Metallobeta-Lactamase Enzymes and Antibiotic Susceptibilities in Strains of *Pseudomonas Aeruginosa* Isolated from Intensive Care Units in Turkey

Türkiye'de Yoğun Bakım Ünitelerinden İzole Edilen *Pseudomonas Aeruginosa* Suşlarında Metallobeta-Laktamaz Enzimlerinin ve Antibiyotik Duyarlılıklarının Araştırılması

Salih CESUR, MD, Assoc.Prof.,[a]
Eda YILDIZ, MD, Msc,[b]
Hasan IRMAK, MD, Assoc.Prof.,[a]
Zeynep GÜLAY, MD, Prof.,[c]
Uğur ARSLAN, MD, Assis.Prof.,[d]
Sepin Özen NEVGUN, MD,[e]
Gülçin BAYRAMOĞLU, MD, Assis.Prof.,[f]
Mustafa BERKTAŞ, MD, Prof.,[g]
Ata Nevzat YALÇIN, MD, Prof.,[e]
Serap GENCER, MD, Assoc.Prof.,[h]
Salih HOŞOĞLU, MD, Prof.,[i]
Ali Pekcan DEMİRÖZ, MD, Prof.[i]

Clinics of
[a]Infectious Diseases and Clinical Microbiology,
[b]Microbiology and Clinical Microbiology
Ankara Training and Research Hospital, Ankara
[c]Department of Microbiology and Clinical Microbiology,
Dokuz Eylül University Faculty of Medicine, İzmir
[d]Department of Microbiology and Clinical Microbiology,
Selçuk University Faculty of Medicine, Konya
[e]Department of Microbiology and Clinical Microbiology,
Akdeniz University Faculty of Medicine, Antalya
[f]Department of Microbiology and Clinical Microbiology,
Karadeniz Teknik University Faculty of Medicine,
Trabzon
[g]Department of Microbiology and Clinical Microbiology
Yüzüncü Yıl University Faculty of Medicine, Van
[h]Clinic of Infectious Diseases and Clinical Microbiology,
Dr.Lütfi Kırdar Kartal Education and
Research Hospital, İstanbul
[i]Department of Infectious Diseases and
Clinical Microbiology,
Dicle University Faculty of Medicine, Diyarbakır

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Yazışma Adresi/*Correspondence:*
Salih CESUR, MD, Assoc.Prof.
Ankara Training and Research Hospital,
Clinic of Infectious Diseases and Clinical Microbiology,
Ankara,
TÜRKİYE/TURKEY
scesur89@yahoo.com

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ABSTRACT Objective: The aim of this study was to determine the frequency of metallobeta-lactamase (MBL) enzyme in *Pseudomonas aeruginosa* strains resistant to carbapenem (imipenem or/and meropenem) in seven regions of Turkey and to assess the minimal inhibitory concentration (MIC) levels of drugs used in treatment such as colistin, aztreonam, polymyxin B and rifampin. Overall 186 *Pseudomonas aeruginosa (P.aeruginosa)* strains resistant to carbapenem from 8 provinces (Ankara, Konya, Antalya, İstanbul, İzmir, Diyarbakır, Van and Trabzon) representing 7 different geographical regions of Turkey were included in the study. **Material and Methods:** The presence of MBL in *P.aeruginosa* strains resistant to carbapenem was investigated by combined disk methods with imipenem and EDTA absorbed imipenem disk. The MBL positivity was determined in the strains. Additionally, susceptibility to aztreonam, colistin, polymyxin B, and rifampin was established by the E-test method. **Results:** MBL enzyme positivity was detected in 58 out of 186 strains (31.2%). There was statistically significant difference between regions in terms of MBL positivity, with the highest rates in Antalya (52%), and İstanbul (50%) and the lowest in Diyarbakır (6%). Aztreonam sensitivity was detected in 134 (72 %) strains; 155 (83.3%) were sensitive to colistin and 148 (79.6%) to polymixine. No strain (0%) was sensitive to rifampin. **Conclusion:** In conclusion, the overall mean rate of MBL positivity was 31.2%, which is quite high. Therefore, it will be beneficial to confirm the MBL positivity of strains with molecular methods, to review regional antibiotic surveillance data at certain intervals and to share the obtained data with relevant institutions in order to prevent the regional spread of these strains. Thus, it is essential to record and monitor systematically the antibiotic surveillance data.

Key Words: *Pseudomonas aeruginosa*; carbapenemase; intensive care units; beta-lactamase

ÖZET Amaç: Bu çalışmanın amacı, Türkiye'nin yedi bölgesinde karbapeneme (imipemen ve/veya meropenem) dirençli *Pseudomonas aeruginosa* suşlarında metallobeta-laktamaz (MBL) enziminin sıklığını belirlemek ve kolistin, aztreonam, polimiksin B ve rifampin gibi tedavide kullanılan ilaçların minimum inhibitör konsantrasyon (MİK) düzeylerini değerlendirmektir. Türkiye'de 7 farklı coğrafi bölgeyi temsil eden 8 ilden (Ankara, Konya, Antalya, İstanbul, İzmir, Diyarbakır, Van ve Trabzon) toplam 186 karbapeneme dirençli *Pseudomonas aeruginosa (P. aeruginosa*) suşu çalışmaya alındı. **Gereç ve Yöntemler:** Karbapeneme dirençli *P. aeruginosa* suşlarında MBL'nin varlığı imipenem ve EDTA emdirilmiş imipenem diski ile araştırıldı. Suşlarda MBL pozitifliği ve negatifliği belirlendi. Ayrıca aztreonam, kolistin, polimiksin B ve rifampine duyarlılık E-test yöntemi ile belirlendi. **Bulgular:** Yüz seksen altı suşun 58 (%31,2)'inde MBL enzimi pozitifliği saptandı. MBL pozitiflik oranları açısından bölgeler arasında istatistiksel olarak anlamlı fark bulundu; enzimin bulunma sıklığının Antalya (%52) ve İstanbul'da (%50) en yüksek, Diyarbakır'da (%6) ise en düşük düzeyde olduğu saptandı. Yüz seksen altı *P. aeruginosa* suşunun 134 (%72)'ünde aztreonama duyarlılık saptandı. Tüm *P. aeruginosa* suşlarının 155 (%83,3)'i kolistine duyarlıydı. Tüm suşların 148 (%79,6)'inde polimiksine duyarlılık saptandı. Suşların hiçbirinde rifampine duyarlılık saptanmadı (%0). **Sonuç:** Sonuç olarak, tüm bölgelerde MBL enziminin ortalama pozitiflik oranının %31,2 gibi yüksek bir düzeyde olduğu belirlenmiştir. Bu nedenle, moleküler yöntemle suşların MBL pozitifliğini doğrulamak, belli aralıklarla bölgesel antibiyotik sürveyansını gözden geçirmek ve bu suşların bölgesel yayılımını önlemek için elde edilen verileri ilgili kurumlarla paylaşmak faydalı olacaktır. Bu amaçla, antibiyotik sürveyans verilerini sistematik olarak kaydetmek ve izlemek gereklidir.

Anahtar Kelimeler: *Pseudomonas aeruginosa*; karbapenemaz; yoğun bakım; beta-laktamaz

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Pseudomonas aeruginosa is an important gram negative bacillus that occurs frequently in the hospital environment. It causes infections with high mortality rates and develops resistance to more than one antibiotic group through different mechanisms. Along with *Acinetobacter* species, it is the most common cause of nosocomial pneumonia and causes a high rate of mortality.[1]

In studies from Turkey, the most common gram negative bacilli in the hospital environment are *Pseudomonas* species, *Klebsiella* spp., and *Acinobater* spp in decreasing order.[2]

Recently, metallobeta-lactamase enzymes, which hidrolyze carbanepem group antibiotics (imipenem, meropenem), in nosocomial agents such as *P. aeruginosa*, *Klebsiella* spp., and *Acinetobacter* spp. have been reported in Turkey and other countries, leading to considerable concern.[3-10]

The aim of this study was to determine the frequency of metallobeta-lactamase (MBL) enzymes in *P. aeruginosa* strains resistant to imipenem in various regions of Turkey using the imipenem and imipenem+EDTA combined disk method. Additionally, to determine the minimum inhibitory concentration (MIC) levels of those strains for aztreonam, polymyxin, colistin, and rifampin with the E-test method.

MATERIAL AND METHODS

This study was carried out between May 2006-May 2007 in the Ankara Training and Research Hospital with the colloboration of Infectious Diseases and Clinical Microbiology Departments. Carbapenem resistant (imipenem and/or meropenem resistance) *P. aeruginosa* strains were obtained from 8 provinces from 7 different geographical regions of Turkey, so that they may be partially representative of Turkey. Overall, 186 *P. aeruginosa* strains were included in the study. The presence of metallobeta-lactamase was screened by imipenem and imipenem+EDTA disk. Distribution of *P. aeruginosa* strains according to clinical samples was shown in Table 1.

TABLE 1: The distribution of *P.aeruginosa* strains according to clinical samples.

Sample	Number	Percentage (%)
Blood	82	44
Tracheal aspirate	58	31
Sputum	16	8.6
Catheter	12	6.4
Urine	10	5.4
Pus	4	2.1
Cerebrospinal fluid	4	2.1
Total	186	

The preparation of Imipenem EDTA combined disk: For this method, 186.1 g disodium EDTA.2H_2O (Merck chemicals, Germany) was disolved in 1000 ml distilled water and 0.5 M EDTA solution was obtained. On imipenem disk (10mg Oxoid, UK) 5 mL (930 mg) 0.5 EDTA solution was added and combined disks were prepared (10mg/930mg). The pH was adjusted to 8.[11,12]

Combined disk synergy test: Bacteria suspension was prepared at 0.5 Mc Farland and was inoculated superficially on Mueller-Hinton medium. After the surface of the medium was dried, imipenem (IMP) and imipenem+EDTA (IMP-EDTA) combined disks were placed on the surface of the medium 22 mm apart. The medium was incubated at 37°C for one night and was evalauted the following day. A 7 mm or higher inhibition zone diameter of the combined disk was considered positive.[12]

P.aeruginosa strains obtained from the Hacettepe University Faculty of Medicine, Department of Pediatrics, Infectious Diseases Unit and VIM-5 metallobetalactamase producing status confirmed with molecular methods (polymerase chain reaction-PCR) were used as control strains. Screening for metallobeta-lactamase with imipenem and imipenem+EDTA combined disks was shown in Figure 1.

ANTIBIOTIC SUSCEPTIBILITY TESTS

Sensitivity to colistin, aztreonam, polymyxin and rifampin was determined with the E-test (AB, Biodisk, Sweden) method using MIC values.

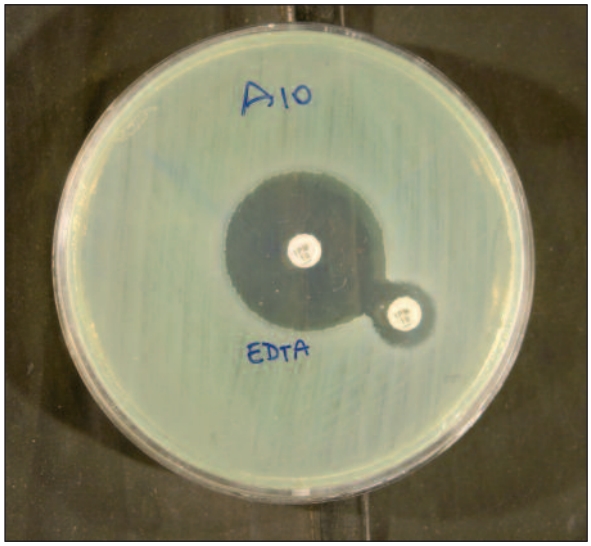

FIGURE 1: Screening for the presence of metallobetalactamase with imipenem and imipenem+EDTA combined disks.
(See for colored form http://tipbilimleri.turkiyeklinikleri.com/)

The E-test catalogue was used to establish MIC values. Susceptibilities of strains to each antibiotic were evaluated in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI).[13] *P.aeruginosa* ATCC 27853 was used as a control strain.

For aztreonam, strains with an MIC value of ≤ 8μg/ml, 16 μg/ml and ≥ 32 μg/mL were considered sensitive, intermediate sensitive and resistant, respectively. For colistin and polymyxin strains with an MIC value of ≤ 2 μg/mL were considered sensitive and those with an MIC value of ≥4 mg/mL resistant. For rifampin, strains with an MIC value of ≥4 μg/mL were considered resistant.[14]

All data were recorded in Excel program. Chi-square test was used for statistical analyses. A p value of ≤ 0.05 was considered statistically significant.

RESULTS

In 58 out of 186 carbapenem resistant strains (31.2%), metallobeta-lactamase positivity was established with the imipenem and imipenem+EDTA combined disk method. The comparison of MBL enzyme positivity between geographical regions and provinces revealed that the highest frequency was in Antalya (Mediterrenean region) (52%) and İstanbul (Marmara region) (50%) while the lowest was in Diyarbakır (6%) (Southeastern Anatolia).

Statistically significant diferences were found between provinces and geographical regions in terms of MBL positivity (p=0.014 and p= 0.010, respectively). Results were demonstrated in Table 2.

Antimicrobial susceptibilities of 186 *P.aeruginosa* strains to aztreonam, colistin, polymyxin, and rifampin were determined with the E-test, establishing their MIC values.

In 186 carbapenem resistant *P. aeruginosa* strains obtained from seven different geographical regions, sensitivities to aztreonam, colistin, polymyxin and rifampin were 72%, 83.35%, 79.6% and 0%, respectively.

MBL positive and negative strains were compared in terms of their sensitivities to aztreonam, colistin, polymyxin and rifampin. Forty-three of the 58 MBL positive strains and 91 (71%) of the 128 MBL negative strains were sensitive to aztreonam but this difference was not statistically significant (p>0.05).

Fifty-two (89.7%) of the 48 MBL positive strains and 103 (80.5%) of the 128 MBL negative strains were sensitive to colistin; the difference between the groups was not significant (p>0.05). While 49 (33%) of the 48 MBL (burada rakamsal hata var) positive strains were sensitive to

TABLE 2: The distribution of metallobeta-lactamase enzyme positivity according to regions and provinces.

Region/Province	Number of strains	MBL positivity	(%)
Marmara/Istanbul	18	9	(50)
Aegean/Izmir	30	7	(23.3)
Inner Anatolia/Ankara	20	6	(30)
Inner Anatolia/Konya	26	11	(42.3)
Mediterrenean/Antalya	25	13	(52)
Black sea/Trabzon	25	6	(24)
Southeastern Anatolia/Diyarbakır	17	1	(6)
Eastern anatolia/Van	25	5	(20)
Overall	186	58	(31.2)

MBL: Metallobeta-lactamase.

polymyxin, polymyxine sensitivity was present in 99 (67%) out of 128 MBL negative strains; again, the difference was not significant (p=0.328). The results were demonstrated in Table 3.

DISCUSSION

Gram negative bacteria develop resistance to carbapenem group antibiotics due to beta-lactamase enzymes hidrolyzing antibiotics. There are two groups of enzymes in gram negative bacteria that hydrolize carbapenem group antibiotics, namely,'serin carbapenamases' and 'metallobeta-lactamases'. Metallobeta-lactamase enzymes require +2 value metal cofactors (usually zinc). The transfer of MBL enzymes may be chromosome mediated or through transferable genes as in all beta-lactamase enzymes. Metallobeta-lactamase enzymes are categorized in three groups according to the classification of Buch. Many different MBL enzymes have been descibed in various Gram negative bacilli such as *Enterobacteriaceae*, *Acinetobacter* species, *P. aeruginosa* and *Klebsiella*.[3]

Recently, carbapemem resistant *P. aeruginosa* strains have been reported globally and in Turkey. This is due to the production of metallobeta-lactamase enzyme.[3-10]

Metallobeta-lactamase enzymes consist of four enzyme groups-IMP, VIM, SPM and GIM series.[3] Especially in Mediterranean countries, enzymes in VIM series are more common than the others are. IMP-1 was first described in a *Serratia marcescens* strain in Japan. Following this, VIM-1 was described in Italy. It is the second transferrable metallobeta-lactamase coded on integron.[3,5,10]

Owing to the coexistence of MBL genes with plasmids, which code for other antibiotic resistance genes, MBL positive strains are usually resistant to aminoglycoside group antibiotics and fluoroquinolones.[3,5] However, MBL producing strains are usually sensitive to polymyxin.[3] In animal experiments, aztreonam was reported to be effective in infections due to MBL positive strains.[15] There are no large-scale surveillance studies aiming to determine the proper treatment in infections due to MBL positive isolates. Therefore, in the treatment of these infections, the choice of antibiotics remains unclear. Moreover, although MBL enzyme inhibitors are effective in suitable conditions, there is no MBL inhibitor that can be used *in vivo*. In infections due to MBL positive strains, aztreonam has been shown to be effective.[3,16,17] The most effective treatment options in MBL positive bacteria infections are polymyxin and colistin. Polymyxin and colistin are effective in the treatment of infections caused by multi drug resistant gram negative bacteria. In addition, rifampicin has also shown to be effective against MBL positive *P. aeruginosa* strains.[3]

Metallobeta-lactamase enzymes have been reported from various geographical regions (South America, South Europa, and Southeastern Asia).[3-6] Lauplan et al. reported that 28 out of 228 patients infected with *P. aeruginosa* (12.8%) had MBL pro-

TABLE 3: Aztreonam, colistin, polymyxin and rifampin sensitivitities of MBL positive and MBL negative *P. aeruginosa* strains

		Sensitive		Resistant		Total	
Antibiotics	**MBL status**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Aztreonam	MBL positive	43	(74)	15	(26)	58	(31)
Aztreonam	MBL negative	91	(71)	37	(29)	128	(69)
Colistin	MBL positive	52	(89.7)	6	(10)	58	(31)
Colistin	MBL negative	103	(80.5)	25	(19.5)	128	(68.8)
Polymyxin	MBL positive	49	(33)	9	(23.7)	58	(31)
Polymyxin	MBL negative	99	(67)	29	(76)	128	(69)
Rifampin	MBL positive	0	(0)	58	(100)	58	(31.2)
Rifampin	MBL negative	0	(0)	128	(100)	128	(68.8)

MBL: Metallobeta-lactamase.

duction and a high rate of multi drug resistance in MBL producing strains and bacteremia. In the same study, mortality rate was higher in patients infected with MBL producing strains.[18] Sanchez et al. found MBL positivity in 4 out of 133 *P. aureginosa* strains resistant to carbapenem with the E- test method.[19] The strains with MBL positivity produce VIP type enzymes as determined with the PCR method.

Phenotypic methods have been developed to detect MBLs in routine microbiology laboratories using the characteristic of MBLs to be inhibited by EDTA, heavy metal salts and thiol compounds.[3,11,12,17]

Young et al. carried out a study on 102 *P. aeruginosa* and 20 *Acinetobacter baumannii* and 3 *Acinetobacter* spp. strains, most of which produced VIM-2 MBL.[12] They considered a difference of more than 7 mm between imipenem and imipenem+EDTA zone diameters as the sign of MBL positivity and reported that this method yielded excellent results for *P. aeruginosa* strains and good results for *Acinetobacter* spp. Walsh et al. reported that the sensitivity and specifity of IP and IP-EDTA E-test methods for MBL enzymes were 94% and 95%, respectively.[20] Toraman et al. established MBL enzyme positivity using E-test in 15 out of 52 *P. aeruginosa* strains, 5 out of 24 *A. baumannii* strains (21%) and 2 out of 2 *Klebsiella* strains (100%).[7] The antibiotic sensitivities in 22 strains producing MBL were isepamisin 73%, ciprofloxacin 64%, amikasin 59%, aztreonam 18%, tobramicin 18%, meropenem 14%, cefoperazone-sulbactam 5% and piperacillin-tazobactam 0%. When MIC values were compared between MBL producing and non-producing strains, the MIC value of meropenem was higher in strains producing MBL. Bayraktar et al. reported MBL positivity with IP/IPI (IP-R-EDTA) E tests in 17 out of 27 *P. aeruginosa* strains isolated from the intensive care unit.[9] They also established that the strains were resistant to other antipseudomonal beta-lactams. In this study, no relation was found between resistance to other beta-lactam phenotypes and MBL positivity.

Limoncu et al. investigated the presence of MBL in *P. aeruginosa* and *Acinetobacter* strains with MIC values for ceftazidim over 64mg/ml using thiol compounds and imipenem+EDTA disk synergy test.[10] In their study, the presence of MBL was detected by imipenem and imipenem+EDTA combined disk synergy test in 12 (32%) out of 38 *Pseudomonas aeruginosa* strains and in 5 (29%) out of 17 *Acinetobacter* spp isolates.

In the present study, the presence of MBL enzyme was established using imipenem and imipenem+EDTA combined disk synergy test in 58/186 (31.2%) carbapenem resistant *P. aeruginosa* strains collected from 7 different geographical regions. The rates we found were similar to those found by Toraman et al.and Limoncu et al.[7,10]

Our study is different from other studies in that it partially represents the population of Turkey. There are two studies carried out in Turkey to investigate MBL with molecular methods (PCR). In one of these studies, Bahar et al. in Ankara reported VIM-5 type MBL enzyme in a *P. aeruginosa* isolate resistant to carbapenem.[8] In the other study, Aktaş et al. reported the presence of VIM-5 type MBL in a *Klebsiella pneumoniae* strain resistant to imipenem by PCR in İstanbul, Turkey.[21] Arabacı and Oldacay reported imipenem resistance in 20 out of 108 *P. aeruginosa* strains isolated from patients hospitalized in the intensive care unit with 14 (70%) being phenotypically metallo-betalactamase positive by double-disk synergy methods.[22] The highest rate of metallobeta-lactamase positivity in carbapenem resistant *P. aeruginosa* strains in Turkey was reported in that study. The results of that study are discrepant with those of other studies and the present study.

Carbapenems consist of imipenem, meropenem, ertapenem etc. and have the broadest antibacterial activity currently available. Carbapenems are the most effective antibiotics against extended spectrum beta-lactamases (ESBL). Metallobeta-lactamase has a large hydrolysis spectrum including carbapenems and broad-spectrum cephalosporins. While IMP series MBLs are frequently reported in gram negative aerobes and

from Southeastern Asia, VIM series MBLs in *P. aeruginosa* have recently been reported from South Europe and Taiwan.[3-6,15]

Antibiotic treatment for infections due to metallobeta-lactamase producing *P. aeruginosa* strains is not clear.[3-5,16,17]

In a study by Yong et al. in Korea, acquired metallobeta-lactamase enzyme has been reported in 36 (6.2%) out of 581 *P.aeruginosa* strains, 4 (9.2%) out of 42 other *Pseudomonas* species and 13 (2.5%) out of 513 *Acinetobacter* spp. isolates.[8] In the study, bla VIM-2 genes were more common in the *Enterobactericeae* family including *Klebsiella pneumoniae*. In a study by Lee et al. in Korea, in 112 out of 1234 carbapenem resistant *Pseudomonas* spp. and *Acinetobacter* spp. strains, the presence of metallobeta-lactamase was detected by imipenem EDTA, imipenem-sodium merkaproacetic acid double disk synergy tests and E- test.[11] In that study, 204 strains (96%) had IMP-1and VIM-2 MBL ezymes detected with PCR.

IMP and VIM series that acquired MBL enzymes are common all around the world and have a widespread distribution. Recently, SPM type MBLs have been reported from Brazil and GIM series from Germany.[3-10] Valenza et al. in their study in Germany, found 8 MBL positive isolates out of 489 *P. aeruginosa* strainsusing the imipenem and EDTA combination test and the strains (one isolate VIM-1, seven isolates VIM-2 MBL) were confirmed by molecular methods.[23]

Gupta et al. in their study in India, reported metallo-betalactamase positivity in 13 (7.5%) out of 75 *Pseudomonas* and *Acinetobacter* strains, which showed decreased sensitivity to imipenem and ceftazidime.[16] The distribution of the strains was as follows: 9 *Pseudomonas aeruginosa*, 2 *Acinetobacter* spp, 1 *Pseudomonas fluorescens* and 1 *Pseudomonas putida*.

All MBL positive strains were resistant to ceftazidim, cefepim, piperacillin-tazobactam, amoxicillin-clavulanate, and cephoperazone-sulbactam, 72% of the MBL producing strains were resistant to amikasin, while 80% were resistant to tobramicin and 75% to ciprofloxacin. All strains were sensitive to aztreonam.

In the study by Navaneeth et al. from India, MBL positivity rate was reported to be 12% in all strains.[17] In their study, all MBL positive isolates were resistant to beta-lactam antibiotics (ceftazidim, cefepim, piperacillin) and all strains were sensitive to aztreonam.

In the present study, 58/186 (31.2%) 186 *P. aeruginosa* strains were MBL enzyme positive. MBL positivity showed significant difference between regions with the highest rates in Antalya (52%) and Istanbul (50%) and the lowest rate in Diyarbakır (6%).

In the present study, the difference in aztreonam, colistin and polymyxin sensitivities and MBL positivity rate in *P. aeruginosa* strains between regions may stem from the clonal and/or horizontal spread of resistance. Fiett J et al. carried out a study based upon molecular methods on *P. aeruginosa* strains resistant to carbapenem in different provinces of Poland and concluded that regional spread of MBL producing strains might originate from clonal spread, horizontal transfer of resitance determinats or from both.[24]

In the present study, in 186 carbapenem resistant *P. aeruginosa* strains, sensitivities to aztreonam, colistin, polymyxin and rifampin were 72%, 83.35%, 79.6% and 0%, respectively.

In conclusion, the overall mean rate of MBL positivity is 31.2% in all regions of Turkey, which is quite high. Therefore, it will be beneficial to confirm the MBL positivity of strains with molecular methods, to review regional antibiotic surveillance data at certain intervals and to share the obtained data with relevant institutions in order to prevent the regional spread of these strains. For this, it is necessary to record and monitor systematically the antibiotic surveillance data.

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REFERENCES

1. Verbist L. Epidemiology and sensitivity of 8625 ICU and hematology/oncology bacterial isolates in Europe. International Study Group. Scand J Infect Dis Suppl 1993;91:14-24.
2. Gür D, Ünal S and Studies Group. [Resistance Rates to Selected Antibiotics in Gram Negative Bacteria Isolated from Intensive Care Units (ICU) in Turkish Hospitals]. Flora Dergisi 1996;1(3):153-9. [Turkish].
3. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? Clin Microbiol Rev 2005;18(2): 306-25.
4. Giske CG, Libisch B, Colinon C, Scoulica E, Pagani L, Füzi M, et al. Establishing clonal relationships between VIM-1-like metallo-beta-lactamase-producing Pseudomonas aeruginosa strains from four European countries by multilocus sequence typing. J Clin Microbiol 2006;44(12):4309-15.
5. Nouér SA, Nucci M, de-Oliveira MP, Pellegrino FL, Moreira BM. Risk factors for acquisition of multidrug-resistant Pseudomonas aeruginosa producing SPM metallo-beta-lactamase. Antimicrob Agents Chemother 2005; 49(9):3663-7.
6. Yong D, Choi YS, Roh KH, Kim CK, Park YH, Yum JH, et al. Increasing prevalence and diversity of metallo-beta-lactamases in Pseudomonas spp., Acinetobacter spp., and Enterobacteriaceae from Korea. Antimicrob Agents Chemother 2006;50(5):1884-6.
7. Toraman ZA, Yakupogulları Y, Kizirgil A. Detection of metallo beta-lactamase production and antibiotic resistance with E-test method in Pseudomonas, Acinetobacter and Klebsiella strains, in Turkey. J Infect Chemother 2004; 10(5):257-61.
8. Bahar G, Mazzariol A, Koncan R, Mert A, Fontana R, Rossolini GM, et al. Detection of VIM-5 metallo-ß-lactamase in a Pseudomonas aeruginosa clinical isolate from Turkey. J Antimicrob Chemother 2004;54(1):282-3.
9. Bayraktar B, Yıldız D, Bulut E. [Investigation of metallobeta-lactamase production in carbapenem resistant Pseudomonas aeruginosa strains isolated in intensive care unit]. Türk Mikrobiyol Cem Derg 2004;34(4):248-52.
10. Limoncu MH, Ermertcan Ş, Çavuşoğlu C, Eraç B. [The investigation of metallo-beta-lactamase enzymes in Pseudomonas aeruginosa and Acinetobacter species isolated from nosocomial infections]. Türk Mikrobiyol Cem Derg 2006;36(3):79-82.
11. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2003;41(10):4623-9.
12. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2002;40(10):3798-801.
13. Clinical and Laboratory Standards Institute (CLSI). [Performance standards for antimicrobial susceptibilities tests]. Onbeşinci Bilgi Eki. Gür D, editör. Ankara: Bilimsel Tıp Yayınevi; 2005; M2-A8 ve M7-A6, S:40-8, 108-9.
14. Timurkaynak F, Can F, Azap OK, Demirbilek M, Arslan H, Karaman SO. In vitro activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of Pseudomonas aeruginosa and Acinetobacter baumannii isolated from intensive care units. Int J Antimicrob Agents 2006;27(3):224-8.
15. Bellais S, Mimoz O, Leotard S, Jacolot A, Petitjean O, Nordmann P. Efficacy of β-lactams for treating experimentally induced pneumonia due to a carbapenem hydroyzing matallo-β-lactamase-producing strains of Pseudomonas aeruginosa. Antimicrob Agents Chemother 2002;46(6):2032-4.
16. Gupta V, Datta P, Chander J. Prevalence of metallo-beta lactamase (MBL) producing Pseudomonas spp. and Acinetobacter spp. in a tertiary care hospital in India. J Infect 2006; 52(5):311-4.
17. Navaneeth BV, Sridaran D, Sahay D, Belwadi MR. A preliminary study on mettalo β-lactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian J Med Res 2002; 116:264-7.
18. Laupland KB, Parkins MD, Church DL, Gregson DB, Louie TJ, Conly JM, et al. Population-based epidemiological study of infections caused by carbapenem-resistant Pseudomonas aeruginosa in the Calgary Health Region: importance of metallo-beta-lactamase (MBL)-producing strains. J Infect Dis 2005;192(9): 1606-12.
19. Sánchez A, Salso S, Culebras E, Picazo JJ. [Carbapenem resistance determined by metalloenzymes in clinical isolates of Pseudomonas aeruginosa]. Rev Esp Quimioter 2004; 17(4):336-40.
20. Walsh TR, Bolmström A, Qwärnström A, Gales A. Evaluation of a new Etest for detecting metallo-beta-lactamases in routine clinical testing. J Clin Microbiol 2002;40(8):2755-9.
21. Aktas Z, Bal C, Midilli K, Poirel L, Nordmann P. First IMP-1-producing Klebsiella pneumoniae isolate in Turkey. Clin Microbiol Infect 2006; 12(7):695-6.
22. Arabacı F, Oldacay M. The investigation of antimicrobial resistance and metallo-beta-lactamase production of Pseudomonas aeruginosa strains isolated from intensive care unit patients. Türk Mikrobiyol Cem Derg 2010;40(1): 37-40.
23. Valenza G, Joseph B, Elias J, Claus H, Oesterlein A, Engelhardt K, et al. First survey of metallo-beta-lactamases in clinical isolates of Pseudomonas aeruginosa in German university hospital. Antimicrob Agents Chemother 2010;54(8):3493-7.
24. Fiett J, Baraniak A, Mrówka A, Fleischer M, Drulis-Kawa Z, Naumiuk Ł, et al. Molecular epidemiology of acquired mettalo-β-lactamase producing bacteria in Poland. Antimicrob Agents Chemother 2006;50(3):880-6.