

Drug Resistant Bacteria are Growing Menace in a University Hospital in Nepal

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Abstract Large amounts of antibiotics used for human therapy has resulted in the selection of pathogenic bacteria resistant to multiple antimicrobial drugs. This has created problems in the treatment of patients. So, this study was carried out to determine multidrug resistant (MDR) bacterial pathogens and their antibiogram in patients with clinically suspected pneumonia attending a tertiary care centre in central Nepal. Specimens representing lower respiratory tract were processed using standard protocol. Antibiotic susceptibility test was performed on bacterial pathogens by Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute guidelines. Fifty-four percent of the total bacterial isolates were MDR. Multidrug resistance was found in *Klebsiella pneumoniae* (23.4%), *Pseudomonads* (20.5%), *Acinetobacter calcoaceticus baumannii* complex (20.6%), *Escherichia coli* (11.6%), *Staphylococcus aureus* (9.1%) and others. Non-fermentative bacteria were more multidrug resistant (MDR) than Enterobacteriaceae (77.8% vs. 68.9%) whereas extended-spectrum beta-lactamase (ESBL) was considerably higher among Enterobacteriaceae (37.27% vs. 10.46%). Resistance was seen even against carbapenems. Only polymyxins were effective against multidrug resistant gram-negative bacterial isolates. This study shows an emergence of MDR bacterial pathogens at an alarmingly high level as the isolates were resistant to almost all antibiotics commonly used in our set-up. There must be prudent use of antibiotics to prevent the emergence of MDR bacterial isolates.

Keywords: carbapenems, multidrug resistant, pneumonia, polymyxins, bacterial pathogens

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1. Introduction

Acute Lower Respiratory Tract Infection (LRTI) is a persistent and pervasive public health problem. In LRTI, pneumonia represents the majority not only because of total number of cases but also due to its high contribution to medical consultation, hospitalization and mortality. In developed countries, pneumonia is the leading cause of morbidity, accounting for 20% of medical consultations, 30% of absences from work and 75% of all antibiotic prescriptions [1]. In Nepal, each year about 40,000 children aged below 5 years are estimated to die from pneumonia. However, this data is based on poorly efficient diagnosis and reporting system by health posts and hospitals to higher authorities and finally to Ministry of Health, Government of Nepal. Therefore, much has to be done to unveil the actual scenario and gravity of problem of pneumonia in Nepal.

Antibiotics remain the front-line therapy to conquer against bacterial infections. Almost three-quarters of all

antibiotic consumption are for the treatment of respiratory tract infections [2]. However, treatment with these drugs is to be acknowledged as a two-edged sword. As antimicrobial agents have been misused and overused, bacteria have fought back with a selection process by which certain strains are no longer susceptible to certain antimicrobial agents with the production of enzyme like extended-spectrum beta-lactamase (ESBL). As a result, bacteria that once seemed to be losing the battle for survival have re-emerged to create therapeutic dilemmas with resulting increased risks of treatment failure and disease complications.

Unless we gather the information about the existing multidrug resistant (MDR) strains, the rate of emergence and spread of antimicrobial resistance can not be reduced [3]. Unfortunately, only few pilot studies have been done regarding the prevalence of MDR pathogens causing pneumonia in Nepal. One study carried out in a tertiary care centre of Kathmandu revealed that 47.5% of the total lower respiratory tract isolates were MDR [4]. The earlier studies may not precisely represent today's scenario since bacterial aetiology and their antibiogram may vary in

different geographical regions and even over time in the same location and population. Hence, this study was intended to address drug resistance among the pathogens causing pneumonia.

2. Methods

A cross-sectional study was carried out at a tertiary care hospital in Nepal on 1120 sputum, endotracheal aspirate and bronchial washing specimens from outpatients and inpatients over a period of six months. The institutional ethical committee clearance was taken from Institutional Review Board of the institute before conducting the study.

Specimen collection, culture, identification tests were done according to the guidelines given by American Society for Microbiology [5]. The antibiotic sensitivity test of the pathogens isolated from the clinical specimen against different antibiotics was done using Mueller Hinton agar (MHA) (Oxoid, United Kingdom) by the standard disk diffusion technique of modified Kirby-Bauer method as recommended by Clinical and Laboratory Standards Institute (CLSI) [6].

2.1. Definition of MDR

If the isolates were resistant to at least three classes of first line antimicrobial agents, they were regarded as MDR [7].

2.2. Test for ESBL [6]

The initial screen test for the production of ESBL was performed by using ceftriaxone (CRO) (30 µg), ceftazidime (CAZ) (30 µg) and cefotaxime (CTX) (30 µg) disks (Oxoid, UK). If the zone of inhibition (ZOI) was ≤ 25 mm for CRO, ≤ 22 mm for CAZ and/or ≤ 27 mm for CTX, the isolate was considered a potential ESBL-producer as recommended by CLSI.

Combination disk (CD) method was used for the phenotypic confirmation of ESBL-producing strains in which CTX and CAZ (30 µg), alone and in combination with clavulanic acid (CA) (10 µg) were used (Becton Dickinson, USA). An increased ZOI of ≥ 5 mm for either antimicrobial agent tested in combination with CA versus its zone when tested alone confirmed ESBL.

2.3. Statistical Analysis

Data were analyzed by Chi Square test using Statistical Package for Social Sciences (SPSS) version 11.5 software and represented as frequency distribution, percentage and *P* value. *P* value less than 0.05 was considered to be significant.

3. Results

Among the 533 bacterial isolates, *Haemophilus influenzae* was the chart-topper, followed by *Klebsiella pneumoniae*, Pseudomonads, *Acinetobacter calcoaceticus baumannii* (Acb) complex, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Citrobacter freundii*, *Enterococcus* spp., *C. koseri*, *Morganella morganii*, *Enterobacter aerogenes*,

Serratia marcescens, *A. lwoffii*, *K. pneumoniae* subsp. *ozanae* and *Streptococcus pyogenes*.

3.1. Antibiogram of *H. Influenzae*

The resistance of *H. influenzae* against different antimicrobials was as follows-chloramphenicol (1.8%), doxycycline (2.7%), amoxicillin-clavulanate (4.5%), ciprofloxacin (8.9%) and amoxicillin (30.4%). No *H. influenzae* isolate was resistant to ceftriaxone.

3.2. Antibiogram of Non-Fermentative Bacteria

For Pseudomonads, the most effective antibiotic was imipenem, followed by cefoperazone-sulbactam, meropenem and piperacillin-tazobactam. The action of later generation cephalosporins were poor which was < 50%. *Acinetobacter* spp. showed relatively lower susceptibility to imipenem (64.5%), meropenem (50.0%), cefoperazone-sulbactam and amikacin, (each 45.2%). For third generation cephalosporins, the resistance they exhibited ranged from 80.7% with ceftazidime to 87.1% with ceftriaxone.

3.3. Antibiogram of *Streptococcus Pneumoniae* and *Staphylococcus Aureus*

In the case of *S. pneumoniae*, chloramphenicol, oxacillin and ceftriaxone were found to be most effective. Nearly ninety-six percent isolates showed susceptibility to penicillin, amoxicillin, erythromycin and ciprofloxacin. Likewise, cloxacillin was found to be effective against 63.6% of *S. aureus* isolates. Only 36.4% of *S. aureus* were susceptible to erythromycin. Of the total 33 isolates of *S. aureus*, 26 (78.8%) were MDR. There was an incidence of inducible clindamycin resistance case (3.0%). Vancomycin and teicoplanin were the most effective antibiotics against MDR *S. aureus*.

3.4. MDR in General

Out of total 533 bacterial isolates, 53.7% were MDR. The highest number of MDR among gram-negative bacteria was seen in *K. pneumoniae* and Pseudomonads (Figure 1).

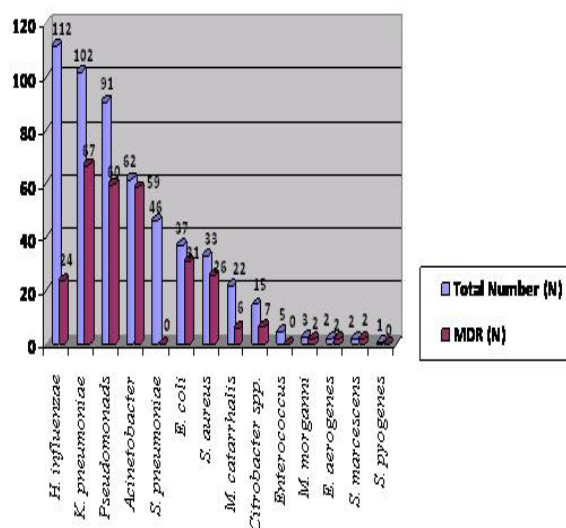


Figure 1. Number of MDR among total bacterial isolates

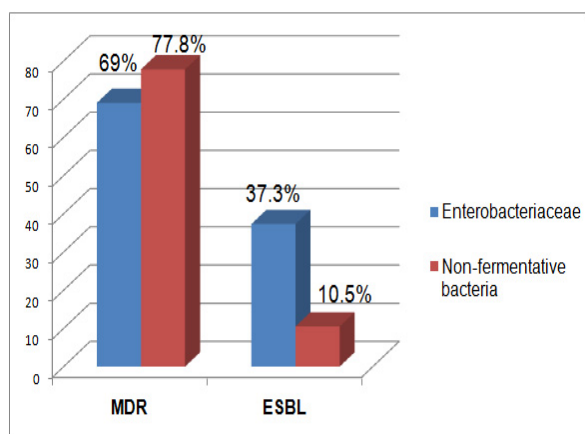


Figure 2. Prevalence of MDR and ESBL in Enterobacteriaceae and non-fermentative bacteria

Non-fermentative bacteria were more MDR (77.8%) than *Enterobacteriaceae* (68.9%) isolates whereas ESBL was considerably higher among *Enterobacteriaceae* (37.27% vs. 10.46%) ($P < 0.001$) (Figure 2). Multiresistance was more common in isolates of *K. pneumoniae* and *Acb* complex from inpatients (Table 1).

Table 1. MDR prevalence in isolates from inpatients and outpatients

Organisms	Inpatient (MDR)		Outpatient (MDR)		Total MDR	
	N	%	N	%	N	%
Gram-Positive Bacteria						
<i>S. pneumoniae</i>	0	0.00	0	0.00	0	0.00
<i>S. aureus</i>	23	100.00	3	100.00	26	9.10
<i>Enterococcus spp.</i>	0	0.00	0	0.00	0	0.00
<i>S. pyogenes</i>	0	0.00	0	0.00	0	0.00
Total	23	100	3	100		
Gram-Negative Bacteria						
<i>H. influenza</i>	1	0.57	23	27.00	24	8.39
<i>K. pneumoniae</i>	52	29.71	15	17.65	67	23.42
Pseudomonads	36	20.57	24	28.23	60	20.98
<i>Acb</i> complex	52	29.71	7	8.23	59	20.63
<i>E. coli</i>	21	12.00	10	11.76	31	11.57
<i>M. catarrhalis</i>	6	3.43	0	0.00	6	2.10
<i>Citrobacter spp.</i>	4	2.29	3	3.53	7	2.45
<i>M. morgani</i>	0	0.00	2	100.00	2	0.70
<i>E. aerogenes</i>	1	0.57	1	1.18	2	0.70
<i>S. marcescens</i>	2	1.14	0	0.00	2	0.70
Total	175	100	85	100	286	100

4. Discussion

The emergence of gram-negative bacterial species with acquired resistance to various broad-spectrum beta-lactams and other classes of antimicrobials is becoming a worldwide clinical problem [8]. The increasing incidence of antibiotics resistance for respiratory pathogens complicates the use of empiric treatment with traditional agents [9].

This study showed that out of 533 bacterial isolates, 286 (53.7%) were MDR. This finding is much higher than a report by Tuladhar et al (25.8%) in the same setting [3]. Regarding the distribution of MDR strains, they were significantly common among inpatients (65.5%) than among outpatients (32.0%). This may be ascribed to exposure of inpatients to different extended-spectrum drugs; besides, multi-resistant isolates are disseminated widely in the hospital setting due to different iatrogenic

mechanism and these patients may not be immunocompetent [4].

Among the bacterial isolates, higher percentage of MDR strains belonged to *K. pneumoniae* (23.4%) followed by Pseudomonads (21.0%) and *Acb* complex (20.6%). These pathogens are more common in hospital-setting and are mainly accountable for nosocomial infections. Besides, the infection by these bacteria is frequently difficult to treat because of their intrinsic as well as acquired resistance to multiple groups of antimicrobial agents. Apart from *K. pneumoniae*, MDR isolates were widely present among other members of *Enterobacteriaceae*, viz., *E. coli*, *Citrobacter* spp., *M. morgani*, *E. aerogenes* and *S. marcescens*. The emergence and increasing trend of MDR among *E. coli* has been reported in other studies too [3,4]. For MDR gram-negative isolates, carbapenems had the widest coverage followed by cefoperazone-sulbactam, amikacin and piperacillin-tazobactam.

Acinetobacter spp. harbor resistance plasmids which enable them to act as reservoir of multidrug resistance. In this study, we found a very high population of MDR *Acinetobacter* spp. isolates. The staggering rate of MDR *Acinetobacter* underscores the need for coherent step in the treatment option. Ling et al [10] reported a higher sensitivity of *Acb* complex against cefoperazone-sulbactam than piperacillin-tazobactam. Complying with the previous studies, this study also showed cefoperazone-sulbactam had comparatively lower resistance rate than piperacillin-tazobactam. Though tigecycline and carbapenems were relatively effective against *Acinetobacter* spp., we encountered many strains resistant to carbapenems at a much higher frequency than some studies in Indian hospitals [11,12].

P. aeruginosa represents phenomenal antibiotic resistance, demonstrating practically all known enzymatic and mutational mechanisms of bacterial resistance. The incidence of MDR *P. aeruginosa* has been found to have increased in our hospital in four-year interval from 55.2% [4] to 65.9%. Such MDR and some extensively drug-resistant strains were more common in intensive care units (ICUs). The increasing rate of these strains determines them as emerging pathogens, especially in ICUs and justifies the necessity for antimicrobial-resistance surveillance.

Considering the antimicrobial agents used against Pseudomonads, piperacillin was not effective in 44.0% of the cases. Meanwhile, no increment in resistance to amikacin was observed in this study as compared to reports of 2006 and 2007 when the pseudomonas isolates showed 21.7% and 22.7% resistance respectively at Tribhuvan University Teaching Hospital (TUTH) [13]. Quinolones, in particular ciprofloxacin, was active against 67.0% of *P. aeruginosa*. A much higher resistance of 15.4% was recorded against imipenem in this study when compared with the resistance rate of 8.2% in a study done in 2006 at the same hospital setting [13]. Numerous mechanisms including loss of the OprD porin, efflux pump overexpression and carbapenemase production can be responsible for the resistance to carbapenems. Class B (Metallo-beta-lactamases-MBLs) and D (Oxacillinases) beta-lactamases are the most important group of enzymes able to hydrolyze carbapenems [14]. It is noteworthy that MBLs have already emerged in Nepal [15]. Because of its

unique antipseudomonal activity, ceftazidime is a reserved drug for *P. aeruginosa* infections. In CLSI guidelines [6] also, this is included in group A drugs. Unfortunately, we found that 58.2% of Pseudomonads exhibited resistance against ceftazidime which is in acquiescence with this finding (57.1%) by Aksaray et al [16].

The reduced susceptibility of gram-negative isolates towards the later generation cephalosporins could be attributable to ESBL or AmpC beta-lactamase production or some other relevant underlying mechanisms. Out of total 314 non-duplicate gram-negative bacteria, 206 isolates across eight different genera of *Pseudomonadaceae*, *Moraxellaceae* and *Enterobacteriaceae* were resistant to ceftazidime. ESBL-production was significantly more common among members of *Enterobacteriaceae* as compared to non-fermenters. It is more difficult to treat infections caused by ESBL producing pathogens. These strains may remain within the environment and patients for a longer period of time and may spread easily within and between hospitals [17].

The finding suggest that MDR *P. aeruginosa* and *Acinetobacter* spp. infections are such that the mainstay antibiotic regimens used to eradicate these bacteria can no longer be relied upon. Presumably this is due to multiple factors, including antibiotic usage, dosing regimens, and local hospital practices concerning isolation of patients with multiresistant pathogens. The extensively drug-resistant gram-negative isolates were sensitive to polymyxin B and colistin sulphate.

The global spread of resistance in *S. pneumoniae* is of particular concern as this organism is an important cause of community-acquired pneumonia, and has a propensity to acquire resistance to multiple classes of antibacterials [18]. However, in the present study there was no MDR *S. pneumoniae*. It is noteworthy that a high incidence of MDR *H. influenzae* was seen in this study. Comparing the sensitivity pattern of *H. influenzae* from this study and a report by Alliance for Prudent Use of Antibiotics (APUA)-Nepal [13], we can conclude that sensitivity of *H. influenzae* has declined moderately for ciprofloxacin and much significantly for amoxicillin and cotrimoxazole.

Today the concern of methicillin-resistant *S. aureus* (MRSA) has reached the pinnacle. It is noteworthy that MRSA can cause both community and hospital-acquired pneumonia. There are only few reports depicting the situation of MRSA among LRTI isolates in Nepal. In this study, 42.4% were identified as MRSA. Among them, 92.9% were isolated from inpatients. Similar high prevalence of MRSA was encountered in another study in Nepal (43.8%) [19].

It is noteworthy that many different definitions have been used by different researchers for the categorization of MDR bacteria [20]. This variability has precluded reliable comparison of surveillance data for MDR bacteria and consequently it has prevented the medical community from having a complete understanding of the extent of the problem of antimicrobial resistance. Therefore, we recommend that there should be a consensus definition of MDR at the global level.

5. Conclusion

Antimicrobial resistance in bacterial pathogens has emerged as a significant public health problem even in our

context as determined by phenotypic methods. Due to resource constrains, genotyping of the resistant isolates could not be done. Polymyxins, carbapenems followed by amikacin and cefoperazone-sulbactam were found to be the most effective antibiotics for gram-negative bacilli. However, these antibiotics should be preserved for treatment of MDR strains only. Although some resistance is inevitable with the use of antibiotics, steps can be taken to curtail practices that cause and propagate resistance. In this way, we will be able to maintain or prolong the efficacy of existing drugs.

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