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Full Length Research Paper

In vitro activity of aminoglycosides, lactam-lactamases inhibitor combinations and tetracyclines against multi-drug resistant *Acinetobacter baumannii*, isolated from a tertiary care hospital

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Acinetobacter baumannii **has emerged as a significant nosocomial pathogen, particularly in intensive care units. Isolates of** ***A. baumannii*** **resistant to major groups of antibiotics have been identified. These multi-drug resistant (MDR) organisms are limiting the treatment options. The study was performed to determine the** ***in vitro*** **activity of aminoglycosides, lactam- lactamase inhibitor combinations and tetracyclines against MDR** ***A. baumannii*****, isolated from a tertiary care hospital. The study was carried out from January, 2009 - August, 2009, at the Department of Microbiology, Army Medical College/National University of Sciences and Technology, Rawalpindi, Pakistan looking after an 1100 bedded tertiary care hospital. Routine clinical specimens were received from various wards.** ***A. baumannii*** **was identified by using standard microbiological procedures. Antimicrobial susceptibility test (gentamicin, amikacin, tobramycin, ampicillin-sulbactam, piperacillin-tazobactam, cefoperazone-sulbactam, tetracycline, doxycycline, minocycline, tigecycline,) was performed according to CLSI guidelines using Kirby- Bauer disc diffusion technique. Resistance to carbapenems, fluoroquinolones and the beta-lactams were observed in significant proportion of fifty isolates. Among the aminoglycosides, the isolates were more susceptible to tobramycin than gentamicin and amikacin. Cefoperazone-sulbactam was superior to piperacillin-tazobactam and ampicillin -sulbactam in activity against MDR** ***A. baumannii*****. Both tigecycline and minocycline were the active agents against most isolates. Multidrug resistant** ***Acinetobacter*** **infections are posing an increasing threat to our population. Minocycline, tobramycin and cefoperazone-sulbactam provide an effective option against infections caused by resistant** ***A. baumannii.***

Key words: Aminoglycosides, -lactam/ -lactamase inhibitor combinations, multi-drug resistant *Acinetobacter*, tetracyclines.

INTRODUCTION

Acinetobacter is a ubiquitous gram negative cocco-bacillus that during the past three decades has emerged as a significant nosocomial pathogen. Its spectrum of illness includes pneumonia, blood stream infections, urinary tract infections, skin and wound infections, meningitis and endocarditis (Munoz-Price and Weinstein, 2008) . Infections with *Acinetobacter* species tend to occur in immunocompromised patients, especially those admitted in the intensive care units. Non-fastidious growth requirements combined with its intrinsic resis-tance to many antimicrobial agents enables it to spread in

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the hospital setting. Risk factors for outbreaks of Acinetobacter in intensive care units include central vascular catheterization, tracheostomy, mechanical ventilation and treatment with broad spectrum antibiotics (Schreckenberger et al., 2007).

Antibiotics which previously showed good results against Acinetobacter infections were the cephalosporins, fluoroquinolones and gentamicin. But now, sensitivity pattern has changed because of the diverse mechanisms of resistance this organism has acquired. Against multi-drug resistant strains (MDR), treatment options are very limited. Aminoglycosides act against Acinetobacter by inhibiting protein synthesis. Resistance to aminoglycosides is encountered by aminoglycoside-modifying enzymes, including acetyltransferases, nucleotidyltrans-ferases, and phosphotransferases (Bonomo and Szabo, 2006). Increased resistance to gentamicin and amikacin has been observed. Tigecycline, a glycylcycline, is a bactericidal against *Acinetobacter*. Unique feature of tigecycline is that it can evade two major resistance mechanisms that are ribosomal protection and efflux pump (Schreckenberger et al., 2007) . Sulbactam, a - lactamase inhibitor, has intrinsic activity against *Acinetobacter*.

Resistance mechanisms that are expressed frequently in nosocomial strains of *Acinetobacter* include – lactamases, alteration in cell wall channels and efflux pumps. There is a multidrug resistant efflux pump in Acinetobacter which is responsible for resistance against tetracyclines, fluoroquinolones, chloramphenicol, - lactam drugs and tigecycline (Schmidt and Hensel, 2004). MDR Acinetobacter is defined according to Centers for Disease Control and Prevention (CDC) guidelines as *Acinetobacter* species resistant to at-least 3 antimicrobial classes including beta-lactams, aminoglycosides, carbapenems and fluoroquinolones (CDC NHSN: Multi-drug resistant organism (MDRO) and *Clostridium difficile* associated disease module(CDAD et et al., 2010). Multidrug resistant strains are involved in hospital outbreaks. Several studies have described the involvement of MDR Acinetobacter in outbreaks (Lolans et al., 2006; Hartstein et al., 1988; Maragakis et al., 2004). As infections due to MDR Acinetobacter are on rise in our hospitals (Irfan et al., 2008; Saleem et al., 2010). So we conducted this study to check the susceptibility pattern of resistant *Acinetobacter baumannii* against commonly available antibiotics in our set up.

MATERIALS AND METHODS

The study was carried out from January, 2009 - August, 2009 at the Department of Microbiology, Army Medical College/ National University of Sciences and Technology (NUST), Rawalpindi, Pakistan affiliated with an 1100 bedded tertiary care hospital.

A total of fifty *A. baumannii* were included in the study. These were isolated from various samples including urine, blood, pus, sputum, nasobronchial lavage (NBL), catheter tips, chest tubes received from patients admitted in Military hospital, Rawalpindi. All the specimens were inoculated on MacConkey's agar (Oxoid, UK), and were incubated at 37°C aerobically for 24 h. *Acinetobacter* species were identified by Gram's staining, catalase test, oxidase test, motility and by using conventional sugar fermentation tests/routine biochemical tests (Schreckenberger et al., 2007). API 20NE (Biomerieux) was used to confirm *A. baumannii*. Antimicrobial susceptibility test (gentamicin, tobramycin, amikacin, ampicillin-sulbactam, piperacillin-tazobactam, cefoperazone-sulbactam, tetracycline, doxycycline, minocycline, tigecycline) was performed by using the Kirby-Bauer disc diffusion techniques according to CLSI guidelines (Wayne, 2009). Inocula were prepared by suspending the isolates in normal saline equal to the turbidity of 0.5 McFarland turbidity standards (10^6 cfu/mL). The lawns of organisms were applied on Mueller Hinton agar (Oxoid, UK) plates. Discs of gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), ampicillin-sulbactam (20 µg), piperacillin-tazobactam (110 µg), cefoperazone-sulbactam (105 µg), tetracycline (30 µg), minocycline (30 µg), doxycycline (30 µg) and tigecycline (15 µg) were placed. Inoculated plates were incubated along with controls overnight at 37°C aerobically. *A. baumannii* ATCC 19606 was used as control strain. The results were interpreted according to criteria set by Clinical and Laboratory Standards Institute (CLSI) (Wayne, 2009). For tigecycline, the interpretation of zone diameters for *Acinetobacter* spp. was done using the US FDA breakpoints (sensitive 19 mm, intermediate 15 - 18 mm, resistant 14 mm) (Wyeth Pharmaceuticals, 2005).

RESULTS

From a total of 78 *Acinetobacter* species isolated during the study period, fifty were multi-drug resistant *A. baumannii*. *A. baumannii* simultaneously resistant to carbapenems, fluoroquinolones and - lactams were defined as MDR in our study. Among the aminoglycosides, high resistance was noted against gentamicin (58%) and amikacin (56%). Tobramycin showed good result, 72% isolates were sensitive to tobramycin. Cefoperazone-sulbactam was superior in activity against MDR *A. baumannii* in this study than ampicillin-sulbactam and piperacillin-tazobactam. Majority of isolates were sensitive to minocycline (88%) and tigecycline (74%). Table 1 shows the resistance pattern of organisms against aminoglycosides, lactam- lactamase inhibitor combinations and tetracyclines.

From a total of fifty MDR *A. baumannii* isolated, 28 were from the patients admitted in ICU, 14 were from various wards (surgical, medical) and 8 were from out-patient department. Majority of the isolates were from nasobronchial lavage specimen (38%) followed by pus specimen (34%). Eight percent MDR *A.baumannii* were isolated from blood specimen, 10% from catheter tip, 4% from sputum, 4% from chest tube and 2% from urine specimen (Table 2). Among the 4 isolates from blood, 2 isolates were resistant to all tested antibiotics except minocycline and cefoperazone-sulbactam.

DISCUSSION

Aminoglycosides

MDR Acinetobacter is a threat for our population. Very

Table 1. Resistance pattern of *A. baumannii* (n = 50) against beta-lactam/beta lactamases inhibitor combinations, aminoglycosides and tetracyclines

Antibiotic	Number of isolates resistant n = 50 (%)
Gentamicin	29 (58)
Amikacin	28 (56)
Tobramycin	14 (28)
Cefoperazone-sulbactam	20 (40)
Piperacillin-tazobactam	36 (72)
Ampicillin-sulbactam	41 (82)
Tetracycline	40 (80)
Doxycycline	18 (36)
Minocycline	06 (12)
Tigecycline	13 (26)

Table 2. Number (%) and site of *Acinetobacter* spp. Isolates.

Specimen	No. of isolates (%)
Nasobronchial lavage	19 (38)
Pus	17 (34)
Catheter tip	5 (10)
Blood	4 (8)
Sputum	2 (4)
Chest tube	2 (4)
Urine	1 (2)

few antibiotics can be reliably used against this resistant organism. In our study, tobramycin showed better activity against *A. baumannii* than gentamicin and amikacin. In a study done in USA, tobramycin was more active against Acinetobacter than gentamicin and amikacin (out of 107 isolates tested, 27.1% were susceptible to tobramycin), which is in concordance to our study (Akers et al., 2010). Susceptibility to aminoglycosides has changed a lot in recent years. Gentamicin used to be the most active agent against Acinetobacter infections. But now this organism has acquired resistance against this antibiotic. According to the results of this study, 21 out of 50 isolates (42%) were sensitive to gentamicin. Dauner et al. (2008) reported reduced susceptibility to gentamicin (40.5%) against Acinetobacter, comparable to our study. In another study, Mordi and Erah (2006) noted that none of the *Acinetobacter calcoaceticus* isolated from urine samples were sensitive to gentamicin. Our study showed that only 44% of isolates were sensitive to amikacin. Previously, Irfan et al (2008) also reported the poor sensitivity of amikacin against *Acinetobacter* species (16.7% in first study period, 1999 - 2000 and 51.9% in second study period, 2001 - 2006). High MIC of amikacin (128 µg/ml) against *Acinetobacter* species were seen in another study from Pakistan (Ahmad et al., 2009).

Beta-lactam/beta-lactamase inhibitor combinations

In the present study, among the three beta-lactam/beta-lactamase inhibitor combinations tested, cefoperazone-sulbactam showed the highest activity against *A. baumannii*. Out of 50 isolates, 30 were sensitive to cefoperazone-sulbactam. 72% isolates were resistant to piperacillin-tazobactam. The high resistance to piperacillin-tazobactam (100% in first study period and 36.5% in second study period) is seen in another study from Pakistan (Irfan et al., 2008). In a study done in Turkey, Alver et al. (2008) noted that in a period of four years, resistance to cefoperazone-sulbactam has reduced from 54.7 to 28.6%. According to present study, ampicillin-sulbactam is least effective against *Acinetobacter* isolates. Only 18% isolates were sensitive to ampicillin-sulbactam. In contrast, Levin et al (2003) tested ampicillin-sulbactam against MDR *A.baumannii* infections. Among 40 patients treated, 27(67.5%) were improved with ampicillin-sulbactam. In another study, out of 27 patients suffering from *A. baumannii* ventilator associated pneumonia, Betrosian et al. (2007) clinical improvement in 66.7% of study population when treated with ampicillin-sulbactam.

Tetracyclines

In this study, we observed that 88% of *A. baumannii* was sensitive to minocycline and 74% to tigecycline. In contrast, Ahmed et al. (2009) reported that all the isolates tested were susceptible to tigecycline. Similarly, Song et al. (2007) noted that tigecycline was bacteriostatic against all the 43 carbapenem-resistant *A. baumannii* tested. Regional data from India showed that 42% of MDR *Acinetobacter* spp. were sensitive to tigecycline (Behera et al., 2009). Tigecycline is the first of the glycylcyclines which has been marketed recently. Though it can evade two major resistance mechanisms that are ribosomal protection and efflux pump, yet organism has developed resistance against this newly introduced antibiotic. In a developing country like us, minocycline which is cost effective can rescue us from resistant Acinetobacter infections. According to the results of this study, minocycline, an older tetracycline, can be a good treatment option against resistant Acinetobacter infections. Doxycycline showed moderate activity against many isolates. Highest resistance to tetracycline (80%) was noted. In contrast, results of a previous study showed that *A. baumannii* is 100% susceptible to doxycycline (Butt et al., 2004). In another study, Mordi and Erah (2006) noted resistance of *Acinetobacter calcoaceticus* isolated from urine samples to tetracycline was 100%. A study from Argentina showed that

doxycycline (4 µg/ml) and tigecycline (8 µg/ml) were bactericidal against 36 and 54% of *A. baumanni,* respectively (Bantar et al., 2008), which is in concordance to our results.

Multidrug resistant *Acinetobacter* infections now are an established threat in our hospitals. Effective antibiotic therapy is required to treat the infections and to prevent its complications. This study will help our clinicians in finding an appropriate therapy against *Acinetobacter* infections. In conclusion, minocycline, tobramycin and cefoperazone-sulbactam provide an effective and inexpensive option against infections caused by resistant *A. baumannii.*

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