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Full Length Research Paper

Emergence of oligoclonal *Acinetobacter baumannii* nosocomial infection in a Hospital in Nepal

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The molecular epidemiology of fifteen clinical strains of *Acinetobacter baumannii* recovered from various clinical specimens from different wards during January to June, 2010 from a hospital in Nepal was evaluated. Kirby-Bauer disk diffusion test was used for determining *in-vitro* activities of antibiotics. Molecular epidemiology was investigated by polymerase chain reaction-randomly amplified polymorphic DNA (PCR-RAPD) and plasmid profiling. *A. baumannii* recovered were multidrug resistant. Isolates represented three antibiotypes (a, b and c). Isolates in antibiotype c (n=12) were resistant to all antibiotics tested while isolates in antibiotype a (n=2) was susceptible to netilmicin and b (n=1) was susceptible to aminoglycosides and fluoroquinolones tested. Four plasmid profiles (i) 1 isolate; (ii) 1 isolate; (iii) 1 isolate; and (iv) 12 isolates and four PCR-RAPD types (I)1isolate; (II) 8 isolates; (III) 1 isolate; (IV) 5 isolates revealed oligoclonal population of *A. baumannii*. Antibiotypes, plasmid profiles and PCR-RAPD types showed no empirical association. *A. baumannii* isolates were oligoclonal and multi-drug resistant. The emergence of multi-drug resistant oligoclonal population of this pathogen in a hospital warrants for development of appropriate antibiotic policies and immediate implementation of infection prevention and control measures.

Key words: *Acinetobacter baumannii*, multidrug-resistant, oligoclonal, Nepal.

INTRODUCTION

Acinetobacter baumannii is emerging as a nosocomial pathogen around the globe. This pathogen is ubiquitous in the hospital environment, is multidrug, pandrug to extensively drug-resistant, can survive wide range of pH, salinity, humidity, and can thrive on almost all nutrient sources. They frequently colonize respiratory and digestive tract, skin, and throat causing wide array of infections especially in immunocompromised and debilitated patients admitted in intensive care units (ICU) (Montefour et al., 2008; Rosenthal and Tager, 1975; Somerville and Noble, 2008). *Acinetobacter* spp. is responsible for 3 to 4% of ventilator associated pneumonia and crude mortality rate due to *A. baumannii* is 30 to 70% (CDC, 1984).

Since its emergence as nosocomial infection in USA in 1991, *A. baumannii* has been isolated in numerous health care facilities and city, country and continent wide outbreak of this pathogen have been documented (Go et al., 1994; Peleg et al., 2008). This pathogen has successfully overcome therapeutic armament by accumulating its innate and acquired resistance repertoire (Peleg et al., 2008). *A. baumannii* resistant to all beta-lactams has already emerged (Peleg et al., 2008). Polymyxins, tigecycline and rifampin are considered as magic bullets to treat *A. baumannii* infections but resistant strains to these antibiotics are emerging (Ko et al., 2007; Thapa et al., 2009a).

Multidrug resistant strains of *Enterobacteriaceae* like, *Escherichia coli Klebsiella pneumoniae*, *Citrobacter* spp., *Proteus* spp., and *Enterobacter* spp., have been the subject of attention in Nepal but nosocomial infection by

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Table 1. Antibiotypes, plasmid profiles and PCR-RAPD types of isolates studied.

Isolates	PCR-RAPD	Plasmid profile	Antibiotypes	AST, sensitive to
104	I	iv	c	-
106, 107, 1011, 1012, 1015, 1016	II	iv	c	-
109				-
1010	II	iv	a	AK, G, K, NT, NX, CF
108	III	iii	c	-
101	IV	i	a	AK, G, K, NT, NX, CF
102	IV	ii	b	NT
105,1013,1014	IV	iv	c	-

AST, Antibiotic susceptibility test; AK, Amikacin; G, Gentamicin; K, Kanamycin; NT, Netilmicin; NX, Norfloxacin; CF, Ciprofloxacin.

non-*Enterobacteriaceae*, like *A. baumannii* is also emerging (Banjara et al., 2003; Gaur et al., 2007; Thapa et al., 2009b). Molecular studies of *A. baumannii* from Nepal are scarce. Here, we studied the molecular epidemiology of nosocomial strains of *A. baumannii* isolated from Nepal.

MATERIALS AND METHODS

Bacterial strains

Out of 36 strains of *A. baumannii* isolated from various specimen sources in Microbiology laboratory of Kathmandu Medical College and Teaching Hospital (KMCTH), Kathmandu, Nepal during 6 months period (January to June, 2010), 15 were studied. The isolates were identified based on the published reports (Malini et al., 2009).

Antibiotic susceptibility test

Antibiotic susceptibilities of these pathogens were tested using Kerby-Bauer disk diffusion assay following CLSI guidelines (CLSI, 2005). The disk containing antibiotics (µg/disk) (HiMedia Pvt. Ltd, India) used were; Amoxicillin (20), Amoxicillin-Clavulanic acid (20+10), Piperacillin (100), Ceftizoxime (30), Ceftriaxone (30), Ceftazidime (30), Cefazolin (30), Cefoxitin (30), Amikacin (30), Gentamicin (30), Kanamycin (30), Netilmicin (30), Norfloxacin(10), and Ciprofloxacin (5).

Genetic analysis

Genomic and plasmid DNA from these isolates were extracted using Genomic DNA extraction Kit (Puregene, Minneapolis, Minnesota, USA) and Plasmid Miniprep (MN, Germany), respectively. polymerase chain reaction-randomly amplified polymorphic DNA (PCR-RAPD) was performed on Genomic DNA extract as described previously (Thapa et al., 2010). Briefly, PCR reaction was carried in 20 µl containing 50 ng of genomic DNA template, 0.2 µM primer (R003, 5' CTTGACGCA 3'), 0.2 mM dNTPs (FINZYMES), 2.5 µl of supplied PCR buffer, and 1.0 U of *Taq* polymerase (FINZYMES). 5% dimethylsulfoxide was added into the reaction. The PCR (PERKIN ELMER) profile used was: initial denaturation at 94°C for 2 min; followed by 40 cycles of 94°C for 10 s, 36°C for 30 s, and 72°C for 1 min; and a final heating at 72°C for 2 min. Amplified products and extracted plasmids were resolved in 1% TAE agarose (Research organics, inc. USA). Plasmid profiles were interpreted on the basis of the number and size of the plasmids. The study was approved by the institutional review board, Kathmandu Medical College, Nepal.

RESULTS

Antibiotic sensitivity test and antibiotype

The *in-vitro* activities of 14 antibiotics were tested against these isolates. All strains were multidrug-resistant (Table 1). Most of the isolates (n=13) were resistant to all antibiotics tested. All isolates were also resistant to beta-lactam antibiotics tested. Aminoglycosides and fluoroquinolones were effective against two isolates (101, 1010) while netilmicin was only effective to an isolate, 102. Based on the antibiotic susceptibility test these isolates were grouped into three antibiotypes, a, b, and c (Table 1). Most isolates (n=12) in antibiotype c were resistant to all antibiotics while antibiotype a (n=2) was sensitive to amino glycosides and fluroquinolones and b (n=1) was sensitive to netilmicin.

Plasmid profile

The size and number of the plasmids were able to categorize isolates into 4 plasmid profiles (i, ii, iii, and iv) (Figure 1 and Table 1). Most isolates (n=12) were grouped into plasmid profile iv while rest of the isolates represented individual plasmid profile type i, ii, and iii (Table 1).

PCR-RAPD

PCR-RAPD analysis of these strains revealed three RAPD types (I, II, and III) (Figure 2 and Table 1). Most of the isolates (n=8) accounted for type II and isolates 104 and 108 accounted for type I and III, respectively. Few

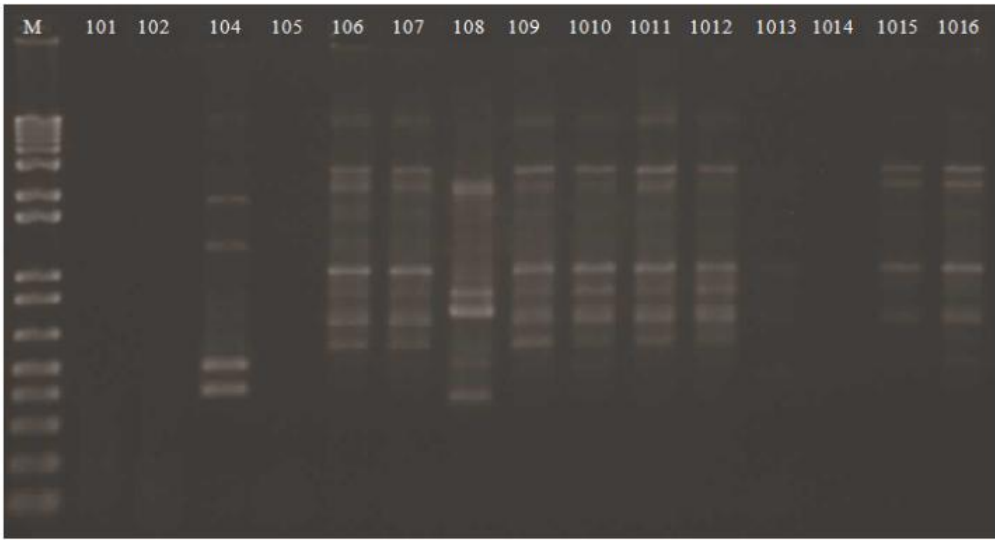

Figure 1. RAPD for *A. baumannii* studied. Lane M, molecular weight marker (1 kb+, Invitrogen); Numbers above lanes (2-17) indicates the isolates.

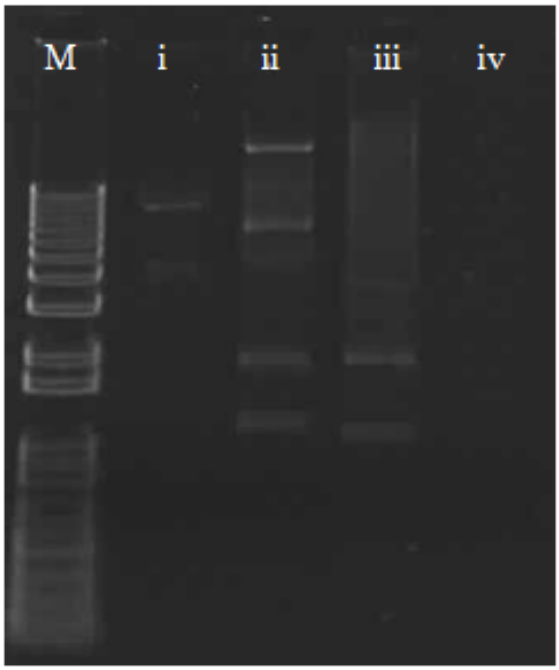

Figure 2. Plasmid profile of *A. baumannii* studied. Lane M, Molecular weight marker (1 kb^{+}, Invitrogen); lanes (2-5) indicates different plasmid profiles.

isolates (n=5) were not amplified and were grouped as type IV.

DISCUSSION

The indiscriminate use of antibiotics has led to the emergence of MDR, PDR and XDR strains of *A. baumannii* which was conventionally considered as less virulent and clinically unimportant. *A. baumannii* infection is a growing concern around the globe but the evidence of the emergence of this pathogen in Nepal is scarce. Out of 195 bacterial isolates obtained from surgical wound infection in Nepal, 13 bacterial species were identified and *Acinetobacter* spp. ranked 5th with the prevalence rate of 7.6%, and 9 strains were MDR (Banjara et al., 2003). In this study, all *A. baumannii* isolates were multidrug resistant. All were resistant to beta-lactam antibiotics tested. Some isolates were resistant to all antibiotics tested (antibiotype c) while others were sensitive to aminoglycosides and fluoroquinolones. Strains resistant to these antibiotics and to carbapenems have already been reported elsewhere (Chaiwarith et al., 2005; Thapa et al., 2010). At the time of conducting this study, carbapenems were just introduced in the clinical practice in Nepal and carbapenem susceptibility was not performed. Carbapenems including polymyxin, tigecycline, and rifampin in combination with other antibiotics which have been recommended for the management of multidrug-resistant *A. baumannii* can be the choices (Kasiakou et al., 2005; Thapa et al., 2009a). There are no current guidelines for treating *A. baumannii* in Nepal and the susceptibility data to these antibiotics should be generated before formulating such guidelines.

The clonality of the isolates was evaluated using antibiotypes, plasmid profiles, and PCR-RAPD. The evaluation of genetic relatedness using PCR-RAPD is an easy, cost effective, and rapid (Thapa et al., 2010). Using this arbitrarily primed PCR, we successfully identified the circulating local oligoclones (I to IV). Similar olioglonal outbreaks of *A. baumannii* have been reported (Thapa et al., 2010; Naas et al., 2005; Jeon et al., 2005). Plasmid

profiling a conventional typing tool grouped these isolates into four types (i to iv), and the isolates were grouped into three antibiotypes (a, b and c). Type II PCR-RAPD clone was most commonly encountered (n=8). These isolates also had similar plasmid profile and antibiotype. Similar plasmid profiles (type iv) and antibiotypes (type a and c) were observed among the isolates in PCR-RAPD types II and IV suggesting transfer of plasmids and resistance genes among different lineages. Two isolates (101 and102) within same PCR-RAPD type IV had independent plasmid profile and antibiotype. This reflected high rate of genetic promiscuity among similar genotypes. The difference of plasmid profiles and antibiotypes among different PCR-RAPD types can be explained by the high transformation capability of *Acinetobacter* spp. to expand its genetic pool of resistance (resistant plasmids and genes) (Metzgar et al., 2004). The same genotype of *A. baumannii* was found circulating in different wards. This suggests that the particular clone is hovering between wards and urge for prompt detection and elimination of the source.

Acinetobacter can be found in normal human skin, nosopharynx and digestive tract of hospitalized patients and infects debilitated and immunocompromised patients (Rosenthal and Tager, 1975). Most of the *A. baumannii* nosocomial outbreaks are also linked to the environmental sources in the hospital like, particles, air, injectable intravenous fluids, hands of medical staffs, and medical equipments (Deitz et al., 1988). These sources must be detected to control the spread of these clones. MDR international *A. baumannii* clones known as European clones I, II, and II have been reported in several European countries and also in United States (Nemec et al., 2004, van Dessel et al., 2004; Wroblewska et al., 2007). The rise in *A. baumanni* in United States has been contributed by the injured military personal returning from war in Iraq and Afghanistan (Scott et al., 2007; Davis et al., 2005). There was also an increase in prevalence of MDR *A. baumannii* between 1997 to 2001 in South American countries like, Argentina, Colombia, Chile, and Brazil (Tognim et al., 2004). Similarly, numerous PDR *A. baumannii* outbreaks have been reported from Asian hospitals (Thapa et al., 2010; Koh et al., 2007; Ying et al., 2006). Multidrug- and pandrug-resistant *A. baumannii* have been reported from almost all continents and is now a global problem.

This study, for the first time showed inter-ward spread of the *A. baumannii* clones and sensitized the need for monitoring of inter-institutional and international clones in Nepal. PCR-RAPD offers a dynamic platform to investigate clones in rapid and cost effective manner, has high sensitivity and high resolution for local epidemiological studies but it lacks reproducibility and produce categorical data that cannot be used to understand global epidemiology (Grundmann et al., 1997). More robust molecular typing tool-multi locus sequence typing-is necessary to establish the spread of these clones outside this institution (Bartual et al., 2005).

In conclusion, oligoclonal multidrug resistant *A. baumannii* has emerged as a successful nosocomial pathogen in this hospital in Nepal and warrants for tracing and elimination of the source. Prudent use of antibiotics, infection control and prevention practices, monitoring of these multidrug oligoclonal *A. baumannii* will help to stop the emergence and spread of the pathogen and its resistance genes across Nepal and internationally.

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