Research Article / Araştırma Makalesi

Investigation of Carbapenemase Production Among Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates

Karbapeneme Dirençli *Pseudomonas aeruginosa* İzolatlarında Karbapenemaz Üretiminin Araştırılması

Shirwan Hussein DARWEESH [1], Umut Safiye ŞAY COŞKUN [2]

[1]Department of Medical Microbiology, College of Health Sciences, Cihan University-Duhok, Duhok, IRAQ
[2]Department of Medical Microbiology, Faculty of Medicine, Tokat Gaziosmanpaşa University, Tokat, TÜRKİYE

Abstract

Background: *Pseudomonas aeruginosa* is an opportunist organism that causes potentially life threatening nosocomial infections, particularly in immunocompromised patients. Carbapenems are regarded to be the last line of treatment against severe infections caused by multi drug resistant *P. aeruginosa* isolates. *isolates.* Production of the carbapenemase enzyme is the primary mechanism of carbapenem resistance and has become a serious health concern worldwide as these enzymes are highly transferable and limit therapeutic alternatives. Rapid detection of carbapenemase production is important for prompt planning the treatment of carbapenemase-producing isolates and preventing the spread of these strains. This study aimed to investigate carbapenemase production in carbapenem resistant *Pseudomonas aeruginosa* isolates by the Carbapenem inactivation method.
Materials and Methods: In this retrospective study a total of 172 *Pseudomonas aeruginosa isolates* were obtained from different samples sent from various clinics to Tokat Gaziosmanpaşa University Research and Application Hospital Microbiology Laboratory between 2016-2019 and were evaluated. Of the 172 isolates, 51 (29.7%) were found to be carbapenem- resistant and included in this investigation. Identification and antibiotic susceptibility tests of the isolates were performed with the Vitek 2 (Biomerieux, France) automated system. Carbapenem sensitivities were also determined by the disc diffusion method. Carbapenemase production in isolates was investigated by the Carbapenem inactivation method.
Results: These samples were sent from clinical units, such as neurology (n =10), general surgery (n =8), internal medicine (n =7), and pediatric (n =6). The isolates were identified from wounds (n = 17), sputum (n = 15), blood (n = 11), urine (n = 5), and cerebrospinal fluid (n = 3) samples. Of all the carbapenem –resistant samples 32 (62.8%) were obtained from male, and 19 (37.3%) from female patients. Of the 51 carbapenem resistant isolates, 38 (74.5%) were found to be resistant to both imipenem and meropenem. Eight (15.7%) isolates were found to be resistant to imipenem only, and five (9.8%) isolates were resistant to meropenem. Carbapenemase production was detected in 31 (60.8%) isolates by using using the Carbapenem inactivation method. The antibiotic resistance rates of the carbapenem-resistant isolates were as follows: piperacillin-tazobactam 65%, amikacin 6.8%, gentamicin 15.2%, ceftazidime 34.6%, cefepime 38.3%, ciprofloxacin 26.7%, levofloxacin 24.2%.
Conclusions: Rapid identification of carbapenemase enzymes among carbapenem resistant *Pseudomonas aeruginosa* isolates using phenotypic and genotypic approaches is important to control the transmission of infection caused by carbapenem-resistant isolates and to control the morbidity and mortality associated with them. In this study, the carbapenem inactivation test was seen as a method that can be preferred in the laboratory in terms of its easy and fast application in the detection of carbapenemase production.

Key Words: *Pseudomonas aeruginosa*, Carbapenem inactivation method, Antimicrobial resistance

Öz

Amaç: *Pseudomonas aeruginosa*, özellikle immünkompromize hastalarda hayati tehlike oluşturan hastane enfeksiyonlarına yol açan fırsatçı bir patojendir. Karbapenemler, çoklu ilaç direnci olan *Pseudomonas aeruginosa* izolatlarının neden olduğu ciddi enfeksiyonlara karşı son tedavi seçeneği olarak kabul edilmektedir. Karbapenemaz enzim üretimi karbapenem direncinin ana mekanizmalarından biridir. Karbapenemaz direnç genleri yüksek oranda aktarılabilir olduğundan ve terapötik seçenekleri sınırladığından dünya çapında ciddi bir sağlık sorunu haline gelmiştir. Karbapenemaz üretiminin hızlı tespiti, karbapenemaz üreten izolatların tedavisinin hızlı bir şekilde planlanması ve bu suşların yayılmasının önlenmesi için önemlidir. Bu çalışmada, karbapenem dirençli *Pseudomonas aeruginosa* izolatlarında karbapenemaz üretiminin Karbapenem inaktivasyon yöntemi ile araştırılması amaçlanmıştır.
Materyal ve Metod: Bu retrospektif çalışmada 2016-2019 yılları arasında Tokat Gaziosmanpaşa Üniversitesi Araştırma ve Uygulama Hastanesi Mikrobiyoloji Laboratuvarına çeşitli kliniklerden gönderilen farklı örneklerden toplam 172 *Pseudomonas aeruginosa* izolatı değerlendirilmiştir. Toplam 172 izolatın 51'i (%29,7) karbapenem dirençli bulunarak çalışmaya dahil edilmiştir. İzolatların tanımlanması ve antibiyotik duyarlılık testleri Vitek 2 (Biomerieux, Fransa) otomatize sistemi ile gerçekleştirilmiştir. Karbapenem duyarlılıkları ayrıca disk difüzyon yöntemiyle de belirlenmiştir. İzolatlarda karbapenemaz üretimi Karbapenem inaktivasyon yöntemi ile araştırılmıştır.
Bulgular: Örnekler nöroloji (n =10), genel cerrahi (n =8), dahiliye (n =7) ve pediatri (n =6) dahil olmak üzere çeşitli klinik birimlerden gönderilmiştir. İzolatlar yara (n = 17), balgam (n = 15), kan (n = 11), idrar (n = 5) ve beyin omurilik sıvısı (n = 3) örneklerinden tanımlanmıştır. Karbapenem dirençli örneklerin 32'si (%62,8) erkek, 19'u (%37,3) kadın hastalardan elde edilmiştir. Karbapeneme dirençli 51 izolatın 38'i (%74,5) hem imipenem hem de meropeneme dirençli bulunmuştur. Sekiz (%15,7) izolat sadece imipeneme ve beş (%9,8) izolat meropeneme dirençli bulunmuştur. Karbapenem inaktivasyon yöntemi ile 31 (%60,8) izolatta karbapenemaz üretimi tespit edilmiştir. Karbapenem dirençli izolatların antibiyotik direnç oranları şu şekildedir: piperacillin-tazobactam %65, amikasin %6.8, gentamisin %15.2, seftazidim %34.6, sefepim %38.3, siprofloksasin %26.7, levofloksasin %24.2.
Sonuç: Karbapeneme dirençli *Pseudomonas aeruginosa* izolatları arasında karbapenemaz enzimlerinin fenotipik ve genotipik yaklaşımlar kullanılarak hızlı bir şekilde tanımlanması, karbapeneme dirençli izolatların neden olduğu enfeksiyonun bulaşmasını kontrol etmek ve bunlarla ilişkili morbidite ve mortaliteyi kontrol etmek için önemlidir. Bu çalışmada karbapenem inaktivasyon testi, karbapenemaz üretiminin tespitinde kolay ve hızlı uygulanabilmesi açısından laboratuvarda tercih edilebilecek bir yöntem olarak görülmüştür.

Anahtar Kelimeler: *Pseudomonas aeruginosa,* Karbapenem inaktivasyon yöntemi, Antimikrobiyal direnç.

Corresponding Author/Sorumlu Yazar

Dr. Umut Safiye ŞAY COŞKUN
Tokat Gaziosmanpaşa Üniversitesi Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Muhittin Fisunoğlu Cad, Ali Şevki EREK Yerleşkesi 60200 Tokat, TÜRKIYE

E-mail: umut.saycoskun@gop.edu.tr

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Introduction

Pseudomonas aeruginosa (P.aeruginosa) is a common gram-negative aerobe from the *Pseudomonadaceae* family. *P.aeruginosa* is rod-shaped and found alone, in pairs, or in short chains. The name aeruginosa refers to the green-blue colour of clinical isolates' colonies. *P. aeruginosa* does not ferment carbohydrates, instead producing acid from sugars (1). This bacterium is a notable model of antimicrobial resistance among bacterial pathogens because it possesses almost all the known mechanisms to resist antibiotic. There are three main mechanisms through which *P.aeruginosa* may develop carbapenem resistance. Including mutations in the outer membrane porin OprD gene, overexpression of efflux pumps, and carbapenemase production (2,3).

Centers for Disease Control and Prevention (CDC) were classified 18 different types of pathogens into three categories: urgent, serious or alarming in 2019. It was determined that the multidrug-resistant *P.aeruginosa* was classified as a serious threat. Some of multidrug-resistant *P.aeruginosa* isolates are resistant to nearly all antibacterial agents. Two to 3 % of *P.aeruginosa* that is resistant to carbapenems have a mobile genetic element that makes the carbapenemase enzyme. CDC also highlighted that drug development should be focused on the most dangerous infections, like those caused by carbapenemase- producing bacteria, including carbapenem-resistant *P.aeruginosa* (4).

Carbapenems are regarded to be the last treatment options against infections caused by multi drug resistant *P. aeruginosa* (2,5). Nevertheless, increased carbapenem resistance has been reported in *P. aeruginosa* all over the world, with carbapenem-resistance prevalence varying from 10-50% in the majority of countries (6). Carbapenem- resistant *P. aeruginosa* is sometimes resistant to nearly all other antibacterials, which makes infections more difficult to treat and increases morbidity and mortality rates among patients that are hospitalized or have compromised immune systems (7).

Production of carbapenemase enzymes by *P.aeruginosa* has become a serious health issue globally as these enzymes inactivate almost all beta-lactam antibiotics, including carbapenems. Antibiotic resistance spreads easily among diverse bacterial species, often carrying multiple resistance determinants, thus limiting treatment options. For these reasons, these enzymes have been studied in numerous studies worldwide, by using different phenotypic and genotypic methods. Finding carbapenemase enzymes by molecular techniques is the gold standard. However, these approaches are more costly, appropriate laboratory requirements are necessary, and unable to identify unknown and novel resistance genes 8). The carbapenem inactivation method (CIM) is one of several phenotypic methods developed to determine carbapenemase production. Especially in the last five years, this method has become popular. The CIM method has become the most commonly used procedures for phenotypically determining carbapenemase production since this method is simple, reliable, and can be carried out in any laboratory (9). In this study, it was aimed to investigate carbapenemase production in carbapenem- resistant *P.aeruginosa* isolates by carbapenem inactivation method.

Materials and Methods

In this retrospective study *P. aeruginosa* isolates were obtained from various samples (blood, wounds, urine, cerebrospinal fluid, and sputum) that were sent to the Tokat Gaziosmanpaşa University Research and Application Hospital Microbiology Laboratory from various clinics between 2016-2019 were evaluated. Only one isolate from each patient was included in the study. A total 51 (29.7%) of 172 isolates were found to be carbapenem-resistant and included in the study. Identification of the isolates were performed with the conventional methods methods and Vitek 2 (Biomerioux, France) automated system. Antibiotic susceptibility of the isolates were done by Vitek 2 (Biomerioux, France) automated system according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (10). The identified *P. aeruginosa* isolates were stored in skim milk at -80℃ till the study.

The stocks of *P.aeruginosa* isolates were revived by culturing onto blood agar (HiMedia Turkey), and eosin methylene blue agar (HiMedia Turkey). The Kirby- Bauer disk diffusion methods were used for both imipenem 10 µg (Bioanalyse) and meropenem 10 µg (Liofilchem) for carbapenem susceptibility. Carbapenemase activity was detected phenotypically by carbapenem inactivation method (9).

Carbapenem Inactivation Method: For each isolate, a loopful of 10 µl µl culture and a disc of 10 g meropenem (Oxoid Ltd, Hampshire, United Kingdom) were suspended in 400 µl of distilled water and incubated at 35°C for four hours. *Escherichia coli* ATCC 0.5% McFarland suspension was distributed onto Mueller-Hinton agar (HiMedia Turkey) and left to dry for 3-10 minutes at room temperature. The meropenem disc was removed from the solution after incubation and streaked onto Mueller-Hinton agar containing *Escherichia coli* ATCC. The plates were incubated for four hours at 37°C. Positive inhibitory zone diameters ranging from 0 to 16 mm were regarded positive, as was satellite expansion of colonies measuring 16-18 mm. An inhibitory zone diameter of 19 mm showed negative results Fig 2 (9).

Statistical analysis: The data was statistically analysed using SPSS Statistical Program Version 21.0. (SPSS Inc., Chicago, Illinois, USA). For the description of quantitative variables with a normal distribution, mean and standard deviation were utilizedMean and range were used to characterize non-normally distributed data. The qualitative characteristics were described using numbers and percentages.

Results

A total of 172 *P. aeruginosa* isolates were isolated from clinical samples of patients from various units. Only 51 (29.7%) samples were resistant to imipenem and/or meropenem. These 51 carbapenem-resistant isolates were included in this study. A total of 38 (74.5%) isolates were resistant to both imipenem and meropenem. Eight (15.7%) isolates were only resistant to imipenem. And five (9.8%) isolates were resistant to meropenem.

Isolates were identified from different clinical specimens including wound (n = 17), sputum (n = 15), blood (n = 11), urine (n = 5) and cerebrospinal fluid (n = 3). In addition, of all the 51 carbapenem resistant isolates, 32 (62.8%) samples were obtained from male patients, and 19 (37.3%) samples were obtained from female patients. These samples were collected from a variety of clinical units, including neurology (n =10), general surgery (n =8), internal medicine (n =7), and pediatric (n =6). The clinics where samples were sent shown in Figure 1.

Carbapenemase enzyme production was determined by CIM in a total of 31 (60.8%) isolates. CIM positive isolates were shown in Figure 2.

Figure 1. Distribution of the clinics in which samples were sent

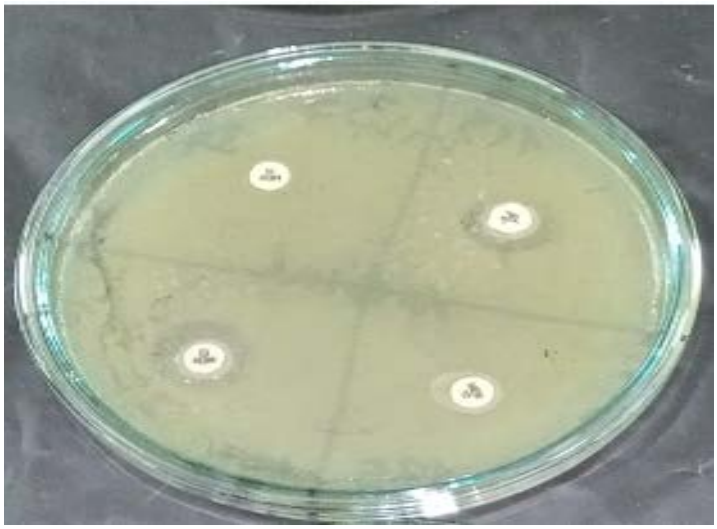

Figure 2. CIM positive isolates

Discussion

Carbapenems are the most efficient antibiotic classes for treating *P. aeruginosa* infections. Also, carbapenems are the last choice for treatment of infections caused by multidrug-resistant *P. aeruginosa* infections (11). Carbapenemase production is a critical factor of carbapenem resistance in *P. aeruginosa*. As a consequence, identifying and monitoring carbapenemase-producing isolates has become critical for the selection of the most effective treatment strategies and the successful execution of infection control measures in hospital settings (12).

Acar et al. performed a meta-analysis in 2019 determined the resistance to meropenem was 30.1% and resistance to imipenem was 28.0%. The researchers concluded that the prevalence of antibiotic resistance in pathogenic strains of *P. aeruginosa* in Turkey is considerably high and continues to rise compared to previous years (13). Imipenem and meropenem resistance rate in *P.aeruginosa* was found as 26.8% and 25.1%, respectively, according to a meta-analysis performed by Işık et al. The authors concluded frequency of *P. aeruginosa* strains resistant to carbapenems has dramatically risen in Turkey as a direct result of the extensive usage of these antibiotics (14). *P.aeruginosa* was determined one of the most frequently bacteriae identified from respiratory tract samples, and 43% of these isolates were susceptible to imipenem and 34.5% to meropenem in 2021 (15). Similarly, the findings of our study showed that resistance to imipenem and meropenem is critically high. And this means that carbapenem resistance is alarmingly prevalent in Turkey.

Production of carbapenemase enzymes by *P.aeruginosa* has become a serious health issue globally as these enzymes inactivate almost all beta-lactam antibiotics, including carbapenems. Their production is one of the main resistance mechanisms of *P. aeruginosa*, and it is easily spread between various bacterial species, which frequently carry multiple resistance determinants, thereby limiting treatment options. For these reasons, these enzymes have been studied in numerous studies worldwide, by using different phenotypic and genotypic methods. (8). In contrast, several phenotypic methods have been developed for the detection of carbapenemase production including the carbapenem inactivation method. The carbapenem inactivation method is one of the most commonly used procedures for phenotypically determining carbapenemase production since this method is simple, reliable, and can be carried out in any laboratory (9). Carbapenem inactivation method has been widely utilized for the identification of carbapenemase enzyme and also it was evaluated in several studies. Akhi et al. evaluated phenotypic methods to investigate carbapenemase production in isolates of carbapenem-resistant *P. aeruginosa.* Carbapenem resistance was found in 121 of the 245 *P. aeruginosa* isolates tested. A total of 35 (28.9%) carbapenem-resistant isolates were positive with CIM. Moreover, CIM has been shown to be highly effective considering its sensitivity, specificity, low cost, and simplicity of result interpretation. CIM was suggested to be used routinely in clinical laboratories for the identification of carbapenemase-producing *P. aeruginosa* (16). Aktaş et al. reported the sensitivity and specificity of the CIM test were 78% and 100%, respectively. Several advantages of carbapenem inactivation method were highlighted, including cost-effectiveness, the use of simple types of equipment that are broadly supplied at laboratories, and interpreting carbapenem inactivation method findings is relatively easy (8) In another study, 84 isolates of carbapenem-resistant *P.aeruginosa* were screened to investigate the presence of the carbapenemase enzyme, but it was detected in only three isolates by carbapenem inactivation method (17). Gutiérrez et al. highlighted that CIM is highly promising method for routine use because of its sensitivity and specificity. It is also inexpensive and simple to use. Furthermore, utilizing the disks to evaluate the inhibition zones simplifies the interpretation of the results and improves both sensitivity and specificity of the test (18).

Beig et al. determined the carbapenemase production and to assess the efficiency of phenotypic methods, including CIM for quick and reliable identification of carbapenemase enzyme in 97 different strains of *P. aeruginosa* that had been obtained from samples collected in hospitals located in Hamadan, Iran in 2021. Their findings showed that 49 isolates were resistant to carbapenems. Carbapenem enzyme production was identified in 46 isolates of carbapenem resistant *P. aeruginosa* by using the CIM. They demonstrated that CIM methods can be used for fast and accurate identification of carbapenemase enzymes. Additionally, this method is suitable to use in medical microbiology laboratories (19). In 2017, Malkoçoğlu et al. indicated carbapenemase production was shown in only three of 84 isolates, and noted that the carbapenem resistance in other strains could be due to another mechanism, for example, porin alterations, and efflux pumps (20). Carbapenemase production and their encoding genes were screened among 70 isolates *P. aeruginosa* in another research by Çiçek et al. They reported that 34.3% of isolates were resistant to carbapenem, also the genes encoding these enzymes were found in 36 isolates (6).

Tufekçi et al. reported 78% (n =57) of isolates produce carbapenemase by CIM (21). Similarly, the results of the present study 60.8% of isolates were found to be positive for carbapenemase activity by the phenotypic CIM test. The results of this study revealed a noticeably high prevalence of carbapenemase-producing *P. aeruginosa* isolates at our hospital.

Early and further investigation of carbapenemase production in *P.aeruginosa* is urgently needed to prevent carbapenemase spread among gram-negative bacteria in healthcare settings.

This research has certain limitations. Infections caused by multidrug-resistant bacteria that have been treated with a wide range of antibiotics and have failed therapies require considerably more detailed guidance to enable the selection of an appropriate antibiotic. Among these microbiological criteria is the antimicrobial's minimum inhibitory concentration

(MIC). However in this study, MIC was not evaluated. Moreover, carbapenemase production has not been studied by molecular methods.

Conclusion

In order to avoid or minimize the development and further spread of bacterial resistance, infection prevention and control and principled antibiotic utilization strategies need to be improved, and antibiotic resistance profiles should be monitored continuously. Reliable and convenient phenotypic laboratory tests are available to detect carbapenemase-producing *P.aeruginosa*. Rapid detection of carbapenemase production is important for prompt planning the treatment of carbapenemase-producing isolates and preventing the spread of these strains. Based on the results of this study, the carbapenem inactivation test was found to be a simple and cost-effective method for the identification of carbapenemase synthesis.

Ethical Approval: *This study was approved by Tokat Gaziosmanpaşa University Ethics Committee for Clinical Research decision number: 21-KAEK-243.*

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Concept: S.H.D., U.S.Ş.C.
Literature Review: S.H.D., U.S.Ş.C.
Design : S.H.D., U.S.Ş.C.
Data acquisition: S.H.D.
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Writing manuscript: S.H.D., U.S.Ş.C.
Critical revision of manuscript: U.S.Ş.C.

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