective antibiotics for *Staphylococcus aureus*.

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