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Multidrug resistance index in *Acinetobacter baumanii/calcoaceticus* complex and antibiotic resistance profile pattern among clinical samples in, Muğla

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Abstract
Background: *Acinetobacter baumanii /calcoaceticus* infections were important for patients.
Objective: the objective of this paper to determine the level of resistance to the widely used antibiotics in clinical isolates of *Acinetobacter baumanii /calcoaceticus* 40 isolates were collected from special hospital in Muğla and recorded at specimens.
Results: The resistance rates for, Ampicillin- Sulbactam, Imipenem, Meropenem, Cefepime, Levofloxacin and Ceftazadime were 95%. In our study, colistin was the lowest resistance antibiotics with12,5%. In our study, 38 isolates showed seven to ten antibiotic resistance. But only two isolates showed no antibiotic resistance any of the antibiotics isolted from urine and ear cultures.
Conclusion: Resistance rates of *Acinetobacter baumanii /calcoaceticus* complex isolates to were tigecyline and colistin very low and the rate of resistant *Acinetobacter baumanii /calcoaceticus* complex to Ampicillin/Sulbactam, Imipenem, Meropenem, Cefepime, Levofloxacin and Ceftazadime was significant. It would be a good idea to consider surveillance of antibiotic usage and restriction of using broad spectrum antibiotics before development of resistance to these agents.

Keywords: *acinetobacter baumanii /calcoaceticus*, antibiotic resistance, clinical isolates

1. Introduction

Acinetobacter calcoaceticus–baumannii (ACB) complex is emerging as one of the most common causes of hospital-acquired infections (HAIs) in intensive care units (ICUs) worldwide and is often resistant to multiple antibiotic classes, complicating treatment [7]. *Acinetobacter baumannii* is a strictly aerobic, non-motile, Gram-negative bacillus belonging to the *A. calcoaceticus–baumannii* complex within the family Moraxellaceae of the order Gammaproteobacteria. Identification by phenotypic methods or DNA–DNA hybridisation does not reliably distinguish *A. baumannii* from other members of the *A. calcoaceticus–baumannii* complex (henceforth referred to as *A. baumannii*) [7]. This poses challenges clinically because *A. baumannii* can be pathogenic whereas *A. calcoaceticus* is environmental. The exact reservoir of *A. baumannii* remains undefined [27]. Members of the *Acinetobacter calcoaceticus–baumannii* complex (ACB complex) are the predominant acinetobacter in clinical settings, and isolates are usually multiresistant, complicating therapy. Carbapenems have become the drugs of choice for serious acinetobacter infections in our country and have retained better activity than other antimicrobials. Nevertheless, reports of carbapenem resistance among Acinetobacter species are accumulating steadily [1]. Some early reports described acinetobacters with -lactamase-independent carbapenem resistance [4, 9, 31]. In contrast to other *Acinetobacter* spp., *A. baumannii* is uncommon in nature compared with the hospital environment [7]. *A. baumannii* is able to survive on dry surfaces in hospital environments for up to 4 months [33].
The aim of this study was to determine the characteristics and patterns of antibiotic resistance among isolates of *Acinetobacter calcoaceticus–baumannii* complex recovered from clinical specimens in Muğla.

2. Materials and Methods

Microbiological analyses and antibiotic susceptibility testing

Bacterial Isolates

40 *ACB* complex were isolated from clinical samples in Muğla. Bacterial isolates were identified to level of species and subspecies by using the morphological and traditional biochemical tests according to standard methods described by [28, 18]. The isolates are defined as the agent of hospital acquired infections, if they emerged after 72 hours of hospitalization. All isolates were obtained from patients at intensive care units. In total, 40 *ACB* complex were isolated from various clinical samples and detected by the VITEK 2 Compact system (BioMerieux, France) at the microbiology laboratory of our hospital between from January to December 2015.

Anti-biogram Pattern of *ACB* complex

Antibiotic resistance was determined by an agar disc diffusion test [2] using Mueller-Hinton agar (Difco) according to Clinical and Laboratory Standards Institute (CLSI) recommendations [5]. Ten different antibiotics were used. For antibiotic resistance determination, the isolates were grown in Luria-Bertani (LB) broth until the turbidity equal to the 0.5 Mc Farland standart (approximately 10^8cfu/ml). Cultures were swabbed on to the Mueller–Hinton agar and all isolates were tested against Ampicillin/Sulbactam (SAM, 20 µg/ml), Imipenem (IPM, 10 µg/ml), Meropenem (MEM, 10 µg/ml),

Cefepime (FEP, 5 µg/ml), Levofloxacin (LVX, 30 µg/ml), Ceftazidime (CAZ, 10 µg/ml), Trimetroprim/Sulfamethoxale (SXT, 30 µg/ml), Amikacin (AN, 30 µg/ml), Tigecyline (TGC, 10 µg/ml) and colistin (CL, 10 µg/ml).

The isolates those grown in inoculation were evaluated as resistant and the others were evaluated as susceptible. The antibiotic discs were dispensed sufficiently separated from each other so as to avoid overlapping of inhibition zones. The plates were incubated at 37°C, and the diameters of the inhibition zones were measured after 18 hr. All susceptibility tests were carried out in duplicate and were repeated twice if discordant results had been obtained.

Multiple Antibiotic Resistance Index:

For all isolates, we calculated the MAR index values (a/b, where a represents the number of antibiotics the isolate was resistant to, b represents the total number of antibiotics the isolate tested against). A MAR index value ≥ 0.2 is observed when isolates are exposed to high risk sources of human or animal contamination, where antibiotics use is common; in contrast a MAR index value <or = 0.2 observed when antibiotics are seldom or never used [17, 20].

3. Results

The results of the antibiotic susceptibility of the isolates are shown in Table 1. The antibiotic susceptibility was determined by the disc agar diffusion method in accordance with the instructions of the antibiotic disc manufacture (Oxoid). The number of isolates are shown in Table 1.

The resistance rates for, Ampicillin/Sulbactam, Imipenem, Meropenem, Cefepime, Levofloxacin and Ceftazadime were 95%. In our study, colistin was the lowest resistance antibiotics with12,5%.

Table 1: Antibiotic suspectibility pattern of *A.baumanii i*solated from clinical samples

Antibiotics	Resistance	Intermediate	Sensitive
SAM	38(95%)	0 (0%)	2(5%)
IPM	38(95%)	0(0%)	2(5%)
MEM	38(95%)	0(0%)	2(5%)
FEP	38(95%)	0(0%)	2(5%)
LVX	38(95%)	0(0%)	2(5%)
CAZ	38(95%)	0(0%)	2(5%)
SXT	36(90%)	0(0%)	4(10%)
AN	35(%87,5)	3(%7,5)	2(%7,5)
TGC	12(30%)	3(7,5%)	25 (62,5%)
CL	5(12,5%)	0(0%)	35 (87,5%)

Abrevations;, SAM: Ampicillin Sulbactam, IPM; Imipenem, MEM;Meropenem, FEP; Cefepime, LVX: Levofloxacin, CAZ: Ceftazadime, SXT: Trimetroprim/ Sulfamethoxale, AN; Amikacin, TGC: Tigecyline

CL: Colistin

In our study, the most resistant antibiotics were Ampicillin/Sulbactam, Imipenem, Meropenem, Cefepime, Levofloxacin, ceftazidime with 95%. Tigecyline and Colistin were the most sensitive antibiotics with 87,5% and 62,5% respectively.

Table 2: Number of clinical samples and Multiple Antibiotic Resistance Index 40 *ACB* complex strains

Source of isolates	Number of isolates	Multiple Antibiotic Resistance Index (MAR)
Tracheal aspirate	16(40%)	0.7 (1isl), 0.8(9 isl), 0.9(3 isl) 1(3isl)
Urine	6(15%)	0.8(5isl), 0(1isl)
Wound	5(12,5%)	0.8(4isl), 0.9(1isl)
Blood	5(12,5%)	0.7(1isl), 0.8(4isl)
Broncoalveolar Lavage	1(2,5%)	0,8(1 isl)
Mucus	2(%5)	0,8(2 isl)
Abces	2(%5)	0.9(1 isl), 0.8(1 isl)
Ear Cultures	1(2,5%)	0(1isl)
Cathather	1(2,5%)	0,8(1isl)
Throat Cultures	1(2,5%)	0,9(1isl)
Total	40	

In the present study, the isolates obtained from Tracheal aspirate(40%), urine(15%), Wound and blood (12,5%). In our study, 38 isolates showed seven to ten antibiotic resistance. But only two isolates showed no antibiotic resistance any of the antibiotics isolated from urine and ear cultures in Table 2.

4. Discussion

Kattel *et al* (2012) [15] reported that All ACB complex were found to be 100% sensitive towards Imipenem. Only half (50%) isolates showed susceptibility towards cotrimoxale, gentamicin, ceftazidime,piperacillin, piperacillin tazobactam, amikacin, and cefepime. All isolates were resistant towards amoxycillin/ampicillin, cephalexin, norfloxacin, nitrofurantoin, nalidixic acid, cefotaxime, amoxycillin plus, clavulanic acid, and all isolates were found MDR.

Colistin was used clinically because of its proven ability to treat infections caused by MDR- *A baumannii* and other MDR organism [22]. Many studies have reported cure rates or improvement with colistin of 57-77% among severely ill patients with MDR Acinetobacter species infection [19]. The ACB complex isolated at various places still show a high susceptibility to colistin. In our study 87,5% were susceptible.

Rifampicin, tigecycline, colistin and polymyxin are considered the last resort drugs. However, increasing resistance rates and higher minimum inhibitory concentration are being reported for tigecycline [23]. Emergence of resistance during treatment has been reported Peleg *et al.,* 2007 [26] probably mediated by efflux pumps [23]. We report a higher percentage (30%) of ACB complex resistant to it than those reported from places where it was introduced earlier, for example, 2.7% from the UK [12] and 26% from Argentina [6]. Tigecyline, a novel broad spectrum glycylcycline was approved by FDA and the European Medicines Agency for the treatment of complicated skin and intraabdominal infections [3].

Aminoglycoside and quinolone resistance are also common place [21]. The bacterium utilizes plasmid or transposon coded aminoglycoside modifying enzymes [24], efflux pumps,

alteration in target ribosomal protein and ineffective transport to interior of bacteria [32]. Amikacin resistance was detected in 87,5% of the ACB complex from our study. Chromosomal mutations altering the target enzymes DNA gyrase and topoisomerise IV; efflux pump and acquisition of mobile genetic element are mechanisms by which ACB complex acquired resistance to quinolones [25].

In the present study, the prevalence of ceftazidime resistance in *A. calcoaceticus-baumannii* was 95%. However, a much lower rate of resistance (6.0%) was reported in Japan [13]. Our rate is higher than those reported in Japan and in the United States and Canada [26, 27].

Carbapenem-resistant *A. calcoaceticus-baumannii* complex has emerged in many parts of the world. The main mechanism of resistance is through the acquisition of B and D class carbapenemases [28]. In a study from Taiwan, the carbapenem resistance of the Acinetobacter species isolated was 10%.8 In another study, Acinetobacter species had a rate of resistance to imipenem that increased from 0% to 42% during the study period [29]. In the present study, imipenem remained the antimicrobial most sensitive (5%) against *A. calcoaceticus-baumannii* complex, and the resistance rates were high (95%). Conversely, a low rate of resistance to imipenem (3.2% of isolates) was reported in Japan [26]. Regional variation in imipenem resistance was noted when North America (4.5% of isolates) and Latin America (11% of isolates) were compared [8].

Jaffar *et al* (2007) [14] reported that none of the isolates showed Trimetroprim/ Sulfamethoxale resistance. Conversely, ın our study, Trimetroprim/ Sulfamethoxale resistance was seen 90%.

In our study the resistance rates were detected as 95% in cefepime and the results were similar to the results of other recent studies in our country [10]. This result might be construed to mean that neither third-generation cephalosporins nor quinolones appear suitable for *A. baumannii* complex infections.

Notably, our findings show that Levofloxacin is ineffective against ACB complex (95% resistance), In the present study, involving 40 clinical isolates of ACB complex, 38 (95%) isolates were detected to have resistant zone sizes for meropenem when tested by disk diffusion method. Indian study by Taneja *et al*. in 2003 [30] reported a high incidence of more than 20% carbapenem resistance among Acinetobacters. As a results a high resistance ratio develops against levofloxacin and meropenem which are the antibiotics commonly used until recent years for *A. baumannii* complex, with a resistance ratio increasing constantly in the whole World [10].

ACB complex is notorious for its multidrug resistance (MDR). The rapid emergence and spread of MDR strains may be due to the combined effect of upregulation of its innate resistance mechanism coupled with gene acquisition following lateral gene transfer and clonal spread of MDR clones [29, 30]. This process is aided by selective pressure exerted by the use of broad spectrum antibiotics and transmission of strains among patients. In the present study, 71.3% ACB complex were MDR. Variable rates of MDR- ACB complex are reported by other researchers from Nepa [21, 16, 32]. Besides the differences in the study setting and study population, non uniformity in the definition of MDR could be the reason for the variation in the percentages of MDR isolates.

In conclusion, the present study recorded data on the rates of antimicrobial resistance observed in *A. calcoaceticus-baumannii* complex isolates from a hospital in Turkey. The antimicrobials most active against these isolates were colistin and tigecycillin. The observed difference in the antimicrobial resistance rates between those observed in this study from Turkey and those reported in other parts of the world may have been related to factors such as antimicrobial use patterns, infection control practices, and climate, as has been suggested in other studies from other parts of the World [11].

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