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The Importance of Acinetobacter Species in the Hospital Environment

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ABSTRACT

Introduction: *Acinetobacter* species is associated with health care associated infections especially in patients on respiratory therapy equipment and indwelling catheters. They are becoming increasingly drug resistant. The knowledge of the prevalence and pattern of antimicrobial susceptibility pattern of Acinetobacter spp. is important. **Aims**: The study is undertaken to estimate the prevalence rate, risk factors and antimicrobial resistance pattern of isolates. in *Acinetobacter* spp. from various clinical samples. **Material and Methods:** The isolates of *Acinetobacter* species obtained from various clinical specimen. Specimens were processed by standard microbiological techniques. Antimicrobial sensitivity tests of the *Acinetobacter* isolates were done by modified Kirby-Bauer disc diffusion method. **Results**: Out of 622 isolates, 399 isolates were from inpatients (62,18%) and 223 were from outpatients (37,82%). More than 90% of isolates displayed resistance to ampicillin, amoxicillin-clavulanic acid, ceftazidime, caftriaxon and amikacin. Resistance to gentamicin, co-trimoxazole and ciprofloxacin were also common. Least resistance was seen to piperacillin-tazobactam and imipenem. A total of 125 Acinetobacter isolates were analyzed, out of which 78.4 % were multi-drug resistant (MDR). Of these MDR isolates, 17.24% were pan-resistant. *A. baumannii* was the most common species responsible for wound infection (84,8%), pneumonia(96,15%), abscess (72.7%), urinary tract infection (85,7%) and septicemia(89,5%). **Conclusion:** Multidrug resistant *Acinetobacter* has emerged as an important nosocomial pathogen. Antibiotic susceptibility testing is critical in the treatment of infections caused by *Acinetobacter.* Continued surveillance of prevalent organisms in ICUs, combined with preventive measures remains absolutely essential in efforts to prevent or limit the spread of *Acinetobacter* infection.

Keywords: *Acinetobacter,* **antimicrobial resistance, hospital.**

1. INTRODUCTION

Acinetobacter, once considered as opportunistic pathogen has recently been emerged as an important nosocomial pathogen world over, mostly involving patients with impaired host defense (1).

Acinetobacter species is associated with health care associated infections especially in patients on respiratory therapy equipment and in dwelling catheters. The infections caused by this pathogen include pneumonia, septicemia, wound sepsis, urinary tract infection, endocarditis, and meningitis. *A. baumannii* is the most common species. *Acinetobacter baumannii*, non-fermenting Gram-negative baciili has become an emerging pathogen especially in the hospitals owing to its ability to survive in adverse environmental conditions (2).

Increasing multidrug resistance pattern by *Acinetobacter* species has narrowed range of drugs for treatment. This leads to use higher antimicrobials like colistin and tigecycline for treatment. The accurate identification and reporting of *Acinetobacter* will help to prevent spread of multidrug resistant organism like colistin and tigecycline for treatment.

With the increase in the use of carbapenems to treat the resistant strains, there is a surge in the rates of carbapenem resistance. Use of polymyxin, colistin, and tigecycline is considered to treat the carbapenem resistant strains.

The knowledge of the prevalence and pattern of antimicrobial susceptibility pattern of *Acinetobacter spp.* is important (3).

2. AIM

Aim of article was to estimate the prevalence rate, risk factors and antimicrobial resistance pattern of isolates in Acinetobacter spp. from various clinical samples.

3. METHODS

A retrospective, hospital record-based was conducted in the Department of Microbiology over a period of 2 years. The isolates of *Acinetobacter* species obtained from various clinical specimen: exudate, urine, and swabs from the patients were included in the study. In addition, the strains were isolated from the cleanses at the purity of the aforementioned period in the anesthesiologic, pediatric, internistic and neurological division of the Cantonal Hospital Travnik, Bosnia and Herzegovina. They covered the wipes of the baby pacifers, the bristle of the auxiliary feeding table, the bed wraps, and the wiper of the mobile aspirator.

Relevant clinical specimens were collected from inpatient departments by standard collection procedures. No specific exclusion criteria envisaged. Specimens were processed by standard microbiological techniques (4). Non-fermenters were initially separated and further identified as *Acinetobacter spp.* In Gram stain of direct smears *Acinetobacter* appeared as tiny, Gram-negative coccobacillary cells often appearing as diplococci (5).

All specimens were inoculated on 10% sheep blood agar and MacConkey agar and incubated at 37°Cfor 18-24 h. Colonies on blood agar were 0.5-2 mm diameter, translucent to opaque (never pigmented), convex and entire. On MacConkey agar a faint pink tint was produced (5).

Gram stain, catalase, oxidase and motility tests were performed. *Acinetobacter*are Gram-negative Coccobacilli, non-motile, strictly aerobic, catalase positive and oxidase negative. Rapid utilization of 10% glucose was seen with O-Fmedium. *Acinetobacter* isolates were differentiated from other oxidase negative, non-motile organisms such as Centers for Disease Control and Prevention NO-1, Bordetella holmessii by nitrate reduction test and presence of brown soluble pigment (5).

Antimicrobial sensitivity tests of the *Acinetobacter* isolates were done by modified Kirby-Bauer disc diffusion method for the following antimicrobial agents according to the Clinical and Laboratory Standards Institutes (CLSI) guidelines (6). The isolates were tested against amikacin (30μg), amoxicillin-clavulanic acid (20/10μg), ceftazidime (30μg), ceftriaxone (30μg), cefepime (30μg), cotrimaxazole (23.75/1.25μg), ciprofloxacin (5μg), gentamycin (10μg), tetracyclines (30μg), piperacillin (100μg), imepenem (10μg), piperacillin and tazobactum (100/10mμg), ofloxacin (5μg).

Statistical analysis

P value was reported and a value of P < 0.05 was considered as a significant. The statistical analysis was performed using the Chi-square test.

4. RESULTS

Out of 622 isolates, 399 isolates were from inpatients (62,18%) and 223 were from outpatients (37,82%). We found, 50.80 % isolates were from females, and 49.18 % were from males. The mean age of the study population was 42,5±23.22 years. In male and female patients it was 42.9±22.3 and 36.3±22.6 years, respectively (p<0.05). The

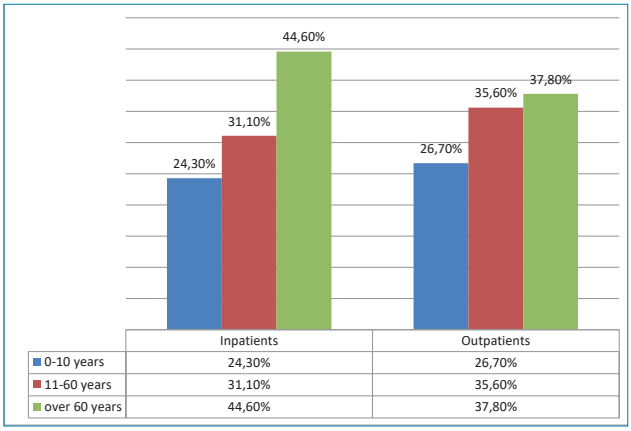

Figure 1. Age-wise distribution of Acinetobacter isolates

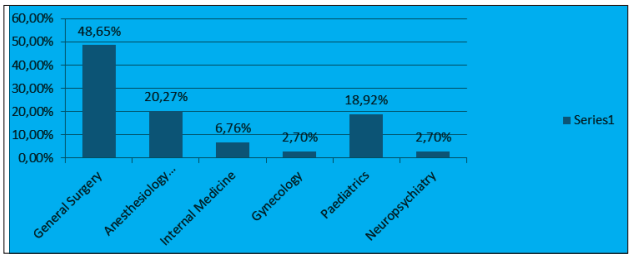

Figure 2. Distribution of Acinetobacter isolates in hospital wards.

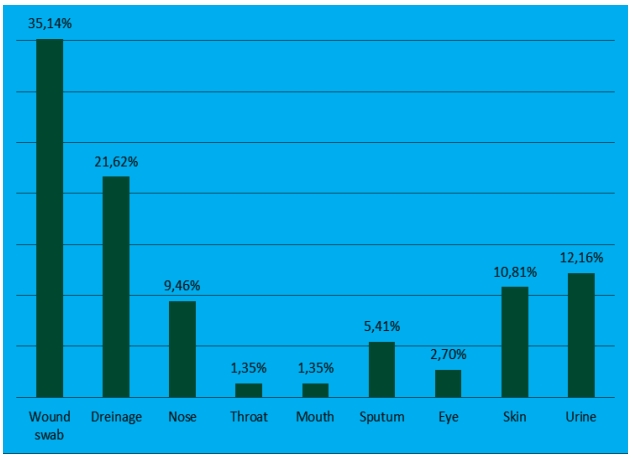

Figure 3. Sample-wise distribution of Acinetobacter isolates

proportion of isolates was more in the age group over 60 (p=0,763) (Figure 1).

A total of 125 *Acinetobacter* isolates were analyzed, out of which 78.4 % were multi-drug resistant (MDR). Of these MDR isolates, 17.24% were pan-resistant. *Acinetobacter spp.* were isolated from different wards in our hospital. Most of the positive isolates, 68.92 %, were from the general surgery (48,65%) and intensive care units (ICU) (20,27%) (p<0.01) (Figure 2).

Acinetobacter spp. were isolated from various clinical samples like wounds swabs, nose, throat swabs, urine, sputum, and other body fluids. Shadow wound samples showed the greatest isolation rate of 35.14%, followed by brush the drainage at 21.62% ((p<0,0001). Sources of isolation of *Acinetobacter spp.* from various clinical samples are shown in Figure 3.

Antibiotic susceptibility testing was carried out by the Kirby-Bauer disc diffusion method. More than 90% of isolates displayed resistance to ampicillin, amoxicil-

lin-clavulanicacid, ceftazidime, caftriaxon and amikacin (Table 1). Resistance to gentamicin, co-trimoxazole and ciprofloxacin were also common. Least resistance was seen topiperacillin-tazobactam and imipenem.

Drug	Non-MDR (n=27)	MDR (98)
Ampicillin	13	98
Amoxicillin-clavulanicacid	7	98
Ceftazidime	5	96
Ceftriaxon	5	95
Cefepime	4	90
Amikacin	1	72
Gentamicin	1	83
Co trimoxazole	3	80
Ciprofloxacin	7	88
Piperacillin-tazobactam	3	56
Imipenem	1	39

Table 1. Comparison of antibiotic resistance pattern of MDR and non-MDR Acinetobacter isolates

Acinetobacter infections	Associated risk factor (%)	A. baumannii (%)	Other A.species (%)	Total (%)
Septicemia	IV catheter (25) Surgery (9)	17 (89,5)	2 (10,5)	19 (15,2)
Wound infection	Trauma (8) Previous infection (14)	28 (84,8)	5 (15,2)	33 (26,4)
Abscess	Post-surgical (48) Diabetes mellitus (11)	24 (72,7)	9 (27,27)	33 (26,4)
Pneumonia/ventilator associated pneumonia	Mechanical ventilation (92) Chronic obstructive pulmonary Disease (5)	25 (96,15)	1 (3,84)	26 (20,8)
Urinary tract infection	Catheterization (47) Prolonged antibiotic use* andhospital stay (>10days) (21)	12 (85,7)	2 (14,28)	14 (11,2)
Total		106 (84,8)	19 (15,2)	125 (100)

Table 2. Distribution of Acinetobacter species, major risk factors and various infections (n = 125). * Antibiotics such as third generation cephalosporins

The most common *Acinetobacter* infection was abscess and wound infections (26.4%), followed by pneumonia (20.8%), septicemia (15.2%), and urinary tract infection (11.2%) (Table 2). *Acinetobacter* infections were more common in males (54.20%) as compared with females (45.80%). Major risk factor associated with Acinetobacter infection were post-surgical (48%), followed by diabetes mellitus (11%), I.V. catheterization (25%), extended hospital stay (21%) and mechanical ventilation (92%). Most common Acinetobacter species isolated was *Acinetobacter baumannii* (84,8%) (Table 2). *A. baumannii* was the most common species responsible for wound infection (84,8%), pneumonia (96,15%), abscess (72.7%), urinary tract infection (85,7%) and septicemia (89,5%).

5. DISCUSSION

Acinetobacter spp. is Gram-negative Coccobacilli that contribute profoundly to the burden of modern medicine. *Acinetobacter spp.* is the second most commonly isolated non-fermenter in human specimens (after Pseudomonas aeruginosa). They rank fourth (after *P. aeruginosa*,

Staphylococcus aureus and *Klebsiella pneumoniae*) among the most frequent hospital- acquired infectious agents (7).

Acinetobacter spp. have emerged as a cause of ICUs infection. Multiresistant *Acinetobacter spp.* have become established as "alert" pathogens, particularly in ICUs and are associated with outbreaks of infection. Their ubiquitous nature in the ICU and surgery environment and inadequate infection control practice have continuously raised the incidence of *Acinetobacter* infections over the past two decades. The understanding and recognition of *Acinetobacter* infections in the ICU and general surgery department are critically needed (8).

Acinetobacter has emerged as an important nosocomial pathogen, especially in the ICU set-up (9).

In our study prevalence was more among the inpatients (98%), which clearly reflects the nosocomial origin of this pathogen. Similar prevalence was observed in other studies (10).

We found no gender difference in Acinetobacter infections. In the present study, *Acinetobacter* infections were more common in females (54.20%) as compared with males. This may be due to the fact that the females report more frequently to the hospitals compared with males. Prashanth and Badrinath (11) reported the infections to be more common in males (58.00%) compared with females (42.00%). Joshi et al. (12) reported 50.20% infection in males.

Currently at least 31 *Acinetobacter* genomo species have been described. *Acinetobacter johnsonii*, *Acinetobacter lwoffii* and *Acinetobacter* radioresistant seem to be natural inhabitants of human skin and commensals in human oropharynx and vagina (5). The digestive tract of patients within ICUs often serve as reservoirs for multiresistant *A. baumannii* strains involved in hospital outbreaks (13).

The most common site for *A. baumannii* infection is the respiratory tract and the most common manifestation is VAP and bloodstream infections. *A. lwoffii* has been more commonly associated with meningitis, *A. junii* rarely causes ocular infection and bacteremia (5). In our study, out of the 125 Acinetobacter isolates, A. baumannii (84.3%) was the most common species to cause Acinetobacter infection (Table 2).

The ability of *Acinetobacter* strains to adhere to surfaces is an important mechanism in the pathogenicity. It frequently causes infections associated with medical devices, e.g., vascular catheters, cerebrospinal fluid shunts or Foley catheters. Biofilm formation is a well-known pathogenic mechanism in such infections. Biofilms have clinical and therapeutic implications, because biofilms preserve bacteria from the action of hosts defensive mechanisms and antimicrobial activity against bacteria in biofilms might be substantially diminished. Rodrí-

guez-Baño et al (14) reported 63% biofilm production in *Acinetobacter* isolates.

In the present study, out of 125 *Acinetobacter* cases major predisposing and associated risk factors were evident in many cases (Table 2). Joshi et al. *(12).* reported existing debilitating chronic illness (20.20%), postoperative surgical (18.50%), trauma (3.30%), urinary catheterization (4.10%) as risk factors associated with *Acinetobacter* infections.

Abbo et al, stated that isolation was more from respiratory tract, which was 32%, followed by wound 19.5%, urine 9%, and blood was 16% (15). In our study increased isolation was from pus samples, followed by endotracheal aspirates, and urine as shown in Figure 3. Mastofi et al. then again in a study showed high isolation rates from blood (5.73%) (16).

As noted by the Infectious Disease Society of America, *Acinetobacter* is "a prime example of mismatch between unmet medical need and the current antimicrobial research and development pipeline." *Acinetobacter spp*. are notorious for their ability to acquire antibiotic resistance (17). Antimicrobial resistance among *Acinetobacter spp*. has increased substantially in the past decade and has created a major public health dilemma. The most potent antibiotic drug class currently available are the carbapenems, but resistant strains have emerged (18). We have studied the antimicrobial resistance pattern among *Acinetobacter* isolates by Kirby-Bauer disc diffusion method. Among 125 *Acinetobacter* isolates, 78.4 % isolates displayed resistance to three or more categories of antibiotics; 17.24% of MDR isolates were resistant to all antibiotics tested (pan-resistant). Increased isolation of this organism was seen in general surgery (48,65%) and intensive care units (ICU) (20,27%). This finding is comparable to other studies.

Acinetobacter is resistant to many antibiotics with more isolations from areas under increased antibiotic pressure such as ICUs. This has decreased the therapeutic options available to treat them. Our isolates showed high resistance to antibiotics from distinct groups especially from Protein synthesis inhibitors, Nucleic Acid Synthesis Inhibitors and Metabolic Inhibitors. Similar findings were reported by Sivaranjani et al. (19).

In the present study, the least resistance was shown to piperacillin-tazobactam and imipenem. Another study reports a resistance percentage of 73.3% to imipenem, increased resistance to piperacillin-tazobactam, and high resistance to third-generation cephalosporins (20).

The above findings clearly show the emerging resistance to co-trimoxazole and ciprofloxacin followed by imipenem and piperacillin-tazobactam, which remain the main stay of treatment for these infections. This is comparable to another study done by Valentia et al. (9). Hence, stringent infection control measures and judicious use of antibiotics are essential for treatment and prevention of *Acinetobacter* infections.

Emerging resistance to antibiotics could not be ascertained by determining the minimum inhibitory concentration (MIC) for the drugs tested. There are several major limitations of this study. Risk factor assessment for the MDR Acinetobacter could not be evaluated. Only 16 isolates could be tested for susceptibility to colistin and were found to be sensitive. This was due to non-availability of the disc for testing. These were the major limitations of this study.

6. CONCLUSION

Acinetobacter are the "super bugs" of the modern hospital environment causing significant proportion of infections in specific patient populations, especially in critically ill patients in the ICU. Multi-drug resistant *Acinetobacter* has emerged as an important nosocomial pathogen. Antibiotic susceptibility testing is critical in the treatment of infections caused by *Acinetobacter,* particularly in those with inadequate response to antibiotic therapy. The development of totally new antibiotics with novel bacterial molecular target sites may constitute therapeutic alternatives within the next few years. Nevertheless, continued surveillance of prevalent organisms in ICUs, combined with preventive measures (e.g., isolation precautions, hand disinfection, efficient sterilization of instruments) remains absolutely essential in efforts to prevent or limit the spread of *Acinetobacter* infection.

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