Phenotypic characterization of multidrug-resistant *Acinetobacter baumannii* with special reference to metallo-β-lactamase production from the hospitalized patients in a tertiary care hospital in Nepal.

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Abstract

Introduction: *Acinetobacter baumannii*is an important cause of nosocomial infection and has been associated with a wide variety of illnesses in hospitalized patients, especially patients in the intensive care units. The emergence of carbapenem-resistant clones of *A. baumannii* has been the most serious problem worldwide. After the carbapenem resistant clones have emerged, leaving the hope of treatment of *A. baumannii* infection is by the last resort of antibiotics such as tigecycline, polymyxin-B and colistin. The purpose of this study is to determine the antibiotic resistance patterns of *A. baumannii* isolates, prevalence of multidrug resistance, extended spectrum beta lactamase production and metallo-beta lactamase production.

Methods: This is a prospective study conducted at the department of Clinical Microbiology, Tribhuvan University Teaching Hospital, from December 2013 to September 2014. Ethical approval was taken from the Institutional Review Board of Institute of Medicine. Two hundred and forty six *Acinetobacter*isolates were identified by standard microbiological testing. Antimicrobial susceptibility testing was performed by Kirby Bauer method as per the CLSI guidelines. Multidrug resistance was determined. ESBL production was detected by combination disc method and confirmed by Clinical and Laboratory Standerd Institute confirmatory test. MBL production was detected by using imipenem and imipenem/EDTA disc.

Result: All 122 Multidrug-resistant *A. baumannii* isolateswere resistant to majority of the drugs used. All the isolates were completely sensitive to polymyxin B, colistin and tigecycline only. Fifteen (12.29%) isolates of *A. baumannii* were extended spectrum beta-lactamase producers and 50 (40.98%) were metallo-beta-lactamase producers. Multidrug resistance was common in *A. baumannii*.

Conclusion: Multidrug resistance in *A. baumannii* is becoming more common ESBL and MBL production should be promptly detected and reported to control the spread of resistant phenotypes to other individuals.

Keywords: *Acinetobacter baumannii*, ESBL, MBL, multidrug-resistance.

Introduction

Acinetobacter baumannii are gram-negative, catalase positive, oxidase negative, non-motile, non-fermenting coccobacilli.[1] It is mostly a cause of wide range of clinical infections such as septicemia, pneumonia, urinary tract infections, wound infections and meningitis[2] in hospitalized patients with more severe illnesses. *A. baumannii* are resistant to all major antibiotic classes normally used to treat infections including β-lactams, aminoglycosides, fluroquinolones, chloramphenicol,

tetracyclines and rifampin. [3]Multi-drug resistant isolates of *A. baumannii* have reportedly been increased during the last decade, probably as a consequence of the extensive use of antimicrobial agents.[4] Multidrug-resistant *A. baumannii* has become a life threatening opportunistic nosocomial pathogens worldwide.[1]The prevalence of these multidrug-resistant *A. baumannii* strains leaves limited clinical options for the treatment, underscoring the need for the development of novel antibiotics for these pathogens.[5] Hospital acquired *A. baumannii* infections prolong the length of hospital stay and subsequent health care costs.[6] Infectious Diseases Society of Americ astated *A. baumannii* as one of the "red alert" pathogens that greatly threaten the utility of our current antibacterial armamentarium.[1]

Acinetobacter species possess a wide array of chromosomal β-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins and carbapenems.[7]

Multidrug resistant *Acinetobacter baumannii* has the ability to develop resistance to all available antibiotics including carbapenems, which are the drugs of choice in the treatment of severe infections. [8]Carbapenem resistance in *A. baumannii* is now being observed increasingly worldwide and it constitutes a sentinel event for the emerging anti-microbial resistance. The main mechanism for carbapenem resistance in *A. baumannii* corresponds to efflux pumps, porin mutations, and the production of acquired carbapenem hydrolyzing class D β-lactamases (CHLDs)[9] as well as class B carbapenemases. Class B carbapenemases including IMP and VIM termed as metallo beta lactamsases (MBLs) have been found so far in *A. baumannii* and these are encoded by different plasmid types.[10] MBL producing strains are frequently resistant to aminoglycosides and fluroquinolones but remain susceptible to polymyxins.Carbapenem resistance due to MBL and other carbapenemase production has a potential for rapid dissemination, as it is often plasmid mediated.[11] The rapid detection of carbapenemase production is necessary to initiate effective infection control measures to prevent their dissemination. [12]

This study aims to determine the antibiotic susceptibility patterns of MDR *A. baumannii* isolates and the prevalence of multidrug resistance, ESBL production and MBL production.

Methods

This study was approved by Institutional review board, Institute of Medicine, Research Department, Kathmandu, Nepal. It is a prospective study, conducted at the department of Clinical Microbiology, Tribhuvan University Teaching Hospital(TUTH).

A total of 246 *Acinetobacter* spp.isolates were obtained from hospitalized patients of different wards of TUTH from December 2013 to September 2014.

Specimen collection, culture, identification tests were performed according to the guidelines given by American Society of Microbiology (ASM).

Phenotypic identification was performed by manual biochemical test. [13] Species identification was confirmed by 16SrRNA sequencing.[14]

Drug susceptibility testing was performed by Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI). [15]

Minimum inhibitory concentration (MICs) were performed by broth micro-dilution method and interpreted according to guidelines of the Clinical and Laboratory Standards Institute (CLSI).[15]

MDR *A. baumannii* strains are defined as isolates not susceptible to at least one agent in three or more antimicrobial categories, including aminoglycosides, antipseudomonal carbapenems, antipseudomonal fluroquinolones, antipseudomonalpenicillins/β-lactam inhibitors, extended-spectrum cephalosporins, folate pathway inhibitors, penicillins/β-lactamase inhibitors, polymyxins and tetracyclines.[16]

Screening of ESBL producers:

ESBL producing strains were screened by using ceftazidime (30μg) or cefotaxime (30μg) disc.

ESBL production was suspected when the zone of inhibition is ≤22 mm for cetazidime (CAZ) and /or ≤27 mm for cefotaxime (CTX).

CLSI phenotypic confirmatory test: ESBL was tested by applying the discs of ceftazidime (30μg) and ceftazidime and clavullanic acid (30μg+10μg) to the lawn culture of the test organism. After incubation for 16 to 18 hours if the zone of inhibition around ceftazidime-clavullanic acid was ≥5 mm than the zone of inhibition around ceftazidime disc, then the test organism was said to be ESBL producer. [15]

The carbapenem resistance was screened by using disk diffusion test with imipenem and meropenem disc following the CLSI guidelines. [15]

Imipenem/ Meropenem resistant isolates were further screened for metallo-β-lactamase production by using imipenem and EDTA.

A colony of the suspected isolate was suspended in Mueller Hinton broth and turbidity was adjusted to 0.5 McFarland opacity standards. Lawn cultures were prepared on Mueller Hinton agar and two 10-µg-imipenem disc were placed on the plate, and the appropriate amounts of an EDTA solution were added to one of them to obtain the desired concentration. The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16-18 h of incubation at 37°C. The test was considered MBL-positive if a >4 mm increase in the zone diameter for imipenem/EDTA was observed. [17]

Results

Of the 246 *Acinetobacter* spp. isolates tested, 129 (52.43%) were multidrug resistant (MDR).Of the 129 MDR isolates, 122 were *A. baumannii*, 6 were *A. calcoaceticus*and 1 was *A. berzinaie*.The majority of MDR*A. baumannii* isolates were resistant to at least one agent in 7 or more antimicrobial categories.

One-hundred-nine and 97 isolates showed high MICs of ≥512 mg/l to amikacin and arbekacin. All isolates were resistant to ceftazidime and all except3 were resistant to meropenem. All were sensitive to colistin with MIC of ≤ 2 mg/l. Ninety isolates showed low MICs of ≤1 mg/l to tigecyline, but 4 isolates showed high MICs of 8 mg/L.

Distribution of MDR *A. baumannii* in various samples:

Among the total bacterial isolates (n=122), majoritywere from respiratory tract (n=60, 49.18%) followed by pus (n=31, 25.40%) and urine (n=13, 10.65%).(Table 1)

Table 1. Distribution of MDR *A. baumannii* in various samples (n=122):

Specimen	No.	%
Respiratory tract	60	49.18
Pus	30	24.59
Urine	13	10.65
Blood	9	7.37
CSF	7	5.73
Others	3	2.45
Total	**122**	**100**

Ward-wise distribution of MDR *A. baumannii*:

Majority of the isolataes were from Intensive care unit (ICU) followed by Surgical ward and Medical ward as dipicted in figure 1.

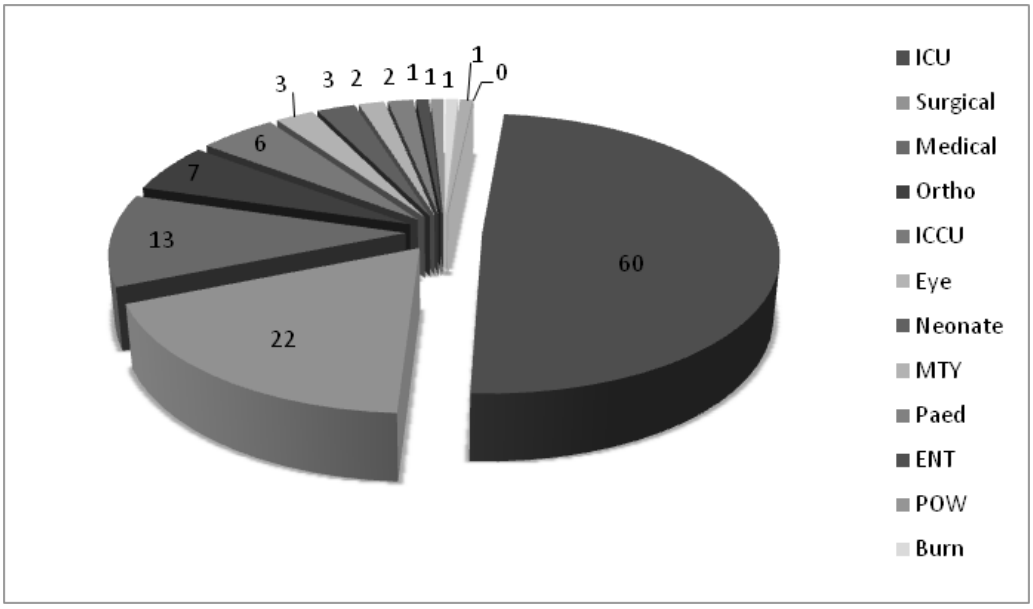

Figure 1. Wardwise distribution of MDR *A. baumannii* (n=122)

Antimicrobial susceptibility profile of MDR *A. baumannii*:

Majority of the isolates were resistant to all the antibiotics tested, which is shown in the table below. All the isolates were sensitive to polymyxin B, colistin and tigecycline.

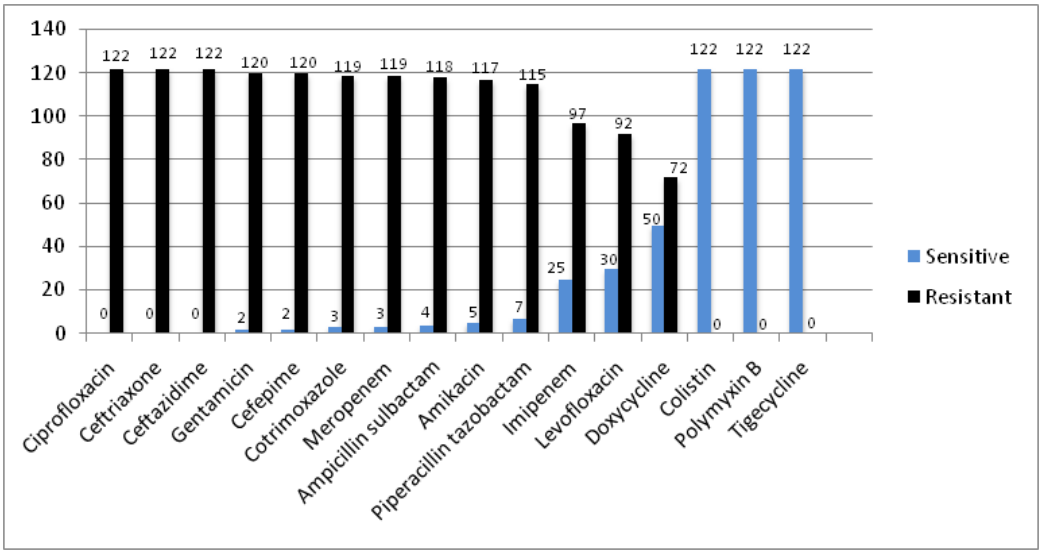

Figure 2. Antimicrobial susceptibility profile of MDR *A. baumannii* (n=122).

ESBL and MBL production inMDR *A. baumanni:* Out of total 122 *A. baumannii*, 50 (40.98%) were MBL producers while 15 (12.29 %) were ESBL producers, which is shown in the figure below.

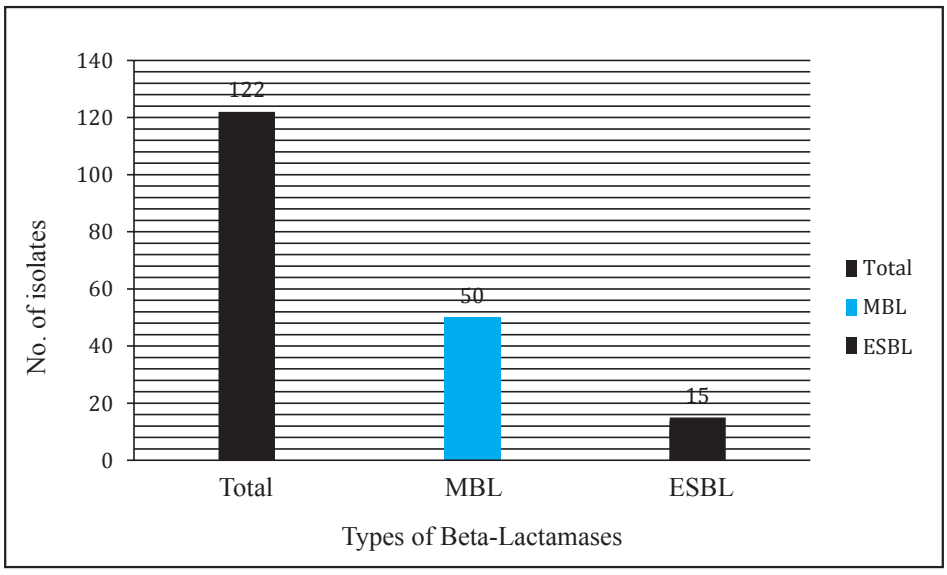

Figure 3. ESBL and MBL production in MDR A. baumannii (n=122)

Discussion

The worldwide emergence of multidrug and carbapenem resistant *A. baumannii* isolate is of great concern.[18] *A. baumannii* is an important nosocomial pathogen associated with a wide variety of illnesses in hospitalized patients especially in the intensive care units imposing greater challenge to the patients management and infection control.

Antimicrobial resistance among *A. baumannii* has substantially increased in the past decade creating a major public health dilemma. Carbapenems are the most potent antibiotic currently available but resistant strains have emerged.[19]

In our study, *A. baumannii* was frequently isolated from respiratory tract (49.18%) followed by pus (24.6%), urinary tract (10.7%), blood (7.40%), CSF (5.7%) and other sources (2.5%). In another study from India, 59.8% *A. baumannii* isolates were reported from respiratory tract followed by 18.6% from blood. [20]

We have studied the antimicrobial resistance pattern of 122 *A. baumannii* isolates by Kirby- Bauer disc diffusion method as recommended by Clinical Laboratory and Standard Institute (CLSI). [15] In our study, *A. baumannii* isolates showed resistance to most of the antibiotic tested. All the isolates were completely sensitive to polymyxin B, colistin and tigecycline only. Maximum sensitivity was observed to doxycycline (40.98%), levofloxacin (24.59%) followed by imepenem (20.49%) and ampicillin- sulbactam (3.27%). All the isolates were completely resistant to ciprofloxacin, ceftazidime, ceftriaxone followed by gentamicin (98.36%), cefepime (98.36%),cotrimoxazole (97.54%) and amikacin (95.90%). Meropenem was resistant to 119(97.54%) of the isolates.

Tripathy et al from India reported maximum sensitivity of *Acinetobacter* spp. to imepenem (57%), amikacin (55.14%) followed by gatifloxacin (44.87%) and tobramycin (41.12%). [11]

Similarly, Kaur et al reported 89% resistance of *A. baumannii* to gentamicin followed by cephalosporin 80%, amikacin 65% and imipenem 40.3%. [21]

A study done in USA detected 79.5% multi-drug resistant *A. baumannii* isolates. Among them 62%were resistant to ceftazidime and 66% were resistant to imipenem. These isolates were also resistant to kanamycin, gentamicin, streptomycin, tetracycline and ciprofloxacin.[22]

A. baumanni has intrinsic resistance to extended spectrum cephalosporins, have an outer membrane with selective permeability to β-lactams and by modification

of outer membrane porins, diminish permeability to other antibiotics. They also have chromosomal β-lactamases. All of these intrinsic mechanism cause resistance to extended spectrum β-lactams antibiotics. [23]Extended spectrum β-lactamase (ESBL) continues to be a major problem in clinical setups and knowledge about their prevalence is essential towards appropriate antibiotic treatment. Significantly high levels of *Acinetobacters* produce ESBL and they are MDR. Routine antimicrobial susceptibility test may fail to detect such ESBL producers but a simple, rapid and inexpensive method like combined disc method may help to screen all the clinical isolates for ESBL production. [24]

In our study, out of 122 *A. baumannii* isolates, 12.29% were ESBL producers. Tripathy et al reported 71.87% of *A. baumannii* were ESBL producers.[11]In a study of Young et al 54.63% of *Acinetobacters* were ESBL producers. [25]The difference in the result may be due to the different screening criteria we have used for ESBL. Tripathy et al tested all the isolates for ESBL production but we have used different criteria for ESBL screening.

Carbapenem resistance in *A. baumannii* is considered an emerging serious public health problem.[9]Resistance to carbapenem may also be explained by modification of penicillin binding proteins (PBPs).

Along with the naturally occcuring β-lactamases, several acquired β-lactamase have been identified as a source of carbapenem resistance in *A. baumannii*. These enzymes belong either to the class B enzymes defined by Ambler et al (also known as metallo beta-lactamases)[26] or to the class D enzymes (also known as oxacillinases). [27]

Although metallo β-lactamases (MBLs) are powerful carbapenemases,[10]oxacillinases possesssing the ability to hydrolyseimepenem (but not always meropenem) are grouped in a particular subgroup of β-lactamases termed carbapenem hydrolysing oxacillinases (CHDLs). Both MBL and CHDL are resistant to inhibition by clavulanate and tazobactam.

MBL are susceptible in vitro to EDTA inhibitors. MBLs mainly of types IMP and VIM are increasingly associated with reduced susceptibility to carbapenems seen in several gram-negative species. Despite the worldwide occurrence of epidemic carbapenem resistant strains, MBL producing *Acinetobacters* have been found to be disseminated only in specific geographic areas. Therefore detection of these enzymes is of major importance in control of *A. baumannnii* hospital infection.These tests take the advantage of Zinc dependence of MBLs by using chelating agents such as EDTA to inhibit the enzyme activity.

In our study, out of 122 MDR *A. baumannii* isolates 40.98% were MBL producers. According to Yong et al MBL production in *A. baumannii* was 6.95% only.[17]In a study by Kumar et al 21% of the isolates of *A. baumannii* were found to be MBL producers. [28]This was in concordance with the result, which was obtained by Lee et al in Korea[29] where 25% of the isolates were found to be MBL producers. According to Kabbaj et al among 57.4% of imepenem non-susceptible isolates of *A. baumanni*, 74% were found to be MBL producers.[30]

In a two recent study in Pakistan by Kaleem et al and Irfan et al, the frequency of MBL in *A. baumannii* is higher by 84% and 96% respectively. [31]The difference in our study may be due to the screening criteria used as we have included only carbapenem resistant isolates for the detection of MBL.

This emergence is a serious epidemiological risk for two reasons. First of all, the MBL does not confer resistance to carbapenem only but to all β-lactams and other classes of antibiotics. Secondly, the genes encoding these enzymes spread easily on plasmids, causing nosocomial infections and outbreaks with rising mortality. These results indicate that the available choices for appropriate treatment for infections caused by *A. baumannii* are currently limited. In vitro, studies reveal that tigecycline and colistin are the antibacterial agents with consistent activity against MBL producing strains.[32]The potential limitation of this study is that molecular epidemiological analysis and characterization of ESBL and MBL types were yet to be carried out.

Early detection of these beta lactamase-producing isolates in routine laboratory could help avoid treatment failure. Furthermore, strict antibiotic policies and measures to limit the indiscriminative use of cephalosporins and carbapenems in the hospital environment should be undertaken to minimize the emergence of this multiple beta-lactamases.

Conclusion

This study demonstrated that multidrug resistant strains of *Acinetobacter baumannii* are common in tertiary care hospitals. Unwarranted and unrestricted usage of

antibiotics is associated with emergence of resistance on nosocomial pathogens. Regular monitoring and documentation of carbapenem resistance is crucial in developing strategies to control infections due to these bacteria.

Production of MBL has tremendous therapeutic consequences since these organisms also carry multidrug resistance genes and the only viable option remains the potentially toxic polymyxin B and colistin. It is important to follow antimicrobial restriction policies to avoid excessive use of carbpaenems and other broad-spectrum antibiotics. To understand the epidemiology, there is a need of genetic analysis and also the typing of metallo-beta-lactamases.

Conflict of interests: None Declared

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