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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (6): 275-280, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.275.280

Antimicrobial Susceptibility Pattern in Nosocomial Infections Caused by *Acinetobacter* Species in Asir Region, Saudi Arabia

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Abstract: This study aimed at evaluating the sensitivity of antibiotics towards nosocomial infections caused by *Acinetobacter* species. The study took place during the period Dec. 2011- Dec. 2012 at Assir Central Hospital in collaboration with the department of microbiology, college of medicine, King Khalid University, Abha. A prospective study involving 150 patients presented with nosocomial infections due to *Acinetobacter* species detected by bacteriological tests; direct microscopy, culture in blood agar media, fermentation test in MacConkey media and MIC (minimum inhibitory concentration) for antibiotics sensitivity using Muller Hinton media and Chemical test using API 20. A 150 nosocomial infections in this study showed gram-negative coccobacilli, non motile, glucose-negative fermentor and oxidase negative. All isolates showed 100% sensitivity to: Imipramine, Meropenem, Colistin. From the rest of tested antibiotics the higher resistant ones were; Nitrofurantoin 87% and Cefoxitin 85%. The least resistant antibiotics; Imipenem 3% and Ticarcillin 7%. While variable resistance in the rest of tested antimicrobials. A 47 patients (31.3%) have used antibiotics prior to this study. The high rate of usage occurred in elder patients. The frequency of *Acinetobacter calcoaceticus baumannii* complex multi-drugs resistance ABCMDR is rising including almost all commonly used antibiotics. Only few antibiotics exert 100% sensitivity towards these bacteria.

Key words: *Acinetobacter calaoaceticus baumani* complex, oxidase-negative, multidrugs resistance, gram negative coccobacillus, negative fermentor

INTRODUCTION

Acinetobacter genus belongs to the gamma subdivision of proteobacteria and to the Moraxellaceae family. They are gram-negative, strictly aerobic, non-motile glucose-non fermentative coccobacilli that are oxidase-negative and catalase-positive. The most common species is *Acinetobacter baumannii*, which is usually disseminated in the hospital and health care units environment (McDonald, 2006). *Acinetobacter baumannii* is an important opportunistic pathogen responsible for a variety of nosocomial infections, including bacteremia, urinary tract infection, secondary meningitis, surgical-site infection and ventilator-associated pneumonia, especially in Intensive-care-unit (ICU) patients. *Acinetobacter baumannii Calcoaceticus* Complex Multiple Drug Resistance (ABCMDR), have shown increasing levels of antimicrobial resistance specially towards the commonly used ones such as; beta-lactamase group (Bou *et al*., 2000; Masuda *et al*., 1976), carbapenem (Afzal-Shah and Livermore, 1998), tetracycline, aminoglcosides, impenem (Clark, 1996; Hornstein *et al*., 1997; Paton *et al*., 1993; Tankovic *et al*., 1994) and other chemotherapies (Seifert *et al*., 1993). Many factors have been incriminated in this phenomenon specially patient's age, immune-suppressive conditions such as diabetes and resistant genes (Sato and Nakae, 1991). ABCMDR have been documented in many countries worldwide (Hart and Kariuki, 1998; Mahmood *et al*., 2002; Vila *et al*., 1993; Wu *et al*., 2006).

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Assir Central Hospital is almost 600 bedded and it is accredited from The Central Board of Arab Health. Moreover, it has the most-recent autoanalyzer in all sections. It is the only tertiary care facility in the southern part of the Kingdom of Saudi Arabia, with a population of well above a million. The laboratory is a regional referral hub that serves the hospital as well providing consultation for the rest of the region. On The other hand, the hospital is affiliated to the medical college of King Khalid University. This study was aimed at evaluating the commonly used antibiotics resistant and the factors affecting the drugs sensitivity of *Acinetobacter* sp. nosocomial clinical isolates from of patients presented at Assir Central Hospital General Lab.

MATERIALS AND METHODS

Collection of samples: This study was conducted between Dec 2011 and Dec 2012, at Assir Central Hospital General Lab. A hundred and fifty patients were involved in this study, including both sexes and ages (children and adults) with variable nosocomial infections; Respiratory Tract Infections (RTI), Urinary Tract Infections (UTI), Blood Stream Septicemia (BSS). The patients were informed about the study content and procedures with preservation of human rights and research ethics according to the requirements the Deanship of Scientific Research, KING Khalid University, Kingdom of Saudi Arabia. Research Center For Medical Colleges (Ref. KKU-MED-11-017).

The laboratory specimens include; nasal swabs (collected from the nares with a dry, unmoistened swab, a tip of the collection swab was inserted approximately. (2.56 cm) into the nares and rolled five times in each nostril), urine (mid stream urine, 20 mL) and blood (venous blood 5 mL). Collected specimens were transported and stored at room temperature. Clinical data, including the inpatient and outpatient categories and patient's history of diabetes was registered. Each sample was examined using all bacteriological tests requirements, including; slides, stains, culture, fermentation test, catalase and antibiotic sensitivity test. Each sample was cultured in two media (Blood agar and MacConkey) for 24 h. Giemsa stained slides were prepared from each cultured sample (24-48 h). All stained slides were examined microscopically. Antibiotics sensitivity was tested by two methods; manual (Muller Hinton) and mechanical by microscan and phonix. The tested antibiotics include: Amikacin, Ampicillin, Imipenem, TMP-SMX (trimethoprim-sulfamethoxazole), Cefoxitin, Ceftazidime, Cefriaxone, Ciprofloxacin, Gentamicin, Nitrofurantoin, Tic-Clav (ticarcillin-clavulamic acid), Ticarcillin, Tobramycin, Cefuroxime, Tetracycline, Imipramine, Meropenem, Colistin.

Microbiological tests: The cultures were carried out on blood agar and MacConkey Media. The plates were incubated for 24-48 h at 35°C and examined for growth. After incubation, each plate was examined to observe the characteristics of colonies morphology and the effect of the organism on culture media. The colonies appeared as medium to large, smooth, entire, slightly raised, translucent, most colonies showed a creamy yellow color with no hemolysis. Confirmation of *Acinetobacter* species was conducted using gram stain test. Fermentation test was detected by observation of growth on MacConkey agar. A 3% catalase, oxidase testing, motility test and API 20 were performed according to the working steps of Kayser, Medical Microbiology©2005 Thieme (Kayser *et al*., 2004).

Preparation of Müeller-Hinton agar culture plates: The dissolved and mixed *Acinetobacter* species culture colonies were distributed evenly in normal saline (1 colony/1 mL of NaCl) and spread on Müeller-Hinton agar plates. The inoculated disks were incubated at 30 to 35°C for 24 h or longer (up to 72 h). The antibiotics' disc filter papers were applied over the growing culture colonies followed by incubation at 30 to 35°C for 6-18 h.

Preparation of antibiotics dried filter paper discs: The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers 0.005 mL of antibiotics to each disc. Whatman filter paper No. 1 was used to prepare discs approximately 6 mm in diameter, which were placed in a petri dish and sterilized in a hot air oven.

Zone diameter interpretive criteria: After application of antibiotics filter paper discs over the growing *Acinetobacter* colonies in Muller Hinton media then 16 to 18 h incubation at 35°C, each plate was examined. The resulting zones of inhibition were uniformly circular and a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc. Zones were measured to the nearest millimeter, using sliding calipers or a ruler. Each antimicrobial sensitivity testing of *Acinetobacter* sp. has a zone size interpreted by reference to the Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints (NCCLS, 1997, 2003). Performance Standards for Antimicrobial Susceptibility Testing: classified as susceptible, intermediate and resistant.

RESULTS

Approximately, 150 nosocomial infections in this study showed gram-negative coccobacilli, non motile, glucose-negative fermentor and oxidase negative. All *Acinetobacter* isolates showed 100% sensitivity to: Imipramine, Meropenem, Colistin. From the rest of tested antibiotics the higher resistant ones were; Nitrofurantoin 87% and Cefoxitin 85%. The least resistant antibiotics; Imipenem 3% and Ticarcillin 7%. While variable resistance in the rest of tested antimicrobials. Percentage of susceptibility of 15 antimicrobial agents tested by MIC against *A. baumannii* complex species in this study compared with previous two studies are shown in Table 1. Some factors affect the sensitivity pattern of the commonly used antibiotics such as age of the patients, presence of chronic disease leading to immune depression such as diabetes have not shown significant effect in this study, while the history of medications specially usage of antibiotics showed that 47 patients (31.3%) have used antibiotics prior to this study. The antibiotics usage prior to the study according to the age groups as shown in (Fig. 1) demonstrates a gradual rise of usage as the age increased, so the high rate of usage occurred in elder patients.

DISCUSSION

A. calcoaceticus-baumannii complex (ABC) accounted for approximately 2.5% of all nosocomial infections while the ABC multiple drug resistance (ABCMDR) showed 10.5% according to the Assir Central Hospital laboratory records. These records could be underestimated as the laboratory diagnosis at the species level is crude as there are no molecular facilities (PCR diagnosis). Assir Central Hospital is a 600 beds; it has strict infection-control measures and practices. There are few data available on the pattern of antimicrobial resistance of *A. calcoaceticus-baumannii* complex in Saudi Arabia and few studies on antimicrobial sensitivity pattern have been conducted in Assir region, south west Saudi Arabia (Abdalla, 2011). Application of MICs to determine the sensitivity profile of antimicrobials have been widely used. International or national standard level of MIC for each antimicrobial which should be applied as shown in Table 2.

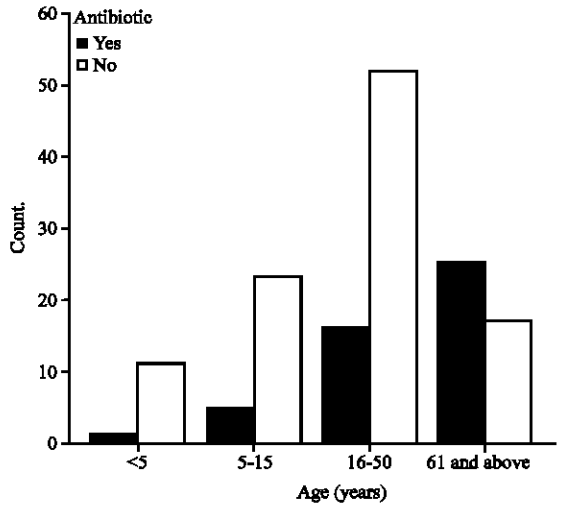

Fig. 1: Antibiotic usage prior to the study according to the age groups

The predominant *Acinetobacter* isolates were *A. baumannii* followed by *A. calcoaceticus baumannii* complex and few isolates were other *Acinetobacter* species. In a previous study; the predominant *Acinetobacter* isolate was *A. baumannii* (60%) followed by *A. calcoaceticus baumannii* complex (25%) and (15%) were other *Acinetobacter* species (Gales *et al*., 2001).

A study performed on *Acinetobacter* Blood Stream Infection in a Teaching Hospital-Riyadh, Saudi Arabia, patients with *Acinetobacter baumannii* blood stream infections were more frequently managed in intensive care units. Approximately, 47.5% had serious underlying illnesses predisposing to *Acinetobacter* blood stream infections, including, cardiac, renal diseases, prematurity

Table 1: Percentage of antimicrobial susceptibility of 15 antimicrobial agents tested by MIC against *A. baumannii* complex species in this study compared with precious two studies

Antimicrobial agent	Al-Tawfiq and Mohandhas (2007)	Babay *et al.* (2003)	This study
Amikacin	34.0	15.4	33
Ampicillin	86.0	84.6	82
Imipenem	3.0	0.0	3
Imipenem	23.0	23.1	24
Cefoxitin	89.0	76.9	85
Ceftazidime	38.0	30.8	39
Cefriaxone	55.0	76.9	17
Ciprofloxacin	30.0	30.8	30
Gentamicin	26.0	30.8	25
Nitrofurantoin	89.0	-	87
Tic-Clav (ticarcillin-clavulanic acid)	16.5	-	17
Ticarcillin	6.0	-	7
Tobramycin	25.0	-	24
Cefuroxime	35.8	92.3	40
Tetracycline	-	53.8	50

Table 2: Standard MIC resistance level of few antimicrobials

Antimicrobial agent	Standard (MIC) minimum inhibition zone diameters (mg L^{-1})
Kanamycin	> or =13
Gentamicin	> or =16
Amikacin	> or =64
Tobramycin	> or = 16
Netilmicin	> or = 32

and severe burns with 25% having a fatal outcome. Risk factors for *Acinetobacter baumannii* blood stream infection included: intravascular catheters, mechanical ventilation, prior antibiotic use and colonization at other body sites. These factors were independently associated with *Acinetobacter baumannii* acquisition in these patients. The study indicated that results of antimicrobial susceptibility tested by disc diffusion method were comparable to those of E test (Babay *et al*., 2003).

A study conducted during the period from 1998 through 2004, involved a total of 476 isolates of *Acinetobacter calcoaceticus-baumannii* complex. The organism showed high rates of resistance to ampicillin (86% of isolates), cefoxitin (89%) and nitrofurantoin (89%). The rate of resistance to imipenem was 3% (similar percentage in this study); ticarcillin-clavulanic acid, 16.5% (higher than this study); gentamicin, 26% and to ceftazidime 38%. Multidrug resistance was observed in 14-35.8% of *Acinetobacter* species isolates. Commonly used antibiotic resistance profile in previous annual estimations (1998-2004); showed 100% resistance to: Cefazolin, Cefoxitin and Nitrofurantoin and rising resistance to: Amikacin 30%, Ceftazidime 80%, Ceftriaxone 75%, Ciprofloxacin 40%, Gentamicin 37% (Al-Tawfiq and Mohandhas, 2007).

Carbapenem-resistant *A. calcoaceticus-baumannii* complex has emerged in many parts of the world. The main mechanism of resistance is thought to be through the acquisition of B and D class carbapenemases (Poirel *et al*., 2003). In a study from Taiwan, the carbapenem resistance of the *Acinetobacter* species isolated was 10%. In a small study from Jeddah, Saudi Arabia, imipenem was the antimicrobial most active against *A. calcoaceticus baumannii* complex. In this study, the rate of resistance to imipenem was as low as 3% of isolate (Eltahawy and Khalaf, 2001). In another study, *Acinetobacter* species had a an increased rate of resistance to imipenem from 0 to 42% during the study period. In the present study, imipenem remained the antimicrobial most active against *A. calcoaceticus-baumannii* complex and the resistance rates were usually low. Similarly, a low rate of resistance to imipenem (3.2% of isolates) was reported in Japan (Ishii *et al*., 2006). Imipenem resistance was estimated in variable countries such as; Spain (Cisneros *et al*., 2005), Regional variation in imipenem resistance was noted as reported in North America (4.5% of isolates) and Latin America (11% of isolates). The rate of resistance to ceftazidime in *A. calcoaceticus-baumannii* complex in this study is 39% while it was higher in Thailand 68.5% (Thongpiyapoom *et al*., 2004) and in North America (Pfaller *et al*., 2006).

Several resistant antimicrobial genes have been reported. The presence of at least one of the following AME genes was detected in 94% of 66 MDR strains: aphA1 (n = 56), aacC1 (n = 52), aphA6 (n = 39) and aadB (n = 20). The presence of these genes and phenotypes of resistance to kanamycin, gentamicin, tobramycin and amikacin were well incorporated. The genes encoding netilmicin-modifying enzymes (aacC2, aacA4) were not detected in any strain. Clones I and II shared all the genes studied (except for aadB that was not detected in clone I). The gene adeB was found in all MDR strains and eight 50% of control susceptible strains. Clinically relevant aminoglycoside resistance of Czech *A. baumannii* strains is significantly associated with the genes encoding enzymatic modification of kanamycin, gentamicin, amikacin and tobramycin. These genes can spread horizontally and emerge in different combinations leading to high-level resistance to multiple aminoglycosides. The AdeABC pump is likely to play a role in the intermediate susceptibility to netilmicin but further study is needed in this regard (Nemec, 2008, Nemec and Maixnerova, 2004). Repetitive-DNA-element PCR fingerprinting and antibiotic resistance of pan-European Multi-resistant *Acinetobacter baumannii* (MAB) clones study, reviled that $(GTG)_5$-PCR fingerprinting is a cost-effective tool for the rapid recognition of new members of major pan-European MAB clones such as clone III. The finding that the oldest clone III strains described so far dated from 1991 indicates that this clone has spread among European hospitals for several years, similar to the case for MAB clones, I and II. Furthermore, the data suggest that the degree of diversification in antibiotic-resistance traits among clone III strains may be higher for tet genes (Tetracycline genes) compared to AG-modifying genes (Aminoglycosides genes), but more strains need to be investigated to support this conclusion. This finding may be associated with the high frequency and efficiency by which tet gene carriers can successfully disseminate throughout MAB populations (Huys *et al*., 2005).

CONCLUSION

The *Acinetobacter baumannii* is the most common *Acinetobacter* species causing significant blood stream infections among patients in intensive care units with serious underlying illnesses. Risk factors studied were independently associated with the disease process in these patients. Imipenem is the most active antimicrobial agent against clinically significant *Acinetobacter baumannii* blood stream infection although the problem of multi-drugs resistance is existing and expanding.

Acinetobacter calcoaceticus baumannii complex accounted for approximately 2.5% all nosocomial infections, mostly in intensive care units (ICU and CCU). Antibiotic sensitivity pattern should be made prior to start treatment.

ACKNOWLEDGMENT

We confer our gratitude to the laboratory members at Assir Central Hospital. Our sincere thanks to all members of the department of microbiology, Ribatt National University, Sudan for their technical support. This study is funded by the college of scientific research, King Khalid University, Abha, Saudi Arabia. Ref. KKU-MED-11-017.

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