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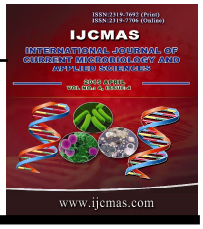

Original Research Article

Acinetobacter species in Health Care setting: Clinical significance and Antimicrobial sensitivity

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Keywords

Acinetobacter, Nosocomial, Drug resistance, Infection control, Antibiotic stewardship

A B S T R A C T

Acinetobacter species has been increasingly reported as the cause of nosocomial infections and possess a serious threat to the health care system because of its multi-drug resistance.The present study was conducted over a period of one year(September 2011 to August 2012) in a tertiary care hospital to isolate and speciate *Acinetobacter* species from clinical samples and to determine their antibiogram. 172 clinical isolates of *Acinetobacter* species were processed for species identification and antimicrobial susceptibility of these isolates was performed by Kirby-Bauer disc diffusion method. *A.baumannii* was the commonest species isolated 150/172 (87.2%), followed by *A. haemolyticus* 16/172 (9.3%) and *A. lwoffii* 6/172 (3.5%). Majority of the isolates were isolated from blood culture 92 /172(53.48%), followed by pus 34/172 (19.76%) and sputum 22/172 (12.8%) Of the total 172 isolates of *Acinetobacter* species, nosocomial isolates from the hospital patients were 131 (76.1%) as compared to the 41 (23.9%) community acquired isolates. Out of 172 *Acinetobacter* isolates, 99 (58%) were extensively drug resistant, 24 (14%) were multi-drug resistant and none of the isolates were pandrug resistant. Proper application of infection control measures and antibiotic stewardship is necessary in order to combat this problem.

Introduction

In 1911 Beijerinck, a Dutch microbiologist working in Delft, isolated and described the organism which is now recognised as *Acinetobacter* (Dijkshoorn L, Nemec A, 2008). Brison and Prevot proposed the generic designation, *Acinetobacter* in 1954. In 1971, the Subcommittee on Taxonomy of Moraxella & allied Bacteria suggested that the genus *Acinetobacter* shall include only oxidase negative bacteria, non-motile, non-fermenting, gram negative cocco-bacilli.

DNA homology has led to recognition of at least 25 genomospecies by various workers. To date only 10 are named.

Genomospecies 1- *A. calcoaceticus*
Genomospecies 2- *A. baumannii*
Genomospecies 4- *A. haemolyticus*
Genomospecies 5- *A. junii*
Genomospecies 7- *A. johnsonii*
Genomospecies 8- *A. lowffii*
Genomospecies 12- *A. radioresistens*

A. ursingii, *A. schindleri*, *A. venctianus* are the others.

The other genomospecies are unnamed. DNA groups 1, 2, 3 and 13 are sachharolytic strains and are collectively referred to as"*Acinetobacter calcoaceticus-A. baumannii complex*"(William Riley,2005).

Acinetobacter species are saprophytic and ubiquitous and can be found in natural (e.g. soil, water, food) and hospital environment. *Acinetobacter* is considered as a part of commensal flora of man (e.g. axillae, groin, digit webs) where they occasionally present as opportunistic pathogens. Cutaneous colonization can be seen in approximately 25% of population. 7% of adults and children show transient pharyngeal colonization. It is often difficult to distinguish between the colonization and the infection with this organism and hence attribute the exact morbidity and mortality associated with infections due to this organism(Larson E et al,1986).

Acinetobacter species are increasingly being recognized as a major pathogen causing nosocomial infections, including bacteremia and ventilator associated pneumonia, particularly in patients admitted to intensive care units. Carbapenems are often used as a last resort for infections due to multidrug resistant gram negative bacilli. However, there is an alarming increase in reports of carbapenem resistance in *Acinetobacter* species.These carbapenem resistant organisms have the ability to rapidly disseminate within an institution and may lead to poor patient outcomes when infection occurs. Therefore, early detection and identification of these multidrug resistant organisms is of great clinical importance (Bonomo RA, Szabo D, 2006).

Most reports in india do not report the species involved in human infections and address the infections at only genus level. Speciation of isolates is important in the epidemiology of *Acinetobacter* infections. In view of the increasing challenges posed by this organism in health care settings, the present study was undertaken to isolate and speciate *Acinetobacter* species from clinical samples and to determine their antibiogram

Materials and Methods

The present prospective study was conducted in the Department of Microbiology at a tertiary level teaching health care facility over a period of one year (September 2011 to August 2012) after approval from the Institutional Ethical Committee

Sample collection:.A total of 172 isolates of *Acinetobacter* species recovered from the urine, pus, blood, respiratory samples such as endotracheal aspirates, bronchoalveolar lavage (BAL), CSF, high vaginal swabs and various body fluids were included in the study.

Operational definitions used in the study were as follows:

Multi-drug resistant (MDR) *Acinetobacter*: *Acinetobacter* isolates resistant to at least three classes of antimicrobial agents- all penicillins and cephalosporins (including inhibitor combinations), fluoroquinolones and aminoglycosides

a. Extensively drug resistant (XDR) *Acinetobacter*: *Acinetobacter* isolates resistant to the three classes of antimicrobials described above(MDR) and shall also be resistant to carbapenems

b. Pan drug resistant (PDR) *Acinetobacter*: *Acinetobacter* isolates resistant to the

three classes of antimicrobials described above (MDR), carbapenems, polymyxins and tigecycline.

c. Community-acquired *Acinetobacter* infection: Infection diagnosed within 48 hours of hospital admission

d. Nosocomial *Acinetobacter* infection: Infection diagnosed >48 hours after hospital admission, based on National Nosocomial Infection Surveillance System (NNIS) criteria

Isolation and identification of *Acinetobacter* species: For the isolation of *Acinetobacter* spp., the clinical samples were inoculated onto blood agar and MacConkey agar. After overnight incubation at 37^0C, the suspected colonies were further processed for identification of *Acinetobacter* species by routine conventional methods. Species differentiation was done on basis of glucose oxidation, gelatin liquefaction, haemolysis, growth at 37°C and 42°C, susceptibility to penicillin and chloramphenicol discs.

Antimicrobial susceptibility testing: The antimicrobial susceptibility testing of all the 172 *Acinetobacter* isolates was carried out by Kirby-Bauer disc diffusion method on Mueller-Hinton agar medium and results were interpreted as per the Clinical and Laboratory Standards Institute guidelines. Antimicrobial discs used in the study were procured from Hi-media Laboratories, Mumbai, India. Antibiotics tested were gentamicin, amikacin, netilmycin, amoxicillin-clavulinic acid, cefotaxime, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, imipenem, meropenem, doxycycline, piperacillin-tazobactum, polymyxin B and colistin. *Escherichia coli* ATCC 25922 strain was employed as a control strain.

The data accrued on all *Acinetobacter* infections was analyzed using SPSS version 17.0.Chi-square test was used in assessing the associations between categorical variables. A p-value of 0.05 or less was considered statistically significant.

Result and Discussion

A total of 172 non-duplicate, non-consecutive *Acinetobacter* isolates were processed for species identification, antimicrobial susceptibility testing and to know the MDR, XDR and PDR pattern of these isolates.

A.baumannii was the commonest species isolated 150/172 (87.2%), followed by *A. haemolyticus* 16/172 (9.3%) and *A. lwoffii* 6/172 (3.5%) (Table 1). *A. baumannii* is among the most common of multi-drug resistant clinical isolates in the United States, Europe and Asia, and is a major threat moving forward.It has already been notified by the Infectious Disease Society of America as a “red alert” pathogen. Larson et al 1986, showed that *Acinetobacter* were the most common gram-negative organisms carried on the skin of hospital personnel. Studies in Germany, London and rural India all found between 42% and 55% of healthy individuals to be colonised with *Acinetobacter* spp., which comprised up to 30% of the total microbiota collected from sites on the forehead, arms and toes. Hospitalised subjects had a higher colonisation rate of ~75%. The majority of skin isolates from both healthy and hospitalised European subjects were *A. lwoffii* (>50%) and *A. johnsonii* (21%), while *A. baumannii* was recovered from <1% of individuals tested. In contrast, the most prevalent strains colonising individuals in rural India were *A. haemolyticus* (41%) and *A. calcoaceticus* (15%).These studies suggest that natural carriage of pathogenic

species including *A. baumannii* by healthy or sick individuals is rare (Mindolli et al; 2010, Oberoi et al;2009).

The pattern of distribution of *Acinetobacter* species from various clinical samples is reflected in Table 2. Majority of the isolates were isolated from blood culture 92 /172(53.48%), followed by pus 34/172 (19.76%) and sputum 22/172 (12.8%). Studies on *Acinetobacter* in various countries have shown a predominance of isolation from urine (21-27%) and tracheo-bronchial secretions (24.8-48.8%).In this study, Blood samples (53.48%) were predominantly received for culture as compared to urine and respiratory secretions.Pus/ burn/ wound swab isolation rate in this study was 19.76% which was comparable to the results of other western countries (Cisneros JM et al;1996, Seifert H et al,1995).

Bloodstream infections due to *Acinetobacter* spp. account for ~1.5–2.4% of all reported BSIs in the United States, and *A. baumannii* (86%) is the most frequently isolated species.Because of the relatively low incidence of *A. baumannii* BSI, empiric therapy for Gram-negative bacteremia is often directed at more common offenders which are predominantly susceptible to b-lactam/b-lactamase inhibitor combination drugs. This has the potential to result in treatment failure and increase morbidity and mortality in patients with *A. baumannii* BSIs.

A. baumannii BSIs are associated with mortality rates as high as 44% to 52%,which is slightly higher than the 20% to 40% mortality reported for Gram-negative sepsis as a whole. However, it can be difficult to unequivocally attribute mortality to *A. baumannii* infection since many of these patients have other underlying diseases (Gaynes R, Edwards JR,2005).

Acinetobacter has been one of the established causes of Ventilator Associated Pneumonia(VAP). About 5-10% cases of all the VAP are due to *Acinetobacter*. In this the predisposing factors are Endo-tracheal intubation, tracheostomy, surgery, previous antibiotic treatment or underlying lung disease. Nosocomial spread of the organism can be due to ventilator equipment, gloves, contaminated parenteral solutions, computer key boards. Mortality decreases once anti-microbial therapy has been instituted for more than 3 days. Secondary bacteremia and sepsis are poor prognostic factors. The presence of *Acinetobacter* is attributable more to ICU patients' inadequate immunity rather than the virulence of the organism (Shete et al,2010).

Skin and soft tissue infections account for a small percentage of infections attributable to *Acinetobacter*, and are primarily restricted to patients suffering severe burns or traumatic injury. This type of skin and soft tissue infection caused by *A. baumannii* can often be recognised by the 'peau d'orange' erythyma preceding development of fasciitis.These infections require extensive debridement in addition to antibiotic treatment to resolve the infection. Dissemination to the bloodstream is also common in this patient group, occurring in 44% of these infections.During the Vietnam conflict the most common gram negative organism to contaminate traumatic extremity injuries was *Acinetobacter* with bacteremia occurring 3-5 days later. This phenomenon was also noted during the Iraq war. But in these cases the *Acinetobacter* were multi-drug resistant(Guerrero DM,2010).

Genito-urinary tract infections in the form of cystitis and pyelonephritis can be seen in case of indwelling catheters or nephrolithiasis. Intracranial infections generally follow head trauma or

neurosurgery and can also be seen in healthy individuals. Petechial rash is seen in 30% of the patients. Waterhouse-Friderichson syndrome has also been documented with *Acinetobacter* meningitis (David M, 2005).

The male to female ratio among patients with *Acinetobacter* infection was 1.7:1(Table 3). The age wise pattern of distribution of *Acinetobacter* species is reflected in Table 4. Most common age groups involved were less than ten 50/172 (29.1%),age group between 20-30 years 35/172(20.4%) and patients of more than 60 years 32/172 (18.1%).The pattern of distribution of *Acinetobacter* species from various hospital units is reflected in Table 5. Majority of the isolates were recovered from the patients admitted in ICUs 64/172(37.2 %) followed by those admitted in the surgical wards 38/172(22.09%) and medical wards 27/172(15.7 %) where a number of risk factors were present, including the fact that patients were hospitalised for very long periods, the moist environment of the catheters/urobags and treatment with antibiotics off and on, all giving an opportunity for the bacilli to colonise various sites and then later turn into a pathogen (Vincent et al; 2009, Lee Sang Oh et al; 2004).

Of the total 172 isolates of *Acinetobacter* species, nosocomial isolates from the hospital patients were 131 (76.1%) as compared to the 41 (23.9%) community acquired isolates, bringing to fore the role of *Acinetobacter* spp as an important nosocomial pathogen, since in most cases the patients were symptomatic. The risk factors associated with community acquired infections are alcoholism, smoking, chronic lung disease, diabetes mellitus and residence in tropical developing community. The risk factors associated with nosocomial infections are length of hospital stay, surgery, wounds, previous infections, indwelling intravenous catheters, mechanical ventilation, parenteral nutrition etc.

In the healthcare setting, resistance to desiccation and disinfectants allows *Acinetobacter* spp. to persist and remain viable on surfaces for 13–27 days (Jawad A et al,1998).The establishment of biofilms can further hinder cleaning and sterilisation, thus providing a persistent source for nosocomial infection(Felföldi T,2010).Surfaces commonly contaminated with *Acinetobacter* include computer keyboards, countertops, laryngoscopes, gloves, patient charts, endotracheal connector tubes and bedding. Specifically, *A. baumannii* was isolated from 4.3% of computer keyboards used by healthcare workers (HCWs) in proximity to patients. Similarly, a survey of patient charts in a surgical ICU unit found *A. baumannii* to be the most prevalent Gram-negative bacterium, present on 5.5% of charts.With so many inanimate reservoirs in addition to colonised and infected patients, it is not surprising that the hands of HCWs are often contaminated leading to nosocomial infections(Morgan DJ,2010).

The antimicrobial susceptibility pattern of *Acinetobacter* species is shown in table 6. Out of 172 *Acinetobacter* isolates, 99 (58%) were XDR as these were resistant to atleast one of the carbapenems, aminoglycosides, fluoroquinolones, β-lactams and β-lactam-β-lactamase inhibitor combinations. About 24 (14%) of the isolates were resistant to other group of antimicrobial agents except carbapenems so, these were categorized as MDR isolates. None of the isolates recovered was resistant to polymyxin B and colistin.Thus, there was no isolate, which was found to be PDR. The mechanisms of resistance generally fall into 3 categories:

(1) antimicrobial-inactivating enzymes, (2) reduced access to bacterial targets, or (3) mutations that change targets or cellular functions .Apart from its intrinsic resistance mainly due to the low permeability of the outer membrane to certain antibiotics as well as constitutive expression of certain efflux pumps, *A. baumannii* is able to easily acquire and incorporate genetic elements such as plasmids, transposons and integrons. (H. Giamarellou,2008). Therefore, *A. baumannii* belongs to a unique class of Gram-negative bacteria that are characterised as "naturally transformable". MDR *A. baumannii* have been reported from hospitals in Europe, USA, China, Hong Kong, Korea and Japan as well as from remote areas such as the South Pacific (F Perez, 2007). A major unwelcome feature of MDR *A. baumannii* is aminoglycoside resistance by modifying enzymes. Topoisomerase mutations lead to quinolone resistance and efflux pumps can actively expel beta lactams, quinolones and even aminoglycosides. The chromosomally encoded beta lactamase, particularly AmpC cephalosporinase, is common to all *A. baumannii* strains. There are also resistance mechanisms common to both *Acinetobacter* species and *Pseudomonas aeruginosa*. These include carbapenemases, which are OXA beta lactamases and metallo- beta lactamases, especially the VIM type which conferred significant carbapenem resistance in isolates from Korea(Bonomo RA,Szabo D,2006) .This propensity for multi drug resistance makes *Acinetobacter* infections problematic and hard-to-treat.

The emergence of *A. baumannii* as a significant nosocomial pathogen is a prime example of an opportunistic pathogen taking advantage of an increasingly at-risk population. Intrinsic resistance to common disinfectants and the ability to persist on surfaces and form protective biofilms make it difficult to eradicate this bacterium from heathcare settings and provides an easy mechanism for patient-to-patient spread and repeated outbreaks. The presence of an array of native antibiotic resistance mechanisms, as well as the acquisition of specific antibiotic resistance genes through lateral transfer, has resulted in strains of *A. baumannii* that are resistant to nearly every type of drug currently available. Thus, the treatment of resistant *A. baumannii* infections will continue to present a challenge. This underscores the importance of proper infection control measures, including screening, contact isolation and good hand hygiene when interacting with patients colonised or infected with *A. baumannii*.

Table.1 Species differentiation of the isolates

	Glucose oxidation	**Gelatin liquefaction**	**Hemolysis**	**Growth At 42°C**	**Penicillin susceptibility**	**Total**
Acinetobacter baumannii complex	+	_	_	+	_	150 (87.2%)
Acinetobacter hemolyticus	+	+	+	-	_	16 (9.3%)
Acinetobacter lowffii	_	_	_	-	+	6 (3.5%)

Table.2 Distribution of the isolates from various specimens

Sample	Number of isolates
Blood	92 (53.48%)
Sputum	22 (12.8%)
Respiratory secretions	7 (4.07%)
Urine	13 (7.6%)
Pus	34 (19.76%)
Fluids	4 (2.3%)

Table.3 Sex wise distribution of isolates

Sex	Number of isolates
Male	109
Female	63

Table.4 Age wise distribution of isolates

Age in years	Number of isolates
0-10	50 (29.1%)
10-20	28 (16.6%)
20-30	35 (20.4%)
30-40	14 (8.3%)
40-50	8 (4.2%)
50-60	5 (3.3%)
More than 60 years	32 (18.1%)

Table.5 Unit wise distribution of isolates

Units	Number of isolates
Intensive care units	64 (37.2 %)
Surgical wards	38 (22.09 %)
Medical wards	27 (15.7 %)
Burn	10 (5.8 %)
Orthopedic	8 (4.6 %)
OPD	25 (14.53 %)

Table.6 Antimicrobial susceptibility pattern of Acinetobacter isolates

Antimicrobial drugs	*Acinetobacter* isolates (n=172) Number (%)
Amikacin	48 (27.9)
Amoxicillin-clavulinic acid	7 (4.06)
Cefotaxime	2 (1.16)
Ceftriaxone	2 (1.16)
Ceftazidime	3 (1.74)
Cefepime	8 (4.65)
Ciprofloxacin	35 (2.03)
Cotrimoxazole	9 (5.23)
Colistin	172 (100)
Doxycycline	18 (1.04)
Gentamicin	36 (2.09)
Imipenem	89 (5.17)
Meropenem	78 (45.34)
Netilmycin	67 (38.95)
Piperacillin-tazobactum	94 (54.65)
Polymyxin B	172 (100)

* Numbers in parenthesis indicate percentage of susceptible Acinetobacter isolates

Recommendations

Today, while clinicians confront the worst situation trying to combat even pan drug resistant isolates such as *Acinetobacter baumannii*, the industry curtails the development of new antibiotics. A concerted effort by industry, government and academies is urgently required to improve the situation. In the meantime, what is left for the clinician? Proper application of infection control measures and particularly of "hand hygiene" as well as better antibiotic stewardship in order to slow the development of resistance and to decrease high resistance rates. There is no doubt that we 'must' explore ways of maintaining the potency of currently available antibiotics. Appropriate cultures should be taken in order to avoid the empiricism of the "experts", and pharmacokinetics/ pharmacodynamics should be exploited, whereas as soon as culture results are ready, de-escalation of the administered antibiotics should be promptly ordered.

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