ORIGINAL ARTICLE

ANTIBIOTIC RESISTANCE PATTERN OF *ACINETOBACTER BAUMANNII* ISOLATED FROM BACTEREMIA PATIENTS IN PAKISTAN

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Background: *Acinetobacter baumannii* causes a number of life-threatening infections in Hospitalized patients attributed to its ability to develop resistance against multiple antibiotics. The current scrutinisation is aimed to observe the prevalence and antibiotic resistance profile of *A. baumannii* strains isolated from blood of tertiary care Hospitalized patients in Lahore, Pakistan. **Methods:** This research is a retrospective study conducted over a period of one year where 1864 blood samples were collected from both male and female patients with septicaemia. Total 156 *A. baumannii* species were identified by conventional method and their antimicrobial resistance pattern against 22 antimicrobials (representing all known classes of antibiotics) was evaluated by Kirby Bauer disc diffusion method. MICs of colistin, polymyxin B and vancomycin against *A. baumannii* were calculated by E test and broth dilution method. **Results:** More males (n=97, 62%) were found infected than females (n=59, 38%). The spreading rate of *A. baumannii* was highest (n=101, 65%) in patients of age ≤20 years, and lowest (n=12, 7%) in the patients with the age of 41–60 years. Most of the strains of *A. baumannii* (n=118, 75.6%) were found to be MDR (multi drug resistant), 37 (23.7%) strains were XDR (Extensively drug-resistant) and only 1 (0.05%) strain was PDR (pandrug resistant). All the strains were sensitive to minocycline and tigecycline whereas highest non-susceptibility (n=144, 92%) was seen against Ampicillin-Sulbactam. Most of the strains demonstrated resistance against carbapenem and cephalosporin beckoning that *A. baumannii* can no longer be considered for salvage therapy by carbapenem. MICs of colistin, polymyxin B and vancomycin against *A. baumannii* divulged polymixin B as the most effective drug. **Conclusion:** Use of wide range of drugs has made *A. baumannii* multidrug resistant. Colistin, polymyxin B and vancomycin are the preferable drugs for the treatment of *A. baumannii* infections.

Keywords: *Acinetobacter baumannii*; Antibiotic resistance; Multiple drug resistance; Polymixin B; Septicaemia

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INTRODUCTION

Acinetobacter baumannii is gram negative coccobacilli and belongs to genus *Acinetobacter*. It is immotile, aerobic, catalase positive, oxidase negative, and lactose non fermenting organism occurring in pairs. This organism mostly occurs in patients with low immunity and acquires opportunity to cause serious infections in them; therefore, it is argued as an opportunistic pathogen. It is widely distributed in soil, water and waste materials. It can also be found in skin, oropharynx, gastro intestinal tract (GIT) and respiratory tract of normal individuals, affirming it as normal flora of human respiratory tract and skin.[1] *A. baumannii* causes a number of infections including urinary tract infections, osteomyelitis, endocarditis, meningitis, scar infections, wound infections, ventilator associated pneumonia (VAP), respiratory tract infections and septicaemia.[2] These infections are major source of life threatening effects in hospitalized patients attributed to its ability to form biofilms and to develop resistance against multiple antibiotic drugs.[3–5]

Different isolation sources of this organism exist including wound swab, pus, sputum, tracheal aspirate, tissue, bronchial washing, blood, cerebrospinal fluid (CSF), and urine.[6] Several drugs are used for the treatment of bacterial infectious diseases but most of these drugs are losing their effectiveness against *A. baumannii*, because of its increasing resistance against extended spectrum drugs therefore, the problems caused by *A. baumannii* are difficult to overcome.[7]

The Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) propounded a standardized terminology, "ESKAPE" (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii*, *Pseudomonas aeuroginosa*

and *Enterobacter* spp.) which are the most common multidrug resistant pathogens.[8] *A. baumannii* is one of these six most important multidrug resistant microorganisms in hospitals all over the world and able to resist multiple antibiotic classes effective against other bacteria.[9] Most of the health care associated infections occur due to *A. baumannii* making it very difficult to select an appropriate drug in order to treat infections caused by *A. baunmannii*.[10]

The major resistance mechanisms of *A. baumannii* to antimicrobial agents are aminoglycoside-modifying enzymes, the alterations in penicillin-binding proteins (PBP), the production of broad-spectrum β-lactamases and changes in outer membrane proteins. Chromosomes, plasmids, and transposons are the other genetic resistance mechanisms.[11] *A. baumannii* are becoming increasingly resistant to nearly all generally prescribed antimicrobial agents, including tobramycin, gentamicin, netilmicin, amikacin, imipenem, doripenem, meropenem, levofloxacin, ciprofloxacin, piperacillin-tazobactam, ceftazidime, cefetrixone, trimethoprim-sulphamethoxazole, cefotaxime, ampicillin-sulbactam, colistin, ticarcillin-clavulanic acid, polymyxin B, doxycycline, tetracycline, cefepime and minocycline. Many bacterial isolates are resistant to antibiotic class cephalosporin, whereas the resistance is increasing against carbapenems due to its ability to produce carbapenem hydrolyzing beta lactamases of ambler classes A, B and E.[12] *A. baumannii* being resistant to multiple antibiotic agents is broadly classified into three major groups, i.e., pandrug-resistant (PDR), multidrug-resistant (MDR) and extensively drug-resistant *(*XDR).[13] The microbe which is resistant to at least 1 drug in three or greater than three antibiotic categories is termed as MDR while the organism which is resistant to at least 1 agent in all but 2 and fewer antibiotics classes is regarded as XDR and the organism resistant to all available antibiotic classes is declared as PDR. The prevalence of antibiotic resistant *A. baumanii* has increased with the passage of time.[14] The major factors causing antibiotic resistance to commonly used drugs are the lack of national guidelines for antibiotic use and absence of proper laboratory practices to do antimicrobial drug susceptibility test.[15] The resistance patterns of *A. baumannii* against different drugs are reasonably variable among different countries attributed to multiple including, altered epidemiological conditions, wide range of antibiotics and their excessive usages. The main objective of the present study is to investigate the prevalence and drug resistance profile of *A. baumannii* isolated from patients of septicaemia in Pakistan.

MATERIAL AND METHODS

A total of 1864 blood samples were collected in six (06) months period (January–June, 2020) from tertiary care hospitals (Mayo, Services, Children and Jinnah hospitals) of Lahore Pakistan under sterilized conditions and transported to laboratory in Amies media for further processing. BACTEC bottles were used for culturing blood specimens and were incubated for seven days at optimum temperature, to see any positive reaction. After incubation period, gram staining was performed on positive samples, and inoculated on blood and MacConkey agar. Patients with symptoms of bacterial infections including urinary tract infections, meningitis, open wound infections, pneumonia, sepsis, abdominal infections, respiratory tract infections and endocarditis were selected with no age restriction. The patients with immune system dysfunction were excluded. Preliminary screening was done by Gram staining, oxidase, catalase, motility and growth at 44 °C. For confirmation API 20NE (Biomerieux, France) was used to check results obtained after sub-culturing by following manufacturer's instructions.

All the patients included in the study were informed not to disclose their identity and usage of their data for research purpose only. The study was approved by the institutional review board (IRB) of the university 04-03-2019 under the approval number IRB-UG-19668.

The susceptibility of isolated strains of *A. baumnnii* to different antimicrobial agents including tobramycin, gentamicin, netilmicin, amikacin, imipenem, doripenem, meropenem, levofloxacin, ciprofloxacin, piperacillin-tazobactam, cefotaxime, cefepime, tetracycline, ceftazidime, cefetrixone, trime-sulphamethoxazole, ampicillin-sulbactam, colistin, polymyxin B, doxycycline, minocycline and ticarcillin-clavulanic acid (Oxoid, UK) was determined by Kirby Bauer Method.[16] Briefly, overnight bacterial culture was picked up with a sterilized loop, suspended in peptone water and incubated at 37 °C for 2 hours. The McFarland's standard of turbidity (0.5) was used to compare the results. The bacterial suspension was spread on Mueller Hinton (MH) agar plates, standard antibiotic discs were placed to check the sensitivity and incubated the plates at 37 °C for 12–16 hours. The inhibition zones that appeared after the incubation were measured and compared to the standards of CLSI guidelines 2018.

Minimal Inhibitory Concentration (MICs) of selected antibiotics *i.e*., polymyxin B, colistin and vancomycin were calculated by broth dilution method.[17] In microbiology reference laboratories micro-broth dilution methods and semi-automated or manual broth dilution methods are used to check the minimal inhibitory concentration (MIC) for epidemiological purposes. It can also be used to measure the lowest concentrations of antimicrobial agents required to kill bacteria which is called as minimum bactericidal concentration (MBC).

The stock solutions of antibiotics were prepared by using the following equation;

$$W = \frac{C.V.1000}{P}$$

Where, P is potency of antibiotics given by the manufacturer, C is final concentration of solution, V is volume in ml required, W is weight (g) of the antimicrobial dissolved in volume V (ml). These solutions were made in high concentrations and stored at -20°C in suitable aliquots. Once taken out they were not reused or not refrozen. To check the turbidity of inoculums for MIC determination, a $BaSO_4$ turbidity standard, equivalent to a 0.5 MacFarland was used. This standard was prepared by adding 0.5 ml aliquot of 0.048 mol/L $BaCl_2$ to 99.5 ml of H_2SO_4. The turbidity of the standard was checked by spectrophotometer (Microlab 300, Merk). This suspension was transferred into 4–6 ml tubes of same size which were tightly sealed and stored in the dark at room temperature. Standardized inoculum of the test organism was added to the dilutions of antimicrobial agents in a broth medium. The suspensions of the tubes were observed for turbidity after incubating at 37 °C for 18–24 hours. The outcomes were interpreted according to the breakpoints provided by CLSI guidelines 2018. SPSS (Statistical Package for the Social Sciences) V24.0 was used for data entry and its statistical analysis.

RESULTS

Overall, 156/1864 patients were found infected with *A. baumannii* and thus the prevalence *A. baumannii* was 8.3%. Moreover, *A. baumannii* infections were found more prevalent in male patients (n=97, 62%) than that of female (n=59, 38%). The gender wise distribution of the infection was statistically significant (p=0.08).

The age of male and female patients was ranged from 1 month to 92 years with the mean age of 18±24.5 years. The mean ages of the male and female patients were 17.24±24.1 and 20.01±24.8 years, respectively. The spreading rate of *A. baumannii* was highest (n=101, 65%) in patients of age ≤20 years, which was seen lowering (n=29, 19%) in age 21–40 years, and has further lowered (n=14, 9%) in age greater than 60 years. In patients of age 41–60 years, the prevalence rate of *A. baumannii* was lowest (n=12, 7%) (Figure-1).

The presented study determined the sensitivity and non-susceptibility pattern of *A. baumannii* isolates against 22 antibiotics. All the isolates were found sensitive against minocycline and tigecycline (n=156, 100%) while highest non-susceptibility (n=144, 92%) was seen against Ampicillin-Sulbactam (Figure-2).

On the basis of antibiotic resistance profile, the prevalence of PDR, MDR and XDR *A. baumannii* was determined. Most of the strains of *A. baumannii* were found to be MDR (n=118, 75.6%) as they were resistant to ≥ two classes of drugs, 37 strains (n=37, 23.7%) were XDR being susceptible to only two antimicrobial classes, and only one strain was PDR as it was resistant to all antimicrobial classes (Figure-3).

MICs of colistin, polymyxin B and vancomycin against *A. baumannii* were calculated. MIC breakpoints for vancomycin, colistin and polymyxin B given by CLSI guidelines 2018 (sensitivity ≤2 µg/ml and resistance ≥4 µg/ml) were used to interpret the results. MICs for vancomycin, colistin and polymixin B ranged 0.38–1.5 µg/L, 0.50–0.75 µg/L and 0.75–4 µg/L, respectively. Highest number of isolates (n=11, 35%) were resistant to polymyxin B in the concentration of 0.75 µg/L (Table-1).

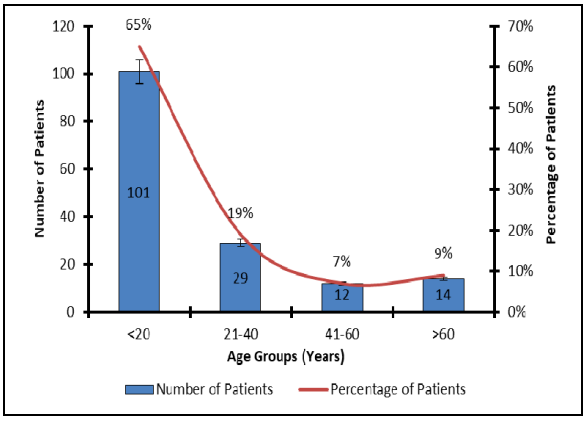

Figure-1: Prevalence of *A. baumannii* in different age groups.

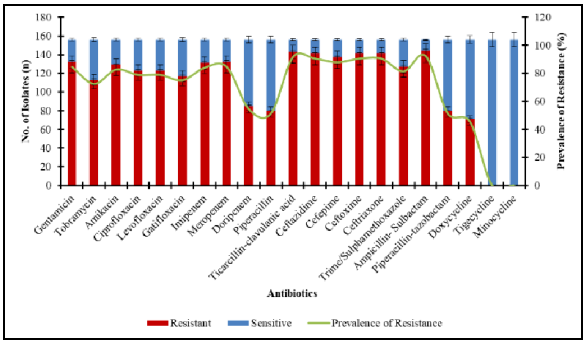

Figure-2: Antimicrobial Profile of *A. baumannii*.

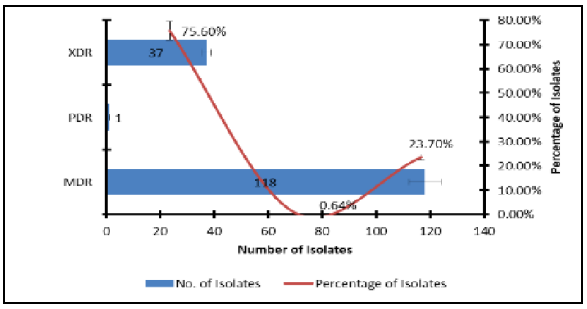

Figure-3: Prevalence of MDR, XDR and PDR *A. baumannii* Isolates.

Table-1: MICs of Various Drugs against *A. baumannii*.

Antimicrobial agents	Breakpoints	MIC (µg/ml)							
		0.25	0.38	0.50	0.75	1	1.5	2	4
Polymyxin B	≤ 2 µg/ml Susceptible ≥ 4 µg/ml Resistant	2	26	38	51	21	16	1	1
Colistin	≤ 2µg/ml Susceptible ≥ 4 µg/ml Resistant	-	16	29	48	41	11	11	-
Vancomycin	≤ 2µg/ml Susceptible ≥ 4 µg/ml Resistant	-	-	12	39	54	30	21	-

DISCUSSION

Acinetobacter baumannii is a gram negative, aerobic, catalase positive, oxidase negative and lactose non fermenting coccobacillus.[18] It is responsible for a variety of infections, especially in hospitalized patients with reduced immunity. Persistence and attachment of *A. baumannii* on dry and moist surfaces, the consequent rescinding of superficial cells, the capability to gain vital nutrients such as iron, and the ability to produce certain enzymes such as, beta lactamase and proteinases that harm the immune-compromised tissues of infected patients are the main factors that contribute to the increased pathogenic behaviour of *A. baumannii*. It usually causes a broad range of infections in patients with reduced immunity including endocarditis, meningitis, ventilator-associated pneumonia, soft-tissue and skin infections, kidney infections, wound, surgical site infections and bacteremia.[19] A variety of attributes are accountable for the spreading of *A. baumannii* in sanatorium location such as, environmental contamination, its ability to utilize a diversity of carbon and energy sources ,and growth at different temperature and affirming it not to be a fastidious pathogen.[20] The infections caused by *A. baumannii* have increased due to the factors including use of multiple and inappropriate antibiotics, use of ventilators in ICU departments of hospitals, use of catheters and contaminated hands of hospital staff.[21] The ability of *A. baumannii* to form biofilms and to develop resistance against multiple antibiotics causes mortal effects in ill sufferers. Infections caused by *A. baumannii* strains can be controlled by adopting some precautionary measures such as restricting the contact of infected person with healthy individuals or by using suitable disinfectants.[22]

In the present study, 156 positive samples were extracted from 1864 blood cultures of septicemia patients. *A. baumannii* caused infection was found more prevalent in male population (n=97, 62%) than in female population (n=59, 38%). Several previous studies have articulated masculine predominance as *A. baumannii* infections are usually associated with alcoholism, smoking, diabetes and other pneumopathies. Moreover, oestrogen (a female sex hormone) has been reported to have pro-inflammatory effects thus minimizing infections in females.[23–25]

The distribution pattern of *A. baumannii* in different age groups has been shown in figure 1. The rate of infection was highest in the age group <20 because of more sensitivity to infections due to poor immunity and aging. There are several studies available that demonstrate that the patients in the age range of 1–29 years are more prone to *A. baumanni* caused infections.[26,27]

The ability to develop resistance against multiple antibiotics is the most common striking feature of *A. baumannii*. This microbe developed resistance against multiple classes of antibiotics by different mechanisms. Resistance against extended spectrum cephalosporin and penicillin is due to reduced permeability, mutation and degradation of the β lactam ring.[28] It developed resistance against vancomycin by an alteration of D alanine (binding site of vancomycin) to D lactate and thus inhibited the binding of vancomycin. Resistance against tetracycline and carbapenems by export of drugs from bacteria and the degradation of β lactam ring by carbapenemases, respectively.[29] It mediates the resistance against sulphonamides either by chromosomal mutation or plasmid encoded transport system.[30] *A. baumannii*, multi-resistant organism, is more resistant to antibiotics as compared to other species. The most common effective drugs used for *A. baumannii* were tigecycline and minocycline. But unfortunately, in the United State, France and Singapore *A. baumannii* developed resistance against imipenem.[31] Current results demonstrate that, 92% isolates were resistant to ampicillin sulbactam thus depicting the increasing ability of *A. baumannii* to establish resistance against tigecycline and minocycline. Ampicillin Sulbactam, beta lactamase inhibitors, was used against *A. baumannii* isolates which were resistant to tigecycline and minocycline.[32] But the present outcomes illustrated that 92% isolates were resistant to sulbactam. From other β lactams only ticarcillin clavulanic acid and ceftzidime showed activity while our outcomes indicate the highest resistance (99%) against ticarcillin-clavulanic acid, while resistance against the extend spectrum of cephalosporins developed due to production of cephalosporinase and the extensive use of these antibiotics reduced effectiveness of this class.[33] In the present study 87–90% isolates were resistant against cephalosporins and

78% demonstrated resistance against fluoroquinolones category. The most effective drugs are tigecycline and minocycline. Tigecycline is derived from tetracycline group and inhibits the protein formation at the ribosomes level. It is the most effective drug used to treat infections caused by MDR-AB.[34]

A. baumannii has become vigorously resistant to various drugs over time. Present research showed that 75.6% (n=118) isolates of *A. baumannii* were MDR, 23.7% (n=37) strains were XDR and 0.64% (n=1) strains were PDR. While previous study indicated that 51% isolates of this microbe were XDR and 11% were MDR.[35] Current study described that the pathogen is establishing resistant to most of the drugs at alarming level. The microbe is spreading and resisting almost more than half of the classes of drugs including carbapenems. Colistin, polymyxin B and vancomycin are the last available antibiotics that can be used as alternative treatments.

The antibiotic polymyxin B is a lipopeptide sequestered from *Bacillus polymyxa*. Polymyxin B contains a peptide ring cationic in nature and a tail of fatty acids with the tripeptide side chain. It usually consists of four closely associated constituents including polymyxin B2, B1 and polymyxin B4 to B1.[36] In 1947, a cationic antimicrobial peptide colistin was obtained from *B. polymyxa*. It has been used clinically since 1958. The interaction between positively charged peptide colistin and the anionic component *i.e*., lipopolysaccharide of the bacterial cell membrane, leads to the transposition of magnesium and calcium. This causes deterioration of the external's heath, thus leading to cell death through the outflow of cell contents. Usually, two formulae of colistin commercially exist; colistin-methane-sulfonate (CMS) for parenteral administration and colistin sulfate for topical and oral use.[37] Colistin and polymyxin B are almost similar in their structure but differ from each other by one amino acid. Colistin is directed as the sodium salt of colistin-methane-sulphonate while Polymyxin B is directed parenterally as the sulphate salt.[38] Sensitive and resistant divisions of vancomycin for *A. baumannii* are variable (Table-1). Tigecycline is derivative of minocycline with the capability to overwhelm the ribosomal protein resistance and active efflux mechanisms.[39] In recent study we analyzed MIC for colistin, vancomycin and polymyxin B by using E-test and broth dilution method. Present results claim that vancomycin and colistin persist as the effective treatment options for infections caused by *A. baumannii*.

CONCLUSION

This scrutinisation concludes that blood provides the ideal atmospheric conditions for the survival of *A. baumannii* as a wide range of strains were obtained from blood. The prevalence of *A. baumannii* in blood is found 8.37% as only 156 *A. baumannii* strains could be isolated and identified from 1864 blood samples. It also divulges that *A. baumannii* infections occur mostly in the young age (<20 years) than in other age groups and males were more affected as compared to females. Use of wide range of drugs has made *A. baumannii* multidrug resistant. Colistin, polymyxin B and vancomycin are the preferable drugs for the treatment of *A. baumannii* infections.

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AUTHORS' CONTRIBUTION

ZK conceived and designed the work and provided logistic support, FJ conducted the lab work, MS collected samples and organized data, AT analysed and interpreted data, FJ and IT were involved in writing final draft and HGMS critically reviewed the manuscript for intellectual contents. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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