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Antimicrobial Sensitivity among Bacterial Isolates from Sputum Samples in a Tertiary Level Hospital

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ABSTRACT

Introduction

Emergence of antimicrobial resistance is a huge challenge in management of pneumonia especially health care associated ones. Culture and sensitivity test of the bacteria from sputum samples is not only useful for rational treatment of respiratory bacterial infections of individual cases but also for surveillance of changing sensitivity status of prevalent organisms. The aim of this study was to find the bacterial sensitivity pattern from the sputum samples and endotracheal aspirates.

Methods

This cross-sectional study was conducted in Shree Birendra Hospital, Kathmandu, Nepal. Standard guideline was followed for sputum culture and identification of the bacterial isolates. The antimicrobial susceptibility test was conducted using the Kirby-Bauer disc diffusion technique. Every bacterial isolate's sensitivity was studies and noted.

Results

The culture positivity rate was 9.87% (99/1003). Predominant isolates were gram-negative bacteria (97.4%). *Pseudomonas spp.* was the most frequent isolate (31%). Among the all isolates, 48.1% of *Pseudomonas spp.*, 81.5% of *Klebsiella pneumoniae*, and 60% of *Escherichia coli* isolates showed meropenem resistance. Imipenem and meropenem resistance was observed in 91.7% of the isolates of *Acinetobacter spp.* Rate of resistance to carbapenems i.e. imipenem and meropenem was higher in comparison to rate of resistance to aminoglycosides in all of the gram-negative isolates.

Conclusion

The isolates belonging to *Enterobacterales* and the *Acinetobacter spp.* have been found to have high incidence of resistance to carbapenems in comparison to other groups of antibacterial agents.

Keywords

Acute respiratory infections; antimicrobial susceptibility; gram-negative organisms; sputum culture

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INTRODUCTION

Worldwide, bacterial pathogen-induced acute respiratory tract infections are among the leading cause of morbidity and mortality.[1] Patients' age, comorbidities, and immunological state are risk factors for a serious and deadly respiratory infection.[2] Ventilator-associated pneumonia and health care associated pneumonia have high morbidity and mortality.[3] Emergence of antimicrobial resistance (AMR) is a huge challenge in management of pneumonia especially health care associated ones.[4] Culture and sensitivity test of the bacteria from sputum samples is very useful as a guideline for rational treatment of respiratory bacterial infections. This also helps to avoid unnecessary use of broad-spectrum antimicrobial agents, thus limiting the selection pressure on the pathogens since empirical therapy respiratory symptoms with broad-spectrum antibacterial agents is a contributory factor for development and spread of AMR. Though international surveillance to the emergence of such multi-drug-resistant strains can provide hints for developing antibiotic policies, local antibiograms with continuous update are required for developing local treatment guidelines and formulating policies for containment of antimicrobial resistance.

This study was undertaken to find the short-term trend in antimicrobial sensitivity among bacterial isolates from sputum samples. Findings from the study may help for surveillance of changing sensitivity status of prevalent organisms

METHODS

The cross-sectional study was carried out in the microbiology laboratory of Shree Birendra Hospital (SBH), Kathmandu which is a tertiary level teaching hospital of Nepalese Army Institute of Health Sciences (NAIHS). The study duration was from October 2021 to February 2022 and ethical approval was obtained from Institutional Review Committee (IRC) of NAIHS (Approval number 459, September 2021).

All culture-positive reports for sputum samples submitted to the Microbiology laboratory from clinically suspected cases of lower respiratory tract infections during the study period were included. The demographic details of the patients with culture positive result for the sputum sample was collected in addition to the sensitivity of each of the bacterial isolates using the predesigned proforma. The anonymity of the patients was ensured by omitting the names of the patients while collecting the data. Number of samples with no growth in sputum sample was also recorded. Sputum samples included spontaneously produced sputum as well as endotracheal aspirate. Laboratory technician collected the sputum samples that were transported to the microbiology laboratory as soon as possible. After receiving them, all the samples were Gram stained to know its appropriateness for culture. Samples with leukocytes count >25/LPF were included for culture. Sputum samples containing epithelial cells >25/LPF were considered as saliva, so those samples were rejected. Samples were inoculated in MacConkey Agar (MA), Blood Agar (BA), and Chocolate Agar (CA) according to standard procedure (Mast Group Ltd, Merseyside UK). Bacitracin discs (10 units) and optochin discs (5µg) were used on CA for the main and secondary inoculations, respectively, to screen for Streptococcus pneumoniae and Haemophilus influenzae. Whereas CA plates were incubated at 37°C in a carbon dioxide (CO_2) incubator with a concentration of 5–10% of CO_2, BA and MA plates were incubated for 24 hours at 37°C in an aerobic atmosphere.

The organism's identification was completed in accordance with standard procedures.[5] Antimicrobial Susceptibility Testing (AST) was conducted in accordance with guideline 2020 of the Clinical and Laboratory Standards Institute (CLSI).[6] Regular quality control was performed on media, reagents, and laboratory equipment. The antibiotic discs and Mueller-Hinton agar (MHA) were examined for correct storage, lot number, manufacturing, and expiration dates. As part of quality control, control strains of *E. coli* American Type Culture Collection (ATCC) 25922, *P. aeruginosa* ATCC 27853, and S. aureus ATCC 25923 were examined in parallel for the purpose of standardizing the Kirby-Bauer disc diffusion test and evaluating the effectiveness of antibiotics and MHA. By keeping the pH between 7.2 and 7.4, as well as the Mueller-Hinton agar thickness at 4 mm, the quality of the sensitivity tests was preserved. The zone of inhibition, in accordance with the manufacturer's sensitivity chart and the CLSI recommendations, was used to establish antibiotic susceptibility.

Data on the bacterial isolates' antimicrobial sensitivity and demographics were entered into Microsoft Excel (Microsoft Office Professional Plus 2013), and the frequency of susceptibility to the tested antimicrobial agents was examined for each bacterial isolate.

RESULTS

The culture positivity rate was 9.87% (99/1003). The distribution of the culture isolates is shown in Figure 1. Predominant isolates were Gram negative bacteria (97.4%). Most common isolate was *Pseudomonas aeruginosa*. Two pathogenic bacteria were isolated in sputum samples from 10 patients. *Candida spp.* (fungus) were isolated in two cases and thus excluded from the study.

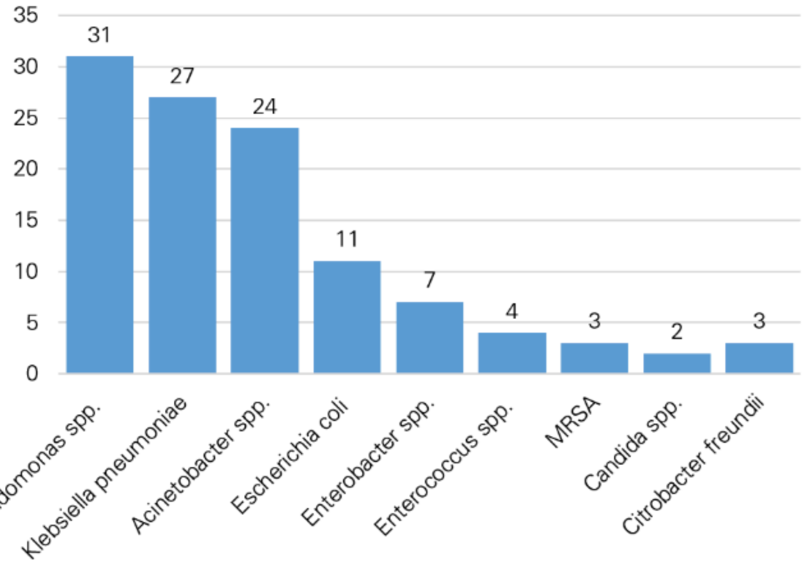

Figure 1. *Distribution of culture isolates (MRSA = Methicillin resistant Staphylococcus aureus)*

The results of the sensitivity tests of the isolated bacteria are summarized in Table 1. The bacterial isolates which were resistant to all the tested antimicrobial agents, comprised of *Acinetobacter* spp. (n=12), *Klebsiella pneumoniae* (n=7), *Pseudomonas aeruginosa* (n=1) and Enterobacter spp.(n=1) which are likely to extended spectrum beta lactamase (ESBL) producers

Among the 99 cases with culture-positive samples, 55 were from male and 44 were from female patients. The mean age was 62.63 years and the highest number of patients (n= 32) belonged to age group of 61-70 year.

DISCUSSION

Low culture positivity rate in sputum samples in our study may have multiple reasons. However, the most important reason may be the use of antibacterial agent(s) before patient reaches to hospital for consultation. Our study too has found high proportion of gram-negative bacteria, *Pseudomonas*, *Klebsiella* and *Acinetobacter spp.* being the three most common isolates. The reason for predominance of gram-negative organisms in sputum culture may again be because of pretreatment with antibiotics with coverage to common gram-positive organisms.

A study including 166 patients with positive sputum cultures conducted at intensive care unit (ICU) setting of the Tribhuvan University Teaching Hospital (TUTH) in Kathmandu showed that bacteria were present in 63% of the samples, with 64% of these cases belonging to the species *Pseudomonas, Acinetobacter, Burkholderia, and Stenotrophomonas.*[7] High proportion of bacterial growth in this study in contrast to our study may be because of the samples taken in the ICU setting before commencement of antibiotic treatment. A study done in SBH during 2013-2014 on patients with acute exacerbation of COPD had also found that 88.76% of isolates to be gram negative bacteria with predominance of same organisms in our study.[8]

Gram-negative isolates were found to be present in 78.1% (25/32) of individuals with acute exacerbations of COPD, according to a 2019 Ethiopian study.[9] *Pseudomonas aeruginosa* (21.9%; 7 out of 32), *Klebsiella pneumoniae* (18.75%; 6 out of 32), and *Staphylococcus aureus* (15.62%; 7/32) were the most common bacterial strains among them.[9] Previous study focusing on sensitivity of *Pseudomonas aeruginosa* done in the same hospital had shown that 46.5% (93/200) of the isolates were from sputum samples.[10]

Though *Pseudomonas* species are known for their noted resistance to multiple classes of antimicrobial agents, *Acinetobacter spp.* and *Klebsiella pneumoniae* exhibit a much higher frequency of resistance to the tested antimicrobial drugs in our study. *Pseudomonas* showed considerable resistance rates to levofloxacin (61%), cefepime (50%), and amikacin (50%), whereas *Acinetobacter* showed strong resistance to cefepime (95%), imipenem (92%), and levofloxacin (86%), according to research conducted by Ghimire and colleagues.[7] For *Pseudomonas spp.*, findings are similar in our study with 51.6% resistance to ofloxacin, 48.4% for cefepime but only 19.4% for amikacin. Susceptibility to cefepime was not tested in for *Acinetobacter spp.* in our study, but resistance to imipenem as well as meropenem was 91.7% and thus alarming. *Klebsiella pneumoniae* isolates in this study showed very low sensitivity to both of the carbapenems tested with 77.8% and 81.5%

Table 1. *Sensitivity of bacterial isolates (percentage) towards antibacterial agents*

Organisms	AMP	PI	PTZ	CTX	CRO	CPZ	CAZ	CPM	CIP	OF	AK	GN	COT	IMP	MR	DOX	VA	LZ	TPL
Pseudomonas spp.(n=31)	ND	77.4	77.4	ND	ND	ND	41.9	51.6	58	48.4	80.6	ND	ND	29	41.9	ND	ND	ND	ND
Klebsiella pneumoniae (n=27)	0	7.4	18.5	18.5	33.3	3.7	ND	ND	3.7	22.2	48.1	48.1	3.7	22.2	18.5	22.2	ND	ND	ND
Acinetobacter spp. (n=24)	0	12.5	16.7	0	0	0	ND	ND	0	16.7	16.7	12.5	4.2	8.3	8.3	16.7	ND	ND	ND
Escherichia coli (n=10)	0	50	60	30	20	10	ND	ND	30	40	60	60	30	10	40	30	ND	ND	ND
Enterobacter spp. (n=7)	0	28.6	42.9	14.3	28.6	0	ND	ND	28.6	42.9	57.1	28.6	0	28.6	14.3	0	ND	ND	ND
Enterococcus spp. (n=4)	0	ND	ND	ND	ND	ND	ND	ND	0	0	ND	75	ND	ND	ND	75	75	100	100
Staphylococcus aureus (MRSA) (n=3)	ND	ND	ND	ND	ND	ND	ND	ND	0	0	66.7	66.7	0	ND	ND	100	ND	100	ND
Citrobacter freundii (n=3)	0	66.7	100	66.7	100	0	ND	ND	33.3	0	100	66.7	0	33.3	33.3	0	ND	ND	ND

Abbreviations:

ND= Not done (Antibiotic disc not used for the organism) , AMP= Ampicillin, PI= Piperacillin, PTZ= Piperacillin+tazobactam, CTX= Cefotaxime, CRO= Ceftriaxone, CPZ= Cefoperazone, CAZ= Ceftazidime, CPM= Cefepime, CIP= Ciprofloxacin, OF= Ofloxacin, AK= Amikacin, GN=Gentamicin, COT= Cotrimoxazole, IMP= Imipenem, MR= Meropenem, DOX= Doxycycline, VA=Vancomycin, LZ= Linezolid, TPL= Teicoplanin

(For MRSA additional antibiotics tested were- azithromycin, clindamycin and chloramphenicol and all the isolates were resistant to them) of isolates showing resistance to imipenem and meropenem respectively which suggests possibly the prevalence of carbapenemase producing strains is high. Unlike the finding of the study examining sputum culture data of more than 10000 patients retrospectively showing upward trend in susceptibilities to third generation cephalosporins amongst *Klebsiella pneumoniae* from 2009 to 2018 (79.1% vs 86.4%),[11] our study has shown low sensitivity rates to the tested third generation cephalosporins as well. A systematic review and meta-analysis was conducted to assess the overall estimates of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-KP) and examine their drug resistance patterns and they examined Nepalese papers on ESBL-KP published between 2011 and 2021. According to the meta-analysis, *Klebsiella pneumoniae* had combined prevalence rates of ESBL and multidrug resistance (MDR) of 23% and 55%, respectively.[12] In vitro evaluations led to the identification of imipenem as the recommended medication for ESBL-KP infection.[12] Though the number of isolates was low, 90% of E.coli isolates (5/10) have also shown resistance to imipenem and 60% (6/10) to meropenem but only 40% of them were resistant to each of piperacillin + tazobactam, amikacin and gentamicin. This is in contrast to a 2015 study conducted in Nepal that found that Escherichia coli and *Klebsiella pneumoniae* isolated from various clinical samples (blood, sputum, urine, pus, and bodily fluids) had a significantly greater rate of resistance to aminoglycosides and a substantially lower rate of imipenem resistance.[13]

A previous report from Nepal revealed a high prevalence of community-acquired multi-drug resistant (MDR) clinical isolates of *Escherichia coli*. Of these isolates, over 78% were found to be multi-drug resistant, and 24%, 15%, and 9% of them produced extended spectrum β-lactamase (ESBL), metallo β-lactamase (MBL), and AmpC β-lactamase (AmpC).[14]

All the isolates belonging to *Enterobacterales* and the Acinetobacter spp. in our study have been found to have high incidence of resistance to carbapenems in comparison to other beta lactam agents i.e. extended spectrum penicillins and third generation cephalosporins. This result is consistent with a large-scale study of hospitalised patients in the United States that looked into trends in resistant *Enterobacteriaceae* and *Acinetobacter* species. The study analysed resistance profiles of MDR and carbapenem-non-susceptible (Carb-NS)*Acinetobacter spp.* and extended-spectrum beta-lactamase (ESBL)-producing, multidrug-resistant (MDR), and Carb-NS *Enterobacteriaceae* phenotypes, as well as MDR and Carb-NS *Enterobacteriaceae*. The study concluded that the number of hospital admissions for both MDR and Carb-NS *Enterobacteriaceae* increased significantly between 2013 and 2017.[15]

Limitations of this study included inability to segregate the data for the sputum samples from the patients who developed the respiratory symptoms after being admitted to the hospital wards including ICU and patients consulting the emergency or out-patient department with respiratory symptoms. Thus, we are unable to analyze the data comparing possible hospital acquired infections and community acquired ones. Sensitivity testing for colistin, tigecycline and combinations of cephalosporins with beta lactamase inhibitors (e.g. ceftazidime-avibactam, ceftalozane-tazobactam) which are important antibacterial agents against highly resistant bacteria were not done during the study period as the recommended method/antibiotic discs for the same was not available in the hospital.

CONCLUSION

The isolates belonging to *Enterobacterales* and the *Acinetobacter spp.* have been found to have higher resistance to carbapenems in comparison to the extended spectrum penicillin, third generation cephalosporins and aminoglycosides thus need to be under surveillance.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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