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Research Article

BEYOND *PSEUDOMONAS*- EMERGING NOSOCOMIAL INFECTIONS

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ABSTRACT
The genus Acenitobacter is widely distributed in nature as well as in the hospital environment. *Acenitobacter baumannii* has emerged as an important and problematic human pathogen as it is the causative agent of several types of infections including pneumonia, meningitis, septicaemia and urinary tract infections. It ranked second after *Pseudomonas aeruginosa* among the nosocomial, aerobic, non fermentative, gram negative bacilli pathogens. Furthermore this organism causes infections associated with medical devices e.g. vascular catheters, cerebrospinal shunts, foley catheters etc. A.baumannii has emerged recently as a major cause of hospital acquired infections because of the extent of its antibiotic resistance and its propensity to cause large, often multi factility nosocomial outbreaks. Mortality in patients suffering from *A. baumannii* infections can be as high as 75%. Infections due to A.baumannii often prove difficult to treat due to high level resistance to multiple antibiotics as a result of both intrinsic and acquired mechanisms.

Keywords*: Nosocomial Infections*

INTRODUCTION
Pathogenic bacteria have increasingly been resisting to antimicrobial therapy. Recently, resistance problem has been relatively much worsened in Gram-negative bacilli (Salyers and Whitt, 2005). An increasing incidence during the 1970s of resistant members of the family Enterobacteriaceae involved in nosocomial infections was followed by the therapeutic introduction of newer broad spectrum antibiotics in hospitals and a subsequent increase in the importance of strictly aerobic gram-negative bacilli, including *Pseudomonas aeruginosa, Stenotrophomonas maltophilia,* and *Acinetobacter* spp.
Of these ‘‘newer’’ pathogens, it is now recognized that *Acinetobacter* spp. play a significant role in the colonization and infection of patients admitted to hospitals (Actis *et al.*, 1993). Risk factors associated with colonization or infection include prolonged hospitalization, intensive care unit admission, recent surgical procedures, antimicrobial agent exposure, central venous catheter use, prior hospitalization, nursing and local colonization pressure on susceptible patients (Maragakis and Perl, 2008; Jang *et al.*, 2009; Mahgoub *et al.*, 2002).
The association *of A. baumannii* with pneumonia, bacteremia, wound infections, urinary tract infections, and meningitis has been well described (Maragakis and Perl, 2008). The ability to survive for extended periods on environmental surfaces is notorious and is likely important for transmission within the health care setting. Degradation enzymes against b-lactams, modification enzymes against aminoglycosides, altered binding sites for quinolones, and a variety of efflux mechanisms and changes in outer membrane proteins have been reported. Essentially, any and all of these elements can be combined to result in a highly drug-resistant, and at times pan resistant, opportunistic pathogen (Peleg *et al.*, 2008).
The challenges of treating multidrug-resistant bacteria continue to be at the forefront of the clinician’s practice in caring for hospitalized patients. *Acinetobacter baumannii* has proven to be an increasingly important and demanding species in health care–associated infections. The drug-resistant nature of the pathogen and its unusual and unpredictable susceptibility patterns make empirical and therapeutic decisions even more difficult (Maragakis and Perl, 2008).
Therefore, it seems to be an appropriate time to review the current status of resistance in general and that of the most feared *Acinetobacter* spp. in a tertiary care hospital in Amritsar in particular to help understand and alleviate this serious problem.

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MATERIALS AND METHODS

A prospective study was carried out from January 2013 to July 2014 in the department of Microbiology at tertiary care centre. Various clinical samples like pus, urine, blood, body fluids, tracheobronchial secretions and others were processed in the laboratory according to the standard procedures (Colle *et al.,* 1996). The isolates were identified as non fermenting Gram negative bacilli (NFGNB) on the basis of colony characteristics, Gram's staining, motility test, oxidase test and alkaline reaction on Triple Sugar Iron agar. All the oxidase negative, catalase positive and nonmotile, gram negative coccobacilli were identified as *Acinetobacter* spp and further species differentiation was done on basis of glucose oxidation, gelatin liquefaction, utilization of 1%glucose on O/F medium, hemolysis, growth at 37°C and 44°C, susceptibility to penicillin and chloramphenicol discs (Table-1) (Colle *et al.,* 1996; Koneman *et al.,* 1997). Detailed clinical history were recorded and associated risk factors and comorbidities were also evaluated. Patients from whom *Acinetobacter* were isolated in the absence of any clinical disease suggesting colonization were excluded from the study.

The antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method using gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), Cefotaxime (30ug), Ceftriaxone (30ug), ceftazidime (30 µg), cefepime (30 µg), piperacillin (100 µg), piperacillin/tazobactam (100/10 µg), and imipenem (10 µg) and polymyxin B (300ug) as per CLSI Guidelines (Wayne, 2013). Imipenem resistant isolates were selected for the detection of MBL production by Imipenem-EDTA combined disc test (Yong *et al.,* 2002).

RESULTS AND DISCUSSION

Results

A total of 407 clinical samples yielded growth of NFGNB and among these 76(18.7%) were found to be *Acinetobacter* spp. From the 76 isolates, majority (84.2%) were detected from the patients admitted in various wards of the hospital as compare to outpatients (15.8%). *Acinetobacter* infection was significantly observed among inpatients admitted in ICU and patients having other co morbidities and associated risk factors (Table 2). Majority of the *Acinetobacter* spp. were isolated from respiratory samples (36.8%), pus (32.8%) and blood/ body fluids (13.1%) (Table 3). *Acinetobacter bauamanii* showed predominance (69.7%) amongst the isolated species. Other species identified were *A lwoffii* (21.0%) *and A. haemolyticus* (9.2%).

Table 1: Risk factors associated with patients for *Acinetobacter* spp infection:

Risk Factor	Number (Percentage)
1.Attended hospital as	
Inpatient	64 (84.2%)
Outpatient	12 (15.8%)
2.Age	
>55 years	41(53.9%)
< 55 years	35 (46.1%)
3.Invasive procedure/ devices	
(Peripheral/central venous catheter/ urinary catheters/ post surgical/ intubation/ventilation)	51(67.1%)
4.Hospital stay	
>7 days	41(53.91%)
< 7 days	23(30.3%)
5.Comorbidities/Chronic illness	
Present	56(73.7%)
Absent	20(26.3%)

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Table 2: Identification of isolates

	Glucose oxidation	Gelatin Liquefaction	Haemolysis	Growth 37^0	Growth 44^0	Susceptibility P	Susceptibility C	Total (n/%) 76/18.8
A.baumanni	+	-	-	+	-	-	-	53/69.7
A.lwoffi	-	-	-	+	-	+	+	16/21.0
A.hemolyticus	+	+	+	+	-	-	-	07/09.2

P-Penicillin, C-Chloramphenicol

Table 3: Distribution of isolates in various clinical samples

Sample	*A.baumannii* (n=53)	*A. lwoffii* (n=16)	*A.hemolyticus* (n=7)
Tracheobronchial secretions/ BAL	22	4	2
Pus	15	7	3
Blood/body fluids	11	2	0
Urine	03	2	1
Others	02	1	1

In the present study, *A.baumannii* showed high level of resistance to penicillins, cephalosporins, flouroquinolones (Table 4). Among aminoglycosides, netilmicin showed lesser resistance (23.08%) than amikacin (46.15%) and gentamicin (61.54%). *A. lwoffii* and *A. hemolyticus* showed lesser resistance to all antibiotics as compared to A. *baumannii*. All isolates of *A. lwoffii* and *A. hemolyticus* were sensitive to Polymyxin B whereas 82.00 % isolates were found to be imipenem sensitive. However, among carbapenems, meropenem showed more resistance as compared to imipenem. MBL activity was seen in 17.5% of the isolates. MBL positive isolates of *A. baumannii* were showing significantly higher resistance to all antimicrobials tested as compared to MBL negative isolates and it was found to be statistically significant ($P < 0.05$).

Table 4: Antimicrobial resistance pattern of isolates:

Antimicrobial agent	*A.baumanni* (n=53)	*A.lwoffi* (n=21)	*A.haemolyticus* (n=07)
Amikacin	24	8	1
Gentamicin	36	10	1
Netlimicin	12	4	1
Ciprofloxacin	40	8	1
Ceftriaxone	51	9	1
Cefotaxim	49	7	1
Ceftazidime	49	7	1
Cefepime	47	7	1
Piperacillin+Tazobactam	32	9	2
Cefoperazone+Sulbactam	35	9	2
Meropenem	32	10	1
Imipenem	12	02	00
Poly B	02	00	00
*MBL	11	2	-

**MBL-Metallo beta lactamase producer*

Discussion

During routine clinical microbiology work in most laboratories, non-fermentative Gram negative bacilli (NFGNB) other than *Pseudomonas aeruginosa* are not taken seriously as a pathogen (Veenu *et al.,* 1999). Most of them are not pursued for identification and are dismissed as contaminants. We took up this study

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when we regularly encountered isolates of NFGNB from various clinical samples and these isolates were identified as *Acinetobacter spp*.

Out of 76 isolates of Acinetobacter species, 64 (84.21%) were nosocomial isolates obtained from patients admitted to various wards, whereas only 12(15.78%) were community acquired from the OPD cases. The overall percentage of isolation in hospitalized cases stands at 84.21% vis-à-vis 15.78% in OPD cases from amongst all bacterial isolates, thereby bringing to fore the role of Acinetobacter spp as an important nosocomial pathogen, since in most cases the patients were symptomatic with fever, leucocytosis, pus discharge / UTI.

Various studies (Villers *et al.,* 1998) have identified various risk factors for *Acinetobacter* infection or colonisation, that include factors related to host like, period of hospitalisation, subject to procedures-indwelling catheters, intubation, catheter lines etc. and previous antibiotic therapy (cephalosporins/fluroquinolones).

Majority (84.21%) of the isolates in this study were from patients of the intensive care unit 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. Husni *et al.*, (1999) found an association between cephalosporins and *Acinetobacter* infection.

The role of exposure to certain antibiotics provides a selective advantage to a small resistant sub population of organisms in patients already colonised, thereby enabling them to turn into pathogens at the opportune moment. In many of the patients *Acinetobacter* spp exhibiting two different antibiograms were isolated from different clinical specimens of the same individual, indicating the necessity to clinically correlate the isolate as a pathogen or commensal.

In our study, the most common Acinetobacter species identified from various samples was *A. baumannii* followed by *A. lwoffii* and *A. haemolyticus*. Similar results had been reported in literature (Kumar and Neelagund, 2004; Shete *et al.,* 2009). Most of the nosocomial infections are caused by *A. baumannii*, whereas other species are considered less virulent. *A. baumannii* isolates were resistant to most of the antibiotics used. Resistance to cephalosporins was observed in >80% isolates and among aminoglycosides, Netilmicin showed higher sensitivity as compared to gentamicin and amikacin in *Acinetobacter* spp. Similar results were observed in study by Malini *et al.*, (2009) and Maria *et al.*, (2004).

In this study, majority of isolates were from intensive care unit hence were treated aggressively since patients were symptomatic and in septicaemia. Tracheal aspirates and sputum sample isolates were treated only on clinical correlation of cases. Cases of pneumonia, especially ventilator associated (VAP) with fever, leucocytosis and lung infiltrates showed *Acinetobacter* in one case. Since this organism is a fast coloniser of the respiratory tract, its percentage can increase from 7% to 45% in healthy subjects to those on ventilators respectively, and all samples from such patients should be scrutinized for this bacilli (Tankovic *et al.,* 1994).

Thus, Overall infections caused by *Acinetobacter* spp provide an impressive demonstration of the increasing importance of this genus as a human pathogen because of the high potential of this genus to develop antibiotic resistance, leading to a considerable selective advantage in environments with widespread and heavy use of antibiotic, especially with relation to hospital environment and nosocomial infections.

To conclude, this study underscore pressing need on early detection and infection control practices as the best defenses against these organisms; therefore, systematic surveillance to detect MBL producers is necessary. It is important to follow antibiotic restriction policies to avoid excessive use of carbapenem and other broad spectrum antibiotics.

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