

Screening of Antibiotype among Environmental Isolates of *Acinetobacter* spp. in Hospital Setting

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Abstract

Cross infection through hospital environment has been a major challenge to control nosocomial infection. This has been worse with the emergence of multidrug resistant strains. The present study was conducted to study the pattern of antibiotic resistant group of *Acinetobacter* spp. in hospital environment to understand possibility of nosocomial infection. The study was conducted for a period of six months during which *Acinetobacter* spp. from 269 samples of hospital environment (air sample, surface swab and hand swab from healthcare workers) were identified by conventional microbiological method and antibiogram was performed by Kirby-Bauer disc diffusion method and NCCLS guidelines. Bacterial isolates obtained from both samples were tested for their relatedness based on their resistivity pattern among the tested antibiotics. Of the total environmental samples 212 samples were found to be positive and a total of 183 gram negative isolates were obtained. Of the total gram negative isolates, 84.2% (154/183) *Acinetobacter* spp. were isolated. Analysis of MDR isolates revealed 70.8% (109/154) *Acinetobacter* spp. which was MDR. A total of 12 different antibiogram patterns were found with five different antibiotic groups tested.

Key Words antibiogram typing, antibiogram type, environment, MDR, nosocomial

Introduction

Acinetobacter are normal environmental dwellers that have a extremely rapid propensity to develop antibiotic resistance (Katsutoshi *et al.* 2003, Stéphane *et al.* 2003). They can be found on a range of dry or moist inanimate surfaces and as commensals on the skin of man and animal (Forbes *et al.* 2007, Peleg *et al.* 2008). These have also been recognized as an important pathogens involved in outbreaks of hospital acquired infection (Buisson *et al.* 1990, Guillou 1991) particularly in high- dependency cases or ICUs (Sague *et al.* 1990). In developed countries many surveys and control programs are implemented so as to prevent transmission of pathogens from hospital environment to the patients (Orsi *et al.* 2005, Wilks *et al.* 2006, Zollidan *et al.* 2005). On the other hand, in the context of resource poor countries like Nepal, there are less

studies carried out to identify the *Acinetobacter* spp. as potential pathogen for nosocomial infection.

Methodology

This study was carried out at 100 bed hospital in Kathmandu. A total of 134 surface swab samples from different locations were taken. Surface swabs was taken from bed fabric, bed bar, equipment rack, wash basin, scissors, etc using sterile cotton swabs soaked in brain heart infusion broth (BHI). About a total of 81 hand swabs were taken, the entire area of palm and between fingers was rubbed with sterile swab, dipped in BHI broth. Air samples were collected by plate exposure technique.

The swab samples were inoculated in NA plates and MacConkey plates and incubated at 37°C for 24hrs.

Identification of isolates done based on the morphological and biochemical characters. Antibiotic susceptibility testing was done to the isolates identified as *Acinetobacter* spp. by Kirby-Bauer disc diffusion method. Ampicillin (10 mcg/disc), Co-trimoxazole (25mcg/disc), Gentamycin (10 mcg/disc), Ciprofloxacin (5 mcg /disc), Ceftazidime (30 mcg/disc), Ceftriazone (30mcg/ disc) and Amoxicillin (10mcg/disc) were used.

Results and Discussion

Of the total 134 swab samples from different inanimate objects close to patient; 103 samples was found to be positive and *Acinetobacter* spp. was found in 77 positive samples. Similarly of total 54 plate exposure sample 43 was found to be positive and *Acinetobacter* spp. was found from 41 different sites. Similarly of the total 81 hand swabs taken from health care workers, 47 swab sample showed positive growth of which in the

36 sample *Acinetobacter* spp. was isolated, the illustration of the *Acinetobacter* isolated from the sample is shown in Fig. 1.

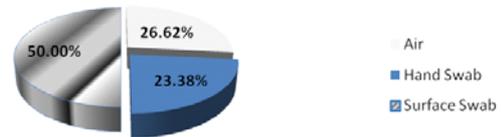


Fig. 1. Occurrence of *Acinetobacter* spp. in Different Samples

Table 2 showed the antibiotic resistivity pattern of the isolated *Acinetobacter* spp. Quinolones (Ciprofloxacin) was the most effective followed by co-trimoxazole and gentamycin while the other aminoglycoside, amikacin was found to be least sensitive.

Table 1. Resistivity Pattern of the isolates

Group number	Antibiotics Group	Antibiotic	Resistance n (%) #
Group 1	Aminopenicillins	Ampicillin	97 (62.98%)
		Amoxicillin	88 (57.17%)
Group 2	Sulphonamides	C o-trim oxazole	34 (22.08%)
Group 3	Quinolones	Ciprofloxacin	15 (9.74%)
Group 4	Aminoglycosides	Gentamycin	35 (22.72%)
		Amikacin	100 (64.93%)
Group 5	Cephalosporins	Cefotaxime	73 (47.40%)
		Ceftriaxone	65 (42.21%)

Each isolates were grouped into different antibiotypic group based on their resistivity pattern. Grouping of antibiotics was done as stated in Table 1. Different antibiotype group is indicated in Roman Numerals.

The 12 different antibiotype groups were found. Analysis of the MDR (resistant to two or more than two groups of antibiotics) showed that 70.8% of the isolated *Acinetobacter* spp.

Table 2. Antibiotyping of the isolates

Antibiotype	Description (Resistant group)	Number	Percent (%)
I	All sensitive	17	11.04
II	Group 1	25	16.23
III	Group 4	1	0.65
IV	Group (1+4)	14	9.09
V	Group 5	1	0.65
VI	Group (5+ 1)	6	3.89
VII	Group (5+ 2+ 1)	3	1.95
VIII	Group (5+ 4)	3	1.95
IX	Group (5+ 4+ 1)	35	22.73
X	Group(5+ 4+ 2+ 1)	29	18.83
XI	Group (1+3+4+5)	8	5.19
XII	All resistant	12	7.79
Total		154	

Acinetobacter spp. has become an increasing problem in health-care-associated infections over the past few years (Bernards *et al.* 2004, Fournier & Richert 2006, Maragaskis *et al.* 2008, Seifert & Dowzicky 2009). The Nation Nosocomial Infection Study (NNIS) reported a 6.9% increase in *Acinetobacter* spp. causing hospital-acquired pneumonias in ICUs in 2003, up from 1.4% in 1975 (Gaynes & Edward 2005). In addition, this organism has the ability to persist and colonize environmental surfaces for prolonged periods of time and biofilm formation, thereby contribute to its pathogenicity, increased transmission rates and association with nosocomial outbreaks (Kunz and Brook 2010, Dallo & Weito 2010).

From several studies it has been shown isolates of *Acinetobacter* spp. particularly *A. baumannii*, those recovered from patients with nosocomial infections, are frequently resistant to multiple antimicrobial agents, including cephalosporins, aminoglycosides, and quinolones (Fournier & Richert 2006, Gaynes & Edward 2005). The report of environmental analysis of occurrence of *Acinetobacter* spp. in hospital environment of Nepal is rarely available while the study of susceptibility pattern of the genus from different clinical samples has been done in different hospitals. In a research done in Eighty-six strains of *Acinetobacter* spp. particularly *A. baumannii* from India and Nepal for the presence of integrons in relation to multiple drug resistance by integrase gene polymerase chain reaction (PCR), integrons were found to be present at a rate of 43.02% (37/86) and integrons were significantly correlated with multidrug resistance to several antibiotics (Gaur *et al.* 2007). 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 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). Similarly occurrence of oligonal *A. baumannii* has been reported from study on nosocomial infection in Nepal (Thapa *et al.* 2011). In the current study also, though the analysis up to molecular basis was not performed but the

antibiotyping revealed the existence of 104 MDR strains which was more than 50% of the total isolates, this showed high occurrence of *Acinetobacter* spp. capable of causing nosocomial infection.

From the study done in BPKIHS, Dharan similar type of result of AST pattern was observed; as in this study gentamicin was found to be most effective than cephalosporins and amikacin and also ciprofloxacin was found to be effective than 3rd generation cephalosporins (Ghimire *et al.* 2007). In another research it was found that 50.0% of *Acinetobacter* spp. was resistant to the imipenem (Tiwari *et al.* 2007). However this differs with the research done in Taiwan where ampicillin was found to be most effective drug than cephalosporins and quinolones while in this study this has been found as one of the least effective drug among tested (Hsueh *et al.* 2002) his difference may be particularly due to difference in hospital settings and use of the antibiotics.

Acinetobacter spp. is often transferred from the hands and nostrils of health workers of hospital personnel to patients and result in significant morbidity, especially in intensive care and rehabilitation units (Ramazanzadeh 2009, Nicasia *et al.* 2008, Perez *et al.* 2007). It was demonstrated that the hands of medical staff and the surface area can be an important source during nosocomial outbreaks (NNIS 2004, Paterson 2008, Perez *et al.* 2007). In our research though the transmission has not yet been established but the isolation of *Acinetobacter* spp. from hand swab shows the possibility of accidental transmission.

Acinetobacter spp. have been isolated from various types of opportunistic infections, including septicemia, pneumonia, endocarditis, meningitis, skin and wound infection, and urinary tract infection (Mittal *et al.* 2003, Prashanth & Badrinath 2005). The occurrence of *Acinetobacter* spp. in hospital air has been related to one of the significant cause of ventilator associated pneumonia (Husni *et al.* 1999). Though in this research no such clinical data of occurrence of complications due to *Acinetobacter* spp. has been recorded but since the bacteria is abundantly present in hospital environment, chances of cross-infection is inevitable.

The true frequency of nosocomial infection caused by *Acinetobacter* spp. is not easy to assess, partly

because the isolation of these organisms from clinical specimens may not necessarily reflect infection but, rather, may result from colonization (Bergogne & Towner 1996). The result obtained from this research therefore cannot be confirmed for the occurrence for the nosocomial infection in the hospital but however maximum occurrence of *Acinetobacter* spp. and their varying antibiotype focus over the need of cleaning, disinfection and sterilization of hospital environment.

The prevention of nosocomial infection demands a thorough knowledge of the infection rates and of the source, type and nature of invading microorganisms along with the risk factors associated with infection (Weinstein 1991). Among the most popular bacteria causing nosocomial infection *Acinetobacter* spp. has emerged as a new challenge to health care workers; the so-called 'super bug' (*A. baumannii*) (CDC 1996). Thus this study focused on the MDR *Acinetobacter* spp. that may cause the health care crisis.

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