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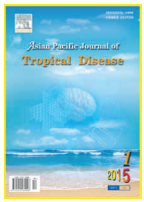

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# The bad, the ugly and the demon: a tale of extensively drug-resistant, extended-spectrum-beta-lactamase- and metallo-beta-lactamase-producing superbugs associated with nosocomial pneumonia

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PEER REVIEW

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**Comments**

The work reports on issue of nosocomial infection with specific use of investigation to support the hypothesis. The work is interesting and can be applicable in tropical medicine. Also, it is useful for clinical pharmacology aspect. The work can be further referenced in the field.
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ABSTRACT

**Objective:** To determine the bacterial etiology of nosocomial pneumonia (NP) and to assess the current levels of antimicrobial resistance with special reference to the status of extended-spectrum-beta-lactamase (ESBL) and metallo-beta-lactamase (MBL)-producing bacterial strains in a university hospital of Nepal.

**Methods:** A total of 60 specimens (sputum and endotracheal secretion) from patients diagnosed of NP were collected and processed following standard methodology. Combined disk and double disk synergy test method were used for the detection of ESBL. Ethylene-diamine-tetraacetic acid-based combined disk method was used for the detection of MBL-producing isolates.

**Results:** Out of total 60 specimens, 85% yielded significant mixed bacterial growth. *Acinetobacter* spp. was the most predominant isolate (30.43%) followed by *Klebsiella* spp. (28.98%), *Pseudomonas aeruginosa* (17.39%), *Escherichia coli* and *Staphylococcus aureus* (*S. aureus*) (8.69% for each). All *Escherichia coli*, *Klebsiella* spp. and *S. aureus* were multidrug resistant. Nearly 76% of *Acinetobacter* spp. were extensively drug resistant. MBL was seen in 25.3% of the Gram-negative isolates. *Acinetobacter* spp. was the most frequent MBL-producer (15.9%). ESBL was present in 41.3% of Gram-negative isolates. Tigecycline and polymyxin B followed by carbapenems, cefoperazone-sulbactam, piperacillin-tazobactam and amikacin were the most effective antibiotics for drug-resistant Gram-negative bacteria. All isolates of *S. aureus* were methicillin-resistant; however, they were susceptible to vancomycin, linezolid, quinupristin-dalfopristin and tigecycline.

**Conclusions:** High prevalence of drug resistance among the isolates of NP has demanded cautious selection of antibiotics. Further studies should be done in our setting to find out genes responsible for drug resistance. Last but not least, we advocate for the development of new antibiotics.

KEYWORDS

Nosocomial pneumonia, Extended-spectrum-beta-lactamase, Metallo-beta-lactamase, extensively drug resistant

## 1. Introduction

Nosocomial pneumonia (NP) is an infection of the lung parenchyma that is neither present nor incubating at the time of hospital admission and which develops after 48 hours of hospital admission. It is the second most frequent nosocomial infection but the first in terms of morbidity, mortality and cost[1]. In the intensive care units (ICU), it is the most frequent nosocomial infection because of the severity of underlying diseases, the frequency of invasive interventions, and the frequent use of broad-spectrum antibiotics[2].

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The nosocomial infections contribute to the emergence of resistant strains like multidrug resistant (MDR), methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA), extended-spectrum-beta-lactamase (ESBL)- and metallo-beta-lactamase (MBL)-producing organisms due to antibiotic selection pressure. As the pathogens causing hospital-based pneumonia become more drug resistant, clinical trial designs become more complex, thus making monotherapeutic protocols nearly impossible and the analyses of trial results extremely difficult[3]. Recently, a high level of antibiotic resistance in lower respiratory tract pathogens, exacerbated by the association of ESBL and MBL, has been seen in Nepal[4]. These strains may be extensively drug resistant (XDR). The emergence of such strains in nosocomial pneumonia drastically compromise effective treatments, bringing us closer to the much feared 'end of antibiotics'.

The incidence of MDR pathogens is not decreasing, despite the attempts of antibacterial stewardship and rigorous endeavor to infection control of MDR bacteria in hospital. Besides, these bad bugs may escape the hospital and join the ranks of the community pathogens[5]. This is a worrying public health issue as infections caused by such enzyme-producing organisms are associated with a higher morbidity and mortality, and greater economic burden to developing countries like Nepal as these enzymes can be carried on bacterial chromosomes, that is, inherent to the organism, or may be plasmid-mediated with the potential to move between bacterial populations.

## 2. Materials and methods

A prospective study was done among the inpatients of Tribhuvan University Teaching Hospital diagnosed with nosocomial pneumonia from May to August, 2012. Endotracheal secretion or sputum sample, as received in the laboratory from patients meeting criteria for NP as defined by Center for Disease Control, was processed following standard methodology[6].

### 2.1. Antimicrobial sensitivity testing

Antibiotic susceptibility test of all the isolates was done by using Mueller Hinton agar (MHA) (Oxoid, UK) by the standard disk diffusion technique of Kirby-Bauer method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) recommendations[7].

### 2.2. MRSA screening

MRSA screening was done using cefoxitin disk (30 μg) method as recommended by CLSI. Organisms were deemed methicillin resistant when zone of inhibition (ZOI) was ≤21 mm for *S. aureus*[7].

### 2.3. Tests for ESBL-production in Gram-negative isolates

#### 2.3.1. ESBL screening test

According to CLSI guidelines, strains showing ZOI of ≤22 mm for ceftazidime (CAZ) (30 μg), ≤27 mm for cefotaxime (CTX) (30 μg), and ≤25 mm for ceftriaxone (CRO) (30 μg) were considered potential ESBL- producer and were selected for confirmational tests of ESBL[7].

#### 2.3.2. ESBL confirmatory tests

##### 2.3.2.1. Combination disk method

CAZ (30 μg) and CTX (30 μg) disks alone and in combination with clavulanic acid (10 μg) were placed 25 mm apart. An increase of ≥5 mm in ZOI for ceftazidime-clavulanic acid (30/10 μg) and cefotaxime-clavulanic acid (30/10 μg) compared to CAZ and CTX alone was confirmed as ESBL producers[7].

##### 2.3.2.2. Double disc synergy test

Three discs including CAZ (30 μg), CTX (30 μg), and CRO (30 μg) were placed around the centrally placed disc of amoxycillin-clavulanic acid (amoxyclav) (20/10 μg) with an edge to edge distance of 15 mm. The isolates showing enhancement of the ZOI and synergy to centrally placed disk of amoxyclav (20/10 μg) for one or more of the discs after overnight incubation at 37°C was considered as the ESBL producer[4].

### 2.4. MBL screening test

The isolates were subjected for MBL detection when the ZOI for CAZ (30 μg) was <18 mm[8].

#### 2.4.1. MBL confirmation by combination disk method

Two imipenem (IPM) disks (10 μg) were used. In one of them, 10 μL of 0.1 mol/L (292 μg) anhydrous ethylenediamine-tetraacetic acid (EDTA) was added. Then the two disks were placed 25 mm apart (center to center). An increase in zone diameter of > 4 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for an MBL[7].

### 2.5. Definition of MDR and XDR

MDR *Acinetobacter* spp. were defined as the isolates of *Acinetobacter* spp. resistant to at least three classes of antimicrobial agents-all penicillins and cephalosporins