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BACTERIAL ETIOLOGY AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF RESPIRATORY ISOLATES AT A TERTIARY CARE HOSPITAL OF EASTERN NEPAL

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ABSTRACT

Background: Lower respiratory tract infections (LRTI) is one of most common infections. Multidrug resistance (MDR) strains of bacteria causing respiratory infections are becoming world-wide problem. The study was designed to determine bacteriological etiology and antibiotic susceptibility pattern of respiratory isolates at BPKIHS. **Methods:** This is a cross-sectional study conducted at Department of Microbiology for one year (1st August 2018 to 31st July2019). A total of 1755 sputum samples of patients suspected of LRTI were obtained in the Department of Microbiology. Culture and bacterial isolates were identified with use of standard method as described by American Society for Microbiology. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion as described by Clinical and laboratory standards institute. **Results:** Out of total 1755 sputum samples, 468 (26.6%) showed significant bacterial growth. Among 468 bacterial growth, 82.9% were gram negative bacilli and 17.1% were gram positive cocci. *Klebsiella pneumoniae* was reported the most common etiological agent (24.35%), followed by *Acinetobacter baumanni complex* (19.23%), *Pseudomonas aeruginosa* (17.09%), and *Staphylocooccus aureus* (14.74%). MDR isolates accounted for 43% of isolates. **Conclusions:** High-level antimicrobial resistance was observed in LRTI with alarming incidence of MDR, ESBL and MRSA. Regular surveillance should be carried out to determine the local prevalence of micro-organisms and antimicrobial susceptibilities in order to guide the proper management.

KEYWORDS: LRTI, MDR, ESBL, MRSA.

INTRODUCTION

Lower respiratory tract infections (LRTIs) are common causes of morbidity and mortality worldwide for all age groups.[1] 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.[2] *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Hemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas species* and *Acinetobacter species* are the common bacterial agents associated with LRTIs. It is not a single disease but a group of specific infections each with a different epidemiology, pathogenesis, clinical presentation and outcome.[3] Age, gender, and season are factors that have been implicated to affect the prevalence of LRTIs. The etiologic agents of LRTIs vary in different geographical area. So the susceptibility profile will also differ between geographical locations.[1]

Infection with multidrug resistant (MDR) bacteria lead to increased morbidity, prolong hospital stays, increased mortality.[4] Multidrug resistance is defined as the resistance to three or more different antimicrobial groups. Extended-spectrum beta-lactamase (ESBL) producing bacteria are major threat to the community placing an extra burden on individual patients and on healthcare system. Monitoring the antimicrobial resistance patterns of the etiological agents is needed for understanding the trend of these infections.[5] Microbiological investigations are of great importance for standardizing empirical therapies to be used in the future.

However, information about the prevalence of respiratory pathogens and their antibiotic resistance pattern is inadequate in our country. Therefore, the aim of this study is to provide updated knowledge about the etiological agents, antimicrobial resistance pattern and prevalence of MDR bacteria associated with respiratory infections at BPKIHS.

METHODS

Study design and study setting

This is a hospital based descriptive cross sectional study conducted in the Department of Microbiology, BPKIHS. Sputum samples of patients received for culture and sensitivity in the Microbiology laboratory during the period of one year (1st August 2018 to 31st July2019) were included in the study. This study included 1755 samples for the study during the allocated research period.

Microbiological methods

Respiratory specimens when received in microbiology laboratory were subjected to gram staining and culture. The specimen is inoculated onto Blood agar and Macconkey agar and incubated aerobically at 35 ºC for 18-24 hours. After incubation, the plates were observed for bacterial growth. Any bacterial colony was identified by using gram staining and biochemical tests following standard microbiological guidelines.[6] After identification, antimicrobial susceptibility testing was performed by modified Kirby Bauer disc diffusion method following clinical and laboratory standards institute guidelines (CLSI).[7]

Definition of MDR and PDR

MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories and PDR is defined as non-susceptibility to all agents in all antimicrobial categories.[8]

Statistical analysis and interpretation

The data was entered in Microsoft excel 2016.

Ethical clearance

Ethical clearance was obtained from Institutional Review Committee, BPKIHS, Dharan, Nepal.

RESULT

During the study period, 1755 respiratory samples were submitted for gram staining, culture and sensitivity. Among them, significant bacterial growth was observed in 468 (26.6%) samples. Normal flora was yielded in 850 samples and 437 samples were found to sterile. Among 468 bacterial growth, 388 (83%) were gram negative bacilli (GNB) and 80 (17.1%) were gram positive cocci (GPC). Among the total bacterial growth 48% of isolates were from outpatient department and 52% of isolates were from inpatient department.

Among 468 bacterial isolates obtained from sputum samples, *Klebsiella pneumoniae* (n=114, 24.35%) was most common organism followed by *Acinetobacter baumanii complex* (n=90, 19.23%), *Pseudomonas aeruginosa* (n=80, 17.09%) and *Staphylococcus aureus* (n=69, 14.74%) [Table 1].

Table 1: Organisms isolated from sputum samples.

Organisms	Number	Growth percentage (%)
Klebsiella pneumoniae	114	24.35
Acinetobacter baumanii complex	90	19.23
Pseudomonas aeruginosa	80	17.09
Staphylococcus aureus	69	14.74
Escherichia coli	65	13.88
Enterobacter aerogenes	21	4.48
Citrobacter freundii	14	2.99
Enterococcus faecalis	11	2.35
Proteus mirabilis	4	0.8
Total	468	100

Antimicrobial susceptibility pattern of the isolates has been tabulated in Table 2. Fifty percentage of *K. pneumoniae* were resistant to Imipenem, while all were sensitive to colistin. Among gram-positive bacteria, 90% of *S. aureus* were resistant to penicillin while all were susceptible to vancomycin and linezolid.

Table 2: Antimicrobial resistance pattern of the isolates (%).

Organism	Amikacin	Ampicillin	Ceftriaxone	Cefoxitin	Levofloxacin	Co-trimoxazole	PIT	Imipenem	Colistin	Penicillin	Vancomycin	Linezolid
Klebsiella pneumoniae	35	-	55	-	42	61	20	50	0	-	-	-
Acinetobacter anitratus	40	83	65	-	45	65	35	65	0	-	-	-
Pseudomonas aeruginosa	40	40	45	-	42	65	15	70	0	-	-	-
Escherichia coli	30	70	70	-	60	65	30	65	0	-	-	-
Enterobacter aerogenes	20	80	50	-	60	55	5	40	0	-	-	-

Citrobacter freundii	30	85	70	-	42	75	30	70	0	-	-	-
Proteus mirabilis	0	100	50	-	0	75	0	25	0	-	-	-
Staphylococcus aureus	30	-	50	30	40	70	-	-	-	90	0	0
Enterococcus faecalis	20	-	-	-	50	-	-	-	-	60	0	0

Note: PIT: Piparacillin-Tazobactam.

Forty three percentage of organisms were MDR. None of the isolates was PDR.

DISCUSSION

In our study, 26.6% of total specimens showed growth on culture. The isolation rate is close to the observation Sharma *et al* (39.46%) and Okesola *et al* (27%)[9,10] while Dawadi *et al* (48.43%) and Egbagbe *et al* (47.2%) reported slightly higher isolation rate.[11,12] Lower yield in the present study may be attributed to various factors. Common pulmonary pathogens such as *Mycobacterium tuberculosis, Mycoplasma, Chlamydia, Pneumocystis, Legionella*, and anaerobes could not be cultured by routine methods. In this study GNB accounted for 82.9% (388) of all bacteria isolated and GPC accounted for 17.1% (80).

The most common isolate was *Klebsilla pneumoniae* (24.35%) among the total isolates, followed by *Acinetobacter baumanii complex* (19.23%) and *Pseudomonas aeruginosa* (17.09%). *P.aeruginosa* is the epitome of an opportunistic pathogen of human and is notorious for nosocomial infections. In the present study, *P aeruginosa* 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.[3] Infections with *Acinetobacter baumanii complex* and *P. aeruginosa* is a serious problem affecting hospitalized patients, particularly those who are critically ill and imunocompromised, such as patients with cystic fibrosis.[2] Some studies have also pointed to the predominance of other gram negative bacilli in lower respiratory tract infection. For example, Sharma et al and Gauchan et al isolated *Hamemophilus influenzae* as the most common isolate.[9,13] While Okesola et al and Egbagge et al reported *Klebsiella pneumoniae* as the most common bacteria associated with LRTI.[10,12] *S. aureus* accounted for 14% of the total isolates in our study, it 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.[3]

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. Antimicrobial susceptibility test performed for bacterial isolates in the current study showed that vancomycin and linezolid (100% sensitivity) was the most effective antibiotic against Gram-positive bacteria and penicillin the least effective one (90% resistance). All gram-negative bacteria, were susceptible to colistin (100% sensivity) while susceptibility pattern to other antibiotics were was variable. *Klebsiella pneumoniae* was most sensitive to PIT (80% sensitivity) while *Acinetobacter* spp was also to PIT (65%). In *Pseudomonas aeruginosa* also PIT was the most effective antibiotic (85% sensitivity). In the similar study conducted by Gauchan *et al* 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.[13] Emergence of serious infections due to multidrug resistant organisms poses a therapeutic challenge.

Forty three percentage of organisms were MDR, a study conducted by Vishwanath *et al* observed MDR in 37% isolates, which is quite similar to the finding of our study. They did not isolate any PDR organisms, which also agrees to our finding.[4] A study conducted by Shrestha A *et al* in Chitwan, Nepal observed 55% MDR isolates from LRTI.[2] Our study also showed high incidence of MDR. Similar results have been reported by studies done worldwide. There has been a worldwide increase in emergence of drug resistant organisms in recent years.[14] Multidrug resistant (MDR) bacteria has been well recognized as one of the most important public health problems in current scenario. Treatment outcomes in patients infected with MDR bacteria tend to be worse as compared to those infected with susceptible organisms.[15] Worldwide, the prevalence of Methicillin-resistant *S. aureus* (MRSA) range from 30% to 90% depending upon the type of infections.[15] The Prevalence of ESBL producer worldwide range from 12 to 80%. The epidemiology of ESBL-producing bacteria is becoming more complex with increasingly blurred boundaries between hospitals and the community.[16]

CONCLUSIONS

The study showed respiratory infection rate of 26.6% among the 1755 sputum samples received. *K.pneumoniae, A. baumanii compex, P.aeruginosa* and *S. aureus* are the common organisms. There is an increasing trend of antimicrobial resistance but still vancomycin is susceptible to all the Gram positive cocci and colistin is susceptible to al gram negative bacilli. Routine surveillance of MRSA, ESBL, and multi-drug resistant organisms is essential in proper management of respiratory infections.

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