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Prevalence and resistance of *Acinetobacter baumannii* isolated at Hyatabad Medical Complex, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Objective: To assess the resistance against widely used antibiotics in case of *Acinetobacter baumannii* (*A. baumannii*) infection.

Methods: A total of 350 samples of pus, urine, swab and others from different patients were examined and the bacterial growth appeared in 50 samples. Each sample was inoculated on blood, MacConkey and cystine lactose electrolyte deficient agar. The antimicrobial susceptibility profile of the isolates was determined using agar plate method/disk-diffusion method (modified-Kirby Baur disc diffusion method).

Results: In current study, a large number of isolates of *A. baumannii* obtained from different specimens were resistant to avelox (56%), followed by tygacil (46%), augmentin (46%), cefspan (38%), cefixime (24%) and ampicillin (20%). However, the antibiogram of *A. baumannii* also showed that most of the isolates (88%) were highly sensitive to cefalexin. Second maximum sensitivity of *A. baumannii* was seen to amikacin (84%). The sensitivity of isolates against amikacin was followed by ticarcillin (80%). Meronem was found highly active against the tested isolates (78%). Sensitivity was observed for tienem (76%), sulzone (72%) followed by azactam (68%), cefobid (66%) to cefotaxime (66%) and ciproxin (62%).

Conclusions: Results elucidate that *A. baumannii* is a severe problem as it has become a highly resistant species in hospitalized patients and resistant *A. baumannii* infection turned out to have increased all-cause mortality.

1. Introduction

Acinetobacter baumannii (*A. baumannii*) is a Gram-negative, rod-shaped bacterium commonly found in the lower intestine of warm-blooded organisms (endotherms) that exist in moist habitat, and found in disinfectant solutions and water, because of its ability to utilize various organic compounds and its perpetuate life in nutrient deficient conditions[1]. This bacterium can grow in different water sources like river water, sea water and bottled mineral water[2-4]. Most pathogenic *A. baumannii* species related to humans are involved in opportunistic infections. *A. baumannii* got importance as a hospital pathogen in the second half of last century[5]. Its nutritional requirements are least. In normal people, it's a commonest human micro flora. Such degree of commensalism accelerates continuously per expanded period of stay at hospital[6]. In hospitalized patients having recurrent infections, it's commonly identified as strategic microorganism and also found to exist in different hospital environments[7,8]. *A. baumannii* is a chief, contrary and hazardous organism infecting burn patients[9].

A. baumannii is convoluted in analysis of many diseases including endocarditic, meningitic, bronchopneumonic, ocular, burnt and wound infections[10-12]. It can infect any exposed part or organ of the body and that is the reason why it can be isolated from various body fluids such as pus, urine, eye, ear swab, sputum and blood, *etc.* [13]. In Nigerian children, wound infection is one of the dominant sources of limb implantation[14].

Most pathogenic *A. baumannii* species related to humans are involved in opportunistic diseases[15]. Patients with weaker immune system like neutropenia and bone marrow graft are commonly affected by opportunistic infections. *A. baumannii* causes 16% of nosocomial pneumonia[16], 12% of hospital-acquired urinary tract infections[17], 8% of surgical wound infections[17] and 10% of bloodstream infections[18,19].

In spite of the appropriate anti-microbial therapy, longer

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hospitalization and high cost evaluation, the mortality rate of patients infected with resistant organism is raised as compared to the patients infected with microbial bacteria[20]. The need of antimicrobials agents is not limited only to the human medicine and these bioactive biomolecules are in increasing demand in several domains including food industries for foodstuffs preservation and in dentistry. Thus, there is a continuous requirement for the discovery of new antimicrobial agents. *A. baumannii* is a chief and usual non-fermentive bacterial species observed in clinical specimens of hospitalized patients[21]. In the list of hospital microorganisms, it ranks the fifth well known pathogen and causes 10% of all hospital acquired infections[22]. In Bangladesh, it is graded the third and becomes the source of large number of diseases[23]. In recent times, this bacterium has emerged remarkably defiant to various antimicrobial agents[24].

The discovery of antimicrobial agents has a good impact on the survival rate from infections. However, the changing capacity of antimicrobial resistance causes a demand for new antibacterial agents. Microbial ability to oppose drugs is a typical complication for human health[25,26]. In recent years, drug resistant bacteria have risen many serious occurrences of infections with many deaths[27]. Meanwhile, a prominent elevation in the existence of multidrug resistance (MDR) in *A. baumannii* has been observed, which corresponds to the high morbidity and fatality[19,28]. In medical practice, it is difficult to treat for its flexibility in physiology[10,19]. It's the major prevalent pathogen in patients with immune suppression, cystic fibrosis and malignancy[17]. The generality of anti-microbial pathogen differs to large extent between communities, hospitals of the same community and among different population of patients[29]. Its general resistance is caused by various factors[30].

The Infectious Diseases Society of America added *A. baumannii* to the list of ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) pathogens that present the public health with formidable threat due to the combination of increasing prevalence and ineffectiveness of existing antibacterial agents[31]. Unfortunately, the microorganisms has shown the parallel progress in developing resistance to the advancement of antibiotics. *A. baumannii* may also proceed with the same speed in varying mechanisms of resistances. Owing to its existence in diverse habitats, its pathological importance and resistance to antibiotics appeared. This study was performed to identify and isolate *A. baumannii* from different clinical samples and determine its antibiotic susceptibility and resistance profile.

2. Materials and methods

The authors followed standard systematic review methods[32]. This study was organized and operated at Department of Microbiology in Hayatabad Medical Complex, Peshawar, Khyber Pakhtunkhwa, Pakistan from February 2016 to June 2016. It was a tertiary care center, referral and teaching hospital.

2.1. Samples collection

The study included 50 positive samples out of 350 samples. They were obtained from different patients who were hospitalized for more than one week duration. Bacterial isolates were gathered from samples of pus, blood, sputum, urine, wounds, burn and swab using conventional sampling method and then submitted for analysis to the Microbiology Department. All the specimens were collected from different hospital wards.

2.2. Isolation and confirmation of A. baumannii

Each sample was inoculated on blood, MacConkey and cystine lactose electrolyte deficient agar. The plates having these media on which samples were applied were incubated at 37 °C for 18–24 h to obtain the bacterial colonies. Positive specimens were then refined further for diagnosis and description using common running operations[33,34]. Gram staining was performed to characterize and determine Gram-positive and Gram-negative bacteria. Stained slides were observed under the microscope for the presence of any rods and cocci, *etc*.

For the confirmation of *A. baumannii*, different biochemical tests were performed like tryptophan hydrolysis by the breakdown of amino acid tryptophan with release of indole, triple sugar iron test for selective isolation, urease production test, citrate utilization test, oxidative-fermentative test for alkaline protease production and motility tests.

2.3. Antibiotic sensitivity test

The antimicrobial susceptibility profile of the isolates was determined using agar plate method/disk-diffusion method (modified Kirby–Baur disc diffusion method) by following the guidelines of Clinical and Laboratory Standards Institute[35].

Bacterial isolates were diagnosed against few antimicrobial drugs including tygacil, amikacin, cefspan, sulzone, cefixime, meronem, tienem, cefobid, ciproxin, augmentin, cefotaxime, ticarcilline, and ampicilin. These antibiotics were selected according to the 2004 National Committee for Clinical Laboratory Standards guidelines. During the use of this method, microbial suspension equal to half of the McFarland scale was prepared and then was planted on plate by Mueller–Hinton agar culture. The plate was moved clockwise and then anticlockwise to create bacterial lawn. Related antibiotic discs were then pasted in culture with 1 cm apart from each other. The diameter of the non-growing areolae of antibiogram was measured after 18–24 h of incubation. After that, zone of inhibition was measured for each antibiotic to declare which are sensitive, resistant or intermediate.

3. Results

Overall 350 different clinical samples including 161 males and 189 females were put to procedure of isolation of *A. baumannii*. Among 350 samples, 50 (14.28%) were positive for *A. baumannii*. Out of these 50 positive samples of *A. baumannii* 27 (54%) were isolated from males, while 23 (46%) from females.

Out of 350 clinical isolates of *A. baumannii,* 156 (45%) were separated from pus, which were found with maximum positive isolates of 25. A total of 102 (29%) were isolated from urine, of

which 7 samples showed growth of *A. baumannii*. Tweenty nine (8%) were isolated from swab, of which 7 were found positive. Tweenty two (7%) samples were isolated from wounds, of which 7 were positive. Six (2%) were isolated from high vaginal swab and 15 (4%) from blood but both were found negative, *i.e.* no growth was observed. And 20 (5%) were isolated from other samples where only 4 showed the growth of *A. baumannii* (Figure 1).

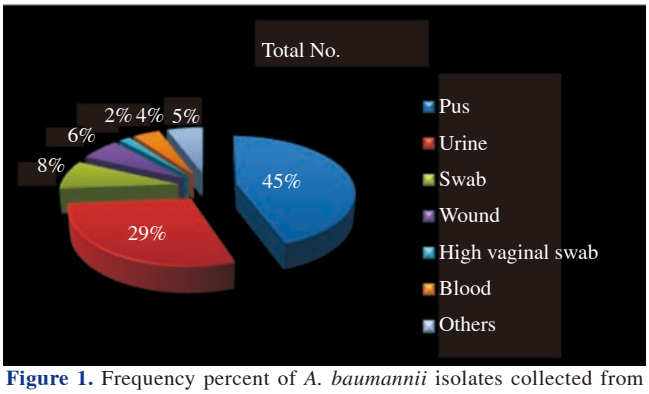

Figure 1. Frequency percent of *A. baumannii* isolates collected from clinical samples.

For age wise distribution of patients infected with *A. baumannii*, the age ranged from 2 years to more than 60 years. Thirteen (26%) isolates belonged to the age group up to 20. Tweenty five (50%) isolates were related to the age group of 21–40. Eight (16%) isolates were collected from the age group of 41–60 and 4 (8%) isolates were from patients above 60 years. This made it certain that *A. baumannii* infection was not limited to particular age.

The highest numbers of patients falled in the range 21–40 years of age with 25 positive MDR *A. baumannii* (Table 1).

Table 1
MDR *A. baumannii* among different age groups.

Age group	MDR	Non-MDR	Total
< 20	13	70	83
21–40	25	104	129
41–60	8	96	104
> 60	4	30	34
Total	50	300	350

The antibiotic susceptibility was determined according to standards of Clinical and Laboratory Standards Institute. The antibiogram of *A. baumannii* revealed sensitivity status of antibiotics as: Most of the isolates (88%) were highly sensitive to tazocin. Second most sensitivity of *A. baumannii* was seen to amikacin (84%). The sensitivity status of isolates against amikacin was followed by ticarcilline (80%). Meronem was found highly active against isolates (78%). Sensitivity was observed for tienem (76%), sulzone (72%), azactam (68%), cefobid (66%), cefotaxime (66%) and ciproxin (62%). Nevertheless, the pathogen tested in this study showed varying degrees of resistance. That is, maximum isolates of *A. baumannii* were resistant to ampicillin (80%), followed by cifixime (76%), cefspan (62%), augmentin (56%), tygacil (56%), avelox (44%), ciproxin (38%), cefobid (34%), cefotaxime (34%), azactam (32%), sulzone (28%), Tienem (24%), meronem (22%), ticarcilline (20%), amikacin (16%) and tazocin (12%) (Figure 2).

4. Discussion

A. baumannii owing to its pathogenic status and involvement in nosocomial infections, leading to raised morbidity and mortality in hospitalized patients, come out as a foremost pathogen. This importance to *A. baumannii* is given to it by its low susceptibility to typical antibiotics, antiseptics and its ability to adapt to diverse hospital environment. It can survive in availability of minimal nutrients, if moisture is present.

A. baumannii is mainly a hazardous microorganism that causes nosocomial infections. It is a frequent source of infections like burn sepsis, urinary tract infection and external ear inflammation. It is commonly found in patients with mucoviscidosis, grafting, and acute leukemia. The infections with high fatality rates are caused by it[13,24]. Currently, our aim is to determine the antibiotic susceptibility profile of 50 *A. baumannii* isolates from various clinical origins. Various studies are accomplished to reveal antibiotic sensitivity pattern for different applicable medicines for *A. baumannii*. Such study can help clinician in providing better management to patients[36].

Therefore, the current study is performed to determine the antibiotic resistant and sensitivity pattern of *A. baumannii* isolated from different clinical specimens. The isolation rate of *A. baumannii* is commensurate with other studies.

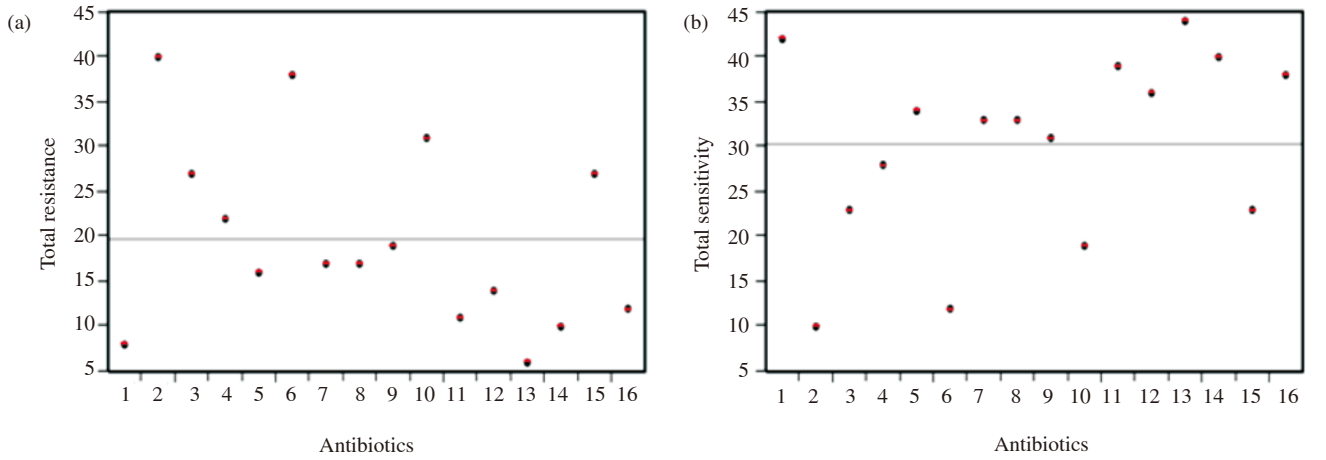

Figure 2. Antibiogram of *A. baumannii* against selected antibiotics.
1: Amikacin; 2: Ampicillin; 3: Augmentin; 4: Avelox; 5: Azactam; 6: Cefixime; 7: Cefobid; 8: Cefotaxime; 9: Ciproxin; 10: Cefspan; 11: Meronem; 12: Sulzone; 13: Tazocin; 14: Ticarcilline; 15: Tygacil; 16: Tienem.

Sex wise distribution of clinical isolates shows that infections induced by *A. baumanni*are majorly occur in males (54%) compared to females (46%). This is comparable with study of Javiya *et al.*[15], Rashid *et al.*[34] and Khan *et al.*[37] This distribution also represents that most of patients 25 (50%) of age ranges from 21 to 40 which resembles with the study of Rashid *et al.*[34].

More isolates of *A. baumannii* were isolated from pus 156 (45%), followed by urine 102 (29%), swab 29 (8%), 22 (7%) from wounds, 6 (2%) from high vaginal swab and 15 (4%) from blood but both were found negative, *i.e.* no growth was observed and 20 (5%) from other samples where only 4 showed the growth of *A. baumannii*. These outcomes are comparable to studies of Khan *et al.*[37] and other studies of Syed *et al.*[5], Shenoy *et al.*[36], Murase *et al.*[38]. The chief proportion of *A. baumannii* diseases are noticed in the surgical ward (48%), pediatric ward (23%) and medical ward (17%). Ubiquity of infection is greater in surgical ward as maximal pus/ swab specimens showed growth of *A. baumannii*[7].

The antibiogram studies showed that *A. baumannii* was highly susceptible to cefalexin (88%) and second most sensitivity was amikacin (84%) followed by ticarcilline (80%). Meronem was found highly active against isolates (78%), tienem (76%) to sulzone (72%) followed by azactam (68%), cefobid (66%) to cefotaxime (66%) and ciproxin (62%). Similar antibiogram was obtained by Ejaz *et al*.[31], which indicated that *A. baumannii* is highly sensitive to imipenem (99%) followed by amikacin (79%) > tobramycin (70%) > ceftazidime (62%) > ciprofloxacin (73%)> cefoperazone (60%) > piperacillin (65%)> gentamycin (34%) and cefotaxime (14%). This difference in response to different antibiotics might be due to frequent exposure of organisms to these antibiotics. It might also be expected that in less developed countries, many pharmaceuticals bargained have no sufficient concentration of main active components declared by companies in stickers on it, despite of at preparation and assembling stages. In our study, maximum isolates of *A. baumannii* were resistant to avelox (56%) followed by tygacil (46%), augmentin (46%), cefspan (38%), cefixime (24%), ampicilin (20%). According to Rashid *et al.*, cefotaxime was highly resistant (93.3%) and trimoxazole (93.5%) and majority was resistant to ceftazidime (86%), gentamycin (77.3%) and ciprofloxacin (75.5%) [34]. According to Amadi *et al.*[35], amoxicillin was highly resistant (88.2%). Studies show that *A. baumannii* isolates are getting resistant to ordinarily used antibiotics and boosting progressive resistance to recent antibiotics[38-42]. The antimicrobial substances are failing their effectiveness due to the transmission of recalcitrant organisms, because of aimless and frequent extensive usage of antimicrobials, absence of attention, patients' refusal and contaminated environments[36].

Keeping in view the resistance pattern of pathogens to frequently used antibiotics, it is also necessary to run a large scale study with newer antimicrobials. This will hopefully reduce the resistant pattern and the treatment cost and initiate the quality of patients' care. It is urgent to design antimicrobials management and administration guidelines and strategies to prevent and overcome this arising obstacle. Also, struggle should be done to keep the organism from spreading. There is an urgent need for the establishment of prevention program to teach patients how to take antibiotics properly as well as raising awareness.

The highly susceptible antibiotic against isolates of *A. baumannii* is cefalexin, which showed maximum sensitivity zone to all the isolates, while the least effective drugs were avelox (56%) and followed by tygacil (46%), augmentin (46%), cefspan (38%), cefixime (24%) and ampicilin (20%). To keep the bacteria from spreading, it is pre-requisite to have tough antimicrobial protocols, while supervision acts and plans for MDR microorganism. Also, infections prevention practices are necessary to be implemented. Currently, the most important thing is that the antibiotic resistance and sensitivity patterns of microorganisms like *A. baumannii* should be regularly monitored in clinical units and results should be easily accessible to clinicians and microbiologists to reduce its resistance.

The problem could be resolved by determined struggle of microbiologist, clinician, pharmacist and community to build up cooperation and understanding of this problem. Regular use of gloves, gowns, masks and washing hand after performing clinical procedures should be brought into practice to avoid transmission of organism. Improved medical care and better treatment facilities should be given to patients all along hospital stay.

Conflict of interest statement

We declare that we have no conflict of interest.

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