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Emergence of Aminoglycoside Resistance Due to armA methylase in Multi-drug Resistant Acinetobacter Baumannii Isolates in a University Hospital in Nepal

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ABSTRACT

Background: The emergence of multidrug-resistant Acinetobacter baumannii associated with hospital-acquired infections has been increasingly reported worldwide. 16S rRNA methylase producing Gram-negative bacteria are highly resistant to all clinically important aminoglycosides. We analyzed A. baumannii clinical isolates resistant to aminoglycosides from hospitalized patients. The objective of this study was to investigate the emergence of armA in A.baumannii species associated with nosocomial infection in a university hospital in Nepal.

Methods: This was a cross-sectional study conducted at the department of Clinical Microbiology, Tribhuvan University Teaching Hospital (TUTH), from December 2013 to December 2014. A total of 246 Acinetobacter species were isolated from different patients were screened for MDR A. baumannii. Identification at the species level was confirmed by 16S rRNA sequencing. Drug susceptibility testing was performed by Kirby- Bauer disc diffusion method and minimum inhibitory concentrations (MICs) were determined using the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Screening for 16S rRNA methylase-production was done for the isolates resistant to gentamicin and amikacin. Detection of 16S rRNA methylase gene was done by PCR.

Results: All 122 multidrug-resistant A. baumanniiisolates were resistant to majority of the antibiotics used except polymyxin and tigecycline. Ninty-six MDR A. baumannii isolates had MICs of > 512 mg/L to amikacin and arbekacin indicating their high resistance to aminoglycosides.Of the 96 pan-aminoglycoside resistant isolates, 75 isolates had 16SrRNAmethylasewith all isolates harboring armA gene.

Conclusions: This is the first report describing multidrug-resistant A. baumannii strains harboring armA from hospitalized patients in Nepal. A methylase gene (armA), conferring high level of resistance to aminoglycosides, was detected in majority of our isolates.

Keywords: Acinetobacterbaumannii; aminoglycosideresistance; 16S rRNAmethylase; multidrug-resistant.

INTRODUCTION

Acinetobacterbaumannii is a gram-negative, non-lactose-fermenting organism recognized as a major pathogen responsible for nosocomial infections.[1] It plays a significant role in the colonization and infection of hospitalized patients implicating variety of nosocomial infections, particularly in patients admitted to intensive care units.[2]

Methylation of 16S ribosomal RNA (rRNA) has emerged as a new mechanism of resistance to clinically important aminoglycosides among gram-negative pathogens of the family Enterobacteriaceae and glucose-non-fermenting organisms including Pseudomonas aeruginosa and Acinetobacter species.[3]

The armA gene encoding a 16S rRNA methylase, was initially identified in Citrobacterfreundii in 2002 in Poland[4] causing global dissemination of hazardous multiple aminoglycoside resistance genes. Although data on the prevalence of aminoglycoside resistance mediated by 16S rRNA methylation among gram-negative bacilli is still scarce, the presence of 16S rRNA methylases has already been reported worldwide

METHODS

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This study was approved by Institutional Review Board, Institute of Medicine, Research Department, Kathmandu, Nepal. It is a cross-sectional study, conducted at the Department of Clinical Microbiology, Tribhuvan University Teaching Hospital (TUTH).

From December 2013 to September 2014, two hundred and forty six Acinetobacter spp.were isolated from non-duplicate, non-consecutive samples of blood, wound, urine, sputum and respiratory tract of patients from different wards of TU Teaching hospital in Nepal.

Specimen collection, culture, identification tests were performed according to the guidelines given by American Society of Microbiology (ASM).[5]

Phenotypic identification was performed by conventional biochemical methods.[6] Species identification was confirmed by 16SrRNA sequencing[7] also confirmed for blaOXA-51 genes by PCR.[8]

Drug susceptibility testing was performed by Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines 2015.[9]Escherichia coli ATCC 25922 was used as the quality control strain.

Minimum inhibitory concentration (MICs) were performed by broth micro-dilution method and interpreted according to guidelines of the CLSI 2015.[9]

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, antipseudomonal penicillins/ß-lactam inhibitors, extended-spectrum cephalosporins, folate pathway inhibitors, penicillins/ß-lactamase inhibitors, polymyxins and tetracyclines.[10]

A. baumannii isolates showing resistance to gentamicin and amikacin in disk diffusion were subjected to screen for producing 16 SrRNA methylase.[3]

PCR analysis was done using 16S rRNAmethylase gene specific primers to detect the armA, rmtA, rmtB, rmtD genes. The PCR primers used were, for armA, 5′-ATTCTGCCTATCCTAATTGG-3′ (forward) and 5′-ACCTATACTTTATCGTCGTC-3′ (reverse), which amplify a 315-bp DNA fragment; for rmtA, 5-CTAGCGTCCATCCTTTCCTC-3′ (forward) and 5′-TTGCTTCCATGCCCTTGCC-3′ (reverse), which amplify a 635-bp DNA fragment for rmtB, 5′-ATGAACATCAACGATGCCCTCACC-3′ (forward) and 5′-TATCAAGTATATAAGTTCTGTTCCG-3′ (reverse), which amplify a 741-bp DNA fragment;and for rmtD, 5′-CGGCACGCGATTGGGAAGC-3′ (forward) and 5′-CGGAAACGATGCGACGAT-3′ (reverse), which amplify a 401-bp DNA fragment.[4]

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. calcoaceticusand 1 was A. berezinaie. The majority of MDR A. baumannii isolates were resistant to at least one agent in 7 or more antimicrobial categories.

One-hundred-nine (89.34%) and 97(79.50%) isolates showed MICs of ≥512 mg/L to amikacin and arbekacin respectively. All isolates were resistant to ceftazidime and 119(97.54%) were resistant to meropenem. All were sensitive to colistin with MICs of ≤ 2 mg/L. Ninety isolates (73.77%) had MICs of ≤1 mg/L to tigecyline, whereas 4 isolates (3.27%) had MICs of 8 mg/L.

Among the total bacterial isolates (n=122), majority was from respiratory tract (n=60, 49.18%) followed by pus/ wounds (n=30, 24.59%) 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

Majority of the isolates were resistant to all the antibiotics tested, which is shown in the table below (Table 2). All the isolates weresensitive to polymyxin,colistin andtigecycline

Table 2. Antimicrobial susceptibility profile of 122 MDR A.baumannii isolates.

Antibiotic	Resistant, number (%)	Sensitive, number (%)
Ciprofloxacin	122 (100)	0
Ceftriaxone	122 (100)	0
Ceftazidime	122 (100)	0
Gentamicin	120 (98.36)	2 (1.63)
Cefepime	120 (98.36)	2 (1.63)

Co-trimoxazole	119 (97.54)	3 (2.45)
Meropenem	119 (97.54)	3 (2.45)
Ampicillin/ sulbactam	118 (96.72)	4 (3.27)
Amikacin	117 (95.90)	5 (4.09)
Piperacillin/ tazobactam	115 (94.26)	7 (5.73)
Imipenem	97 (79.50)	25(20.49)
Levofloxacin	92 (75.40)	30 (24.59)
Doxycycline	72 (59.01)	50 (40.98)
Colistin	0	122 (100)
Polymyxin	0	122 (100)
Tigecycline	0	122 (100)

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

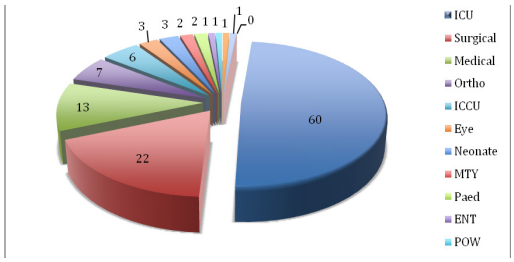

Fig 1. Wardwise distribution of MDR A. baumannii (n=122).

Ninety-six of the 122 MDR A. baumannii isolates had MICs of > 512 mg/L to amikacin and arbekacin which indicates their high resistance to aminoglycosides. 9 isolates had MICs of >512mg/L to amikacin but <64mg/L to arbekacin. 3 isolates had MICs of 512mg/L to amikacin but <16mg/L to arbekacin. The remaining 14 isolates had MICs of <512mg/L to amikacin and< 64mg/L to arbekacin.

Of the 96 pan-aminoglycoside resistant isolates, 75 isolates had 16SrRNAmethylasewith all isolates harboring armA gene. None of the isolates harbored genes other than armA. (Figure 2)

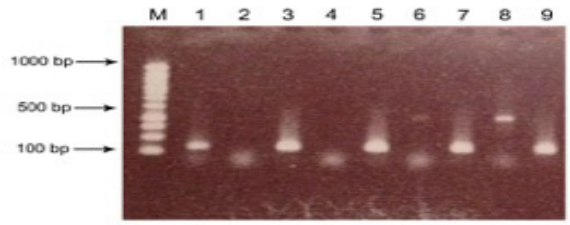

Fig 2. Electrophoresis profile of the PCR product of armA genes: laneM- marker, lane1- positive control, lane 2- negative control, lane 3, 5, 7 and 9 - armA positive.

DISCUSSION

Multi-drug resistantA. baumannii has been the cause of an increasing threat in hospitals and a global challenge nowadays. 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. In our study also majority of the isolates were recovered from patients from ICU (60, 49.18%) followed by surgical ward (22, 18.03%). 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.[11]

In our study, A. baumannii was frequently isolated from respiratory tract (49.18%) followed by pus (24.59%), urinary tract (10.65%), blood (7.37%), CSF (5.73%) and other sources (2.45%). In another study from India, 59.8% A. baumannii isolates were reported from respiratory tract followed by 18.6% from blood.[12]

We have studied the antimicrobial resistance pattern of 122 A. baumannii isolates. In our study, A. baumannii isolates showed resistance to most of the antibiotic tested. All the isolates were completely sensitive to polymyxin, colistin and tigecycline only.

Aminoglycosides continue to play an important role in the management of serious infections caused by gram-negative pathogens, often in combination with broad-spectrum beta-lactams but the activity of aminoglycosides is lower for MDR isolates of A. baumannii compared with non-multiresistant ones. To survive in this niche, A. baumannii developed a large variety of resistance traits including production of 16S rRNA methylases with the major representative armA.

Incidence of infection by A. baumannii with armA 16S rRNAmethylase has increased, leading to reports of high-level resistance to most aminoglycosides.13In our study, the prevalence of armA among MDR A. baumannii was 61.47% and the isolates with armA in our study were highly resistant to all available aminoglycosides. According to a study done in Korea, the prevalence of armA among MDR A. baumannii was 97.8%[13] which is

quite higher than our study. Another study done in Egypt found 94% prevalence of armA in clinical isolates of A. baumannii.[14]

Antibiotics are among the most commonly prescribed drugs in hospitals. In developed countries around 30% of the hospitalized patients are treated with these drugs.[15] Among the hospitalized patients in a medical settings in Vietnam, 67.4% received antibiotics including 18.9% receiving aminoglycosides with 30.8% inappropriately prescribed.[16]A study done in Nepal documents 29.5% of the patients were prescribed antibiotics. This is very similar to the reports from developed countries.[15]A study done in outpatients of Nepal concludes antibiotics were the most frequently prescribed therapeutic class with 16.54% of the total prescribed drugs.[17]

Nosocomial infections caused by multidrug-resistant, gram-negative bacteria have become a serious problem in clinical facilities. P. aeruginosa and Acinetobacter spp. have been especially efficient at developing multidrug- resistance against broad-spectrum ß-lactams, fluoroquinolones, and aminoglycosides. The identification of armA and rmtB genes in Europe and East Asia suggests that we must pay consistent attention to prevent further global proliferation. If 16S rRNA methylase positive bacterial isolates disseminate widely and extensively, the high level of pan-aminoglycoside resistance will undoubtedly have an impact on illness, deaths, and costs of care.[18]

To our knowledge, this is the first report of 16S rRNA methylase in A. baumannii isolates causing high level of resistant to aminoglycosides in medical settings in Nepal. Some of the A. baumannii strains were simultaneously resistant to other classes of antimicrobials including carbapenems. The high prevalence of 16S rRNA methylase producing MDR A. baumannii in our hospital would have resulted from the high rate of the use of aminoglycosides.

Our study strongly suggests that A. baumannii isolates producing a 16S rRNA methylase, armA, have emerged and disseminated in Nepal. A methylase gene (armA), which confers high levels of resistance to aminoglycosides, was detected in the majority of our isolates. This suggests that aminoglycosides may no longer be recommended as a first-line treatment for multidrug resistant A. baumannii infections in our settings.

CONCLUSIONS

This is the first report describing the presence of methylase producing MDRA. baumannii in medical settings in Nepal. Multidrug resistantA. baumannii were common in our hospital and they usually harbored 16S rRNA methylase gene, predominantly armA.

The high prevalence of armA can threaten the existing therapeutic qualities for such infections.Strict surveillance and more rapid detection are essential to reduce the spread of MDRA. baumannii including 16S rRNA methylase.

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REFERENCES

1. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: Emergence of a successful pathogen. Clin Microbiol Rev. 2008;21(3):538-582. doi:10.1128/CMR.00058-07.

2. Bergogne-Bérézin E, Towner KJ. Acinetobacter spp. as nosocomial pathogens: Microbiological, clinical, and epidemiological features. Clin Microbiol Rev. 1996;9(2):148-165.

3. Doi Y, Arakawa Y. 16S Ribosomal RNA Methylation : Emerging Resistance Mechanism against Aminoglycosides. 2007;45. doi:10.1086/518605.

4. Tada T, Miyoshi-Akiyama T, Shimada K, Shimojima M, Kirikae T. Dissemination of 16S rRNA methylase armA-producing Acinetobacter baumannii and emergence of OXA-72 carbapenemase co-producers in Japan. Antimicrob Agents Chemother. 2014;58(5):2916-2920. doi:10.1128/AAC.01212-13.

5. Isenberg HD. Clinical Microbiology Procedures Handbook.2nd ed. Washington D.C., ASM Press.

6. Shahcheraghi F, Abbasalipour M, Feizabadi MM, Ebrahimipour GH, Akbari N. Isolation and genetic characterization of metallo-β-lactamase and carbapenamase producing strains of Acinetobacter baumannii from patients at tehran hospitals. Iran J Microbiol. 2011;3(2):68-74.

7. Clarridge JE, Alerts C. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical

microbiology and infectious diseases. Clin Microbiol Rev. 2004;17(4):840-862. doi:10.1128/CMR.17.4.840.

8. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by detection of the bla OXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol. 2006;44(8):2974-2976. doi:10.1128/JCM.01021-06.

9. CLinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility testing, 17th informational supplement. Wayne, PA, M100-S17, 2007

10. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-281. doi:10.1111/j.1469-0691.2011.03570.x.

11. Shrestha S, Tada T, Shrestha B, Kirikae T, Pokhrel BM, Sherchand JB. 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 . J Inst Med (Nepal) 2015;37 (3):3-10

12. Jaggi N, Sissodia P, Sharma L. Acinetobacter baumannii isolates: epidemiology, antibiogram and nosocomial status studied over a 25 month period in a tertiary care hospital in India. BMC Proc. 2011;5(Suppl 6):P291. doi:10.1186/1753-6561-5-S6-P291.

13. Lim J, Cho HH, Kim S, et al. The genetic characteristics of multidrug-resistant Acinetobacter baumannii coproducing 16S rRNA methylase armA and carbapenemase OXA-23. J Bacteriol Virol. 2013;43(1):27-36. doi:10.4167/jbv.2013.43.1.27.

14. Ahmed ES, Amin MA, Tawakol WM, Loucif L, Bakour S, Rolain JM. High prevalence of blaNDM-1carbapenemase-encoding gene and 16S rRNA armA methyltransferase gene among Acinetobacter baumannii clinical isolates in Egypt. Antimicrob Agents Chemother. 2015;59(6):3602-3605. doi:10.1128/AAC.04412-14.

15. Shankar RP, Partha P, Shenoy NK, Easow JM, Brahmadathan KN. Prescribing patterns of antibiotics and sensitivity patterns of common microorganisms in the Internal Medicine ward of a teaching hospital in Western Nepal: a prospective study. Ann Clin Microbiol Antimicrob. 2003;2:7. doi:10.1186/1476-0711-2-7.

16. Tada T, Akiyama TM, Kato Y, Ohmagari V, Takeshita N, Kirikae T. Emergence of 16S rRNA methylase-producing Acinetobacter baumannii and Pseudomonas aeruginosa isolates in hospitals in Vietnam. BMC Infect Dis. 2013;13(1):1-6. doi:10.1186/1471-2334-13-251.

17. Kumar J, Shaik M, Kathi M, Deka A, Gambhir S. Prescribing indicators and pattern of use of antibiotics among medical outpatients in a teaching hospital of Central Nepal. J Coll Med Sci. 2010;6(2):7-13. doi:10.3126/jcmsn.v6i2.3610.

18. Yamane K, Wachino J, Suzuki S, Shibata N, Kato H, Arakawa Y.16S rRNA methylase-producing, gram-negative pathogens, Japan. Emerg Infect Dis. 2007;13. doi:10.3201/eid1304.060501.