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Investigation of Metallo-Beta-Lactamase Production in Carbapenem-resistant Clinical *Acinetobacter baumannii* Isolates

Karbapenem Dirençli Klinik *Acinetobacter baumannii* İzolatlarında Metallo-Beta-Laktamaz Üretiminin Araştırılması

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ABSTRACT

Introduction: *Carbapenem resistance generally emerges from carbapenemase production in Acinetobacter baumannii isolates. It is known that prognosis is adversely affected, and mortality rate increases in infections caused by Metallo-beta-lactamase (MBL) type carbapenemase-producing isolates. This study aimed to investigate MBL production in carbapenem-resistant A. baumannii isolated from inpatients in Kastamonu Training and Research Hospital (TRH), Türkiye.*

Materials and Methods: *A total of 110 non-duplicated carbapenems (imipenem and meropenem) resistant A. baumannii isolates between July 2020 and July 2021 were included in the study. Identification of the isolates was performed by conventional methods, VITEK 2 Compact automated system, and amplification of the OXA-51-like gene region. Antibiotic susceptibility tests were conducted and evaluated with EUCAST criteria using VITEK 2. Carbapenemase production of the isolates was tested using the modified Hodge test. MBL production was screened using imipenem-EDTA double-disc synergy test. The presence of* bla_{IMP}, bla_{VIM}, bla_{GIM}, *and* bla_{NDM} *genes was investigated using polymerase chain reaction (PCR) to confirm the MBL production.*

Results: *All isolates were confirmed to be A. baumannii. All isolates were resistant to imipenem, meropenem, ciprofloxacin, and levofloxacin. The susceptibilities to trimethoprim-sulfamethoxazole, tobramycin, amikacin, and gentamicin were 0.9% (n= 1), 1.8% (n= 2), 2.7% (n= 3), and 2.7% (n= 3), respectively. All isolates had carbapenemase activity. However, the MBL phenotype was present in none of the strains. Also, MBL genes were not detected in the isolates.*

Conclusion: *MBL production was not detected in carbapenem-resistant A. baumannii isolates obtained from Kastamonu TRH. Carbapenem resistance in these isolates may be due to the production of non-MBL carbapenemases such as OXA-type carbapenemases.*

Key Words: *Acinetobacter baumannii; Antibiotic resistance; Carbapenems; Metallo-beta-lactamases; PCR*

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ÖZ

Karbapenem Dirençli Klinik *Acinetobacter baumannii* İzolatlarında Metallo-Beta-Laktamaz Üretiminin Araştırılması

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Giriş: *Acinetobacter baumannii izolatlarında karbapenem direnci genellikle karbapenemaz üretimine bağlıdır. Metallo-beta-laktamaz (MBL) tipi karbapenemaz üreten izolatlardan kaynaklanan infeksiyonlarda prognozun olumsuz etkilendiği ve mortalite oranının yükseldiği bilinmektedir. Bu çalışmada Kastamonu Eğitim ve Araştırma Hastanesinde (EAH) yatan hastalara ait çeşitli klinik örneklerden izole edilmiş karbapenem dirençli A. baumannii izolatlarında MBL üretim sıklığının belirlenmesi amaçlanmıştır.*

Materyal ve Metod: *Çalışmaya Temmuz 2020-2021 tarihleri arasında farklı hastalardan izole edilmiş 110 adet karbapenem (imipenem ve meropenem) dirençli A. baumannii izolatı dahil edilmiştir. İzolatların tanımlanması konvansiyonel yöntemlerin yanı sıra VITEK 2 kompakt otomatize sistemi ve OXA-51-benzeri gen bölgesinin amplifikasyonu ile gerçekleşmiştir. Antibiyotik duyarlılık testleri VITEK 2 otomatize sistemi ile EUCAST kriterleri doğrultusunda çalışılmış ve değerlendirilmiştir. İzolatların karbapenemaz üretimi modifiye Hodge testi ile belirlenmiştir. İzolatların MBL üretimi imipenem-EDTA çift disk sinerji testi ile araştırılmıştır. MBL üretimini doğrulamak için* bla_{IMP}, bla_{VIM}, bla_{GIM} *ve* bla_{NDM} *genlerinin varlığı polimeraz zincir reaksiyonu (PZR) ile araştırılmıştır.*

Bulgular: *Tüm izolatlar A. baumannii olarak doğrulanmıştır. İzolatların tamamı imipenem, meropenem, siprofloksasin ve levofloksasin antibiyotiklerine dirençli bulunmuştur. İzolatların trimetoprim-sulfametoksazol, tobramisin, amikasin ve gentamisin antibiyotiklerine duyarlılıkları sırasıyla %0,9 (n= 1), %1,8 (n= 2), %2,7 (n= 3) ve %2,7 (n= 3) olarak tespit edilmiştir. Tüm izolatların karbapenemaz enzimine sahip oldukları belirlenmiş ancak hiçbir izolatta MBL fenotipi saptanmamıştır. İlave olarak izolatlarda MBL geni tespit edilmemiştir.*

Sonuç: *Kastamonu EAH'den elde edilen karbapenem dirençli A. baumannii izolatlarında MBL üretimi tespit edilmemiştir. Çalışmaya dahil edilen izolatlarda karbapenem direnci, OXA tipi karbapenemazlar gibi MBL olmayan karbapenemazların üretiminden kaynaklanmış olabilir.*

Anahtar Kelimeler: *Acinetobacter baumannii; Antibiyotik direnci; Karbapenemler; Metallo-beta-laktamazlar; PZR*

INTRODUCTION

Carbapenems (imipenem, meropenem, etc.) are preferred antibiotics, especially in the treatment of infections caused by antibiotics resistant gram-negative pathogens, with their strong and broad-spectrum effects[1]. However, the increase in carbapenem resistance in *Acinetobacter baumannii* has reached an alarming level[2].

Carbapenem resistance in *A. baumannii* is mainly mediated by Ambler's Class D (OXA-type) carbapenemases)[3]. OXA-51-like, one of the subgroups of OXA enzymes and encoded by the $bla_{OXA\text{-}51\text{-}like}$ gene, is intrinsically expressed at the basal level in *A. baumannii* strains. However, the presence of ISAba1 or ISAba9 insertion sequences initiates overexpression of the $bla_{OXA\text{-}51\text{-}like}$ gene[4]. Another carbapenemase enzyme group reported in *A. baumannii* strains is Metallo-beta-lactamases (MBLs) found in Ambler's Class B. MBLs including IMP (imipenemase), VIM (Verona integron-encoded metallo-beta-lactamase), GIM (German imipenemase), NDM (New Delhi metallo-beta-lactamase), and SIM (Seoul imipenemase) have been identified in *A. baumannii* isolates[5].

MBLs hydrolyze all beta-lactam antibiotics except monobactam and are not inhibited by beta-lactamase inhibitors. MBLs are inhibited by metal chelators such as EDTA (Ethylene diamine tetra-acetic acid) because they need zinc ions for their activation[6]. Thus, MBL production of the isolates can be screened by several phenotypic tests (combined disc diffusion test, double-disc

synergy test, gradient diffusion method) using EDTA. However, the results must be confirmed using molecular tests[7].

Although MBLs are not as common as OXA-type carbapenemases in *A. baumannii* isolates, their hydrolytic activities are more powerful than OXA-type carbapenemases[8]. MBL-producing bacteria are multidrug-resistant because the genes encoding MBLs are carried together with other antibiotic resistance genes on integrons[3]. In addition, MBL-producing strains adversely affect the prognosis of patients and increase mortality(9). Therefore, early detection of MBL production in *A. baumannii* isolates and taking necessary precautions are significant. This study aimed to investigate MBL production in carbapenem-resistant *A. baumannii* isolated from inpatients at Kastamonu Training and Research Hospital (TRH).

MATERIALS and METHODS

Bacterial Isolates

Carbapenems (imipenem and meropenem) resistant 110 *A. baumannii* isolates obtained from Kastamonu TRH between July 2020 and July 2021 were included in the study. The isolates belonging to the same patient were excluded from the study. The isolates were identified using conventional methods (determination of colony morphology, gram and oxidase reactions) and VITEK 2 Compact automated system (BioMérieux, France). The identification of *A. baumannii* was confirmed by PCR amplification of the $bla_{\text{OXA-51-like}}$ gene region[10]. The isolates were named with KA (K: Kastamonu, A: *Acinetobacter*) code and isolate number. The isolates were stored at -80°C in Mueller-Hinton broth (Merck, Darmstadt, Germany) containing 20% glycerol (v/v).

Antibiotic Susceptibility Profile

The antibiotic susceptibility of the isolates to antibiotics was studied and evaluated using EUCAST standards by VITEK 2 Compact automated system. The antibiotics used in this study were: imipenem (IPM) and meropenem (MEM) as carbapenems; ciprofloxacin (CIP) and levofloxacin (LEV) as fluoroquinolone; amikacin (AK), gentamicin (CN), and tobramycin (TOB) as aminoglycosides; trimethoprim-sulfamethoxazole (SXT) as folate pathway inhibitors. The minimum inhibitory concentration (MIC) values of >4, >8, >1, >1, >8, >4, >4, and >4 mg/L were considered to be the resistance breakpoints for IPM, MEM, CIP, LEV, AK, CN, TOB, and SXT, respectively[11]. *Pseudomonas aeruginosa* ATCC 27853 was used as the quality control strain.

Phenotypic Detection of Carbapenemases

Carbapenemase production of all isolates was determined using the modified Hodge test (MHT). The test was done by CLSI guidelines[12]. Briefly, a 0.5 McFarland turbidity of *Escherichia coli* ATCC 25922 was prepared with the direct colony suspension method in serum physiological (SF) solution and diluted 1:10 in SF. Then, the prepared suspension was seeded in three directions on Mueller-Hinton agar (MHA), and the plate was allowed to dry for five minutes. One imipenem (IPM; 10 µg $disc^{-1}$, Oxoid, UK) disc was placed on the center of the inoculated plate. Three to five colonies were taken from each isolate with a 10 µL loop and streaked in a straight line from the edge of the IPM disc to the edge of the plate. After 20 hours of incubation at 37°C, the presence of a cloverleaf-type indentation at the intersection of isolates and *E. coli* within the inhibition zone formed by the disc indicated the positivity of the test.

MBL production of the isolates was screened using the imipenem-EDTA double-disc synergy test (DDST) as described by Picro et al[13]. Briefly, fresh cultures of the isolates grown on MHA (Merck) were adjusted to 0.5 McFarland turbidity and inoculated on MHA plates. IPM (10 µg $disc^{-1}$) and EDTA (1900 µg $disc^{-1}$; Sigma-Aldrich, USA) discs were placed at a distance of 20 mm apart from each other on the MHA plates and incubated for 24 hours at 37°C. The enhancement of the inhibition zone diameter of the imipenem disc towards the EDTA disc or the presence of a phantom zone between the discs were considered as indicative of MBL production.

Klebsiella pneumoniae NCTC 13440 and a *P. aeruginosa* strain previously tested and confirmed not to produce carbapenemases was used positive and negative controls, respectively, in the tests.

PCR Assays for MBL Genes

The presence of bla_{IMP}, bla_{VIM}, bla_{GIM}, and bla_{NDM} as MBL genes in all isolates was investigated by PCR using the specific primers listed in Table 1. Total DNA extraction from the isolates was done using the boiling method as described previously[14]. PCR assays were performed in a thermal cycler (Techne TC-512, Techne Co., UK) cycling conditions were as follows: initial denaturation at 94°C for five min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at specific temperatures, extension (at 72°C for 30 sec for bla_{NDM}, for 50 sec for bla_{IMP}, bla_{VIM}, and bla_{GIM}); final extension at 72°C for eight min for bla_{NDM} and five min for bla_{IMP}, bla_{VIM}, and bla_{GIM}[15,16]. PCR reactions were prepared to contain the following ingredients; 2 µL DNA template, 0.4 µL 10 pmol/µL of each primer (forward and reverse), 4 µL 5x FIREPol® Master Mix (SolisBioDyne, Tartu, Estonia), and ultra-pure water up to 20 µL. Sterile ultra-pure water was used as the negative control, while total DNA of *Klebsiella pneumoniae* NCTC 13440 (bla_{VIM}), *K. pneumoniae* NCTC 13443 (bla_{NDM}), and clinical *P. aeruginosa* (bla_{IMP}) and *A. baumannii* (bla_{GIM}) strains served as the positive controls. The clinical control strains have been previously confirmed by DNA sequencing to contain the bla_{IMP} and bla_{GIM} genes. PCR products were electrophoresed on a 1.5% agarose gel stained with RedSafe™ Nucleic Acid Staining Solution (20.000x) (iNtRON Biotechnology, Inc. Seoul, Korea) in 1x Tris-acetate-EDTA buffer. The results were evaluated in the presence of a DNA size marker (GeneRuler 100 bp, Thermo Scientific, CA, USA), visualized using a gel image analysis system (Quantum, Vision-Capt., Vilber Lourmat, Collegien, France).

Statistical Analysis

The data were examined for normality using the Wilks-Shapiro test. Statistical analysis was done by Mann-Whitney U test using the SPSS version 23 (IBM Inc., Armonk, NY, USA) for Windows, and $p< 0.05$ was considered statistically significant.

RESULTS

All isolates were found to carry the $bla_{OXA\text{-}51\text{-}like}$ gene and were confirmed to be *A. baumannii*. Of the isolates, 104 (94.5%) were isolated from intensive care units (ICUs) and six (5.5%) from inpatient services. Distribution of the isolates among the clinical samples was as follows: respiratory secretions 83 (75.5%), blood 19 (17.3%), urine six (5.5%), and wound two (1.8%).

All isolates were resistant to IPM, MEM, CIP, and LEV. The susceptibilities to SXT, TOB, AK, and CN were 0.9% (n= 1), 1.8% (n= 2), 2.7% (n= 3), and 2.7% (n= 3), respectively. There was no significant difference in SXT, TOB, AK, and CN susceptibilities among the isolates obtained from ICUs and inpatient services ($p> 0.05$). All isolates were detected as carbapenemase positive by MHT (Figure 1). However, MBL phenotype was present in none of the strains. Also, the bla_{IMP}, bla_{VIM}, bla_{GIM}, and bla_{NDM} genes were not detected in the isolates (Figure 2).

Table 1. The specific primers used in PCR

Gene	Sequence (5′→3′)	Annealing Temperature	Product Size	Reference
$bla_{OXA\text{-}51\text{-}like}$	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACTTCATCTTGG	at 52°C for 40 sec	353 bp	10
bla_{IMP}	F: GGAATAGAGTGGCTTAAYTCTC R: CCAAACYACTASGTTATCT	at 51°C for 40 sec	188 bp	15
bla_{VIM}	F: GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG	at 52°C for 40 sec	390 bp	
bla_{GIM}	F: TCGACACACCTTGGTCTGAA R: AACTTCCAACTTTGCCATGC		477 bp	
bla_{NDM}	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	at 55°C for 30 sec	621 bp	16

Table 2. Sources and antibiotic susceptibility profiles of the isolates

Isolate Name	Isolation Source	IPM	MEM	CIP	LEV	AK	CN	TOB	SXT
KA-1	Blood	R	R	R	R	**S**	**S**	R	R
KA-2	Respiratory secretions	R	R	R	R	R	R	R	R
KA-3	Respiratory secretions	R	R	R	R	R	R	R	R
KA-4	Blood	R	R	R	R	R	R	**S**	R
KA-5	Respiratory secretions	R	R	R	R	R	R	R	R
KA-6	Respiratory secretions	R	R	R	R	R	R	R	R
KA-7	Respiratory secretions	R	R	R	R	R	R	R	R
KA-8	Respiratory secretions	R	R	R	R	R	R	R	R
KA-9	Respiratory secretions	R	R	R	R	R	R	R	R
KA-10	Respiratory secretions	R	R	R	R	R	R	R	R
KA-11	Respiratory secretions	R	R	R	R	R	R	R	R
KA-12	Respiratory secretions	R	R	R	R	R	R	R	R
KA-13	Respiratory secretions	R	R	R	R	R	R	R	R
KA-14	Respiratory secretions	R	R	R	R	R	R	R	R
KA-15	Respiratory secretions	R	R	R	R	R	R	R	R
KA-17	Respiratory secretions	R	R	R	R	R	R	R	R
KA-18	Respiratory secretions	R	R	R	R	R	R	R	R
KA-19	Respiratory secretions	R	R	R	R	R	R	R	R
KA-20*	Urine	R	R	R	R	R	R	R	R
KA-23	Respiratory secretions	R	R	R	R	R	R	R	R
KA-24	Respiratory secretions	R	R	R	R	R	R	R	R
KA-25	Respiratory secretions	R	R	R	R	R	R	R	R
KA-26	Respiratory secretions	R	R	R	R	R	R	R	R
KA-27	Respiratory secretions	R	R	R	R	R	R	R	R
KA-28	Blood	R	R	R	R	R	R	R	R
KA-29	Wound	R	R	R	R	R	R	R	R
KA-30	Respiratory secretions	R	R	R	R	R	R	R	R
KA-31	Respiratory secretions	R	R	R	R	R	R	R	R
KA-32	Respiratory secretions	R	R	R	R	R	R	R	R
KA-33	Respiratory secretions	R	R	R	R	R	R	R	R
KA-34	Blood	R	R	R	R	R	R	R	R
KA-35	Blood	R	R	R	R	R	R	R	R
KA-37	Respiratory secretions	R	R	R	R	R	R	R	R
KA-38	Respiratory secretions	R	R	R	R	R	R	R	R
KA-39	Respiratory secretions	R	R	R	R	R	R	R	R
KA-40	Blood	R	R	R	R	R	R	R	R
KA-41	Respiratory secretions	R	R	R	R	R	R	R	R
KA-42	Respiratory secretions	R	R	R	R	R	R	R	R
KA-43	Respiratory secretions	R	R	R	R	R	R	R	R
KA-44	Respiratory secretions	R	R	R	R	R	R	R	R

Table 2. Sources and antibiotic susceptibility profiles of the isolates (continue)

Isolate Name	Isolation Source	IPM	MEM	CIP	LEV	AK	CN	TOB	SXT
KA-45	Respiratory secretions	R	R	R	R	R	R	R	R
KA-46	Respiratory secretions	R	R	R	R	R	R	R	R
KA-47	Respiratory secretions	R	R	R	R	R	R	R	R
KA-48	Respiratory secretions	R	R	R	R	R	R	R	R
KA-49	Blood	R	R	R	R	R	R	R	R
KA-50	Respiratory secretions	R	R	R	R	R	R	R	R
KA-51	Respiratory secretions	R	R	R	R	R	R	R	R
KA-53	Blood	R	R	R	R	**S**	**S**	**S**	**S**
KA-55	Respiratory secretions	R	R	R	R	R	R	R	R
KA-57	Respiratory secretions	R	R	R	R	R	R	R	R
KA-58	Respiratory secretions	R	R	R	R	R	R	R	R
KA-59	Respiratory secretions	R	R	R	R	R	R	R	R
KA-60	Respiratory secretions	R	R	R	R	R	R	R	R
KA-61	Blood	R	R	R	R	R	R	R	R
KA-62	Blood	R	R	R	R	R	R	R	R
KA-63	Blood	R	R	R	R	R	R	R	R
KA-65	Respiratory secretions	R	R	R	R	R	R	R	R
KA-66	Respiratory secretions	R	R	R	R	R	R	R	R
KA-67	Blood	R	R	R	R	R	R	R	R
KA-68	Blood	R	R	R	R	R	R	R	R
KA-69	Blood	R	R	R	R	R	R	R	R
KA-70	Urine	R	R	R	R	R	R	R	R
KA-71*	Respiratory secretions	R	R	R	R	R	R	R	R
KA-72	Respiratory secretions	R	R	R	R	R	R	R	R
KA-73	Respiratory secretions	R	R	R	R	R	R	R	R
KA-74*	Respiratory secretions	R	R	R	R	R	R	R	R
KA-76	Respiratory secretions	R	R	R	R	R	R	R	R
KA-77	Respiratory secretions	R	R	R	R	R	R	R	R
KA-78	Respiratory secretions	R	R	R	R	R	R	R	R
KA-79	Respiratory secretions	R	R	R	R	R	R	R	R
KA-80	Respiratory secretions	R	R	R	R	R	R	R	R
KA-81	Respiratory secretions	R	R	R	R	R	R	R	R
KA-82	Respiratory secretions	R	R	R	R	R	R	R	R
KA-83	Urine	R	R	R	R	R	R	R	R
KA-84	Respiratory secretions	R	R	R	R	R	R	R	R
KA-85*	Urine	R	R	R	R	R	R	R	R
KA-86	Blood	R	R	R	R	R	R	R	R
KA-87	Blood	R	R	R	R	R	R	R	R
KA-88	Respiratory secretions	R	R	R	R	**S**	**S**	R	R
KA-89	Respiratory secretions	R	R	R	R	R	R	R	R

Table 2. Sources and antibiotic susceptibility profiles of the isolates (continue)

Isolate Name	Isolation Source	IPM	MEM	CIP	LEV	AK	CN	TOB	SXT
KA-90	Blood	R	R	R	R	R	R	R	R
KA-91	Respiratory secretions	R	R	R	R	R	R	R	R
KA-92	Blood	R	R	R	R	R	R	R	R
KA-93	Respiratory secretions	R	R	R	R	R	R	R	R
KA-94	Respiratory secretions	R	R	R	R	R	R	R	R
KA-95	Respiratory secretions	R	R	R	R	R	R	R	R
KA-96	Respiratory secretions	R	R	R	R	R	R	R	R
KA-97	Respiratory secretions	R	R	R	R	R	R	R	R
KA-98	Respiratory secretions	R	R	R	R	R	R	R	R
KA-99	Respiratory secretions	R	R	R	R	R	R	R	R
KA-101	Respiratory secretions	R	R	R	R	R	R	R	R
KA-102	Respiratory secretions	R	R	R	R	R	R	R	R
KA-103*	Wound	R	R	R	R	R	R	R	R
KA-104	Respiratory secretions	R	R	R	R	R	R	R	R
KA-105	Respiratory secretions	R	R	R	R	R	R	R	R
KA-106*	Urine	R	R	R	R	R	R	R	R
KA-107	Urine	R	R	R	R	R	R	R	R
KA-108	Respiratory secretions	R	R	R	R	R	R	R	R
KA-109	Respiratory secretions	R	R	R	R	R	R	R	R
KA-110	Respiratory secretions	R	R	R	R	R	R	R	R
KA-111	Respiratory secretions	R	R	R	R	R	R	R	R
KA-112	Blood	R	R	R	R	R	R	R	R
KA-113	Respiratory secretions	R	R	R	R	R	R	R	R
KA-114	Respiratory secretions	R	R	R	R	R	R	R	R
KA-115	Respiratory secretions	R	R	R	R	R	R	R	R
KA-116	Respiratory secretions	R	R	R	R	R	R	R	R
KA-117	Respiratory secretions	R	R	R	R	R	R	R	R
KA-118	Respiratory secretions	R	R	R	R	R	R	R	R
KA-119	Respiratory secretions	R	R	R	R	R	R	R	R
KA-120	Respiratory secretions	R	R	R	R	R	R	R	R

KA: Kastamonu *Acinetobacter*, *: Isolated from inpatient services, IPM: Imipenem, MEM: Meropenem, CIP: Ciprofloxacin, LEV: Levofloxacin, AK: Amikacin, CN: Gentamicin, TOB: Tobramycin, SXT: Trimethoprim-sulfamethoxazole, R: Resistant, S: Susceptible. There was no significant difference in antibiotic susceptibility among isolates obtained from ICUs and inpatient services ($p > 0.05$).

DISCUSSION

A. baumannii is a nosocomial pathogen often isolated from inpatients. In particular, patients in ICUs are at risk since intensive use of broad-spectrum antibiotics, mechanical ventilators, and catheters in ICUs predispose inpatients to infections caused by *A. baumannii*[17]. In this study, 94.5% of the isolates collected in Kastamonu TRH within one year were isolated from patients in ICUs. The others were isolated from various inpatient services. Among the clinical samples, they were mostly isolated from respiratory secretions, followed by blood, urine, and wound cultures. *A. baumannii* is one of the leading etiological agents of ventilator-associated pneumonia so they are frequently isolated from

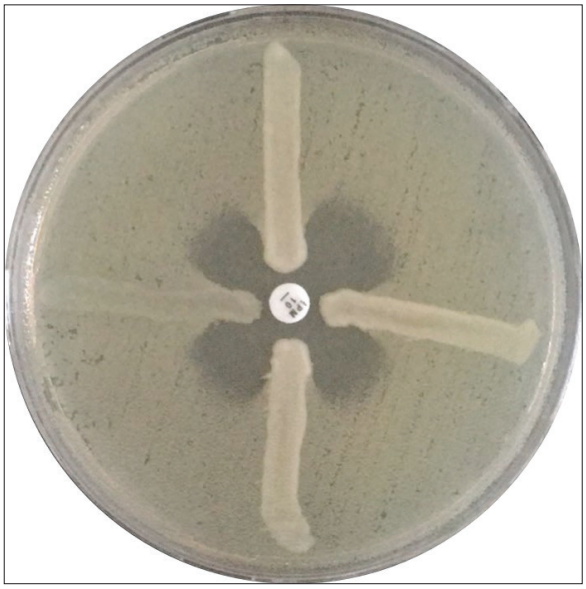

Figure 1. Representative image showing the results of the modified Hodge test in four isolates (KA-27, KA-28, KA-29, KA-30; K: Kastamonu, A: *Acinetobacter*).

the respiratory secretions of inpatients. It can also cause bloodstream, urinary tract, and wound infections[18], and the findings of our study are also consistent with this information.

In the current study, all isolates were resistant to carbapenem and fluoroquinolone antibiotics. Only a few isolates were susceptible to CN (n= 3), AK (n= 3), TOB (n= 2), and SXT (n= 1). *A. baumannii* isolates have intrinsic or acquired resistance to many antibiotics. In particular, it is known that carbapenem-resistant *A. baumannii* isolates are multidrug-resistant[19]. Carbapenem resistance can occur in *A. baumannii* by the following mechanisms: alterations in penicillin-binding proteins (PBPs), decreased membrane permeability, over expression of efflux pumps, and synthesis of carbapenemases[8]. The main mechanism responsible for carbapenem resistance in *A. baumannii* was the synthesis of carbapenemases in this study since all of the isolates (100%) had carbapenemase enzyme based on MHT. The frequency of carbapenemase production in carbapenem-resistant *A. baumannii* isolates in Türkiye has been reported in the range of 39-96%[20,21]. OXA-type carbapenemases are known to be the most common carbapenemases in *A. baumannii*. However, MBLs come to the fore due to their strong hydrolytic activities on carbapenems and their rapid spreading potential among bacteria. So, it is important to detect these enzymes and prevent their spread[8]. In this study, MBL production in carbapenem-resistant *A. baumannii* isolates was investigated phenotypically and genotypically. However, MBL production was not detected in any of the isolates. The frequency of MBL production in carbapenem-resistant *A. baumannii* isolates has been reported phenotypically in the range of 3.7-44.7% in Türkiye in recent years[20-22]. Moreover, bla_{IMP}, bla_{VIM}, bla_{GIM}, and bla_{NDM} genes encoding IMP, VIM, GIM, and NDM type MBL enzymes were not found in any isolates in the present study. The bla_{IMP}, bla_{VIM}, bla_{GIM}, and bla_{NDM} genes have been reported in MBL-producing *A. baumannii* isolates worldwide[8,23].

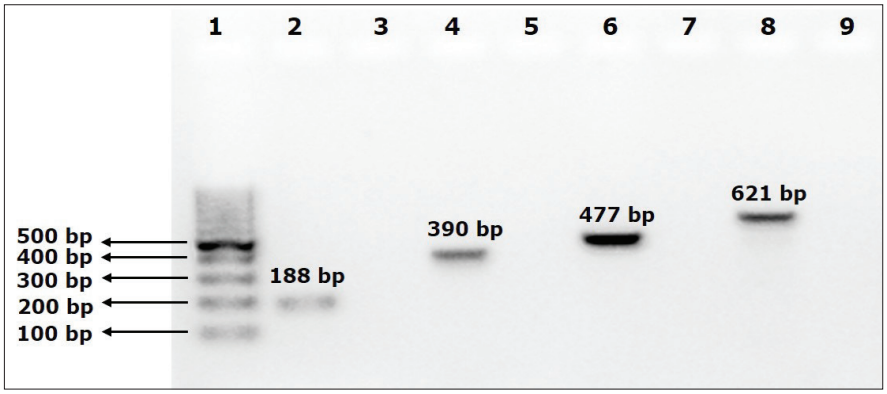

Figure 2. PCR products on agarose gel electrophoresis for MBLs genes. Lane 1: DNA size marker (GeneRuler 100 bp); lane 2, 4, 6, 8: Positive controls for bla_{IMP}, bla_{VIM}, bla_{GIM}, and bla_{NDM} genes, respectively; lane 3, 5, 7, 9: Negative controls for bla_{IMP}, *bla*VIM, bla_{GIM}, and bla_{NDM} genes, respectively.

However, it is stated that MBL genes are not commonly found in carbapenem-resistant *A. baumannii* isolates in studies conducted in different regions of Türkiye[20,22-27]. Moreover, it has been reported that VIM (3.2%) and SPM (4%) type MBL genes were detected in *A. baumannii* isolates from Niğde[28] and Van[29] provinces in Türkiye, respectively. OXA-type carbapenemases were a major factor for carbapenem resistance nonetheless in those studies.

CONCLUSION

All isolates had carbapenemase enzyme. However, no isolate produced MBL. These results showed that carbapenem-resistant *A. baumannii* isolates did not produce MBL in Kastamonu TRH. Carbapenem resistance in these isolates may be due to the production of non-MBL carbapenemases such as OXA-type carbapenemases. Periodic investigation of MBL production in hospitals is important for infection control and public health.

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ETHICS COMMITTEE APPROVAL

Ethics approval for this study was obtained from Karabük University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Decision no: 2020/212, Date: 15.05.2020).

CONFLICT of INTEREST

The authors declare that they have no know competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: EFT, NÇ

Analysis/Interpretation: EFT, AA

Data Collection or Processing: ÇK, MYD

Writing: EFT, AA, MCB, YÇA

Review and Correction: NÇ, MCB, YÇA

Final Approval: NÇ

REFERENCES

1. *Bush K, Bradford PA. β-lactams and β-lactamase inhibitors: An overview. Cold Spring Harb Perspect Med 2016;6(8):a025247. https://doi.org/10.1101/cshperspect.a025247*
2. *Nikibakhsh M, Firoozeh F, Badmasti F, Kabir K, Zibaei M. Molecular study of metallo-β-lactamases and integrons in Acinetobacter baumannii isolates from burn patients. BMC Infect Dis 2021;21(1):782. https://doi.org/10.1186/s12879-021-06513-w*
3. *Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: Mechanisms and epidemiology. Clin Microbiol Infect 2006;(9):826-36. https://doi.org/10.1111/j.1469-0691.2006.01456.x*
4. *Vázquez-López R, Solano-Gálvez SG, Juárez Vignon-Whaley JJ, Abello Vaamonde JA, Padró Alonzo LA, Rivera Reséndiz A, et al. Acinetobacter baumannii resistance: A real challenge for clinicians. Antibiotics (Basel) 2020;9(4):205. https://doi.org/10.3390/antibiotics9040205*
5. *Ibrahim ME. Prevalence of Acinetobacter baumannii in Saudi Arabia: Risk factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance. Ann Clin Microbiol Antimicrob 2019;18(1):1. https://doi.org/10.1186/s12941-018-0301-x*
6. *Ju LC, Cheng Z, Fast W, Bonomo RA, Crowder MW. The continuing challenge of metallo-β-lactamase inhibition: Mechanism matters. Trends Pharmacol Sci 2018;39(7):635-47. https://doi.org/10.1016/j.tips.2018.03.007*
7. *The European Committee on Antimicrobial Susceptibility Testing. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, version 2.0, 2017. Available from: http://www.eucast.org/resistance_mechanisms/*
8. *Nowak P, Paluchowska P. Acinetobacter baumannii: Biology and drug resistance - role of carbapenemases. Folia Histochem Cytobiol 2016;54(2):61-74. https://doi.org/10.5603/FHC.a2016.0009*
9. *Amin M, Navidifar T, Saleh Shooshtari F, Goodarzi H. Association of the genes encoding Metallo-β-Lactamase with the presence of integrons among multidrug-resistant clinical isolates of Acinetobacter baumannii. Infect Drug Resist 2019;12:1171-80. https://doi.org/10.2147/IDR.S196575*
10. *Ahmed SS, Alp E, Ulu-Kılıç A, Dinç G, Aktaş Z, Ada B, et al. Spread of carbapenem-resistant international clones of Acinetobacter baumannii in Turkey and Azerbaijan: A collaborative study. Eur J Clin Microbiol Infect Dis 2016;35(9):1463-8. https://doi.org/10.1007/s10096-016-2685-x*

11. *The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 11.0, 2021. Available from: http://www.eucast.org/clinical_breakpoints/*
12. *Clinical and Laboratory Standards Institute CLSI. Screening and Confirmatory Tests for Suspected Carbapenemases Production. 2010;20 supplemental table 2A-S2.*
13. *Picão RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, et al. Metallo-beta-lactamase detection: Comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. J Clin Microbiol 2008;46(6):2028-37. https://doi.org/10.1128/JCM.00818-07*
14. *Tufekci, EF, Alkateeb A, Kılınç C, Gürbüz M, Altunoğlu YC, Baloğlu MC, et al. Investigation of metallo-beta-lactamase production in carbapenem-resistant Pseudomonas aeruginosa isolated in Kastamonu Training and Research Hospital, Turkey. Malays J Microbiol 2021;17(5):593-600. https://doi.org/10.21161/mjm.211128*
15. *Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother 2007;59(2):321-2. https://doi.org/10.1093/jac/dkl481*
16. *Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70(1):119-23. https://doi.org/10.1016/j.diagmicrobio.2010.12.002*
17. *Brady MF, Jamal Z, Pervin N. Acinetobacter. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430784/ (Accessed date: 13.08.2021).*
18. *Obeidat N, Jawdat F, Al-Bakri AG, Shehabi AA. Major biologic characteristics of Acinetobacter baumannii isolates from hospital environmental and patients' respiratory tract sources. Am J Infect Control 2014;42(4):401-4. https://doi.org/10.1016/j.ajic.2013.10.010*
19. *Centre for Disease Control. Antibiotic resistance threats in the United States, 2019. U.S. Department of Health and Human Services, Atlanta, GA. Available from: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf (Accessed date: 20.01.2022).*
20. *Aksoy MD, Çavuşlu Ş, Tuğrul HM. Investigation of metallo beta lactamases and oxacilinases in carbapenem resistant Acinetobacter baumannii strains isolated from inpatients. Balkan Med J 2015;32(1):79-83. https://doi.org/10.5152/balkanmedj.2015.15302*
21. *Cesur S, Kınıklı S, Doğan K, Büyükdemirci A, Yağcı S, Ataman Hatipoğlu Ç, et al. Klinik örneklerden izole edilien karbapeneme dirençli Acinetobacter baumannii suşlarında metallo-beta-laktamaz enzimi varlığının iki farklı fenotipik yöntemle araştırılması. J Health Sci Med 2018;1(1):9-12. https://doi.org/10.32322/jhsm.410890*
22. *Ocak M, Özer B, İnci M, Duran N. Klinik örneklerden izole edilen Acinetobacter kökenlerinde antibiyotik direnci ve IMP-1, IMP-2, VIM-1, VIM-2 tipi Metallo-β-Laktamazların araştırılması. Klimik Derg 2015;28(1):23-7. https://doi.org/10.5152/kd.2015.05*
23. *Girija SA, Jayaseelan VP, Arumugam P. Prevalence of VIM- and GIM-producing Acinetobacter baumannii from patients with severe urinary tract infection. Acta Microbiol Immunol Hung 2018;65(4):539-50. https://doi.org/10.1556/030.65.2018.038*
24. *Boral B, Ünaldı Ö, Ergin A, Durmaz R, Eser ÖK, Zarakolu P, et al. A prospective multicenter study on the evaluation of antimicrobial resistance and molecular epidemiology of multidrug-resistant Acinetobacter baumannii infections in intensive care units with clinical and environmental features. Ann Clin Microbiol Antimicrob 2019;18(1):19. https://doi.org/10.1186/s12941-019-0319-8*
25. *Gözalan A, Ünaldı Ö, Kırca F, Çöplü N, Müderris T, Açıkgöz ZC, et al. Yoğun bakım ünitelerinde kan dolaşımı enfeksiyonu etkeni karbapenem dirençli Acinetobacter baumannii izolatlarının moleküler yöntemlerle karakterizasyonu. Turk Hij Den Biyol Derg 2020;77(1):15-24. https://doi.org/10.5505/TurkHijyen.2019.53323*
26. *Kostakoğlu U, Ertürk A, Yıldız İE, Özyurt S, Çopur Çiçek A, Karataş M, et al. Antibiotic resistance and integron gene cassettes in acinetobacter baumannii isolates produced in lower respiratory tract samples taken from the intensive care unit. Kırıkkale Üni Tıp Derg 2020;22(3):450-60.*
27. *Telli M, Eyigör M, Korkmazgil B, Aydin N, Atalay MA. Acinetobacter spp. klinik izolatlarında karbapenem direncinin moleküler epidemiyolojisi. Türk Mikrobiyol Cem Derg 2017;47(4):190-6.*
28. *Beriş FŞ, Budak EE, Gülek D, Uzun A, Çizmeci Z, Mengeloğlu FZ, et al. Türkiye'nin farklı bölgelerinden toplanan klinik Acinetobacter baumannii izolatlarında beta-laktamaz gen sıklığı ve dağılımının araştırılması: Çok merkezli bir çalışma. Mikrobiyol Bul 2016;50(4):511-21. https://doi.org/10.5578/mb.29176*
29. *Rağbetli C, Güdücüoğlu H, Parlak M. Yoğun bakım ünitelerinden izole edilen çok ilaca dirençli Acinetobacter ve Pseudomonas izolatlarında karbapenem direncinin araştırılması. J Contemp Med 2019;9(3):275-9. https://doi.org/10.16899/jcm.609560*

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