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# Çeşitli Klinik Örneklerden Elde Edilen Klebsiella pneumoniae'de Karbapenemaz Üretimi ve Tiplendirilmesinde Fenotipik ve Genotipik Yöntemlerin Değerlendirilmesi

## Evaluation of Phenotypic and Genotypic Methods in Carbapenemase Production and Typing in Klebsiella pneumoniae Obtained from Various Clinical Samples

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**ÖZ**

**Amaç:** Karbapenemaz üreten Enterobacteriaceae kaynaklı enfeksiyonlar tüm dünyada halk sağlığını tehdit eden güncel sağlık sorunlarındandır. Tedavisinde dakikaların bile önemli olduğu bu bakterilerde karbapenemaz tespiti için çeşitli hızlı tanı testleri kullanılmaktadır. Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yöntemi; yaklaşık bir saatte, *in vitro* ortamda bakterideki karbapenemazın karbapenemi hidroliz etmesiyle ortam pH'ını düşürerek fenol kırmızısının renginin değişmesi prensibiyle çalışır. Çalışmamızda karbapenem dirençli *Klebsiella pneumoniae* izolatlarında karbapenemaz varlığı, bu yöntemle tespit edilerek yöntemin duyarlılık ve özgüllüğünün araştırılması amaçlanmıştır.

**Gereç ve Yöntem:** Çalışmamıza hastanemiz mikrobiyoloji laboratuvarına 2018-2023 yılları arasında gönderilmiş çeşitli klinik örneklerden elde edilen rutin laboratuvar testleriyle tanımlanıp antimikrobiyal duyarlılıkları belirlenmiş karbapenem dirençli 100, karbapenem duyarlı 25 *K.* pneumoniae izolatı dahil edilmiştir. İzolatların tümünde enzim-substrat ilişkisine dayalı reaksiyon temelli yöntem ile karbapenemaz varlığı araştırılmıştır. Yöntemin duyarlılık ve özgüllüğü, bu tanıda altın standart olan PZR ile karbapenemaz genlerinin (oxa-48, ndm, kpc, imp ve vim) tespitiyle belirlenmiştir.

**Bulgular:** PZR ile karbapenem dirençli izolatların 97'sinde (*oxa-48* n=58, *ndm* n=16, *oxa-48+ndm* n=15 ve *kpc* n=8) karbapenemaz geni tespit edilmiştir. Bu izolatların 94'ü enzim-substrat ilişkisine dayalı reaksiyon temelli yöntem ile karbapenemaz pozitif saptanmıştır. Karbapenem duyarlı izolatların tümü hızlı tanı yöntemiyle karbapenemaz negatif saptanmıştır. Hızlı tanı yönteminin; duyarlılığı %96,9, özgüllüğü %100, pozitif prediktif değeri %100, negatif prediktif değeri ise %90,3 olarak hesaplanmıştır.

**Sonuç:** Karbapenemaz üretiminin hızlı tespiti için kullanılan testlerde maliyet, zaman ve uzman personel gereksinimi gibi birtakım sorunlar bulunmaktadır. Çalışmamızda test ettiğimiz yöntem uygun maliyetli, kolay uygulanabilir ve ortalama 1 saatte karbapenemaz varlığını yüksek duyarlılık ve özgüllükte tespit etmektedir. Kısa sürede doğru ve güvenilir sonuç veren bu yöntemin rutin laboratuvarlarda kullanımı değerlendirilmelidir.

**Anahtar kelimeler:** Klebsiella pneumoniae, karbapenem direnci, karbapenemaz, hızlı tanı testi



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**ABSTRACT**

**Objective:** Infections caused by carbapenemase-producing Enterobacteriaceae are among the current health problems that threaten public health all over the world. Various rapid diagnostic tests are used to detect carbapenemase in these bacteria, for which even minutes are important in their treatment. Reaction-based colormatic rapid diagnostic method based on enzyme-substrate interaction works on the principle of changing the color of phenol red by lowering the pH of the environment when the carbapenemase in the bacteria hydrolyzes the carbapenem. In our study, we aimed to detect the presence of carbapenemase in carbapenem-resistant *Klebsiella pneumoniae* isolates with this method and to investigate the sensitivity and specificity of the method.

**Methods:** Our study included 100 carbapenem-resistant and 25 carbapenem-sensitive *K. pneumoniae* isolates, whose antimicrobial susceptibility was determined by routine laboratory tests obtained from various clinical samples sent microbiology laboratory between 2018 and 2023. The presence of carbapenemase was investigated in all isolates with a reaction-based method based on enzyme-substrate interaction. The sensitivity and specificity of the method were determined by the detection of carbapenemase genes (*oxa-48, ndm, kpc, imp* and *vim*) by PCR that used as the gold standard for this diagnosis.

**Results:** Carbapenemase gene was detected in 97 of the carbapenem-resistant isolates (*oxa-48,* n=58; *ndm,* n=16; *oxa-48+ndm,* n=15 and *kpc,* n=8) by PCR. Ninety-four of these isolates were detected as carbapenemase positive by the reaction-based method based on enzyme-substrate interaction. All carbapenem-susceptible isolates were detected as carbapenemase negative by rapid diagnostic method. For rapid diagnostic method; ~~its~~ sensitivity was calculated as 96.9%, specificity as 100%, positive predictive value as 100%, and negative predictive value as 90.3%.

**Conclusion:** There are problems in the tests used for the rapid detection of carbapenemase production, such as cost, time and the need for expertised personnel. The method tested in our study is cost-effective, easily applicable, and detects the presence of carbapenemase with high sensitivity and specificity in approximately 1 hour. The routine utilization of this method which provides accurate and reliable results in a short time period should be evaluated in clinical laboratories.

**Keywords**: Klebsiella pneumoniae, carbapenem resistance, carbapenemase, rapid diagnostic testenhancing awareness of a healthy lifestyle.

**Keywords:** Anxiety, COVID-19, Mental health, Healthy lifestyle, Stress.

## 1. GİRİŞ

Dünya'da ve ülkemizde en önemli halk sağlığı sorunlarından biri antibiyotiklere dirençli bakterilerin yol açtığı enfeksiyon hastalıklarıdır. Antibiyotiklere dirençli patojenler, tedaviyi zorlaştırarak morbidite ve mortalitede artışa yol açmaktadır. Gram negatif bakterilerden Enterobacteriaceae ailesine üye Klebsiella pneumoniae, hastane kaynaklı enfeksiyonlarda en sık karşılaşılan patojenlerdendir. K. pneumoniae, başta beta-laktam antibiyotikler olmak üzere florokinolonlar ve aminoglikozitler gibi birçok antibiyotik grubuna karşı direnç göstermektedir (Ferreira ve ark., 2019).

Üçüncü kuşak sefalosporinler dahil çoğu antibiyotiğe dirençli K. pneumoniae kaynaklı enfeksiyonların tedavisinde kurtarıcı antibiyotik olarak kullanılan karbapenem grubu antibiyotiklere karşı direnç tüm dünyada olduğu gibi ülkemizde de ciddi oranlarda saptanmaya başlamıştır. Dünya Sağlık Örgütü'nün 2020 yılı Orta Asya ve Avrupa Antimikrobiyal Direnç Gözetimi Raporu'nda (CAESAR) Türkiye'de invaziv örneklerden elde edilen *K. pneumoniae* izolatlarında karbapenem direncinin %39-51 arasında değiştiği bildirilmiştir (WHO, 2020).

Gram negatif bakterilerde karbapenemlere direnç, başta antibiyotiğin yıkımına yol açan karbapenemaz enzimlerinin üretimi olmak üzere, porin kaybına bağlı azalmış membran geçirgenliği veya efluks pompası yoluyla antibiyotiğin dışa atımı gibi mekanizmalara bağlı gerçekleşmektedir (Stuart ve ark., 2010). K. pneumoniae karbapenem direncini genellikle çeşitli karbapenemazlar (Klebsiella pneumoniae carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Imipenem-resistant Pseudomonas (IMP), Verona integron-encoded metallo-β – laktamase (VIM), Oxacillinase-48 (OXA-48) vb.) üreterek sağlamaktadır. Hastalık Kontrol ve Önleme Merkezi'nin (CDC) yayınladığı raporda karbapenemaz üreten Enterobacteriaceae üyeleri, "Acil Tehdit Oluşturan Bakteriler" listesinde yer almaktadır (CDC, 2019). Dolayısıyla bu bakteride karbapenemaz üretiminin tespiti gerekli olup bu amaçla kullanılacak yöntemin seçimi, hızlı ve doğru sonuçlar elde edilmesi açısından büyük önem taşımaktadır.

Bu amaca yönelik olarak karbapenemaz üretimini tespit etmek için çeşitli hızlı tanı yöntemleri geliştirilmiş ve kullanılmaktadır. Kullanılacak yöntemin yüksek duyarlılık ve özgüllüğe sahip olması, yöntem tercihinde dikkat edilen öncelikli faktörlerdir. Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yöntemi; in vitro ortamda bakterideki karbapenemazın imipenemi hidroliz etmesiyle ortam pH'sını düşürerek fenol kırmızısının renginin değişmesi prensibiyle çalışmaktadır. Bu bağlamda; karbapenemaz üreten K. pneumoniae kaynaklı enfeksiyonların sıklıkla karşılaşıldığı ülkemizde, bahsi geçen hızlı tanı yönteminin duyarlılık ve özgüllüğünün belirlenmesi yaygın kullanıma girebilmesi açısından önemlidir.

Çalışmamızın amacı çeşitli klinik örneklerden elde edilen K. pneumoniae izolatlarında karbapenemaz varlığını enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yöntemi ve polimeraz zincir reaksiyonu (PZR) ile karşılaştırmalı şekilde tespit ederek yöntemin duyarlılık ve özgüllüğünü değerlendirmektir.

## 2. GEREÇ VE YÖNTEM

### 2.1. Bakteri izolatlarının belirlenmesi ve canlandırılması

Çalışmaya, Marmara Üniversitesi Pendik Eğitim ve Araştırma Hastanesi Mikrobiyoloji Laboratuvarı'na 2018-2023 yıllarında gönderilmiş çeşitli klinik örneklerden elde edilen, rutin laboratuvarda tür düzeyinde tanımlaması (MALDI-TOF MS, BioMerieux, Fransa) yapılmış ve antimikrobiyal duyarlılık

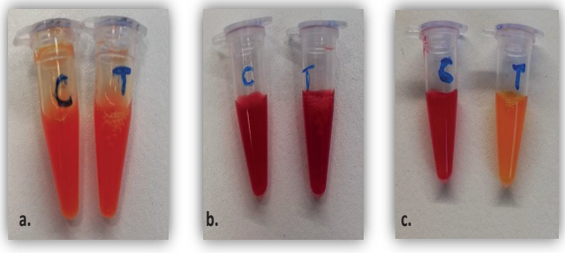
testleriyle (disk difüzyon ve/veya VITEK 2 otomotize sistem) karbapenemlere duyarlılıkları belirlenmiş 125 adet K. pneumoniae izolatı dahil edilmiştir. İzolatlardaki karbapenemaz enziminin klonal kökenli olmaması ve farklı türdeki enzimlerin test edilebilmesi amacıyla; yakın tarihlerde aynı servisten alınmış olmaması ve aynı hastadan alınan örnekler olmaması gibi kriterlere dikkat edilerek seçilmiştir. Antimikrobiyal duyarlılık sonuçları "European Committee on Antimicrobial Susceptibility Testing" (EUCAST) kriterlerine göre değerlendirilmiştir (Versiyon 13.1, Haziran 2023).

Skim milk besiyeri (gliserollü) içerisinde – 80ºC'lik derin dondurucuda saklanan izolatlar MacConkey agar besiyerine (Biomeriux, Fransa) ekilmiş ve 37ºC'de 18-24 saat aerobik ortamda inkübe edilmiştir. Üreyen mikroorganizmaların tür tanımlaması MALDI-TOF MS (BioMerieux, Fransa) ile doğrulandıktan sonra enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yönteminde kullanılmak üzere Mueller Hinton agar besiyerine pasajlanmıştır.

### 2.2. Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yöntemi ile karbapenemaz varlığının belirlenmesi

Test edilecek izolatta hücre içinde bulunan karbapenemazın serbest kalması ve testte çalışır hale gelebilmesi için bakteri, lizis amacıyla 1:10 oranında Tris HCl (20 mmol/L) içeren ortamda 30 dakika boyunca inkübe edilmiştir. Test aşamasında test edilecek her bir bakteri izolatı için 2 tüp hazırlanmıştır. Kontrol tüpünde pH 7,8'e ayarlanmış 100µL fenol kırmızısı + $ZnSO_4$ solüsyonu (10mM) bulunmaktadır. Test tüpünde ise 100µL fenol kırmızısı + $ZnSO_4$ + 6 mg/mL imipenem-silastatin solüsyonu bulunmaktadır. Kontrol ve test tüplerinin hazırlanmasından sonra her iki tüpe de 30 µL lizis edilmiş bakteri süspansiyonundan eklenmiştir. Ardından tüpler 1 saat boyunca oda sıcaklığında bekletilmiştir. Süre sonunda tüplerdeki renk değişimi incelenmiştir.

Her çalışmada kontrol amacıyla karbapenemaz geni taşıdığı bilinen bir pozitif kontrol suşu ve negatif kontrol amacıyla karbapenem duyarlı *Escherichia coli* ATCC 25922 suşu test edilmiştir. Ayrıca test sonuçlarının geçerli kabul edilebilmesi için kontrol tüpünde renk değişikliği meydana gelmemelidir. Kontrol tüpü kırmızı renkte olup, test tüpü sarı veya turuncuya dönmüş izolatlar karbapenemaz pozitif şeklinde yorumlanmıştır. (Resim 1)




**Resim 1**. *Test sonuçlarının yorumlanması (a. Kontrol tüpündeki renk değişikliği sebebiyle geçersiz test sonucu, b. Karbapenemaz negatif test sonucu, c. Karbapenemaz pozitif test sonucu) C: Kontrol tüpü, T: Test tüpü*

### 2.3. Karbapenemaz direnç genlerinin belirlenmesi

İzolatlarda PZR amacıyla kullanılacak genomik DNA ekstraksiyonu kaynatma yöntemi ile yapılmıştır. Steril ependorf tüpünde 100µL distile su ile beş öze dolusu (10µL'lik öze) koloni karıştırılmıştır. Ardından tüp, 1 dk boyunca vortekslenmiştir. Homojenizasyonu sağlanan bu süspansiyon 95ºC'de 10 dk boyunca ısı bloğunda bekletilmiştir. Süspansiyon 15000 rpm'de 2 dk santrifüj edilmiştir. DNA bulunan süpernatant kısmı alınarak steril başka bir ependorf tüpe aktarılmıştır.

PZR ile karbapenemaz direnç genlerinin (oxa-48, ndm, kpc, imp ve vim) belirlenmesi işlemleri tablo 1'de verilen primerler ve reaksiyon koşulları kullanılarak gerçekleştirilmiştir. PZR işlemi sonrası oluşan ürünler, %1 konsantrasyonda hazırlanan agaroz jele yükleme sonrası elektroforez ile saptanmıştır. Bu amaçla örnekler 25 dakika boyunca 80 V altında yürütülmüştür. Yürütme işlemi sonrası elde edilen bantlar UV görüntüleyicide incelenmiştir. Gözlenen bant profilleri DNA ladder ve pozitif kontrol ile karşılaştırılarak değerlendirilmiştir. (Tablo 1) (Resim 2)


***Resim 2.*** *blaOXA-48 pozitif izolatların ve karbapenem duyarlı izolatın jel görüntüsü [blaOXA-48 pozitif örnekler: KLB 10/22, 61/22, 2/23, 3/23, 37/23, 44/23, 56/23, 62/23, 76/23, 116/23, 247/23, 277/23, 331/23, karbapenem duyarlı örnek: KLB18/21; PK: blaOXA-48 pozitif kontrol suşu, DL: 100-1000 bp DNA Ladder (GeneMark, ABD)]*

***Tablo 1.*** *Araştırılan karbapenemaz genlerine ait primer dizileri ve döngü koşulları*

| Primer | Dizi | Boyut (bp) | Amplifikasyon Koşulları |
|---|---|---|---|
| OXA-48 R<br>OXA-48 F | 5' TTG GTG GCA TCG ATT ATC GG 3'<br>5' GAG CAC TTC TTT TGT GAT GGC 3' | 743 | 94ºC 5dk, 35 döngü (94ºC 60sn,56ºC 45sn,72ºC 60sn), 72ºC 7 dk |
| NDM R<br>NDM F | 5' GGG CAG TCG CTT CCA ACG GT 3'<br>5' GTA GTG CTC AGT GTC GGC AT 3' | 475 | 95ºC 5dk, 30 döngü, (95ºC 30sn,60ºC 40sn,72ºC 50sn), 72ºC 6 dk |
| İMP R<br>İMP F | 5' GAA GGY GTT TAT GTT CAT AC 3'<br>5' GTA MGT TTC AAG AGT GAT GC 3' | 587 | 95ºC 5dk, 35 döngü, (95ºC 45sn,60ºC 45sn,72ºC 60sn), 72ºC 8 dk |
| VIM R<br>VIM F | 5' GTT TGG TCG CAT ATC GCA AC 3'<br>5'AAT GCG CAG CAC CAG GAT AG 3' | 389 | 95ºC 5dk, 35 döngü, (95ºC 45sn,60ºC 45sn,72ºC 60sn), 72ºC 8 dk |
| KPC R<br>KPC F | 5' TCT GGA CCG CTG GGA GCT GG 3'<br>5' TGC CCG TTG ACG CCC AAT CC 3' | 399 | 95ºC 2dk, 35 döngü, (94ºC 2sn,62ºC 10sn,72ºC 15sn) |

*KPC: Klebsiella pneumoniae carbapenemase, NDM: New Delhi metallo-β-lactamase, IMP: Imipenem-resistant Pseudomonas, VIM: Verona integron-encoded metallo-β – laktamase, OXA-48: Oxacillinase-48*

Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı testi ile karbapenemaz negatif sonuç veren 3 izolatta PZR çalışması ile blaOXA-48 geni varlığı tespit edilmiştir. Bu izolatlar için öncelikle test aynı koşullarda tekrar edilmiştir. Ancak sonuçlar değişmemiştir. Daha sonra bu izolatlar için iki farklı yaklaşımla, antibiyotik miktarı 8 mg/mL'ye çıkarılarak ve bakteri yoğunluğu 2 katına çıkarılarak test yinelenmiştir. Sonuçlarda herhangi bir değişiklik gözlenmemiştir. Ancak dikkat çekici olarak, bakteri yoğunluğu arttırıldığında pozitif kontrol olarak kullanılan OXA-48 enzimine sahip izolatın çok daha hızlı (normalde 30-60 dk; bu şartlarda 2-3 dk) renk değişimi gösterdiği gözlenmiştir.

### 2.4. Verilerin istatistiksel analizi

Verilerin istatistiksel analizi SPSS sürüm 26.0 (IBM, Armonik, NY, ABD) kullanılarak yapılmıştır. Çalışmamızda kullandığımız enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yönteminin duyarlılık, özgüllük, pozitif prediktif değeri (PPV) ve negatif prediktif değeri (NPV) altın standart olarak kullanılan PZR testi ile karşılaştırılarak hesaplanmıştır. İki yöntem arasındaki niteliksel uyum, Phi Cramer's korelasyonu kullanılarak belirlenmiştir.

## 3. BULGULAR

Çeşitli klinik örneklerden elde edilmiş, rutin antibiyotik duyarlılık test sonuçlarına göre karbapenemlerden en az birine dirençli olduğu saptanan 100 adet ve karbapenemlere duyarlı olduğu saptanan 25 adet K. *pneumoniae* izolatı çalışmamıza dahil edilmiştir.

Karbapenem dirençli K. pneumoniae suşlarının (n:100) elde edildikleri kliniklere göre dağılımı incelendiğinde, yoğun bakım ünitelerinden (n:52), dahiliye servislerinden (n:7) ve değişen oranlarda farklı bölümlerden (n:41) izole edildikleri belirlenmiştir. İzolatların elde edildiği örneklerin dağılımı incelendiğinde ise, sırasıyla; derin trekeal aspirat (n:33), idrar (n:24), kan (n:16), balgam (n:5), yara (n:5) ve diğer örnekler (n:17) olarak dizildikleri belirlenmiştir.

Test edilen K. pneumoniae suşlarının enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yöntemi ve PZR karşılaştırmalı sonuçları tablo 2'de gösterilmiştir. Altın standart yöntemle karşılaştırma sonucu elde ettiğimiz bulgulara göre enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yönteminin; duyarlılığı %96,9, özgüllüğü %100, pozitif prediktif değeri %100, negatif prediktif değeri ise %90,3 olarak hesaplanmıştır. Ayrıca kullandığımız yöntem ile PZR testi arasında uyumluluk açısından çok güçlü anlamlı bir ilişki saptanmıştır (Phi Cramer's p= 0.936, p<0.001). (Tablo 2)

***Tablo 2.*** *Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik hızlı tanı yöntemi ve PZR testi karşılaştırmalı sonuçları*

| Karbapeneme Duyarlılık Durumu | PZR Sonucu | Enzim-Substrat Etkileşimine Dayalı Reaksiyon Temelli Kolormatik Hızlı Tanı Yöntemi | |
|---|---|---|---|
| | | Karbapenemaz Pozitif | Karbapenemaz Negatif |
| Dirençli (n=100) | OXA-48 (n=58) | 55 | 3 |
| | OXA-48+NDM (n=15) | 15 | 0 |
| | NDM (n=16) | 16 | 0 |
| | KPC (n=8) | 8 | 0 |
| | Gen Saptanmadı (n=3) | 0 | 3 |
| Duyarlı (n=25) | Gen Saptanmadı (n=25) | 0 | 25 |

*PZR: Polimeraz Zincir Reaksiyonu, KPC: Klebsiella pneumoniae carbapenemase, NDM: New Delhi metallo-β-lactamase, IMP: Imipenem-resistant Pseudomonas, VIM: Verona integron-encoded metallo-β –laktamase, OXA-48: Oxacillinase-48*

## 4. TARTIŞMA VE SONUÇ

"Karbapenem dirençli enfeksiyonlar" tüm dünyada olduğu gibi ülkemizde de önemli sağlık sorunları arasındandır. Bu enfeksiyonlar, mortalite ve morbidite oranlarında artış, hastanede kalış süresinin uzaması, hasta başı maliyetin artması ve iş gücü kaybı gibi sosyo-ekonomik açıdan birçok soruna yol açmaktadır. Ayrıca antibiyotik direnci sebebiyle de böyle enfeksiyonlarda tedavi zorlaşmaktadır. Antibiyotik dirençli bakterilerin neden olduğu enfeksiyonlarda hastalara en kısa zamanda, etkili antibiyotik tedavisinin verilmesi hayat kurtarıcıdır. Kritik hastalarda uygun antibiyotik tedavisinin verilmesindeki 1 saatlik gecikmenin hastanın ölüm riskini %20 oranında artırdığı gösterilmiştir (Kumar ve ark., 2006).

K. pneumoniae'de karbapenem direnci esas olarak antibiyotiği parçalayarak etkisiz kılan karbapenemaz enzimlerinin üretiminden kaynaklanmaktadır. Bu durum aynı zamanda hastada mortalite ve morbiditede artışa yol açmaktadır. Dolayısıyla enfeksiyon kontrolü ve halk sağlığını koruma açısından K. pneumoniae'de karbapenemaz varlığının hızlı tespiti gereklidir ve yaşamsal öneme sahiptir.

Karbapenemaz tespiti için geliştirilmiş birçok fenotipik (Modifiye Hodge testi (MHT), Karbapenem İnaktivasyon metodu (CIM) vb.) ve genotipik yöntem bulunmaktadır (Osei Sekyere ve ark., 2015). Bu yöntemler genel olarak değerlendirildiğinde, testlerin sonuçlanmasının uzun sürmesi, bazı direnç enzimlerini saptamada duyarlılığın ve özgüllüğün düşük olması gibi birtakım sorunlar göze çarpmaktadır. Örneğin; MHT, çoğu karbapenemaz için, özellikle KPC enzimleri için kabul edilebilir duyarlılık (>%90) gösterirken, NDM ve IMP gibi karbapenemazları saptamada düşük duyarlılığa sahiptir (Girlich ve ark., 2012). Ayrıca test maliyetinin düşük ve uygulanabilirliği kolay olmasına rağmen testin sonuçlanması için 24 saate ihtiyaç duyulmaktadır. Karbapenem inaktivasyon metodu da (CIM), MHT yöntemine benzer şekilde, sonuçlanması için bir gecelik inkübasyona ihtiyaç duymaktadır. Bunlara ek olarak, yapılan çalışmalarda bu yöntemin OXA-48 ve NDM üreten izolatları saptamada yetersiz olduğu gösterilmiştir (Tekintaş ve ark., 2017; Gelmez ve ark., 2021). Çalışmamızda kullandığımız

yönteme benzer bir metodoloji kullanan CARBA NP testi diğer fenotipik yöntemlere kıyasla ortalama 2 saat gibi kısa sürede sonuçlanması sebebiyle daha avantajlı görülmesine karşın yapılan çalışmalarda testin duyarlılığı %73 ile %100 arasında rapor edilmiştir (Vasoo ve ark., 2014; Yusuf ve ark., 2014). Bununla birlikte hem ülkemizde endemik olan OXA-48 benzeri karbapenemazlara karşı duyarlılığı önemli ölçüde düşüktür hem de maliyeti oldukça yüksektir (Poirel ve ark., 2012; Gelmez ve ark., 2020). Karbapenemaz genlerini saptamaya yönelik kullanılan genotipik yöntemler ise altın standart olmasına rağmen maliyet yüksekliği, deneyimli personel ve donanımlı laboratuvar altyapısı gereksinimi gibi nedenler dolayısıyla tercih edilmemektedir. Çalışmamızda kullandığımız enzim-substrat ilişkisine dayalı reaksiyon temelli hızlı kolormatik yöntem düşük maliyetli, kolay uygulanabilir, 1-2 saat gibi çok kısa sürede sonuç verebilen bir test olmasının yanı sıra ülkemiz açısından önem taşıyan OXA-48 tipi karbapenemazları yüksek duyarlılıkla (%95,9; 55/58) saptamaktadır.

Kullandığımız enzim-substrat ilişkisine dayalı reaksiyon temelli hızlı kolormatik yöntem, bu alanda yaygın kullanımda olan ticari CARBA NP testinden bazı basamaklarda avantaj sağlayan farklılıklar içermektedir. Bunlar bakteri lizis aşamasında santrifüj işlemine gereksinim duyulmaması, testin direkt inokülasyon ile gerçekleştirilmesi, saf imipenem yerine ticari olarak kolaylıkla temin edilebilen imipenem-silastatin antibiyotiğini içermesi, reaksiyonun 37ºC'lik etüv içerisinde değil 25ºC'lik oda sıcaklığında gerçekleşmesi, 2 saat yerine 1 saatlik inkübasyon ile testin sonuçlanması ve ülkemizde endemik olan OXA-48 enzimini saptamada yüksek duyarlılığa (%95,9) sahip olması şeklinde sıralanabilir. Ayrıca kullandığımız yöntemin test başı maliyeti 5 TL iken ticari CARBA NP testinin test başı maliyeti 4 dolar olarak hesaplanmıştır (120 TL, Şubat 2024).

Test ettiğimiz yönteme benzer olarak literatürde in-house CARBA NP testi olarak geçen bazı biyokimyasal testler mevcuttur. 2016 yılında Österblad ve ark. tarafından yapılan, karbapenemaz pozitif 57 – karbapenemaz negatif 37 gram negatif basilin değerlendirildiği çalışmada karbapenemaz pozitif izolatların 41'i in-house CARBA NP testi ile karbapenemaz pozitif bulunmuştur (Österblad ve ark., 2016). Karbapenemaz negatif suşların tamamı aynı test ile negatif bulunmuştur. Yazarlar, çalışmalarındaki yanlış negatif sonuç veren izolatların çoğunluğunun karbapenemleri zayıf hidrolize ettiği bilinen OXA-48 veya OXA-181 üreticisi olduğunu saptamış ve yanlış negatif sonuçların bundan kaynaklı olabileceğini belirtmiştir. Aynı tarihli Pires ve ark. tarafından yapılan karbapenemaz pozitif 30 – karbapenemaz negatif 33 Enterobacteriaceae izolatının değerlendirildiği başka bir çalışmada ise in-house CARBA NP testinin %100 duyarlılık ve %98,9 özgüllüğe sahip olduğu gösterilmiştir (Pires ve ark., 2016). Çalışmada OXA-48 enzimi bulunan izolatlarda zayıf karbapenem hidrolizi sebebiyle renk değişiminin daha zayıf olduğunu ancak bu durumu da pozitif olarak kabul ettikleri ifade edilmiştir. Ayrıca yanlış pozitif sonuç veren izolatlarda AmpC enzimi varlığını gerekçe göstermiş ve bu enzimin düşük düzeyde de olsa imipenem hidrolizine yol açmasını söz konusu uyumsuz sonuçlarla ilişkilendirmişlerdir. 2019 yılında Bir ve ark. tarafından yapılan bir çalışmada, karbapenemaz pozitif 32 – karbapenemaz negatif 5 ve karbapenemlere artan dozda duyarlı 3 Enterobacteriaceae izolatı ile elde edilen veriler ışığında, in-house CARBA NP testinin duyarlılığı %93,9 ve özgüllüğü %71,4 olarak hesaplanmıştır (Bir ve ark., 2019). Çalışmada OXA-48 enzimi taşıyan bir izolatta alınan yanlış negatif sonucun enzime bağlı zayıf karbapenemi hidrolizinden kaynaklı olabileceği belirtilmiştir. Ancak yanlış pozitif bulunan izolatlarla ilgili bir yorum yapılmamıştır. Akyar ve ark. tarafından karbapenemaz pozitif 153 – karbapenemaz negatif 16 Klebsiella spp. ve E. coli izolatının değerlendirildiği çalışmada, in-house CARBA NP testinin %96,7 duyarlılık ve %100 özgüllüğe sahip olduğu gösterilmiştir (Akyar ve ark., 2019). Çalışmalarında yanlış negatif sonuç veren izolatlarda inkübasyon süresi arttırıldığında (4 saat), duyarlılığın %100'e yükseldiğini belirtilmiştir. Bu izolatlardaki sonucu, düşük karbapenemaz aktivitesine sahip enzimlerle, özellikle de OXA grubu enzim üreticileriyle ilişkilendirmişlerdir. Çalışmamızda da kullandığımız yöntemle yanlış negatif olarak saptanan 3 izolatta PZR ile OXA-48 enzimi varlığı saptanmıştır. Bu izolatlar için test aynı koşullarda inkübasyon süresi 2 saate uzatılarak tekrar edilmesine rağmen sonuç değişmemiştir.

Yukarıda paylaştığımız literatürdeki in-house CARBA NP testlerinin çoğunun özgüllüğü yüksektir ancak özgüllüğün düşük olarak saptandığın çalışmalarda suşlardaki AmpC enziminin yanlış pozitifliğe neden olabileceği yorumu yapılmıştır (Pires ve ark., 2016; Österblad ve ark., 2016). Ayrıca in-house CARBA NP testlerinin duyarlılık oranları da çoğu çalışmada yüksek bulunmuş ve oranı düşüren neden var ise zayıf karbapenem hidrolizi dolayısıyla OXA tipi karbapenemazlar ile ilişkilendirmişlerdir (Österblad ve ark., 2016; Akyar ve ark., 2019). Çalışmamızda da literatürle uyumlu olarak enzim-substrat ilişkisine dayalı reaksiyon temelli hızlı kolormatik yöntem ile duyarlılık %96,9, özgüllük ise %100 bulunmuştur.

Çalışmamızda hem PZR (taranan *bla*OXA-48, blaNDM, blaKPC, blaVIM ve blaIMP için) hem de enzim-substrat ilişkisine dayalı reaksiyon temelli hızlı kolormatik yöntem ile karbapenemaz negatif sonuç veren 3 izolat bulunmaktadır. Yukarıda bahsi geçen çalışmalarda olduğu gibi çalışmamızda da kullandığımız yöntemin duyarlılık oranını düşüren temel faktör, OXA-48 enzimi taşıyan bu 3 izolatın yanlış negatif olarak saptanmasıdır. OXA-48 benzeri enzimlerin karbapenemler üzerinde zayıf hidrolitik etki sergilediği dikkate alındığında, bu 3 izolatta elde ettiğimiz yanlış negatif sonuçların *bla*OXA-48 alt tipleri ile ilişkili olabileceğini düşünmekteyiz.

K. penumoniae'de karbapenem direncine neden olan en temel mekanizma karbapenemaz üretimi olmasına rağmen başka mekanizmaların da dirençte rol oynadığı bilinmektedir (Alizadeh ve ark., 2020; Ranjbar ve ark., 2019). Ülkemizde diğer tip karbapenemazların görülme sıklığı ve fenotipik test ile de karbapenemaz negatif bulunmaları gibi nedenler düşünüldüğünde, bu izolatlardaki karbapenem direncinin karbapenemaz üretimi yoluyla değil, porin kaybına bağlı (OmpK35 ve OmpK36) membran geçirgenliğinin azalması

veya efluks pompaları ile ilacın hücre dışına atılımının sağlaması yoluyla olabileceği öngörülmektedir.

Sonuç olarak, karbapenem direnci ülkemizde ve dünyada halk sağlığını tehdit eden ciddi bir sağlık sorunudur. EUCAST'a göre; karbapenem direnci tespit edilen izolatlarda, enfeksiyon kontrolü ve halk sağlığı açısından karbapenemaz tespitinin hızlı yapılması gerekli ve önemlidir. Yaygın kullanılan fenotipik karbapenemaz doğrulama testlerinde (CIM, MHT vb.) sonuçlanma sürelerinin 48 saatten uzun sürmesi, enzim tipine göre duyarlılık ve özgüllük oranlarının düşük olması gibi birtakım sorunlar bulunmaktadır. Genotipik testler altın standart olmasına rağmen maliyetinin yüksek olması, deneyimli personel ihtiyacı ve donanımlı laboratuvar altyapısı gereksinimi gibi sebepler dolayısıyla rutin laboratuvar süreçlerinde yer alamamaktadır. Bu sorunlardan yola çıkarak çalışmamızda maliyeti uygun, kısa sürede sonuçlanan ve laboratuvarda kolay uygulanabilecek enzim-substrat ilişkisine dayalı reaksiyon temelli hızlı kolormatik "in-house" yöntemi değerlendirdik. Ülkemiz açısından ele alındığında, karbapenemaz tespitinde kullanılan hızlı tanı testi seçiminde temel faktör, kullanılacak testin ülkemizde yaygın görülen karbapenemazları saptamadaki duyarlılık ve özgüllüğüdür. Çalışmamızda kullandığımız enzim-substrat ilişkisine dayalı reaksiyon temelli hızlı kolormatik test, %96,9 duyarlılık ve %100 özgüllüğe sahip olmasının yanı sıra, benzerlerinden farklı olarak, ülkemiz açısından büyük önem taşıyan OXA-48 tipi karbapenemazları yüksek duyarlılık (%95,9) ile kısa sürede, doğru ve güvenilir şekilde saptayabilmektedir. Bu yöntemin klinik laboratuvarlarda rutin kullanımı değerlendirilmelidir.

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# Comparison of Two Different Proprioception Measurement Methods in the Shoulder Joint

## Omuz Ekleminde İki Farklı Propriyosepsiyon Ölçüm Yönteminin Karşılaştırılması

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**ABSTRACT**

**Aim:** Proprioception assessment is important in shoulder rehabilitation. Proprioception sense can be evaluated with different methods in the clinical setting. The aim of this study was to compare shoulder proprioception measurements made with universal goniometer and isokinetic system.
**Methods:** A total of 52 healthy individuals with a mean age of 24.6 ±4.29 years were included in the study. Shoulder proprioception was evaluated three times with a universal goniometer and isokinetic device at 30, 45 and 60 degrees shoulder flexion angles with eyes closed in a sitting position and mean values were recorded.

**Results:** When the results obtained with the two measurement methods were compared, it was determined that there was a significant difference between the mean values (for 30, 45 and 60 degrees shoulder flexion angles, p=0.003, 0.005, 0.000, respectively) and there was no correlation relationship between the results of the two measurement methods (p<0.05). However, when the mean deviation from the target angle was compared with both measurement methods, it was determined that there was no significant difference between the measurement methods (p<0.05).

**Conclusion:** The findings of this study demonstrated that when shoulder proprioception was measured using a goniometer or an isokinetic dynamometer at various angles, different values could be obtained. However, since the differences compared to the target angle are similar for the two measurement methods, both assessment methods can be used for proprioception evaluation.

**Keywords:** Assessment, shoulder, proprioception, rehabilitation

**ÖZ**

**Amaç:** Omuz rehabilitasyonunda propriyosepsiyon değerlendirmesi önem taşımaktadır. Propriyosepsiyon duyusu klinik ortamda farklı yöntemlerle değerlendirilebilir. Bu çalışmanın amacı universal gonyometre ve izokinetik sistem ile yapılan omuz propriosepsiyon ölçümlerini karşılaştırmaktı.
**Gereç ve Yöntem:** Çalışmaya yaş ortalaması 24,6 ± 4,29 yıl olan toplam 52 sağlıklı birey dahil edildi. Omuz propriosepsiyonu, oturur pozisyonda gözler kapalı iken 30, 45 ve 60 derece omuz fleksiyon açılarında universal gonyometre ve izokinetik cihaz ile üç kez değerlendirildi ve ortalama değerler kaydedildi.

**Bulgular:** İki ölçüm yöntemi ile elde edilen sonuçlar karşılaştırıldığında, ortalama değerler arasında anlamlı fark olduğu (30, 45 ve 60 derece omuz fleksiyon açıları için sırasıyla p=0,003, 0,005, 0,000) ve iki ölçüm yönteminin sonuçları arasında korelasyon ilişkisi olmadığı tespit edildi (p<0,05). Ancak hedef açıdan ortalama sapma her iki ölçüm yöntemi ile karşılaştırıldığında ölçüm yöntemleri arasında anlamlı bir fark olmadığı tespit edilmiştir (p<0,005).

**Sonuç:** Bu çalışmanın bulguları, omuz propriyosepsiyonu gonyometre veya izokinetik dinamometre kullanılarak çeşitli açılarda ölçüldüğünde farklı değerler elde edilebileceğini göstermiştir. Bununla birlikte, hedef açıya kıyasla farklılıklar iki ölçüm yöntemi için benzer olduğundan, her iki değerlendirme yöntemi de propriyosepsiyon değerlendirmesi için kullanılabilir.

**Anahtar Kelimeler:** Değerlendirme, omuz, propriosepsiyon, rehabilitasyon



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## 1. INTRODUCTION

The sense of position and motion of the limbs was first referred to as proprioception by Sir Charles Bell in the early 1830s as the "sixth sense" (1). Sherrington (2) went into greater detail about it at the start of the 20th century.

Proprioception, which means "belonging to oneself," is derived from the Latin word proprius. The full Turkish translation is "perception of one's own self" (3). Thermoreception, nociception, equilibrioception, mechanoreception, and proprioception are among the somatosensory senses, also referred to as the sixth sense. Proprioception includes the senses of passive and active joint position, kinesthesia, force or tension, and feeling of speed change (3-5).

Our capacity to sense where our limbs and joints are in relation to our bodies and environments (both in position and while moving) without visual feedback is known as proprioception. Sensorimotor control depends on proprioception. Proprioception is crucial for movement acuity, joint stability, coordination, and balance, as well as for sensorimotor control and regulation of muscle tension based on feedback and feedforward feedback (2,4).

Proprioception or proprioceptive acuity is a complex system that requires both peripheral and central systems to work in harmony with each other. Since sensory information is derived from changes in internal structures, it is also recognised as interoceptive information. Evidence for the major proprioceptive receptor supports muscle afferent input, particularly from muscle spindles. These receptors are specialised fibres within the muscle that detect the change in muscle length as well as the rate of contraction. They also detect body part movement as a first derivative of length, i.e. the rate of change in length. During contractions, the muscle spindle is under fusimotor (gamma system) control, which has the capacity to change the calibration or sensitivity of the receptor by changing its internal length (3-7).

According to Proske and Gandevia, the perception of joint position and movement is also influenced by cutaneous receptors found in the skin, particularly those found in the fingers, elbow, and knee (6). Joint structures also contain receptors that resemble cutaneous receptors. It is possible to detect static joint position, intra-articular pressure, and possibly joint motion in terms of amplitude and velocity thanks to ruffini bodies found in the joint capsule, ligaments, and menisci. Deeper tissues' Pacinian corpuscles are responsive to changes in velocity. Movement restrictions affect the Golgi tendon organ, which is a part of the ligaments and menisci (5). These sensory inputs from within the body contribute to proprioception (5).

Tests to assess joint position sense, kinesthesia, or force sense should be used in clinical proprioception assessments. Custom-built instruments or expensive computer interfaces are frequently used in laboratories. It can be difficult to use specialised and computerised systems during clinical practice and to access these devices at all times. In a clinical setting, goniometers, inclinometers, pressure sensors, and laser pointers are accessible and simple to use. There is also potential for new, reasonably priced, and precise technology, such as cellphones with integrated gyros and accelerometers, Wii Balance Board, Kinect and other tecnologic systems (4).

A goniometer is a tool used to measure the angle of a joint in the body. It is a simple and inexpensive device that is widely used in physical therapy and sports medicine to assess an individual's range of motion and joint stability. In addition to measuring joint angles, a goniometer can also be used to assess proprioception. The goniometer is a useful tool for assessing proprioception as it provides objective data that can be used to guide the development of effective rehabilitation plans. Its low cost and ease of use make it a valuable tool in both clinical and research settings (8,9). Isokinetic testing is a type of physical performance test that measures an individual's strength and muscular endurance through controlled joint movements. The Isokinetic Dynamometer System is also used to assess proprioception, which is the ability of an individual to perceive the position and movement of their body in space. The system measures the individual's ability to control their movements and maintain balance during exercises that simulate real-life movements (10,11). With the hypothesis that proprioception measurements with goniometer and isokinetic system will be similar, the aim of this study was to compare shoulder proprioception measurements made with universal goniometer and isokinetic system.

## 2. METHODS

The study was carried out between April 2023 and May 2023 at Marmara University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation Laboratory. *The ethics approval for the study was obtained from the Ethics Committee of Marmara University, Faculty of Health Sciences (30.03.2023/49)* and it was conducted in accordance with the Declaration of Helsinki. Each participant signed informed consent forms after informing about the study. A descriptive and cross-sectional study was conducted.

### 2.1. Participants

The study sample was selected from healthy young individuals who are university students attending face-to-face education. Participants were invited to the study via email and whatsapp groups. Being over 18 years of age and healthy were the inclusion criteria. Injury or surgical operation related to the shoulder joint, any congenital or orthopedic problem, presence of neurological and rheumatic diseases, and pregnancy were determined as exclusion criteria.

### 2.2. Assessment

To assess proprioception using a goniometer and the isokinetic system the individual was asked to perform movements such as flexion and extension of a joint while blindfolded. The same physical therapist then measured the joint angle using the goniometer or isokinetic device and

compared it to the target angle. The difference between the actual angle and the target angle provides information about the individual's ability to perceive the position of their joint in space (4,9,10). Resting the participant for 2 – 3 minutes between two different measurements requested.

### *Goniometric Measurement*

To perform goniometric measurements, the participant was asked to sit on a chair with back support. They were instructed to sit with their feet flat on the ground and their knees flexed at 90 degrees. The measurements were initiated by placing the pivot point of the goniometer (Baseline®, 12-inch plastic goniometer, Fabrication Enterprises, Inc: White Plains, NY / USA) on the acromion of the participant's shoulder joint. The fixed arm of the goniometer was positioned parallel to the participant's midaxillary line. The movable arm was fixed parallel to the humerus and followed the humerus during shoulder flexion to measure the angles. Prior to measurements, the participant was asked to perform angular movements with their eyes open and the positions of the angles were taught in three repeated cycles. After the learning period, the participant was asked to close their eyes and three repetitions of 30, 45, and 60-degree shoulder flexion angles were performed and the mean values were recorded.

### *Isokinetic Dynamometer Measurement*

Isokinetic Dynamometer assessment was performed with Biodex® device (Biodex System 3 Pro Multi Joint System®, Biodex Medical Inc, Shirley, NY / USA) which can be used for many different tests and are designed to measure parameters such as weight lifting capacity, force generating capacity, range of motion, muscle strength, endurance and proprioception.

For measurements, the participant was seated on the Isokinetic Dynamometer device with their feet in full contact with the ground. Then they were asked to position their knees at 90 degrees of flexion. After the positioning process was completed, the relevant arm apparatus of the device was adjusted to fit the individual. During measurement, the test procedure was carried out with the participant's arm moving in full extension. First, the participant was taught the activity they were going to perform. The activity consisted of the participant performing 30, 45, and 60 degrees of shoulder flexion angles while the movement was monitored on the device screen and the participant's eyes were kept open to observe the movement. The learning process of each angle consisted of three repetitions. After the teaching process was completed, the participant was asked to close their eyes and sequentially find these angles by flexing their shoulders with the relevant apparatus of the device (Figure 1). These measurements were repeated three times, and the measurement averages were recorded.

***Figure 1.*** *Proprioception assessment with Biodex® Isokinetic Device.*

### 2.3. Statistical Analysis

SPSS (Statistical Package for Social Sciences) Windows v22.0 (SPSS Inc, IBM Corp, Armonk, New York) was used for all statistical analyses in the study. Mean and standard deviation (SD) were used for quantitative results, and percentage (%) values were used for qualitative results. Normal distribution of data was assessed by the "One-Sample Kolmogorov-Smirnov Test" and by examining histograms. Pearson correlation analysis was used to evaluate the relationship between parameters. We evaluated the difference between measurement methods with the Mann-Whitney U test. The level of statistical significance was set at $p<0.05$.

## 3. RESULTS

This study included 19 male and 33 female participants with a mean age of 24.6 ± 4.29 years. The mean height was 165.7cm, mean weight was 64.7 kg and mean BMI was 25.3.

The evaluations conducted with Goniometer and Isokinetic Dynamometer assessment system at 30, 45, and 60 degrees of shoulder flexion angles are presented in Table 1.

***Table 1.*** *Differences between angular measurements.*

| Target Shoulder Flexion Angles (n=52) | Goniometer Measurements Mean (SD) Median (min – max) | Isokinetic Dynamometer Measurements Mean (SD) Median (min – max) | p value |
|---|---|---|---|
| 30 degrees | 32.1 (3.4)<br>31.1 (25.6 – 50) | 30.4 (2.2)<br>30.6 (25 – 35.6) | **0.003** |
| 45 degrees | 45.6 (2.1)<br>45.8 (41 – 51.6) | 44.1 (2.6)<br>44.1 (37.9 – 54.8) | **0.001** |
| 60 degrees | 60.1 (4.6)<br>60.6 (33.6 – 66.6) | 58.5 (2.4)<br>58.7 (52.7 – 63.3) | **0.000** |

*SD: Standart Deviation, min:minimum, max:maximum, Statistical Method: Mann-Whitney U Test*

The box plot and violin plot images of the angular measurements are shown in Figure 2. The box plot and violin plot images present the graphical representations of the means of the measurements and the numerical values of the outliers. Additionally, the plots provide insights into the normal distribution of the measurements. Upon examining the graphs, it is anticipated that the angular measurements do not adhere to a normal distribution.

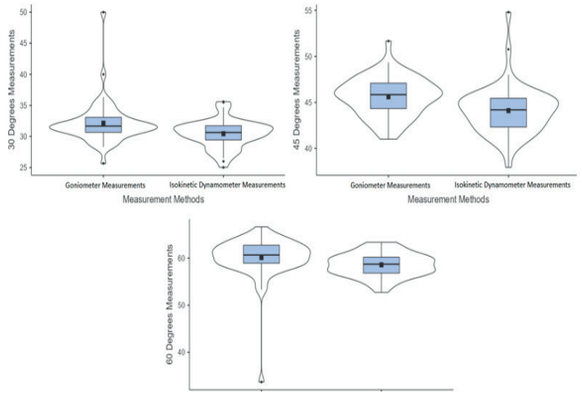


***Figure 2.*** *Violin and box plots of angular values measured in the study.*

The deviations of the angular measurements from the target angle form the basis of the study. Therefore, the distances between the obtained values from the target angular value were calculated. After determining the distances to the angular target, the absolute values were taken for the negative values. Through the descriptive analysis conducted based on the absolute values, it was observed that the distance values from the target angles did not conform to a normal distribution, as indicated by the Shapiro-Wilk test results ($p<0.05$).

Due to the non-normal distribution of the measurements, the Mann-Whitney U test was employed to evaluate the significance level of the differences between the measurements. According to the test results, no significant difference was found between the goniometer measurements and the measurements conducted with the Isokinetic Dynamometer device ($U = 1114$, $p>0.05$ for 30-degree measurements; $U = 1317$, $p>0.05$ for 45-degree measurements; $U = 1311$, $p>0.05$ for 60-degree measurements; Table 2; Figure 3).

***Table 2.*** *Evaluation of the difference between the distance values to the target angles.*

| | Statistic | p | Mean Difference | Effect Size |
|---|---|---|---|---|
| MD in 30 Degrees | 1114 | 0.122 | 0.37 | 0.1764 |
| MD in 45 Degrees | 1317 | 0.822 | 0.06 | 0.0259 |
| MD in 60 Degrees | 1311 | 0.79 | 0.1 | 0.0307 |
| *Note H0 μ0 ≠ μ1* | | | | |

*MD: Measurements Distance, Statistical Method: Mann-Whitney U Test*

There was no significant difference found between the medians of the distance values from the target angle in the goniometric measurements (30 degrees = 1.67; 45 degrees = 1.67; 60 degrees = 1.67) and the medians of the measurements conducted with the Isokinetic Dynamometer device (30 degrees = 1.61; 45 degrees = 1.55; 60 degrees = 2.29) (Table 2, Figure 3).

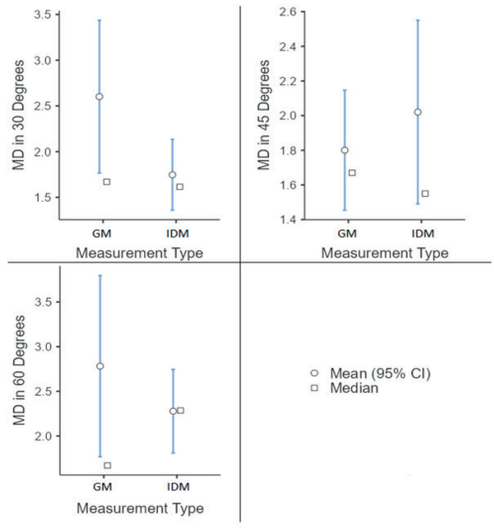


***Figure 3.*** *Descriptive plots of distances to target angles according to measurement methods.*

*(*MD: Measurements Distance; GM: Goniometric Measurements; IDM: Isokinetic Dynamometer Measurements; CI: Confidence Interval)*

Non-parametric Spearman's rho correlation analysis was conducted to assess the relationship between the distances from the target angle in the goniometric measurements and the measurements performed with the Isokinetic Dynamometer device for the target shoulder flexion angles in the correlation analysis considering the differences in measurement methods for the measured angular values, no significant relationship was found ($r = – 0.003$, $p>0.05$ for 30-degree measurements; $r = – 0.216$, $p>0.05$ for 45-degree measurements; $r = – 0.181$, $p>0.05$ for 60-degree measurements). The correlation plot for the respective measurements is provided in Figure 4.

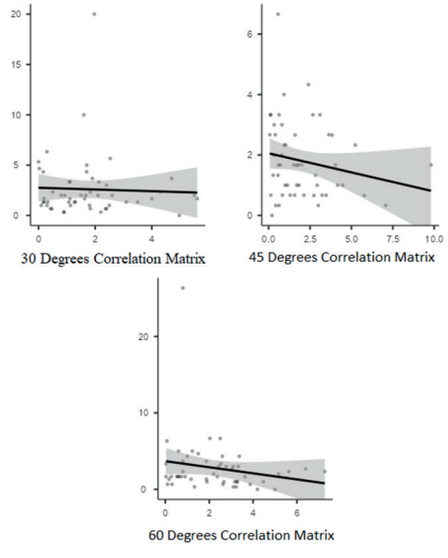


***Figure 4.*** *Correlation matrix of distance to target angle according to measurement methods.*

## 4. DISCUSSION

The results of this study showed that different values can be obtained when shoulder proprioception was evaluated at different angles using a goniometer or an isokinetic dynamometer. It was also found that the results obtained with two different proprioception assessment methods were not correlated. When the mean deviation from the target angle was compared with both measurement methods, it was determined that there was no significant difference between the measurement methods.

According to the median values, the results of proprioception assessment with isokinetic dynamometer were closer to the target angle at 30 and 45 degrees shoulder flexion, while the results of proprioception assessment with goniometer were closer to the target angle at 60 degrees shoulder flexion.

The proprioceptive mechanism integrates the static and dynamic functions of joint stabilizers.

Both passive (bony structures, capsule, and ligaments) and active (muscles) stabilizers contribute to the stability of the shoulder. A watertight capsule, corresponding surfaces, and joint fluid all work together to create negative pressure, which is what gives an object its stability at rest. The joint maintains its stability while in motion by balancing muscle activity and by capsular and ligamentous restraints in extreme motion. The central nervous system is in charge of stabilizing the system [12-16]. Shoulder proprioception has been shown to be affected after surgical treatments and shoulder problems (15,16). Therefore assessment of shoulder proprioception is of clinical importance for physiotherapists.

In a systematic analysis of shoulder proprioception assessment methods in the literature, 21 studies were included (17). The researchers reported that the most reliable movement in the evaluation of shoulder proprioception was internal rotation in 90° abduction and the device was isokinetic dynamometer (17). Shoulder proprioception with shoulder flexion movement was evaluated in 6 studies (17). However, isokinetic evaluation was preferred for evaluation in most of the studies. Only one study examined the validity and reliability of using a goniometer for shoulder proprioception assessment (17,18).

Vafadar et al. (2016) reported that in shoulder proprioception evaluations performed with a goniometer, there was more error at small range of motion values of shoulder flexion, while this margin of error decreased at medium and high angle values (18). In our study, the maximum difference between the target angle and the shoulder flexion angle reached by the subject was 30 degrees, but the difference between the target angle and the achieved angle was smaller for 60 and 90 degrees of shoulder flexion. Vafar et al. (2016) reported interrater and intrarater intraclass correlation coefficients for the goniometer as .60 and .50, respectively; and the authors did not recommend goniometry for shoulder proprioception assessment in the clinic (18).

In a recent study, shoulder internal rotation and external rotation position sense were evaluated with an isokinetic dynamometer and the researchers reported that the intra-rater and inter-rater reliability of the internal rotation position sense tests were moderate to good, and the intra-rater test reliability of external rotation was poor and inter-rater reliability was moderate to good (19). Inter-rater and inter-rater agreement for shoulder flexion position sense assessment was not examined in the present study.

Batista et al. (2006) evaluated the range of motion of the knee joint in 38 healthy subjects with a universal goniometer and isokinetic dynamometer and reported that the results were correlated (0.90) and reliable. The difference between the results of this study and other studies may be due to the evaluation of the knee joint or the range of motion of the joint.

To the best of our knowledge, there is no study in the English and Turkish literature that measured shoulder proprioception with isokinetic dynamometer and goniometer and compared the results obtained.

Another important finding of this study is that there was no correlation between the measurements made by goniometer and isokinetic dynamometer. There was a difference of 0.37, 0.06 and 0.1 between the mean values obtained with both measurement methods at 30, 60 and 90 degrees of shoulder flexion, respectively, so we think that if the number of individuals included in the evaluation increases, there will be agreement between the two measurement methods.

Goniometer assessment results may vary depending on the skill and experience of the assessor. On the other hand, isokinetic dynamometer devices offer a more technological and standardized approach. These fundamental differences between these two techniques may lead to a certain lack of correlation between the measurement processes, even though the overall measurement values are similar. This result of the present study shows that more care should be taken when using different measurement techniques. An important issue to consider is that it is not possible for clinicians to perform isokinetic device assessments for every individual undergoing shoulder rehabilitation.

Vafadar et al. (2016) recommended clinicians to use an inclinometer and laser pointer for proprioception measurement during shoulder rehabilitation.

### Limitations

In this study, only proprioception assessment of shoulder flexion movement was performed, and proprioception assessment of other range of motion positions of the joint was not performed. Another limitation of our study is that intrarater and interrater reliability assessments were not performed for the two measurement methods. We suggest that in future studies, intra – and interrater reliability analyses of different methods should be performed with a larger sample size including different age groups so that physiotherapists

working in the field of shoulder rehabilitation can be provided with methods that they can apply in the clinic.

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# Çeşitli Klinik Örneklerden İzole Edilen Pseudomonas aeruginosa'nın Karbapenemaz Üretimi ve Tiplendirilmesinde Fenotipik ve Genotipik Yöntemlerin Değerlendirilmesi

## Evaluation of Phenotypİc and Genotypic Methods for Carbapenemase Production and Typing in Pseudomonas aeruginosa Isolates from Various Clinical Samples

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**ÖZ**

**Amaç:** Son on yılda karbapenem dirençli *Pseudomonas aeruginosa* izolatlarının artışı enfeksiyon tedavisinde önemli bir sağlık sorunu haline gelmiştir. Rutin laboratuvarda karbapenem direncinin saptanması için gereken süre yaklaşık 48 saattir. Bu durum, özellikle kritik hastalarda etkili tedavinin başlatılmasında gecikmeye neden olur. Bu nedenle çalışmalar antimikrobiyal direncin daha erken tespit edilmesi için yeni yöntemlere odaklanmaktadır. Bu çalışmada, karbapenem dirençli *P. aeruginosa* izolatlarında karbapenemaz üretimine bağlı direncin fenotipik ve genotipik yöntemlerle araştırılması amaçlanmıştır.

**Gereç ve Yöntem:** Marmara Üniversitesi Pendik Eğitim ve Araştırma Hastanesi Mikrobiyoloji Laboratuvarı'nda Ocak 2018 ile Şubat 2023 tarihleri arasında çeşitli klinik örneklerden izole edilen 120 *P. aeruginosa* izolatı rutin antibiyogram sonuçlarına bakılarak çalışmamıza dahil edilmiştir. Karbapenemaz üretimi fenotipik test olarak enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik yöntem ve genotipik test olarak polimeraz zincir reaksiyonu ile tespit edilmiştir.

**Bulgular:** Bu çalışmada karbapenem dirençli 100 izolatın 46'sında (%46) genotipik yöntemle karbapenemaz kodlayan genler tespit edilmiştir. Fenotipik test ile karbapenemaz enzimi taşıyan 46 izolatın 31'inde (%67) 1 saat içinde pozitif sonuçlar kaydedilmiştir. Geriye kalan 13 izolat yanlış negatif olarak; moleküler yöntem ile direnç genlerini taşımadığı belirlenen 2 izolat ise yanlış pozitif olarak değerlendirilmiştir. Fenotipik testin duyarlılık ve özgüllüğü sırasıyla; %67.4 ve %97.3 ($p<0.0001$) olarak bulunmuştur.

**Sonuç:** Sonuç olarak, karbapenem dirençli *P. aeruginosa* izolatlarının hızlı ve doğru tanımlanması, zamanında uygun tedavinin verilmesi ve enfeksiyon kontrol önlemlerinin başarılı bir şekilde uygulanması açısından oldukça önemlidir. Çalışmamızda kullandığımız enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik testin performansının değerlendirilebilmesi için daha fazla örnek içeren *in vitro* çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler:** *Pseudomonas aeruginosa*, antibiyotik direnci, karbapenem, karbapenemaz, fenotipik test



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**ABSTRACT**

**Objective: The** increase in carbapenem-resistant Pseudomonas aeruginosa isolates has become an important health problem in infection treatment in the last decade. The time required to detect carbapenem resistance in the routine laboratory setting is about 48 hours. So, it causes a delay in the initiation of effective treatment, especially in critically ill patients. For this reason, studies focus on new methods to detect antimicrobial resistance earlier. It aimed to investigate carbapenemase-dependent resistance by phenotypic and genotypic methods in carbapenem-resistant clinical P. aeruginosa isolates in this study.

**Methods: A** hundred twenty P. aeruginosa isolates obtained from clinical samples between January 2018 to February 2023 in the Microbiology Laboratory of Marmara University Pendik Training and Research Hospital, were included in our study based on routine antibiogram results. Carbapenemase production was detected by enzyme-substrate reaction-based colorimetric method as a phenotypic test and polymerase chain reaction as a genotypic test.

**Results: In** this study, carbapenemase-coding genes were detected in 46 (46%) of 100 carbapenem-resistant isolates by genotypic method. With the phenotypic test, positive results were recorded within 1 hour in 31 of 46 isolates (67%) carrying the carbapenemase enzyme. The remaining 13 isolates were false negatives; 2 isolates determined not to carry the resistance genes by molecular method were evaluated as false positives. The sensitivity and specificity of the phenotypic test were 67.4% and 97.3%, respectively ($p<0.0001$).

**Conclusion:** In conclusion, rapid and accurate identification of carbapenem-resistant P. aeruginosa isolates is very important for the timely administration of appropriate treatment and successful implementation of infection control measures. In vitro studies with a larger number of samples are needed to evaluate the performance of the enzyme-substrate reaction-based colorimetric test that we used in our study.

**Keywords:** Pseudomonas aeruginosa, antibiotic resistance, carbapenem, carbapenemase, phenotypic test

## 1. GİRİŞ

*Pseudomonas aeruginosa*, karbapenem grubu da dahil birçok sınıfta yer alan antibiyotiklere karşı hızla geliştirdiği direnç nedeni ile tedavide önemli bir sağlık sorunu haline gelmiştir (Zhang et al., 2016). Antibiyotik direnci, ulusal ve uluslararası kuruluşların raporlarına göre, enfeksiyon hastalıklarının tedavi etkinliğini büyük ölçüde sınırlamıştır. Dünya Sağlık Örgütü (DSÖ)'nün 2017 yılında yayınlanan raporuna göre, karbapenem dirençli *P. aeruginosa'*nın antibiyotik direnç sonuçlarının 20 bakteri türü arasında ikinci sırada yer aldığı ve kritik öncelikli bir bakteri olduğu bildirilmiştir (Tacconelli et al., 2018). Ek olarak, *P. aeruginosa* Amerikan Hastalık Önleme ve Kontrol Merkezi'nin 'İnsan Sağlığı için Ciddi Tehdit Oluşturan Bakteriler' listesinde yer almaktadır (CDC, 2019).

*P. aeruginosa*'da karbapenemlere direnç gelişiminde OprD porin proteini kaybı, dışa atım pompalarının aşırı ekspresyonu ve karbapenemaz üretimi gibi farklı direnç mekanizmaları rol oynar (Jean et al., 2022). Son yıllarda *P. aeruginosa*'da karbapenemlere karşı gelişen direnç oranının hızla arttığı bildirilmiştir. Bununla birlikte, karbapenemaz üretimine bağlı direnç gelişimi de sık sık rapor edilmektedir (Çopur ve ark. 2021).

Karbapenem direnci daha yüksek morbidite ve mortalite oranları, maliyetin artması ve hastanede yatış süresinin uzaması ile sonuçlanmaktadır (Shaaban et al., 2017). Dolayısıyla karbapenemaz enzimlerinin varlığının hızlı tespit edilmesi antimikrobiyal yönetimin ve enfeksiyon kontrol önlemlerinin erken aşamada etkili olabilmesi için önemlidir (Osei et al., 2015). Genotipik testler, direnç genlerinin tespit edilmesinde altın standarttır. Genotipik yöntemler deneyimli personel gerektirmesi ve pahalı olması gibi dezavantajlara sahiptir (Malkoçoğlu ve ark., 2017). Bu nedenle rutin laboratuvarda uygulanabilmesi için karbapenemaz üretimini hızlı tespit eden, basit, güvenilir ve uygun maliyetli testlere ihtiyaç vardır (Aktaş ve ark., 2017 ve Osei et al., 2015). Son yıllarda, karbapenemazların fenotipik olarak saptanması amacıyla birçok farklı yöntem (Modifiye Hodge testi, Karbapenem İnaktivasyon Metodu, Carba NP test vb.) geliştirilmiştir. Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik test, karbapenemaz aktivitesinin saptanması için geliştirilen fenotipik yöntemlerden biridir. Bu yöntem karbapenemlerin beta-laktam halkasının enzimatik hidrolizi sonucu oluşan pH değişikliğine bağlı olarak pH indikatöründe renk değişimi ile karbapenemaz üretiminin tespitini sağlar (Nordmann et al., 2012).

Çalışmamızda klinik *P. aeruginosa* izolatlarında karbapenem direncinde öne çıkan mekanizma olan karbapenemaz varlığının enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik yöntem ve polimeraz zincir reaksiyonu (PZR) ile değerlendirilmesi amaçlanmıştır.

## 2. YÖNTEM VE GEREÇLER

Bu çalışma, Marmara Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu tarafından (03.02.2023 tarih ve 265 kayıt numarası) onaylanmıştır.

### 2.1. Bakterilerin Seçimi

Çalışmamızda, son 5 yıla ait (2018 Ocak-2023 Şubat) Marmara Üniversitesi Pendik Eğitim ve Araştırma Hastanesi Mikrobiyoloji Laboratuvarı'nda çeşitli klinik örneklerden etken olarak izole edilen ve rutin antibiyogram sonuçlarına bakılarak imipenem, meropenem veya doripenemden en az birine dirençli olduğu saptanan 100 *P. aeruginosa* izolatı kullanılmıştır. Negatif kontrol grubu olarak karbapenem duyarlı olduğu saptanan 20 *P. aeruginosa* izolatı çalışmaya dahil edilmiştir. Bakteriler –80°C derin dondurucudan çıkartılarak, pasajlanmış ve Avrupa Antimikrobik Duyarlılık Testleri Komitesi (EUCAST) standartlarına göre disk difüzyon yöntemi ile doripenem (10 µg), imipenem (10 µg) veya meropenem (10 µg) diskleri kullanılarak test edilmiştir. İnhibisyon zon çapları ölçümünde doripenem için <22 mm, imipenem için <22 mm ve meropenem için <14 mm dirençli kabul edilmiştir.

### 2.2. Karbapenemaz Üretiminin Fenotipik Tespiti

Karbapenemaz üretiminin saptanmasında laboratuvarda hazırlanan enzim-substrat etkileşimine dayalı reaksiyon temelli fenotipik yöntem kullanılmıştır. Test edilecek her izolat için bir kontrol tüpü (sadece pH indikatörü içeren) ve bir test tüpü (antibiyotik ve pH indikatörü içeren) hazırlanmıştır. Kontrol tüpü 100 µL fenol kırmızısı (Sigma, ABD) solüsyonu (%0.5, pH 7.8) ve 10 mmol/L $ZnSO_4$ (Merck, Almanya) çözeltisi içermektedir. Test tüpünde, kontrol tüpü içeriğine ek olarak 6 mg/mL imipenem/silastatin (Tüm Ekip İlaç, Türkiye) içermektedir.

Çalışmaya dahil edilen *P. aeruginosa* izolatları – 80°C derin dondurucudan alınmış ve MacConkey agara (bioMérieux, Marcy-l'Étoile, Fransa) ekilerek canlandırılmıştır. Ardından bakteriler Mueller Hinton agarda (Oxoid/İngiltere) 37°C'de 18-24 saat inkübasyon ile üretilmiştir. Test edilen izolattan 4 öze dolusu alınarak 400 µL Tris–HCl (HiMedia, Hindistan) lizis tamponunda (20 mmol/L) süspanse edilmiştir. Karışım homojen hale gelene kadar vortekslenmiş, oda sıcaklığında 30 dakika inkübe edilmiştir. Süspansiyondan 30 µL kontrol ve test tüplerine eklenmiş ve oda sıcaklığında 1 saat inkübe edilmiştir. Tüpler 15,30,45 ve 60. dakikalarda renk değimi için değerlendirilmiştir. İnkübasyon süresi 2 saate uzatılmış sonuçlarda değişiklik olup olmayacağı değerlendirilmiştir. Kırmızıdan sarıya renk değişimi olan tüpler pozitif sonuç olarak değerlendirilmiştir (Resim 1). Çalışmamızda pozitif kontrol kökenleri olarak; *Klebsiella pneumoniae* CCUG 56233 (KPC pozitif köken), *Escherichia coli* NCTC 13476 (İMP pozitif köken), *K. pneumoniae* NCTC 13440 (VİM pozitif köken), *K. pneumoniae* NCTC 13443 (NDM-1 pozitif köken), *K. pneumoniae* NCTC 13442 (OXA-48 pozitif köken), negatif kontrol kökenleri olarak da *E. coli* ATCC 25922 ve *P. aeruginosa* ATCC 27853 kullanılmıştır (EUCAST, 2017).

***Resim 1.*** *Enzim-substrat ilişkisine dayalı reaksiyon temelli fenotipik yöntemde pozitif izolat içeren solüsyonun zamana bağlı değişen görüntüsü*

### 2.3. Karbapenemaz Üretiminin Genotipik Tespiti

Bakteri DNA izolasyonu için, Mueller-Hinton agarda 18-24 saat inkübasyon sonunda üreyen bakterilerden birkaç koloni alınmış ve 250 µl steril distile su ile homojenize edilmiştir. Karışım kuru blok ısıtıcıda 95°C'de 15 dk tutulmuş ve 13000 rpm'de 5 dk santrifüj edildikten sonra süpernatant kısmı alınarak polimeraz zincir reaksiyonunda (PZR) kalıp DNA olarak kullanılmıştır (Doyle et al., 2012). PZR amplifikasyonları, 12.5 µL 2x PZR Master Miksi (Thermo Scientific, ABD), 1 µL her bir primer, 2 µL hedef DNA ve steril su eklenerek toplam 25 µL hacimde PZR karışımı içinde gerçekleştirilmiştir. Ambler sınıflandırmasına göre en sık gözlemlenen karbapenemazlar olan sınıf A (KPC, GES), sınıf B (NDM, İMP, VİM) ve sınıf D (OXA-48) beta-laktamazları kodlayan genler spesifik primerler ve amplifikasyon koşulları kullanılarak PZR ile tespit edilmiştir (Tablo 1). Tüm PZR ürünleri, boyut belirteci olarak 1 kbp DNA Ladder (Thermo Scientific, ABD) ile 1x TBE tamponu ve etidyum bromür içeren %1.5'luk agaroz jelde yürütülmüş, ultraviyole ışığı altında görüntülenmiştir.

***Tablo 1.*** *PZR'de kullanılan primerler ve amplifikasyon koşulları*

| Primer | Dizi | Boyut(bp) | Amplifikasyon Koşulları | Referans |
|---|---|---|---|---|
| İMP-R | 5'-GAAGGCGTTTATGTTCATAC-3' | 587 | 95°C 5 dk 35 siklus (95 °C 45 sn, 60 °C 45 sn, 72 °C 1 dk), 72 °C 8 dk | Pitout et al., 2005 |
| İMP-F | 5'-GTACGTTTCAAGAGTGATGC-3' | | | |
| KPC-R | 5'-TCTGGACCGCTGGGAGCTGG-3' | 399 | 95°C 2 dk 35 siklus (94 °C 2 sn, 62 °C 10 sn, 72 °C 15 sn) | Poirel et al., 2011 |
| KPC-F | 5'-TGCCCGTTGACGCCCAATCC-3' | | | |
| NDM-R | 5'-GGGCAGTCGCTTCCAACGGT-3' | 475 | 95°C 5 dk 30 siklus (95 °C 30 sn, 60 °C 40 sn, 72 °C 50 sn), 72 °C 6 dk | Perry et al., 2011 |
| NDM-F | 5'-GTAGTGCTCAGTGTCGGCAT-3' | | | |
| OXA-48-R | 5'-TTGGTGGCATCGATTATCGG-3' | 743 | 94°C 5 dk 35 siklus (95 °C 1 dk, 56 °C 45 sn, 72 °C 1 dk), 72 °C 7 dk | Aktaş ve ark., 2008 |
| OXA-48-F | 5'-GAGCACTTCTTTTGTGATGGC-3' | | | |
| VİM-R | 5'-GTTTGGTCGCATATCGCAAC-3' | 389 | 95°C 5 dk 35 siklus (95 °C 45 sn, 60 °C 45 sn, 72 °C 1 dk), 72 °C 8 dk | Pitout et al., 2005 |
| VİM-F | 5'-AATGCGCAGCACCAGGATAG-3' | | | |
| GES-CASA | 5'-ACAAAGATTTCCATCTCAAGGGAT-3' | 860 | 94°C 5 dk 35 siklus (95 °C 45 sn, 56 °C 45 sn, 72 °C 1 dk), 72 °C 7 dk | Çelik, 2007 |
| GES-CASB | 5'-GTTTTAGACGGGCGTCAACT-3' | | | |

### 2.4. Verilerin İstatistiksel Analizi

Verilerin istatistiksel analizi SPSS sürüm 26.0 (IBM, Armonik, NY, ABD) kullanılarak yapılmıştır. Çalışmamızda kullanılan enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik testin duyarlılık, özgüllük, pozitif prediktif değeri (PPV) ve negatif prediktif değeri (NPV) altın standart olarak kullanılan PZR testi ile karşılaştırılarak hesaplanmıştır. İki yöntem arasındaki niteliksel uyum, Phi Cramer's korelasyonu kullanılarak belirleniştir.

## 3. BULGULAR

Çalışmamıza dahil edilen karbapenem dirençli 100 *P. aeruginosa* izolatının genotipik test sonuçlarına göre 46'sının (%46) bir veya daha fazla direnç geni taşıdığı, negatif kontrol grubunun test edilen hiçbir karbapenemaz genini taşımadığı tespit edilmiştir. Fenotipik test ile karbapenemaz enzimi taşıyan 46 izolatın 31'i (%67) pozitif saptanmıştır. Geriye kalan 13 izolat yanlış negatif olarak; moleküler yöntem ile araştırılan direnç genlerini taşımadığı belirlenen 2 izolat ise yanlış pozitif şeklinde değerlendirilmiştir. Negatif kontrol grubunun tümünde fenotipik test negatif sonuçlanmıştır. 46 izolatta genlerin dağılımı, $bla_{NDM}$ (n=36), $bla_{GES}$ (n=2), $bla_{VİM}$ (n=2), $bla_{İMP}$ (n=2), $bla_{OXA-48}$ (n=1), $bla_{VİM}$ ile $bla_{NDM}$ birlikteliği(n=1), $bla_{GES}$ ile $bla_{NDM}$ birlikteliği (n=1) ve $bla_{NDM}$ ile $bla_{OXA-48}$ birlikteliği (n=1) şeklinde saptanmıştır. Hiçbir izolatta $bla_{KPC}$ geni tespit edilmemiştir. Çalışılan *P. aeruginosa* izolatlarının izole edildikleri klinik örneklere göre dağılımı Tablo 2'de verilmiştir.

***Tablo 2.*** *P. aeruginosa izolatlarının izole edildikleri klinik örneklere göre dağılımı (n=120)*

| Klinik Örnek | YBÜ n(%) | YBÜ Dışı n(%) |
|---|---|---|
| Solunum yolu | 15(12.5) | 25(20.8) |
| İdrar | 6(5) | 12(10) |
| Katater ucu | 7(5.8) | 6(5) |
| Sürüntü | 6(5) | 8(6.7) |
| Kan | 4(3.3) | 4(3.3) |
| Diğer steril örnekler | 12(10) | 15(12.5) |
| Toplam | 50(42) | 70(58) |

**YBÜ: Yoğun bakım ünitesi*

Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik testin pozitif ve negatif prediktif değerleri sırasıyla; %93.9 ve %82.8 olarak hesaplanmıştır. Testin duyarlılık ve özgüllüğü sırasıyla; %67.4 ve %97.3 (p<0.0001) olduğu bulunmuştur. Korelasyon analizi sonucunda fenotipik test ile PZR testi arasında çok güçlü anlamlı bir ilişki olduğu saptanmıştır (Phi Cramer's p=0.704, p<0.001) (Tablo 3).

***Tablo 3.*** *Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik testin PZR sonucu ile karşılaştırılması*

| | | PZR Sonucu | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Negatif | Pozitif | Duyarlılık % | Özgüllük % | PPD % | NPD % | p değeri |
| Enzim-Substrat Etkileşimine Dayalı Reaksiyon Temelli Yöntem | Negatif | 72 | 15 | 67,4 | 97,3 | 93,9 | 82,8 | <0,001 |
| | Pozitif | 2 | 31 | | | | | |

**PZR: Polimeraz zincir reaksiyonu, PPD: Pozitif prediktif değer, NPD: Negatif prediktif değer*

## 4. TARTIŞMA VE SONUÇ

*P. aeruginosa* sağlık bakımıyla ilişkili enfeksiyonları olan hastalarda en sık izole edilen patojenlerden birisidir ve çok sayıda antibiyotiğe karşı doğal dirençlidir (Halat and Moubareck, 2022). Bununla birlikte pseudomal enfeksiyonların tedavisinde son seçenek olarak kabul edilen karbapenemler de dahil pek çok antibiyotik grubuna karşı direnç kazanması küresel bir sağlık sorunu haline gelmiştir (Zhang et al., 2016). Bu durum enfeksiyonların tedavisini zorlaştırmaktadır ve hastaneye yatırılan veya bağışıklık sistemi zayıflamış hastalar arasında morbidite ve mortalite oranlarını arttırmaktadır (Shaaban et al., 2017). DSÖ, *P. aeruginosa'yı* sağlık bakımıyla ilişkili enfeksiyonlarda ve salgınlarda öncelikli tehdit oluşturan altı mikroorganizmadan biri olarak göstermektedir (Tacconelli et al., 2018).

Avrupa Hastalık Önleme ve Kontrol Merkezi'nin 2022 sürveyans verilerine göre dünya genelinde *P. aeruginosa'*da karbapenem direnç oranının %18.1, Türkiye'de ise %39 olduğu bildirilmiştir (ECDC, 2022).

*P. aeruginosa*'da karbapenem direncinin gelişmesinde OprD porin kaybı, dışa atım pompalarının aşırı ekspresyonu ve karbapenemaz üretimi gibi çok sayıda mekanizma rol oynar (Jean et al., 2022). Karbapenemazlar, plazmid ve transpozon gibi mobil genetik elemanlar aracılığıyla çeşitli bakteri klonları arasında hızla aktarılıp yayılabildiğinden bu mekanizmalar içinde kritik önem taşımaktadır (Henry et al., 2011). *P. aeruginosa*'da Ambler sınıf A (KPC ve GES tipi beta-laktamazları), sınıf B (İMP, VİM ve NDM gibi farklı metalo-β-laktamazlar) ve sınıf D (OXA-48) gibi çok sayıda karbapenemaz tanımlanmıştır. Karbapenemazların dağılımı ülkeler arasında farklılık göstermektedir. VİM, İMP ve NDM metalo-beta-laktamazlar en yaygın olanlardır (Walsh et al., 2005). Bunun yanı sıra dünya genelinde; KPC, GES ve OXA gibi beta-laktamaz türlerinin varlığının giderek arttığı rapor edilmiştir (Potron et al., 2015).

Karbapenemaz üretimine bağlı direnç, dünya çapında *P. aeruginosa*'nın karbapenemlere karşı direnç geliştirdiği belirtilen vakaların %39'unu oluşturmaktadır. Avrupa'da ise bu oran %30,6'dır (Rizek, C et al., 2014; Castanheira, M et al., 2014). Çalışmamızda karbapenem dirençli 100 izolattan 46'sının (%46) genotipik yöntemle bir veya daha fazla karbapenemaz geni taşıdığı tespit edilmiştir. Karbapenem

dirençli olan fakat direnç geni saptanmayan 54 izolatın ise diğer mekanizmaları kullanarak direnç kazandığı düşünülmektedir.

*P. aeruginosa*'da karbapenem direnci prevalansı pek çok ülkede %10-50 arasında değişmektedir. Son yıllarda karbapenemlere karşı gelişen direncin tüm dünyada giderek artması nedeniyle bu dirençli mikroorganizmanın direnç mekanizmasının erken saptanmasına yönelik yeni yöntemlerin geliştirilmesine ihtiyaç doğmuştur (Çopur ve ark., 2021).

Karbapenemaz genlerinin genotipik tekniklerle tespit edilmesi altın standarttır. Fakat bu yöntem yüksek maliyetlidir, uzmanlık ve uygun laboratuvar olanakları gerektirmektedir (Malkoçoğlu ve ark., 2017). Ek olarak bilinmeyen ve yeni direnç genlerinin varlığı tespit edilemeyeceğinden yanlış negatif sonuç riski bulunmaktadır (Aktaş ve ark., 2017). Karbapenemazların saptanması amacıyla çeşitli fenotipik yöntemler (Modifiye Hodge testi, karbapenem inaktivasyon metodu, immünokromatografik testler, Carba Np testi vb.) geliştirilmiştir. Tüm bu fenotipik testlerin hız, maliyet, duyarlılık ve özgüllükleri yönünden sınırlamaları bulunmaktadır. Dolayısıyla rutin laboratuvarda uygulanabilmesi için karbapenemaz üretimini hızlı tespit eden, basit, güvenilir ve uygun maliyetli yeni testlere ihtiyaç vardır (Osei et al., 2015; Aktaş ve ark., 2017). Son yıllarda, Nordmann ve çalışma arkadaşları tarafından karbapenemazların hızlı ve ucuz tespiti için Carba NP (Carbapenemase Nordmann-Poirel) testi geliştirilmiş ve ticari olarak pazara verilmiştir (Nordman et al.2012; Dortet et al., 2014). Carba NP, karbapenem grubu ilaçların beta-laktam halkasının enzimatik hidrolizi sonucu pH indikatörünün renk değişikliğine dayanan kolorimetrik bir testtir (Dortet et al., 2014). Klinik ve Laboratuvar Standartları Enstitüsü (CLSI) ve EUCAST kılavuzlarında yer alan Carba-NP testi, yüksek doğruluk oranlarına sahip olması ve 2 saat gibi kısa süre içinde karbapenemaz aktivitesini saptamasıyla diğer fenotipik yöntemlere göre büyük bir avantaj sağlamıştır (CLSI, 2015; EUCAST, 2017). Carba NP testinin, karbapenemaz üreten *Pseudomonas spp.* için %100 spesifik ve %94.4 duyarlı olduğu bildirilmiştir (Nordmann ve Poirel, 2013). Fakat mukoid izolatlarda ve OXA-48 gibi zayıf karbapenemaz aktivitesi gösteren izolatları tespit etmede zorlanması gibi dezavantajları vardır. (Tijet et al., 2013).

Carba NP testinin sınırlamalarının üstesinden gelmek, maliyeti azaltmak ve işlem süresini kısaltmak amacıyla çeşitli modifikasyonları geliştirilmiştir. Çalışmamızda kullandığımız enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik test, CLSI'de açıklanan Carba NP testinin bir modifikasyonudur (CLSI, 2015). Kullandığımız enzim-substrat etkileşimine dayalı reaksiyon temelli yöntemde Carba NP 'den farklı olarak bazı değişiklikler yapılmıştır: Bakteri inokülüm miktarı arttırılmış ve Tris-HCl lizis tamponunda süspanse edilmiştir. Bakteri süspansiyonu santrifüjleme yapılmadan homojen hale gelene kadar vortekslenmiş ve testte bütün bakteri lizatı kullanılarak, etüv yerine oda sıcaklığında 30 dakika inkübe edilmiştir. Ek olarak imipenem monohidrat (3 mg/ml) yerine ticari olarak bulunabilen imipenem-silastatin (6 mg/ml) kullanılmıştır (Abdel Ghani et al., 2015). Çalışma için 1,5 ml'lik mikrotüpler kullanılmış ve sonuçlar 2 saat yerine 1 saat sonunda değerlendirilmiştir (Dortet et al., 2014; Nordmann et al., 2012). Bu değişiklikler yapılarak testin yüksek maliyetinin ve testin uygulama süresinin azaltılması hedeflenmiştir.

Çalışmamızda 36 izolat blaNDM ve 3 izolat çoklu karbapenemaz genleri ($bla_{VİM}$ + $bla_{NDM}$, $bla_{GES}$ + $bla_{NDM}$ ve $bla_{NDM}$ + $bla_{OXA-48}$) varlığı moleküler yöntemle tespit edilmiştir. Çoklu karbapenemazların görüldüğü ve aynı protokoller uygulanarak Modifiye Carba NP testinin değerlendirildiği bir çalışmada 25 *P. aeruginosa* izolatı çalışılmıştır. Yedi izolatta blaNDM direnç geni ve 6 izolatta çoklu karbapenemaz direnç geni (blaNDM + blaOXA 48 benzeri, blaNDM + blaVİM, blaNDM + blaİMP, blaVİM + blaOXA 48 benzeri gen) dahil edilerek toplam izolat 16 karbapenemaz pozitif saptanmıştır. Çalışmamıza benzer şekilde hiçbirinde blaKPC geni saptanmamıştır. Karbapenemaz geni saptanmayan 9 örneğin tamamı fenotipik yöntemlerle dirençli olarak tanımlanmıştır. Modifiye Carba NP testi NDM üreten izolatlardan yalnızca 3'ünde (%43) ve çoklu karbapenemaz üreten izolatlardan 3'ünde (%50) doğru sonuç vermiştir. Çalışmamızda çoklu enzim içeren izolatların tamamı (n=3) fenotipik yöntem ile doğru tanımlanmıştır. NDM üreten izolatların %75'inde (n=27) fenotipik yöntem ile pozitif sonuç gözlemlenmiş olup bu sonuçlar, çalışmamızda çoklu enzim veya NDM üreten izolatların daha iyi tanımlandığını göstermektedir (Rudresh et al., 2017).

Yapılan çalışmalarda, enfeksiyon etkeni olan *P. aeruginosa'*da karbapenem direncinin hızlı tespiti özellikle vurgulanmaktadır (Nordmann et al., 2012; Nordmann 2014). Çalışmamızın önemli sonuçlarından biri; NDM, VİM, İMP, OXA-48 VE GES tipi karbapenemazların fenotipik test kullanılarak 1 saat gibi kısa sürede tespiti sağlanmıştır. İnkübasyon süresi 2 saate kadar uzatılarak sonuçlar tekrar değerlendirilmiş ve tüplerde herhangi bir renk değişimi gözlemlenmemiştir. Kritik hastalarda uygun antibiyotik tedavisinin verilmesindeki 1 saatlik gecikmenin hastanın ölüm riskini %20 artırdığı dikkate alındığında, testin inkübasyon süresinin 2 saatten 1 saate düşmesi hastaya zamanında uygun tedaviyi vermek açısından büyük önem taşımaktadır (Kumar et al., 2006). Buna ek olarak 3 izolatta testin ilk 20 dakika içinde pozitif sonuç verdiği tespit edilmiştir. PZR analizlerinde bu izolatların blaNDM geni taşıdığı belirlenmiştir. Negatif kontrol grubunda fenotipik testle de negatif saptanmıştır. Buna karşın PZR ile karbapenemaz geni saptanan 13 izolat fenotipik testle yanlış negatif sonuç vermiştir. Benzer bir çalışmada karbapenemaz üreticisi olduğu saptanan fakat fenotipik test ile negatif sonuçlanan izolatların, direnç genlerinin ekspresyonunun hiç olmadığı veya düşük seviyede olduğu için fenotipik yöntem ile karbapenemaz varlığı tespit edilemediği bildirilmiştir (Dortet et al., 2014).

Literatürdeki bir çalışmada bakteri ekstraktı miktarının arttırılmasıyla duyarlılık ve özgüllüğün sırasıyla %72,5 ve %69,2'den %80 ve %77,3'e arttığı gösterilmiştir (Tijet et al., 2013). Özellikle OXA-48 ve GES enzimlerinde bakteri inokülüm miktarının artışıyla duyarlılığın yükseldiği gösterilmiştir

(Aguirre-Quiñonero, ve Martínez-Martínez, 2017). Çalışmamızda yanlış negatif sonuç veren izolatlardan bakteri inokülüm miktarı ve antibiyotik düzeyi arttırılarak deney tekrar edildiğinde test sonucumuzda herhangi bir değişiklik gözlemlenmemiştir. Ayrıca çalışmamızda süspanse edilmesi zor olan çok sayıda mukoid izolat ile karşılaşılmıştır. Mukoid izolatlar kullanılan yöntemin en önemli dezavantajlarından biridir. Yanlış negatif sonuçlar, OXA-48 alt tipleri gibi düşük hidrolitik karbapenemaz aktivitesine sahip enzimlerin varlığına ya da mukoid yapıdaki izolatlarla ilişkilendirilebilir (Yusuf et al., 2014). Daha önce yapılan çalışmalarda mukoid izolatların yanlış negatif sonuçlara yatkın olduğu belirtilmiştir (Tijet et al., 2013; Tijet et al., 2014).

Karbapenemaz aktivitesinin enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik test ile tespiti için standart bir protokol bulunmasa da benzer prosedürlerin uygulandığı birçok çalışma yapılmıştır. Farklı çalışmalarda testlerin duyarlılıkları ve özgüllükleri %94-100 ve %98-100 aralığında bulunmuştur (Mangayarkarasi et al., 2018; Yusuf et al., 2014; Tamma et al., 2017; Bayramoğlu ve ark., 2016). Çalışmamızda karbapenemaz üretimi için fenotipik testin duyarlılık ve özgüllüğü sırasıyla %67,4 ve %97.3 olarak bulunmuştur. Duyarlılık oranımız hem ülkemizdeki diğer çalışmalarda hem de dünya literatüründe verilen oranlardan düşüktür (Thomson et al., 2017; Aguirre-Quiñonero, ve Martínez-Martínez, 2017;, Bayramoğlu ve ark., 2016). Öte yandan suşlardaki karbapenemaz enzimlerinin dağılımı ve mukoid izolatlar çalışmalar arasındaki farlılıkları açıklayabilir. Çalışmamızda kullanılan izolatların çoğunluğu NDM taşıyıcısıdır.

Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik testin en önemli avantajlarından biri uygulanmasının kolay olması ve uzmanlık gerektirmemesidir. Rutin laboratuvarda bulunan ekipmanlarla üreme gözlemlenen klinik örneklerden kısa sürede karbapenemaz aktivitesi hakkında bilgi sahibi olunabilir.

DSÖ, karbapenemaz aktivitesini saptamada uygun maliyetli testlerin geliştirilmesini önermektedir. Çalışmamızda test başına maliyet yaklaşık 5 TL (0.14 Euro, 26.01.2024) olarak hesaplanmıştır. Benzer bir çalışmada bir izolat için test maliyeti yaklaşık 0.31 euro, farklı bir çalışmada ise test başına maliyet <0.2 dolar olarak belirtilmiştir (Yusuf et al., 2014; Rudresh et al., 2017).

Enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik testin bir diğer avantajı moleküler yöntemlerle tespit edilemeyenler de dahil olmak üzere tüm karbapenemaz türlerini tespit edebilmesidir (Akhi et al., 2017; Nordmann, 2017). Buna karşın test çıplak gözle subjektif olarak yorumlandığı için küçük renk değişiklikleri yanlış veya belirsiz sonuçlara yol açabilmektedir (Simner et al., 2018).

Sonuç olarak, karbapenem dirençli *P. aeruginosa* izolatlarının hızlı ve doğru tanımlanması, zamanında uygun tedavinin verilmesi ve enfeksiyon kontrol önlemlerini başarılı bir şekilde uygulanması açısından çok önemlidir. Diğer fenotipik yöntemlerle karşılaştırıldığında, enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik test, sonucun hızlı elde edilmesi, kolaylıkla uygulanabilmesi ve kısıtlı bütçeye sahip laboratuvarlarda kullanılabilmesi gibi avantajları nedeniyle öne çıkmaktadır. Çalışmamızda kullandığımız enzim-substrat etkileşimine dayalı reaksiyon temelli kolormatik testin performansının değerlendirilmesine yönelik daha fazla sayıda örnek içeren *in vitro* çalışmalara ihtiyaç olduğunu düşünüyoruz.

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# Evaluation of Nutritional Status of Children with Autism Spectrum Disorder Receiving Daytime Rehabilitation

## Gündüzlü Rehabilitasyon Gören Otizm Spektrum Bozukluğu Olan Çocukların Beslenme Durumlarının Değerlendirilmesi

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**ABSTRACT**

**Objective:** The aim of this study was to evaluate the nutritional status, eating habits and behavior of children with autism spectrum disorder.

**Methods:** This observational study included 109 individuals from Istanbul diagnosed with autism spectrum disorder aged 3-18 years; the patients were receiving daytime rehabilitation. A questionnaire including socio-demographic characteristics, nutritional behavior and eating habits of participants was applied by face-to-face interview method, anthropometric measurements (body weight and height) and food consumption records were taken, using a 24-hour dietary recall taken by the researchers.

**Results:** Of all, 66.1% (n=72) of the participants were male. It was determined that 16.2% (n=6) of the female participants were overweight and 27.0% (n=10) were obese, while 33.3% (n=24) of the male participants were overweight and 29.2% (n=21) were obese. The rate of food selectivity was found to be 59.6%; 28.4% of the participants received additional support during mealtimes, 13.8% followed various special diets, including gluten-free and casein-free diets. Most of the participants' energy intake (60.6%), vitamin D (100.0%) and calcium (71.6%) were below reference values. The majority of the participants' vitamin A (92.7%) and sodium (92.7%) intakes were above the recommended values.

**Conclusion:** The findings of the study reveal that children and adolescents with autism spectrum disorder have high rates of obesity and food selectivity. The need for additional support at mealtimes, and the inadequate or excessive intake of nutrients are among other nutrition-related problems faced by this population.

**Keywords:** Nutrition, children, autism spectrum disorder

**ÖZ**

**Amaç**: Bu çalışmada, otizm spektrum bozukluğu olan çocukların beslenme durumlarının, yeme alışkanlıklarının ve davranışlarının değerlendirilmesi amaçlanmıştır.

**Gereç ve Yöntem:** Bu gözlemsel çalışmaya İstanbul'da gündüzlü rehabilitasyon gören, 3-18 yaş arası, otizm spektrum bozukluğu olan 109 birey dahil edilmiştir. Katılımcılara yüz yüze görüşme metoduyla sosyo-demografik özellikleri, beslenme davranışları ve yemek yeme alışkanlıklarını içeren bir anket uygulanmış, antropometrik ölçümleri (vücut ağırlığı ve boy uzunluğu) ve 24 saatlik hatırlatma yöntemi ile besin tüketim kayıtları araştırmacılar tarafından alınmıştır.

**Bulgular:** Yüz dokuz katılımcının %66,1 (n=72)'inin erkek olduğu saptanmıştır. Kızların %16,2 (n=6)'sinin hafif şişman, %27,0 (n=10)'sinin obez olduğu, erkek katılımcıların ise %33,3 (n=24)'ünün hafif şişman ve %29,2 (n=21)'sinin obez olduğu belirlenmiştir. Katılımcılarda, besin seçicilik oranı %59,6 olarak bulunmuş ve katılımcıların %28,4'ü öğün zamanlarında ek destek aldığı, %13,8'i glütensiz ve kazeinsiz diyet de dahil olmak üzere çeşitli özel diyetler uyguladığı belirlenmiştir. Katılımcıların çoğunun enerji (%60,6), D vitamini (%100,0) ve kalsiyum (%71,6) alımları referans değerlerin altında; A vitamini (%92,7) ve sodyum (%92,7) alımları önerilen değerlerin üzerinde bulunmuştur. Sonuç: Çalışmanın bulguları, Otizm Spektrum Bozukluğu olan çocuk ve adölesanlarda yüksek oranda obezite ve besin seçiciliği olduğunu ortaya koymaktadır. Öğün zamanlarında ek desteğe ihtiyaç duyulması, yetersiz veya fazla besin ögesi alımı, bu popülasyonun karşılaştığı diğer beslenme ile ilintili sorunlar arasındadır.

**Anahtar Kelimeler:** Beslenme, çocuk, otizm spektrum bozukluğu



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## 1. INTRODUCTION

Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disorder that can generally be diagnosed before the age of three. According to the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5), some criteria have been determined for a person to be diagnosed with ASD. Examples of these criteria include deficits in social communication and interaction, persistent behavior in monotonous tasks, stereotyped and repetitive motor movements, excessive perception of sensory stimuli, overly rigid constancy in one's interests, and social and sensory contrasts (APA, 2013). In March 2023, the Centers for Disease Control and Prevention (CDC) proclaimed that according to the Autism and Developmental Disabilities Monitoring Network, the prevalence of ASD was at a rate of 1/36 (CDC, 2023).

There are medical, nutritional and behavioral contextual factors that affect the nutritional status of individuals with ASD. There are three main subcategories of behavioral contextual factors; problem eating behaviors, sensory processing difficulties, and family factors. The most frequent problematical eating behaviors include food selectivity, food refusal and sometimes overeating, chewing difficulty and eating inedible substances (Özeren, 2013). In the literature, there are significant differences in the incidence of these eating problems and food selectivity in this population (Gilger & Redel, 2009).

When the metabolic and nutritional status of individuals with ASD were compared with neurotypical individuals, many differences were found between the two groups (Adams et al., 2011). It is stated that children with ASD are more prone to being overweight and obese between the ages of 2-5 and being underweight between the ages of 5-11. It is also emphasized that among individuals with ASD obesity increases after childhood (Li et al., 2020).

The incidence of gastrointestinal system (GIS) symptoms in individuals with ASD has been reported as between 9-70%, and common GIS problems include chronic diarrhea, chronic abdominal pain, gastroesophageal reflux disease (GERD), and constipation (Buie et al., 2010). Some types of medications used in individuals with ASD, including antiepileptic agents, stimulants, and atypical antipsychotic medications may have side effects that can affect nutrition (i.e., increasing appetite or malabsorption of some specific nutrients) (West et al., 2009). Furthermore, it has been emphasized that there is insufficient (suboptimal) nutritional intake in children with ASD, and attention should be paid especially to bone health, focusing on calcium and vitamin D (Hyman et al., 2012; Geraghty et al., 2010). Nutritional deficiencies in ASD are thought to be related to inadequate digestion and absorption of nutrients caused by GIS problems, anomalies in metabolic utilization of nutrients, elimination diets and eating problems. Nutrient deficiencies can be associated with food selectivity if one of the food groups is completely skipped or food variety decreases (Kirby & Danner, 2009).

In this study, it was aimed to evaluate the nutritional status of children with ASD who received daytime rehabilitation, as well as to investigate eating problems in these individuals and to determine the effects of these problems on their nutritional status.

## 2. MATERIALS AND METHODS

### 2.1. Study Design and Participants

This observational cross-sectional study was conducted with volunteer parents/caregivers of children with autism spectrum disorder (ASD) who received daytime rehabilitation in Istanbul.

This study was conducted between February and June 2018. Four daytime rehabilitation centers located on the Anatolian side of Istanbul were selected randomly. The study population was 152 children receiving outpatient treatment in the four rehabilitation centers. The sample size was calculated using the Epi Info program. In this calculation, the sample size was determined as n=109 when the frequency of the event was taken as 50%, the level of error as 5%, the pattern effect as 1, and the 95% confidence interval. A total of 115 children with ASD between the ages of 3-18 were invited to the study and the study was completed with 109 children. The children whose anthropometric measurements could not be taken (n=4) or who had problems with oral intake (n=2) were excluded from the study. Ethical approval for the study was obtained from the Clinical Research Ethics Committee of Marmara University Faculty of Medicine, decision number 09.2017.045 on 06.01.2017. Prior to the application of the questionnaire and measurements, written consent was obtained from the legal guardians of the participants with a voluntary information form.

### 2.2. Data Collection

Anthropometric measurements of the participants were measured by researchers in the presence of parents/caregivers and educators working in rehabilitation centers. Body Mass Index (BMI) was calculated with the formula "body weight (kg)/height (m)²".

The 24-hour dietary recalls were taken from the parents/caregivers of participants. The amount of ingredients included in the meals were calculated by using the "Standard Recipes" book, and the measurement amounts were calculated using the "Food Photo Catalog" book.

Socio-demographic information, nutritional behavior and eating habits of the participants were investigated with the face-to-face interview method.

### 2.3. Data Analyses

World Health Organization – Multicenter Growth Reference Study (WHO-MGRS) Growth Curves were used for children aged 0-5 (WHO, 2007; WHO, 2006). Body weight for age,

height for age and body mass index for age were evaluated according to Z-scores for children aged 5-19 years, using the AnthroPlus Computer program, based on the reference values created by WHO in 2007 (WHO, 2023).

The food consumption records obtained were analyzed using the "Nutrition Information Systems Package Program (BeBiS) Version 7.0" (EBISpro, 2023). The intake of energy, macronutrients and micronutrients of the participants were analyzed and compared to the Estimated Average Requirement (EAR) and Adequate Intake (AI) values for the Turkish population (TÜBER, 2019). The food consumption of each participant was calculated according to the reference values of their age groups. Macro – and micronutrients that are frequently inadequate or over-intake in children with ASD were evaluated (Bicer & Alsaffar, 2016). Percentages of children with inadequate and excess intakes of energy and macro – and micronutrients were calculated in comparison with EAR/AI reference values.

All data were evaluated by using the "SPSS 21.0 Computer Package Program". The distribution of data is expressed as numbers (n) and percentages (%).

## 3. RESULTS

One hundred and nine children aged between 3 and18 participated in the present study. Of these, 66.1% (n=72) of the participants were male. Table 1 presents the distribution of BMI classifications of participants by gender. Of all the male participants, 33.3% (n=24) were overweight, and 29.2% (n=21) were obese. In female participants, 46.0% (n=17) were in the normal range of BMI, and 27.0% (n=10) were obese.

***Table 1.*** *BMI classification by gender*

| BMI Classification | Male (n=72) | | Female (n=37) | |
|---|---|---|---|---|
| | n | % | n | % |
| Underweight | 2 | 2.8 | 4 | 10.8 |
| Normal | 25 | 34.7 | 17 | 46.0 |
| Overweight | 24 | 33.3 | 6 | 16.2 |
| Obese | 21 | 29.2 | 10 | 27.0 |

*BMI: Body Mass Index*

The eating and mealtime behaviors of the participants are given in Table 2. It was stated that out of 15 (13.8%) children followed a special diet; 8 followed a gluten-free and casein-free diet, 3 a diabetic diet, one DASH diet, one a low-cholesterol diet, one a lactose-free diet, and one a protein-based diet (not shown in the table).

The frequency of participants' energy and nutrient intakes being below or above the EAR/AI values according to the recommended values for their age is given in Figure 1. It was found that the majority of the participants' energy (60.6%), fiber (51.4%), vitamin D (100.0%) and calcium (71.6%) intakes were below the EAR/AI values. The vitamin A (92.7%) and sodium (92.7%) intakes of most of the participants were above the EAR/AI reference values.

***Table 2.*** *Eating and mealtime behaviors*

| Variables | | n | % |
|---|---|---|---|
| **Food selectivity** | Yes | 65 | 59.6 |
| | No | 44 | 40.4 |
| **Eating place** | Table | 83 | 76.1 |
| | In front of TV | 10 | 9.2 |
| | Couch | 6 | 5.5 |
| | Standing | 5 | 4.6 |
| | Wandering around | 5 | 4.6 |
| **Eating with** | Alone | 19 | 17.4 |
| | Family | 77 | 70.6 |
| | Friends | 3 | 2.8 |
| | Caregiver | 10 | 9.2 |
| **Getting additional support** | Yes | 31 | 28.4 |
| | No | 78 | 71.6 |
| **Special Diet** | Yes | 15 | 13.8 |
| | No | 94 | 86.2 |

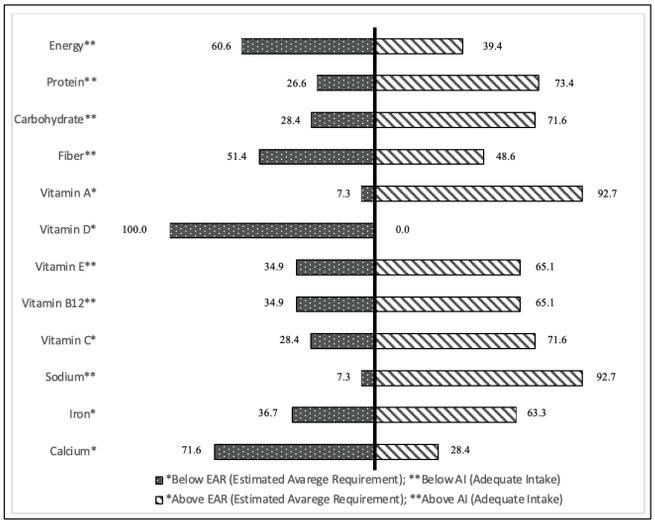


***Figure 1.*** *Energy and nutrient intakes below or above the EAR/AI (%)*

## 4. DISCUSSION

In this study, which aimed to evaluate the nutritional status of individuals with ASD who were receiving daytime rehabilitation, the rate of overweight and obese participants was found to be high; 59.6% of them had food selectivity, and most of the participants' energy, fiber, vitamin D, and calcium intakes were found to be below the recommended values.

According to a CDC press release in March 2023, ASD is nearly four times higher in boys than girls (CDC, 2023). In the study by Shmaya et al., with 91 individuals, 80.4% of the participants were male (Shmaya et al., 2017). Similarly, the rate of male participants was found to be higher (66.1%) in this study.

Recent studies have reported that psychiatric disorders such as attention-deficit/hyperactivity disorder and ASD in children may be related to obesity and being overweight (Kummer et al., 2016). A meta-analysis compiling 15 studies with a total

of 1,045,538 ASD cases showed that obesity, not overweight, was associated with ASD (Zheng et al., 2017). According to the study by Egan and colleagues, 17.16% of 273 children with ASD were overweight and 21.89% were obese (Egan et al., 2013). In a study conducted with 51 children with ASD, it was found that 44.9% of the children were within the normal BMI range and 16.8% were obese (Molina – López et al., 2021). In the study of Bicer & Alsaffar, 32.3% of individuals with ASD were found to be obese (Bicer & Alsaffar, 2013). Considering the general population in Turkey, 17.9% of children aged 0-5 are overweight and 8.5% are obese; 14.3% of children aged 6-18 years are overweight and 8.2% are obese; and in the 18-30 age group 26.9% are overweight and 10.2% are obese (TBSA, 2014). In this study, it was determined that 16.2% of female participants were overweight, 27% were obese and 33.3% of male participants were overweight, 29.2% of them were found to be obese. These results show that, in parallel with the literature, the rates of being overweight and obesity are higher in individuals with ASD than in the general population.

Studies have reported the prevalence of mealtime eating problems in individuals with ASD as being between 43.6% and 96% (Mayes & Zickgraf, 2019; Gray et al., 2018; Seiverling et al., 2018), and the most frequently seen eating problem was food selectivity. For instance, in the study of Cornish et al., it was stated that 70% of children with ASD have food selectivity (Cornish, 1998); in a more recent study, this rate was found to be 72% (Schreck & Williams, 2006). In the study conducted by Williams et al., 67% of parents stated that they faced the problem of food selectivity. In addition, the factors affecting this selectivity were texture (69%), appearance (58%), taste (45%), smell (36%), and temperature (22%). Moreover, it has been emphasized that individuals with ASD have a small repertoire of foods (60%) (Williams et al., 2000). In the study of Schreck et al., it was also stated that 57% of individuals with ASD exhibited food refusal (Schreck & Williams, 2006). In the current study, similar to these studies, it was found that 59.6% of participants exhibited food selectivity.

Among other atypical eating behaviors, it has been shown that individuals with ASD have difficulty sitting at the table until mealtime is over (Gray et al., 2018; Attlee et al., 2015). In the current study, 76.1% of participants sat at the table until the end of mealtime, whereas 4.6% stood and 4.6% wandered around during mealtimes. In a study, mothers who have children with ASD emphasized that it was difficult to eat together as a family at mealtimes and important for their children to eat with at least one family member (Ausderau & Juarez, 2013). It was also emphasized that individuals with ASD needed additional support at mealtimes (Marquenie et al., 2011; Williams & Seiverling, 2010). In the current study, the rate of participants who ate alone was 17.4%, and of all, 28.4% of the participants received help during mealtimes. These results show similarities with previous studies.

Although the gluten-free and casein-free (GFCF) diet does not cause a significant improvement in ASD symptoms or ASD-related nutritional or eating problems, it is the most studied diet in the literature and is the most preferred one by families of individuals with ASD (Cornish, 2002). In the current study, it was found that 7.3% of participants followed the GFCF diet.

Food selectivity that restricts food consumption is common in individuals with ASD, and this may lead to nutrient deficiencies (Esteban-Figuerola et al., 2019). Many studies have reported various nutrient deficiencies in individuals with ASD (Molina – López et al., 2021; Esteban-Figuerola et al., 2019; Stewart et al., 2015; Hyman et al., 2012). In a study with 288 children with ASD between the ages of 2 and 11 it was stated that more than 40% of children were at risk of insufficient intake of vitamins D and E, calcium, choline, potassium, and pantothenic acid (Stewart et al., 2015). In another study with a control group paired with ASD, children with ASD consumed fewer calories, vitamins A and C, and zinc; the majority met the recommended reference values for vitamins K and E. Certain age groups consume excessive amounts of sodium, folate, manganese, zinc, vitamin A, selenium, and copper (Hyman et al., 2012). In another case-control study, a greater percentage of children with ASD were below the RDA for energy and fiber intake (for energy, ASD group: 34.9% and control group: 15.3%; for fiber, ASD group: 37.2% and control group: 8.5%). Although no significant difference was observed between the groups in terms of vitamin and mineral intakes, more children with ASD showed greater deficiencies in vitamins such as vitamin B2 and retinol, and minerals such as calcium, magnesium, iron, selenium, and iodine (Molina – López et al., 2021). A meta-analysis stated that children with ASD consumed less protein, calcium, phosphorus, selenium, vitamin D, thiamine, riboflavin, and vitamin B12 and more polyunsaturated fatty acids and vitamin E than the control group (Esteban-Figuerola et al., 2019). Contrary to the results of those studies that found insufficient calcium intake in children with ASD, Plaza-Diaz et al. found that approximately 79% of children with ASD had adequate calcium intake, which was a higher rate than neurotypical children (Plaza-Diaz et al., 2021). In this study, especially energy, vitamin D, and calcium intake were found to be below the recommended values in most children with ASD. The results of different studies on nutrient intakes are affected by many environmental and cultural factors. Different results in the literature may differ depending on conditions such as special diet applications and food selectivity in the sampled participants.

Limitations of this study include the participants being from one city of Turkey, which makes it difficult to represent the whole population, and not having a control group to compare. Moreover, taking 24-hour dietary recalls may not be representative for their general nutritional intakes, but individuals with ASD have a routine eating pattern so it is thought that 24-hour dietary recalls might actually represent their daily nutrient intakes. In addition to their anthropometric measurements and food records, mealtime behaviors of participants were also assessed; this is a strength of this study.

## 5. CONCLUSION

In conclusion, most of the participants were overweight or obese, and had a high rate of food selectivity, one of the major eating problems of this population. Some of the participants received additional support during mealtimes and followed a specific diet (i.e., gluten-free and casein-free diet). Fiber, calcium and vitamin D intakes were some of the nutrients which were below the recommended values as well as energy intakes. Further research with a wider sample size is needed to explore additional factors contributing to nutritional challenges and eating problems faced by this population.

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# Microfluidic Technology for Detection

## Mikroakışkan Teknolojisinin Tanıda Kullanımı

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**ABSTRACT**

Microfluidics focuses on the movement and interactions of fluidic substances, such as liquids or gases, on a microscopic scale. In general, it provides to take controls and handles fluids by utilizing devices and systems that possess microscale structures within 1-500 micrometer. Microfluidic technology has emerged as a powerful tool for detecting various substances, including pathogens, biomarkers, pesticide residues, gases, and airborne microorganisms. Detection is a key aspect of microfluidic devices by quantifying analyte concentrations on the order of micrometers or determining the mere absence or presence of an analyte.

In microfluidic systems, the detection of various analytes can be achieved through various integrated modules that utilize different physical principles, including electrochemical, optical, magnetic, and thermal methods. The versatility of these integration modules enables researchers to develop their detection strategies to suit diverse application requirements in biomedical, clinical, environmental monitoring, and food safety fields. The benefits of microfluidic systems include rapid detection, ease of use, cost-effectiveness, and high accuracy for the identification of infectious diseases.

In this review, the utilization of detection methods within microfluidic systems and their applications across various domains including biomedical, clinical, environmental monitoring, food safety, and point-of-care diagnostics are being extensively explored by searching English articles published between 2001-2023 in academic databases Pub Med and Google scholar .The review aims to provide a comprehensive understanding of how microfluidic technology enhances detection capabilities, thereby contributing to advancements in healthcare, environmental science and food safety.

**Keywords:** Microfluidics, Detection, Electrochemical detection, Optical detection, Magnetic detection.

**ÖZ**

Mikroakışkanlar, sıvılar veya gazlar gibi akışkan maddelerin mikroskobik ölçekteki hareketi ve etkileşimleri üzerine odaklanır. Genel olarak 1-500 mikrometre arasında mikro ölçekli yapıya sahip olan bu cihazlar ve sistemler, sıvıları kontrol etmeyi ve işlemeyi sağlar. Mikroakışkan teknolojisi patojenler, biyobelirteçler, pestisit kalıntıları, gazlar ve havadan yayılan mikroorganizmalar gibi çeşitli maddeleri tespit etmek için güçlü bir araç olarak ortaya çıkmıştır. Mikroakışkan cihazların tespit özelliği, analit konsantrasyonlarını mikrometre düzeyinde ölçerek veya bir analitin basit varlığını veya yokluğunu belirleyerek anahtar bir özelliktir.

Mikroakışkan sistemlerde, çeşitli analitlerin tespiti, elektrokimyasal, optik, manyetik ve termal yöntemler gibi farklı fiziksel prensipleri kullanan çeşitli birleştirilmiş modüller aracılığıyla gerçekleştirilebilir. Bu entegrasyon modüllerinin çok yönlülüğü, araştırmacılara biyomedikal, klinik, çevresel izleme ve gıda güvenliği alanlarında çeşitli uygulama gereksinimlerine uygun tespit stratejileri geliştirmelerini sağlar. Mikroakışkan sistemlerin faydaları, enfeksiyon hastalıklarının tanımlanması için hızlı tespit, kullanım kolaylığı, maliyet etkinliği ve yüksek doğruluk içerir.

Bu derlemede, mikroakışkan sistemlerdeki tespit yöntemlerinin kullanımı ve biyomedikal, klinik, çevresel izleme, gıda güvenliği ve bakım noktası tanıları gibi çeşitli alanlardaki uygulamaları kapsamlı bir şekilde incelenmektedir. Bu inceleme için PubMed ve Google Scholar akademik veri tabanlarında 2001-2023 yılları arasında yayınlanmış İngilizce makaleler taranmıştır.

Derleme, mikroakışkan teknolojisinin tespit yeteneklerini nasıl geliştirdiğine dair kapsamlı bir anlayış sağlamayı ve böylece sağlık hizmetleri, çevre bilimi ve gıda güvenliği alanlarındaki gelişmelere katkıda bulunmayı amaçlamaktadır.

**Anahtar Kelimeler:** Mikroakışkan, Tespit, Elektrokimyasal Tespit, Optik Tespit, Manyetik Tespit.



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## 1. INTRODUCTION

### Microfluidics in detection

Microfluidic devices have gained significant attention for their potential detection applications owing to their ability to manipulate small volumes of fluids with high precision and sensitivity (Zimmerman et al., 2006; Nasseri et al., 2018). It has the potential to integrate all experimental processes in a research laboratory (sample preparation, reaction, separation, and detection) into a microscale device (Wang et al., 2018). The detecting system is the essential component that is in charge of signal acquisition ever since the micro total analysis system (mTAS) was originally presented by Manz et al. in 1990 in order to read out the analytical data acquired by a microchip (Jin et al., 2018). These devices typically consist of microchannels, chambers, valves, pumps and integrated components such as sensors, detectors and actuators. One of the most promising applications of microfluidics is its use in detection. Microfluidic devices can accurately and precisely identify a wide range of substances, including biomarkers, drugs, toxins, and pathogens. Furthermore, these devices can integrate various detection techniques, such as optical, electrical, and chemical sensing, into a single device, enhancing their capabilities in detection applications. Such as, programmable microfluidic devices based on paper are a well-known type of microfluidic device. It provides improved control over the manipulation of fluid samples, allowing for the automation of single – and multi-step assays as well as the very sensitive detection of a variety of biomarkers (Soum et al., 2019).

Microfluidic electrochemical devices offer numerous advantages in the realm of detection, primarily due to their small sample volume requirement. This feature not only reduces the amount of sample needed for analysis but also enables rapid and high-throughput detection. Additionally, these devices possess on-chip sample preparation capabilities, which streamline the overall detection process by minimizing manual sample handling and reducing the risk of contamination. Furthermore, the benefits of microfluidic electrochemical devices include multiplexed detection, on-chip sample preparation, reduced sample volume needs, and miniaturization, making them particularly suitable for heavy metal detection applications (Li et al., 2019).

The integration of microfluidic systems with on-chip pumping and detection functionalities has led to the development of innovative platforms for long-term perfusion cultures and real-time monitoring of tissue models. These integrated microfluidic devices have the capacity for online and continuous detection applications, allowing for the long-term dynamic investigation of tissue model cellular responses and metabolic processes. For long-term perfusion culture and online tissue model monitoring, integrated microfluidic devices with on-chip pumping and detection capabilities have been created, indicating the possibility for continuous and real-time detection applications (Kimura et al., 2008).

Electrode placement may be precisely controlled to enable sensitive and specific detection with the use of 3D printed electrodes in microfluidic systems, providing an affordable and adaptable solution (Erkal et al., 2014). The integration broadens microfluidic detection, analyzing diverse analytes with precision. Using 3D printed microfluidic devices with integrated electrodes is a major step forward in efficient analyte analysis, with implications for biomedical research, diagnostics, and environmental monitoring. Furthermore, the use of microfluidic systems has demonstrated potential for label-free in-flow detection of individual DNA molecules, presenting fresh possibilities for the detection and identification of individual molecules in microfluidic devices (Gong e al., 2014). Despite the many benefits that microfluidic devices provide for detection applications, issues have been noted with low sample volume and the requirement for sensitive analytical procedures (Chabinyc et al., 2001). Overall, they offer a wide range of detection capabilities, from biomarkers to heavy metals, and from single DNA molecules to tissue models. The integration of various methodologies and the development of sensitive analytical techniques have expanded the potential of microfluidic devices for detection applications.

The aim of this review is to look at the improvements and possible uses of microfluidic devices in detecting procedures with investigating how these devices, with their ability to precisely manipulate small fluid volumes, integrate various experimental processes, and incorporate diverse detection techniques, can revolutionize the field of detection in a variety of domains including biomedical research, diagnostics, and environmental monitoring.

### Types of detection modules in microfluidic

#### *Electrochemical Detection*

Electrochemical microfluidics integrates electrochemical detection into microfluidic systems. This involves incorporating sensors and electrodes in microfluidic channels for precise analyte detection. Key components include microfluidic chambers, channels, electrodes, sensors, control systems, and data gathering systems. Channels and chambers are designed for specific functions, while electrodes facilitate electrochemical detection, typically using a working and reference electrode setup. To complete the electrochemical circuit and significantly boost the system's sensitivity without compromising the microfluidic device's physical design, a coulter counter electrode may also be added (Murali et al., 2009). It's employed in microfluidic systems for impedance cytometry (Brazey et al., 2018). Numerous methods, including amperometric (AD), conductivity-based, voltametric, electrochemical impedance spectrometry, chronocoulometric, or redox cycling, can be used for electrochemical detection in microfluidics devices (Gencoglu et al., 2014).

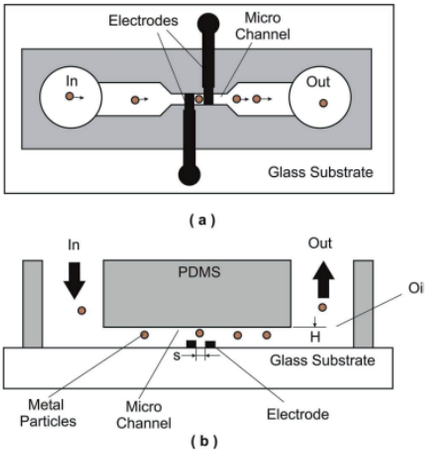


***Figure 1.*** *Schematic of the electrochemical based microfluidic sensor design for metal wear detection with top view (a) and sectioned front view (b) (Murali et al., 2009).*

Amperometric analysis measures current under a constant electric potential between electrodes. The current reflects analyte flux to the electrode surface. Initially high, it declines as analyte depletion extends beyond the surface, reducing the concentration gradient and current. Time-dependent behavior of current can be used to infer the concentration of analyte in the solution. A polymer-based microfluidic device with an amperometric system, for example, is presented by Ruecha et al. for the very sensitive detection of cholesterol (Ruecha et al., 2011).

Voltammetry, a widely used technique, assesses system reversibility. By applying varying electrical potential between electrodes, it measures resulting current over time, unlike amperometry's constant potential. Jiang et al. demonstrated voltammetry's effectiveness in a published paper. They create a microfluidic gene device that uses cyclic voltammetry to detect DNA electrochemically (Jiang et al., 2012). Automated As determination in water using an electrochemical sensor incorporated into a modular microfluidic system is a well-known example of voltametric approaches (Gimenez-Gomez et al., 2019).

Electrochemical impedance spectroscopy has gained popularity in biosensing due to its capability to detect binding events occurring on the surface of a transducer. In electrochemical impedance spectroscopy (EIS), a small sinusoidal AC perturbation is applied along with a DC potential between working electrode (WE) and reference electrode (CE). Using Ohm's law, impedance may be calculated by logging the resultant current's magnitude and phase angle as a function of AC frequency (Rackus et al., 2015). The article by Ali et al. provides a clear illustration to find biomarkers for breast cancer, they developed a microfluidic immuno-biochip (Ali et al., 2016).

Microfluidic types including impendance-based, discrete (like droplet-based) and paper-based (Lindsay et al., 2007) have several benefits, such as downsizing, low sample volume needed, and the possibility of multiplexed detection and on-chip sample preparation. It has been demonstrated that electrochemical detection in microfluidic devices provides real-time detection for a variety of analytes, such as heavy metal ions, biomarkers, and DNA sequences, along with great sensitivity, exceptional selectivity, remarkable stability, and repeatability (Li et al., 2013; Hong et al., 2016; Ming et al., 2021).

### *Optical Detection*

The widespread use of optical instruments in laboratories has made optical detection the most widely used method for quantitative proteome analysis and the diagnosis of infectious diseases. Two main types exist for optical detection in microfluidic devices: "off-chip" with separated detection units, and "on-chip" merging fluidic and optic components. The detection units such as light source, mirrors, and detectors are separated from the Microfluidic platforms. Other approach is the "on-chip" paradigm, where fluidic functional units are produced alongside or merged with the optics. The term "optofluidic" is also used to describe this kind of Microfluidic (Gai et al., 2011).

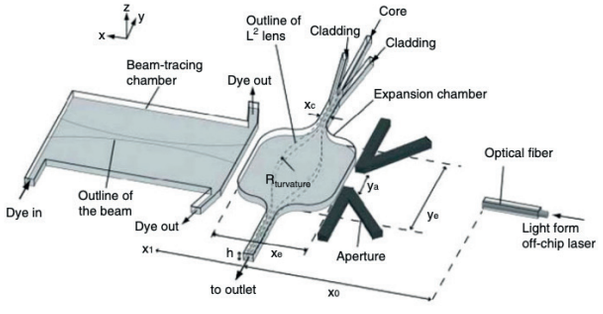


***Figure 2.*** *Experimental setup for focusing light exiting an optical fiber in optofluidic microfluidic devices (Gai et al., 2011).*

Free-space optical detection techniques encompass surface-plasmon resonance, chemiluminescence, absorbance, fluorescence, and Raman spectroscopy/imaging. Microfluidic biosensors similarly utilize these techniques, showcasing their versatility in detection within microfluidic systems (Pires et al., 2014). Case in point, Wang and team workers demonstrates a SERS – microfluidic device using nanotechnology techniques. To regulate the nanogroove characteristics, the device is created by means of tip-based nano scratching utilizing atomic force microscopy (AFM). The synthesized nanostructures are utilized to detect the Raman spectra of rhodamine 6G (R6G). The results indicate possible uses for these kinds of microfluidic devices in chemical or biological molecular detection applications (Wang et al., 2023). A different example is provided by Wang et al. They mentioned a thin-film organic photodiode for microscale chemiluminescence that is based on solutions. These photodiodes are easily incorporated into planar chip-based systems due to their straightforward layered nature. And they were assessed for microscale chemiluminescence detection (Wang et al., 2007). Additionally, the outcome demonstrates

outstanding linearity and a detection limit of 10 micrometers for hydrogen peroxide.

Optofluidic chips combine microfluidics with optical components, achieving higher miniaturization as more functions are integrated onto the chip. Unlike free-space optic systems, integrated optical microfluidic systems don't require alignment and are technician-independent. Khosla et al.'s work demonstrates a microfluidic whispering gallery mode (WGM) biosensing device that effectively delivers and detects target molecules (Khosla et al., 2014). Since their mode volume is so small, WGM resonators are optical sensors that are highly sensitive. The researchers also discovered that when microfluidics and WGM sensing are combined, a highly customizable system with a yield for a certain concentration and the capacity to improve sensing time by modifying the microfluidic system's input power and flow characteristics is produced. Particles and molecules as small as a single BSA protein (about 6 nm in radius) may be detected and analyzed for femtomolar concentrations by combining microfluidics and WGM.

Generally, there are several key components to optical detection within optofluidic, planar optical waveguides, micro lenses, optical lasers, and optical detectors. An optical waveguide is a physical structure designed to transmit light along axis, typically consisting of a "core/cladding" composition. To illustrate, Parker et al. demonstrates the generation of uniform droplets within a uniaxial optofluidic lab-in-fiber setup (Parker et al., 2022). They provide reliable and compact alignment by combining droplet microfluidics with laser-induced fluorescence (LIF) detection through the use of an optical side-coupling fiber known as a periscope fiber. In addition, they demonstrate the usefulness of the apparatus by identifying reverse-transcription loop-mediated isothermal amplification (RT-LAMP) products for COVID-19 diagnostic purposes.

### *Magnet Beads Detection*

Magnetic beads microfluidic detection involves the use of magnetic beads, which are small, functionalized beads that can bind to the specific target molecules such as protein, nucleic acids, or cells within microfluidic systems. These magnetic beads are often coated with ligands that can selectively bind to the target molecules of interest. Typically, magnetic bead microfluidic detection system involves a sample containing the target molecules that as the sample flows in Microfluidic channels, an external magnetic and integrated various detection systems such as fluorescence or electrochemical methods can be used to detect the presence or concentration of the bound molecules on the surface of the bead. A new cell detection device has been presented by Liu et al. that combines microfluidic Coulter counting technology with the magnetic bead cell assay (Liu et al., 2016). The device accurately recognizes certain target cells and measures concentration and cell size distribution. Using resistive pulses from the counters, the transit time and cell size are precisely determined. According to the research, the transit time delay rises linearly as the target cell ratio increases, with an estimated 5.6% detection limit. The gadget may help with stem cell extraction and characterization by detecting target cells quickly and reliably, as shown by its straightforward setup and simple sample preparation.

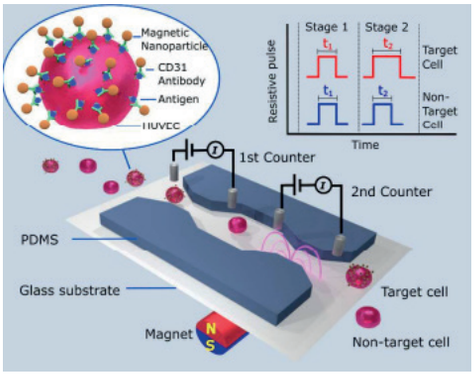


***Figure 3.*** *Schematic of the magnetic bead based microfluidic cell assay which is based on two micro-Coulter counters (Liu et al, 2016).*

### *Thermal Detection*

Among the various methods for counting and detecting particles that have been examined, heat has not yet been thoroughly explored. Through microfabrication, it is now possible to produce low-cost but highly sensitive thermometers capable of detecting tiny fluctuations in the thermal characteristics of a liquid within a microchannel. As particles suspended in a fluid influence the thermal properties of the fluid, a sensitive thermometer essentially serves as a particle counter by detecting these changes. A microscale thermometer could analyze the thermal properties of cells and other particles or droplets, aiding in their identification and characterization. One of the brilliant examples of this type of detection in microfluidic is developed by Liu et al. (Liu et al., 2014). Using hexavalent chromium [Cr (VI)] as a model analyte, a microfluidic flow injection analysis (μFIA)–TLM device was created for the quick detection of contaminants by colorimetric reactions. Additionally, contamination-free, instrument-free, visual detection of SARS-CoV-2 has been made possible by the combination of isothermal amplification, CRISPR cleavage, and lateral flow detection in a single, closed microfluidic device. A unique method for the quick and accurate detection of SARS-CoV-2, the virus that causes COVID-19, is presented by Li et al. (Li et al., 2022). This novel technique makes use of a self-contained microfluidic system, which is straightforward, sensitive, and doesn't require specialist tools. The microfluidic device combines lateral flow sensing, CRISPR cleavage, and isothermal amplification to provide visible, contamination-free viral detection. Without the requirement for power, the microfluidic chip may be incubated using a cheap, portable hand warmer. The technique has been clinically verified using nasopharyngeal swab samples and has demonstrated good sensitivity, specificity, and accuracy in detecting SARS-CoV-2 RNA down to 100 copies.

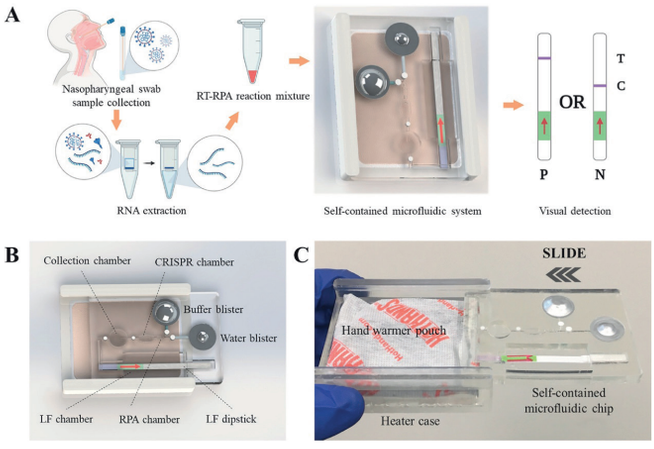


***Figure 4.*** *Self-contained microfluidic design for detection of SARS-CoV-2 by Li et al. ((Li et al., 2022).*

## Applications of microfluidic based detection

### *Biomedical Applications*

Microfluidic detection has gained significant attention in biomedical applications, particularly in isolating and detecting biomarkers such as exosomes and circulating tumor cells. In the realm of biomedical field, microfluidic devices have been applied to the detection of biomarkers and cancer cells at incredibly low concentrations, including micro-RNA, indicating their promise for sensitive and accurate detection in biomedical settings and treatment monitoring (Chen et al., 2012).

The integration of magnetic nanoparticles in microfluidic systems has enabled the development of highly sensitive and specific detection methods. To define the inductance of electrical resonant circuits in magnetometers, for example, the magnetic characteristics of particles in microfluidic chambers have been used, leading to a shift in the resonance frequency (Abedini-Nassab et al., 2021).

Further benefits for identifying active chemical and biological species come from Chandrasekaran et al.'s investigation of an integrated microfluidic biphotonic device intended for laser-induced fluorescence detection (Chandrasekaran et al., 2010). The chip combines an opto-microfluidic chip made on a silicon-polymer hybrid platform with a Spectrometer-on-Chip device, designed for multiple fluorescence detections at different emission wavelengths. The device's potential for high-throughput detection of chemical and biological materials is demonstrated through experimental validation utilizing antibody particles labeled with Alexafluor 647. Another brilliant example is microfluidic paper-based wearable electrochemical biosensor for reliable cortisol detection by Fiore et al. (Fiore et al., 2023). A new paper-based microfluidic system for reagent-free cortisol analysis in perspiration is presented. The device combines capillary-driven microfluidics and filter paper to regulate reagent flow. It detects cortisol using magnetic beads with monoclonal antibodies, facilitated by acetylcholinesterase enzyme-mediated competitive interaction. It can detect cortisol concentrations from 10-140 ng/mL and is integrated with a wireless Near-Field Communication module for wearable cortisol analysis. Its accuracy was validated during real-time sweat cortisol analysis on a volunteer during physical activity.

Microfluidic techniques have also been employed for the detection of cancer cells, demonstrating the versatility of microfluidic platforms in various biomedical applications (Nguyen et al., 2017). To illustrate, a study introduces an electrochemical Lab-on-a-Disc (eLoaD) platform designed for automated quantification of ovarian cancer cells (SKOV3) from whole blood by Nwankire et al. (2015). The platform combines label-free electrochemical impedance for sensitive detection and targeted capture with advanced sample processing techniques like blood separation and cancer cell extraction. It has a wide dynamic linear range and can perform five parallel tests, making it a promising tool for biomedical applications such as cancer cell identification.

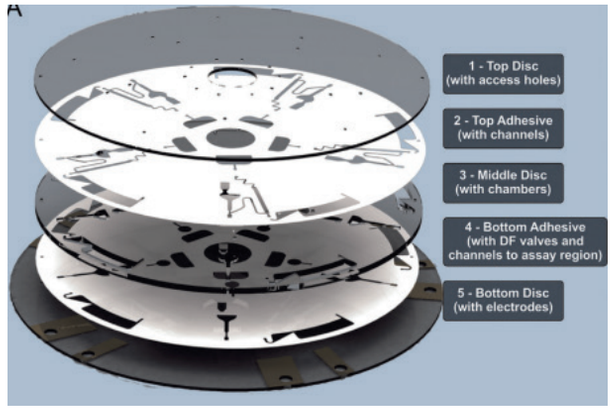


***Figure 5.*** *The 5-layer microfluidic disc platform which is developed by Nwankire et al. (2015)*

### *Clinical Applications*

Within clinical applications, microfluidic devices play a crucial role in various diagnostic procedures, including the detection of the pathogens, monitoring of disease progression, and screening for biomarkers indicative of specific health conditions. By integrating various sensing mechanisms, such as optical, electrical or chemical sensors into Microfluidic devices, research can detect specific biomolecules or cells with high accuracy and speed. A new microfluidic device detects Cry1Ab protein with high sensitivity and selectivity. It contains a microfluidic flow cell and a printed gold electrode chip. Anti-Cry1Ab aptamer-coated magnetic beads bind to Cry1Ab protein, forming modified beads. These are then introduced into the flow cell for measurement, showing a detection limit of 0.015 nm and good linearity from 0 to 0.2 nm concentration at 358.3 Hz. In support of food safety studies, this method provides a sensitive, quick, and targeted substitute for identifying the transgenic protein Cry1Ab (Jin et al., 2017).

Another example is a reproducible microfluidic device utilizing surface-enhanced Raman spectroscopy (SERS), composed of a disposable SERS substrate and a reusable microfluidic channel by Lee and team (Lee et al., 2021). The SERS substrate is created using electrodeposition and nanoimprint lithography, while the microfluidic channel is made through mechanical processing. Assembly is facilitated and secured with screws. The SERS substrate guarantees reliable and sensitive detection due to its excellent enhancement factors, signal consistency, and repeatability. Target molecules can be accurately detected using the disposable substrate. The successful identification of miR-34a at a low concentration of 5 fM showcases its practical utility.

Consequently, the use of microfluidics in clinical settings offers several advantages, including minimally invasive sampling, reduced sample consumption, and rapid results which can lead to timely and effective patient care. Case in point, Yao et al. introduce a telemedicine system employing an integrated microfluidic chip for rapid insulin detection (Yao et al., 2013). The system uses the internet to remotely transmit test results to off-site medical specialists for diagnosis. It employs a microfluidic chip with pneumatic micropumps, a micromixer, and microvalves to automate insulin detection via a double-antibody sandwich chemiluminescence immunoassay. This microfluidics-based telemedicine system shows promise for future diabetes point-of-care screening.

Moreover, the incorporation and implementation of optical detection and microfluidics in immunosensors have been a subject of research, highlighting the potential of microfluidic technology in medical diagnostics (He et al., 2016). And advances in microfluidic platforms have enabled multiplex detection of infectious diseases, including microfluidic immunosensors and microfluidic nucleic acid sensors (Chen et al., 2023).

### *Environmental Monitoring*

The integration of microfluidic technologies in environmental monitoring has garnered significant attention due to their potential to revolutionize detection and analysis processes. Microfluidic systems offer precise manipulation of fluid streams in microscale dimensions, making them valuable tools for on-site environmental monitoring and detection (Yew et al., 2019). Furthermore, the ability of microfluidic platforms to preconcentrate samples from dilute solutions has implications for environmental monitoring, as it enables the detection of contaminants in various environmental matrices (Fu et al., 2018). The versatility of microfluidic platforms has led to their widespread application in the field, signifying their importance in addressing environmental challenges. Additionally, microfluidic detection strategies, including optical and electrochemical techniques, have been explored for the analysis of environmental pollutants, highlighting the potential of microfluidic systems in environmental monitoring for instance. An autonomous microfluidic detection device was designed by Milani et al. to determine iron (Fe II) and manganese levels in water using colorimetric detection (Milani et al., 2015). The device featured a cylindrical housing with a PMMA microfluidic chip, LEDs, lithium batteries, a microprocessor, and custom syringe pumps. Iron levels were measured with ferrozine and manganese levels with the PAN technique. The on-chip optofluidic cell's dimensions allowed sensitive detection with reduced reagent and sample use. Mn Limit of detection (LOD) was 28 nM, and for Fe was 27 nM, with linear ranges of 27-200 nM and 0.28-6 µM, respectively. Iron analysis took five minutes, and manganese took 10 minutes. The method successfully analyzed seawater samples, unaffected by high salt concentrations. The industrialization of microfluidic chip technology is anticipated to drive advancements in environmental sensing, emphasizing the role of microfluidics in future environmental monitoring endeavors (Gao et al., 2020).

As the field of microfluidics continues to advance, it presents new opportunities for enhancing environmental analysis, with the potential for low-cost, high-sensitivity, and rapid detection of environmental contaminants. The combination of microfluidic systems with advanced detection methods, such as real-time fluorescence detection, further enhances their utility in environmental monitoring, offering improved capabilities for detecting contaminants and biological specimens. Moreover, the development of low-cost microfluidic platforms has the potential to democratize environmental monitoring, making it more accessible and affordable. I.e. using a vortex t-structure microfluidic sensor chip, Li et al. have devised a novel approach to real-time water quality monitoring that overcomes the shortcomings of current COD (chemical oxygen demand) measurement techniques (Li et al., 2022). Real-time monitoring of river contaminations is challenging due to complex and lengthy procedures. The new system, based on microfluidic technology and ozone chemiluminescence, offers benefits like ease of use, rapid testing, and minimal environmental impact. Tests confirm the chip's ability to generate measurable ozone bubbles, aiding online river water quality monitoring. Microfluidics, coupled with gold nanoparticles' optical properties, show promise in enhancing environmental monitoring methods. The promise of microfluidics in environmental monitoring and public health has been demonstrated by studies on the application of microfluidic-based systems for the detection of gases and airborne diseases (Kaaliveetil et al., 2022). Overall, the integration of microfluidic technologies in environmental monitoring holds great promise for revolutionizing the detection and analysis of environmental pollutants, offering new avenues for addressing environmental challenges.

### *Food Safety*

Food contamination, a global concern, particularly affects developing nations and poses significant health risks due to harmful substances in food. These contaminants include chemicals and microorganisms, categorizing contamination into chemical and biological types. Rapid and accurate detection methods are crucial to mitigate the threats posed

by food contamination to human well-being. Microfluidic approaches for controlling and detecting chemical contaminants like heavy metals, pesticides and antibiotic residues in food samples. For instance, Li et al. (2021) developed a rapid detection of methyl parathion pesticide by using Microfluidic paper-based chip. A technique developed to detect pesticides like parathion-methyl (PM) utilizes high sensitivity and selectivity. The method employs silver terephthalate metal-organic frameworks and carbon quantum dots with Fe3O4 nanozyme for amplification in a dual catalytic technique. An electrochemical microfluidic paper-based chip integrates a molecularly imprinted polymer (MIP) and Fe3O4/C-dots@Ag-MOFs on its surface. PM absorption by the MIP in the reaction zone reduces the current response, leading to a low detection limit of 1.16×10−11 mol L−1 and good recovery rates in environmental and agricultural samples. This approach improves sensitivity and selectivity and holds potential for detecting various target analytes using microfluidic paper-based chips.

Microfluidic paper-based analytical devices (μPADs) are cost-effective tools for in-field diagnosis, highlighting microfluidics' role in food safety. Microfluidic technologies are crucial for digital immunoassays with multiplexed capacity and ultrahigh sensitivity in food safety and environmental monitoring. Rapid quantification of contaminants in food products, like milk, is essential for addressing food safety incidents, showcasing microfluidics' pivotal role in ensuring food quality. Standalone devices for food safety testing, along with advancements in microencapsulation and microfluidic-based bio species sensing, underscore microfluidic detection technologies' transformative potential in food safety. Microfluidics' progress offers opportunities for enhanced food safety analysis, providing low-cost, high-sensitivity, and rapid detection of food hazards to mitigate safety risks in the food industry.

***Point-of-care Diagnostic***

Microfluidic detection technologies have significantly advanced the landscape of point-of-care diagnostics, offering rapid, accurate, and cost-effective solutions for disease diagnosis and pathogen detection. Microfluidic devices have demonstrated their potential in enabling rapid and fully automated detection of infectious diseases, originated from Ebola virus and African swine fever virus, with median times to threshold as low as 10 minutes, highlighting their applicability in addressing urgent public health challenges (Qin et al., 2019; GaoYe et al., 2019). Further, the creation of microfluidic platforms for recombinase-aided amplification (RAA) and loop-mediated isothermal amplification (LAMP) has made it easier to identify and quantify multiple pathogens simultaneously, opening up a promising path for the quantitative point-of-care detection of a variety of infectious diseases (Fang et al., 2010). The versatility of microfluidic platforms has been exemplified in the development of a portable smartphone-based platform for real-time particle detection, offering potential for real-time point-of-care detection in resource-limited settings (Salafi et al., 2019). Microfluidic tech aids chronic illness diagnosis like diabetes via automated chemiluminescence immunoassays for insulin levels. These devices can detect fluid viscosity without labels, showcasing their point-of-care potential. This presents prospects for the quick and affordable characterization of biological samples in point-of-care situations (Jun Kang et al., 2013). The ability of multiplex microfluidic LAMP chips to properly forecast viruses has shown how microfluidic technology may improve point-of-care diagnostics' specificity and accuracy. As a result, the addition of microfluidic detection technologies has greatly improved point-of-care diagnostics' capabilities and provided cutting-edge approaches to illness diagnosis and healthcare monitoring.

In the examination of spectrum of scholarly investigations spanning biomedical, clinical, environmental, food safety, and point-of-care diagnostic applications, this compilation emerges as a distinctive and comprehensive exposition of multifaceted realm of microfluidic-based detection. By synthesizing a wide range of findings and developments across multiple domains, this article contributes to the existing literature by providing valuable insights into the potential and challenges of microfluidic technology. Its holistic approach, organization, and presentation serve to highlight not only the current state of field but also the future prospects and opportunities for advancement. Meticulous attention has been devoted to ensuring the relevance and currency of the sources. Through a rigorous selection process, emphasis has been placed on incorporating scholarly works that represent the latest advancements and insights in the microfluidic-based detection. Therefore, the originality of this compilation lies in its comprehensive coverage, synthesis of existing research, and insights into the diverse applications and potential of microfluidic-based detection across disparate fields.

## 2. METHODS

The review was conducted using Pub Med and Google Scholar pages by using document analysis method. The search was limited to English articles published between 2001 and 2023 in the academic databases of Pub Med and Google Scholar. The search was provided by searching for 10 keywords by 5 in Turkish and 5 in English. These are mainly; Microfluidics, Detection, Electrochemical detection, Optical detection, Magnetic detection, Mikroakışkan, Tespit, Elektrokimyasal Tespit, Optik Tespit, Manyetik Tespit. As a result of readings and re-elimination, the number of articles were determined as the most suitable and qualified ones.

## 3. CONCLUSION

In summary, microfluidic technology has shown great promise in various detection applications, ranging from medical diagnostics to environmental monitoring. The integration of advanced detection technologies into microfluidic systems

has enabled rapid, sensitive, and specific detection of a wide range of substances, making microfluidics a valuable tool in diverse fields. They offer a wide range of advantages for detection in various applications, including biomedical, clinical, environmental monitoring, food safety, and point-of-care diagnostics. These advantages include precise manipulation of fluids, low sample and reagent consumption, high sensitivity, rapid detection, and portability. In biomedical and clinical applications, microfluidic devices have shown potential in liquid biopsy, disease biomarker detection, and point-of-care diagnostics, offering the benefits of rapid analysis and reduced sample volumes. In environmental monitoring and food safety, microfluidic devices have demonstrated advantages in the rapid and sensitive detection of contaminants and pathogens, contributing to improved safety and quality control. However, challenges exist in the miniaturization of traditional laboratory processes, the integration of complex detection methods, and the development of specific point-of-care microfluidic diagnosis devices. Addressing these challenges will be crucial in fully realizing the potential of microfluidic devices for detection in biomedical, clinical, environmental, and food safety applications. The integration of advanced detection methods, such as surface plasmon resonance, fluorescence, and impedance spectroscopy, and the development of specific point-of-care microfluidic diagnosis devices will be essential for overcoming these challenges and further advancing the field of microfluidic detection.

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