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Prevalence of Multidrug Resistant *Acinetobacter baumannii* in Hospitalized Patients in Lahore, Pakistan

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ABSTRACT

Acinetobacter baumannii is an opportunistic nosocomial pathogen that can be isolated from various clinical specimens. In the present study, specimens were collected from different tertiary care hospitals of Lahore, Pakistan. Among them, pus showed highest frequency of *A. baumannii* (31.25%) followed by CSF (25%), blood (17.5%), CVP tips (13.75%), wound swabs (5%), whereas one isolate each was observed in pleural fluid, urine, HVS, sputum, throat and tracheostomy secretions (1.25%). Routine microbiological procedures were employed to isolate and biochemically characterize *A. baumannii*. It was confirmed by API testing system. The multidrug resistance pattern showed maximum resistance to cephalosporins *i.e*., 98.75% for ceftazidime and cefepime, 97.5% for cefotaxime), 96.25% for trimethoprim-sulphamethoxazole, 88.75% for aztreonam, 86.25% for gentamicin, 77.5% for imipenem, 72.5% for piperacillin-tazobactam and 72.05% for doxycycline. In short, *A. baumannii* has achieved resistance against cephalosporin third and fourth generations, monobactam antibiotic, aminoglycoside, carbapenem, β-lactam antibiotic and tetracycline. It showed sensitivity to tetracycline derivative *i.e*., tigeycycline (52.5%). *A. baumannii* has attained multidrug resistance; tigecycline can be administered to avoid *A. baumannii* infections. It is concluded that antibiotic administration should be administered only under expert opinion in order to avoid multidrug resistance.

Keywords: *Acinetobacter baumannii*, antibiotic, multidrug resistance, nosocomial infections, resistance, sensitivity.

INTRODUCTION

Hospital-acquired infections are big threat to the overall health of an individual and our population. In United States, hospital acquired infections are the sixth leading cause of death (Begum *et al*., 2013). Common multi drug resistant (MDR) pathogens responsible for these infections *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp abbreviated as ESKAPE (Garnacho-Montero and Amaya-Villar, 2010; Begum *et al*., 2013). Among ESKAPE, *Acinetobacter* holds the leading position globally (Cisneros and Rodíguez-Baño, 2002; Wisplinghoff *et al*., 2004; Bonomo and Szabo, 2006). Emergence of MDR *A. baumannii* is reported in France, Belgium Argentina, China, Italy, Netherlands, Japan and Bolivia (Peleg *et al*., 2008; Begum *et al*., 2013). It is an opportunistic nosocomial pathogen owing its ability to colonize the hospital environment and developing resistance becoming MDR (Opaz *et al*., 2012; Begum *et al*., 2013). The most common sites of *A. baumannii* colonization includes respiratory tract, urinary system, gastrointestinal system, surgical sites, catheter-related blood circulatory infections, etc., (Allen and Hartman, 2010; Begum *et al*., 2013). In a typical hospital set up, it is a common and well known pathogen to invade immune-compromised patients admitted in intensive care units (ICUs) or

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using invasive devices which result in mortality (Perez *et al*., 2007; Sinha *et al*., 2013). Previous studies (Perez *et al*., 2007; Maragakis and Perl, 2008; Peleg *et al*., 2008; Begum *et al*., 2013) reported different mechanisms used by *A. baumannii* in achieving resistance which includes (i) changes in porin proteins present in outer membrane of bacterial cell, (ii) performance of efflux pumps, (iii) alterations in penicillin binding proteins (PBPs) and (iv) their breakdown by β-lactamases. Due to these resistance mechanisms, *A. baumannii* was reported to resist a wide range of antibiotics including β-lactams, cephalosporins (up to fourth generation), carbapenems and tetracyclines.

The objective of the present study was to find out the prevalence of *A. baumannii* from patients during hospitalization and establishing their antibiotic resistance pattern.

MATERIALS AND METHODS

Collection of samples

Samples for bacterial isolation were collected from various tertiary care hospitals during July, 2013 – June, 2014 from the patients of all ages and both sexes. Samples included blood, urine, pus, cerebrospinal fluid (CSF), central venous puncture (CVP) tip, pleural fluid, wound swab, high vaginal swab (HVS), sputum, throat swab and tracheostomy secretions were collected from different tertiary care hospitals of Lahore, Pakistan. All these samples were taken though routine clinical technique, aseptically under sterilized condition by the trained staff of the hospital.

All the collected samples were immediately brought to laboratory for further processing. Any delay in processing of samples would result in growth of normal flora especially in urine and sputum samples.

Processing of samples

Cheesbrough (2000) was followed for preparation of different media for growth of bacteria, according to which Tryptic Soy Agar - TSA (general purpose medium used for aerobic pathogenic bacteria), MacConckey agar (differentiates lactose fermenters from non-lactose fermenters), and blood agar (to differentiate between hemolytic and non-hemolytic colonies) were used. For urine samples, cysteine lactose electrolyte deficient (CLED) agar medium was used. Blood samples were processed on tryptic soy broth (TSB), blood agar and MacConkey agar medium.

Isolation and biochemical characterization of *A. baumannii*

After isolation and purification of *A. baumannii*, it was initially identified by performing biochemical tests as catalase, citrate, oxidase and motility. Further confirmation was done by using Analytical Profile Index (API) 20 E kit (Bio Merieux; USA) which is a standardize system for identification of Enterobacteriaceae (Begum *et al*., 2013).

Antibiotic sensitivity testing

The antibiotic sensitivity was determined by disc diffusion Kirby Bauer method (Cheesbrough, 2000). Mueller Hinton agar plates were prepared. The lawn of test organism was prepared with the help of sterilized wire loop. Antibiotics discs were placed on the agar surface. The zone of inhibition /clearance of test organism growth were referred to as organism sensitivity against that antibiotic whereas organism growth was called as resistance of organism for that antibiotic. The antibiotics used to establish antibiotic sensitivity pattern were ampicillin-sulbactam, piperacillin-tazobactam, imipenem, aztreonam, cefoparazone-sulbactam, ceftazidime, cefotaxime, cefepime, gentamicin, and tigecycline, doxycyclines, ciprofloxacin and trimethoprim sulphamethoxazole. The zones of inhibition were recorded according to Anonymous (2006).

Statistical analysis

The means and percentages were calculated wherever applicable.

RESULTS AND DISCUSSION

Isolation and biochemical characterization of *A. baumannii*

During twelve months period, *A. baumannii* was isolated from eighty samples (Table 1). The biochemical features of *A. baumannii* are given in Table 2.

Table 1: ***A. baumannii*** **from various specimens.**

Types of specimen	*A. baumannii* growth
Pus	25
CSF	20
Blood	14
CVP tip	11
Wound swab	4
Pleural fluid, urine, HVS, sputum, tracheostomy secretions, throat swab	1 each
Total	80

Table 2: **Biochemical characterization of** ***A. baumannii***

S. No.	Biochemical tests	Status
1	Catalase	Positive
2	Citrate	Positive
3	Oxidase	Negative
4	Motility	Non-motile

Antimicrobial susceptibility testing

The antibiotic sensitivity pattern showed resistance to cephalosporin third and fourth generation, monobactam antibiotic, aminoglycoside, carbapenem, β-lactam antibiotic and tetracycline. It showed sensitivity to tetracycline derivative *i.e*., tigeycycline (Table 3).

Hospital acquired infections are increasing at an alarming rate because pathogens responsible for them have developed resistance mechanisms to counter the effect of drugs like antibiotics (Opaz *et al*., 2012; Begum *et al*., 2013). Pathogens in the hospital atmosphere remain in search for optimum substrate where they can land and flourish easily. Pus is a protein rich fluid, an exudate that is formed at the site of infection by the collection of dead leukocytes. Nosocomial pathogens are mostly isolated from pus as it is easy sight for microorganisms to colonize (Ali *et al*., 2007). In this study out of 80, 25 *A. baumannii* isolates were recovered from pus sample. According to Agamanolis (2014), CSF is considered as an ideal medium for bacteria because it contains less phagocytes, antibodies and is nutrient rich. About 20 isolates were obtained from CSF samples in this study. Least number of isolates was obtained from HVS, urine, throat, sputum, tracheostomy secretions and pleural fluid (Table 1).

Table 3: **Percentage of** ***A. baumannii*** **isolates showed resistance against the given antibiotics.**

S. No.	Antibiotics	Resistant isolates (%) n=80
1	Cefoparazone-sulbactam (Scf)	66.25
2	Piperacillin-tazobactam (Tzp)	72.5
3	Ampicillin-sulbactam (Sam)	52.5
4	Cefotaxime (Ctx)	97.5
5	Cefepime (Cfp)	98.75
6	Ceftazidime (Caz)	98.75
7	Trimethoprim-sulphamethoxazole (Sxt)	96.25
8	Gentamicin (Cn)	86.25
9	Ciprofloxacin (Cip)	88.75
10	Doxycyclines (Dox)	72.05
11	Imipenem (Imp)	77.5
12	Aztreonam (Atm)	88.75
13	Tigecyclines (Tgc)	47.5

Gram negative bacteria possess β-lactamases which belongs to Ambler classes A to D (Lowings *et al*., 2015). The class D oxacillinases (OXA) belongs to OXA-51 like enzyme group and it is chromosomally encoded (Héritier *et al*., 2005; Chen *et al*., 2010; Gordon and Wareham, 2010). The carbapenems (imipenem, meropenem) resistance of *A. baumannii* is due to this OXA-51 enzyme. The β-lactamases of *A. baumannii* inactivates the β-lactam antibiotics (monobactams, carbapenems, cephalosporins). These β-lactamases are called as extended spectrum β-lactamases, cabapenemases, AmpC-type (ampicillin class C) enzymes (Livermore and Woodford, 2006; Queenan and Bush, 2007; Maamoun, 2013; Sonnevend *et al*., 2013).

According to Chu *et al*. (2013), bacteria produces β-lactamase enzymes that confer them resistance to β-lactam containing antibiotics *e.g*., penicillins. These β-lactamases can be destroyed by sulbactam that is mostly administered in combination with ampicillin (Lode, 2008). Previous studies (Williams, 1997; Corbella *et al*., 1998; Rafailidis *et al*., 2007; Lode, 2008) reported that if sulbactam antibiotic is given to patients alone, it did not show much antimicrobial effect. Although sulbactam is a β-lactamase inhibitor, it plays its role by binding to penicillin binding proteins (Chu *et al*., 2013). Ampicillin-sulbactam is a broad spectrum antibiotic belonging to penicillin group. Its mode of

action is inhibition of bacterial cell wall synthesis. In this study, 52.5% resistance pattern was observed to ampicillin-sulbactam. The study of Lowings *et al*. (2015) showed 100% resistance of *A. baumannii* to ampicillin. Piperacillin-tazobactam is a broad spectrum β-lactam antibiotic that plays its role in bacterial cell wall synthesis inhibition. It was observed in this study that 72.5% isolates were resistant while 27.05% were sensitive to it. In short words, *A. baumannii* showed resistance to piperacillin-tazobactam (Henwood *et al*., 2002). Imipenem is a broad spectrum β-lactam antibiotic belonging to carbapenem that inhibits bacterial cell wall synthesis. According to previous researches (Lautenbach *et al*., 2009; Dizbay *et al*., 2010), *A. baumannii* exhibited imipenem resistance. The resistance pattern of *A. baumannii* to imipenem was 77.5% isolates were resistant to it while 18 (22.5%) were sensitive to it. Overall, *A. baumannii* showed resistance to imipenem. Our study was in accordance with the study of Lowings *et al*., (2015) which reported that more than 67% resistance of *A. baumannii* to imipenem. Aztreonam is a monobactam antibiotic that is narrow spectrum and shows its activity against Gram negative bacteria only. Its mode of action is also inhibition of bacterial cell wall synthesis. This study revealed that *A. baumannii* showed resistance in 88.75% isolates.

Cefoparazone-sulbactam belongs to third generation cephalosporins which inhibits bacterial cell wall synthesis. Out of 80 isolates, 66.25% were resistant while 33.75% were sensitive to this antibiotic. Other third generation cephalosporins used in this study included ceftazidime and cefotaxime. Their sensitivity pattern were same *i.e*. 98.75% isolates were resistant to these drugs while only one isolate (1.25%) showed sensitivity. Resistance of *A. baumannii* to third generation cephalosporins was already reported (Henwood *et al*., 2002; Thomson and Bonomo, 2005; Bonomo and Szabo, 2006; Maragakis and Perl, 2008). One fourth generation cephalosporin (Endimiani *et al*., 2008) was also used here, *i.e*. cefepime which also inhibits cell wall synthesis. It showed almost same result as were obtained with ceftazidime and cefotaxime *i.e*. 97.5% isolates showed resistance. Hakyemez *et al*. (2013) found that 95% isolates were resistant to cefepime. Gozutok *et al*. (2013) reported 91-100% resistance against it. These studies showed that *A. baumannii* isolates are not affected by cefepime *i.e*., fourth generation cephalosporins (Henwood *et al*., 2002; Endimiani *et al*., 2008; Tian *et al*., 2011). The resistance pattern of the isolate against fluoroquinolone (ciprofloxacin) was similar to third generation cephalosporins; 88.75% isolates were resistant to this antibiotic. Our results were in agreement with the studies of Gozutok *et al*. (2013) who observed 32-100% studied resistance in various isolates of *A. baumannii* against ciprofloxacin. Hakyemez *et al*. (2013) observed 84.9% resistance against ciprofloxacin. This elevated level of resistance made ciprofloxacin an undesired drug to cure *A. baumannii*. Gentamicin is a broad spectrum antibiotic belonging to aminoglycosides. Its mode of action is inhibition of protein synthesis. *A. baumannii* showed resistance (86.25%) to it as well. Our findings were in agreement with Hakemyez *et al*. (2013) where 76.5% resistance was noticed against gentamicin. *A. baumannii* showed resistance to gentamicin (Henwood *et al*., 2002). Two antibiotics (tigecyclines and doxycyclines) were also evaluated for resistance against tetracycline group. Their resistance patterns were very different. For doxycyclines, 72.05% isolates showed resistance that was similar to tigecyclines which showed resistance in 47.5% isolates. Previously, tigecycline resistance was found as 7-20.5% by Baadani *et al*. (2013), whereas Hakyemez *et al*., (2013) observed it as 0-12% which was slight less than found in our study. Trimethoprim-sulphamethoxazole was used as a metabolic antagonist. It interferes with bacterial folate production thereby, causes bacterial death. *A. baumannii* showed resistance to it as well. Our findings were also in agreement with the previous study of Begum *et al*., (2013).

CONCLUSION

In conclusion, it was observed that pus samples yielded more *A. baumannii* isolates followed by CSF, blood and CVP tips. One isolate was obtained from urine, HVS, sputum, tracheostomy secretions, pleural fluid and throat. Out of thirteen drugs tested, *A. baumannii* showed

sensitivity to only one drug *i.e*., tigecyclines. Multidrug resistance pattern in *A. baumannii* is alarming for scientists related to health care department. The foremost reason for this is self medication and over-usage of antibiotics (Maragakis and Perl, 2008; Fishbain and Peleg, 2010). Strict measures should be taken by the health care personnel's to stop this practice.

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