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Changes in Antimicrobial Susceptibility Patterns of Microorganisms Isolated from Urine Cultures in the Last Decade

İdrar Kültürlerinden İzole Edilen Mikroorganizmaların Antimikrobiyal Duyarlılık Paternlerinin Son On Yıldaki Değişimi

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Abstract

Aim Urinary tract infections are the infections for which antibiotics are most frequently prescribed to outpatients. The purpose of this study is to evaluate the ten-years change in antibiotic resistance profiles of microorganisms grown in urine cultures.

Material and Method The results of urine cultures that were sent to the Microbiology Laboratory between 01.01.2013 and 31.12.2022 were evaluated retrospectively. Identification and antimicrobial susceptibility of microorganisms were performed with the BD Phoenix 100 (Becton Dickinson, Maryland, USA) device between 2015-2016, and with the VITEK-2 compact system (BioMérieux, France) between 2013-2014, 2017-2022. While the antibiotic susceptibility results of the isolates were interpreted using the clinical breakpoints defined by the Clinical Laboratory and Standards Institute (CLSI) between 2013 and 2015, they were evaluated according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria after 2015.

Results The most common microorganism is *Escherica coli* with 49%(n=4.898), while the second most common agent is Klebsiella spp. with 13.8% (n=1.380) were gram-negative microorganisms. While the most sensitive antibiotics were carbapenem and aminoglycoside groups in *Escherichia coli* and *Klebsiella spp.*, the resistance to ampicillin, ciprofloxacin and cephalosporins in *Escherichia coli* was over 60%, while this rate was around 80% in Klebsiella spp. In our study, resistance rates of PIP-TZP, ceftazidime, carbapenems, aminoglycosides and quinolones were higher against *Acinetobacter spp*. than *Pseudomonas spp*. In general, resistance to *Acinetobacter spp. and Klebsiella spp.*, and there was a tendency for the resistance rates of all microorganisms to increase over the years.

Conclusion Creating more detailed cumulative antibiogram reports, sharing these reports with relevant clinics within specified periods, establishing antimicrobial management teams that will work in harmony with infection control committees in hospitals, structuring a nationwide surveillance program; It will contribute to the determination of empirical treatment, prevention of the development of antimicrobial resistance and control of hospital-acquired infections.

Keywords Antimicrobial resistance, *Escherichia coli,* Urine culture.

Özet

Amaç *İdrar yolu enfeksiyonları, polikliniklere başvuran hastalara en sık antibiyotik reçete edilen enfeksiyonlardır. Bu çalışmanın amacı idrar kültürlerinde üreyen mikroorganizmaların antibiyotik direnç profillerindeki on yıllık değişimi değerlendirmektir.*

Gereç ve Yöntem *Mikrobiyoloji Laboratuvarı'na 01.01.2013 ile 31.12.2022 tarihleri arasında gönderilen idrar kültürü sonuçları retrospektif olarak değerlendirildi. Mikroorganizmaların tanımlanması ve antimikrobiyal duyarlılıkları iki ayrı sitemle, 2015-2016 yılları arasında BD Phoenix 100 (Becton Dickinson, Maryland, ABD) cihazıyla 2013-2014 ve 2017-2022 yılları arasında ise VITEK-2 kompakt sistemiyle (BioMérieux, Fransa) yapıldı. İzolatların antibiyotik duyarlılık sonuçları 2013-2015 yılları arasında Klinik Laboratuvar ve Standartlar Enstitüsü (CLSI) tarafından tanımlanan klinik sınır değerleri kullanılarak yorumlanırken 2015 sonrası Avrupa Antimikrobiyal Duyarlılık Testi Komitesi (EUCAST) kriterlerine göre değerlendirildi.*

Bulgular *En sık görülen mikroorganizma %49(n=4,898) ile Escherica coli olurken ikinci en sık etken %13,8(n=1,380) ile Klebsiella spp. gibi gram negatif mikroorganizmalardı. Escherichia coli ve Klebsiella spp.'de en duyarlı antibiyotikler karbapenem ve aminoglikozid grubuyken Escherichia coli'de ampisilin, siprofloksasin ve sefalosporinlere direnç %60'ın üzerindeyken bu oran Klebsiella spp.'de %80 civarında tespit edildi. Çalışmamızda, piperasilin tazobaktam, seftazidim, karbapenemler, aminoglikozidler ve kinolonların Acinetobacter spp.'ye karşı direnç oranları Pseudomonas spp.'ye göre daha yüksekti. Genel olarak direnç Acinetobacter spp. ve Klebsiella spp.'da daha ciddiydi ve tüm mikroorganizmaların direnç oranlarında yıllar içinde artış eğilimi vardı.*

Sonuç *Daha detaylı kümülatif antibiyogram raporlarının oluşturulması, bu raporların belirlenen sürelerde ilgili kliniklerle paylaşılması, hastanelerde enfeksiyon kontrol komiteleri ile uyum içerisinde çalışacak antimikrobiyal yönetim ekiplerinin oluşturulması, ülke çapında sürveyans programının yapılandırılması; ampirik tedavinin belirlenmesine, antimikrobiyal direnç gelişiminin önlenmesine ve hastane kaynaklı enfeksiyonların kontrolüne katkıda bulunacaktır.*

Anahtar Kelimeler *Antimikrobiyal direnç, Escherichia coli, İdrar kültürü*

INTRODUCTION

According to the World Health Organization's 2019 report, antimicrobial resistance is one of the biggest threats to public health.[1] Urinary tract infections (UTI) are the most common infections both in the community and in the healthcare settings and are caused by major uropathogens such as *Escherichia coli (E. coli)*, *Klebsiella pneumoniae (K. pneumonia), Proteus mirabilis (P. mirabilis), Enterococcus faecalis (E. faecalis)* and *Pseudomonas aeruginosa (P. aeruginosa)*.[2] When the results of the "Global Prevalence of Infections in Urology (GPIU)" study covering 70 countries were examined, it was seen that most of the uropathogens had high rates of antibiotic resistance and multidrug resistance was especially striking.[3] UTI is one of the most common indications for outpatient empirical antibiotic prescribing.[4]

International guidelines recommend fosfomycin and nitrofurantoin as the first-line treatment of acute uncomplicated cystitis. Trimethoprim-sulfamethoxazole (TMP-SXT) can only be considered as the drug of first choice if local resistance to *E. coli* does not exceed 20 percent. Due to high resistance to aminopenicillins and long-term side effects of fluoroquinolones, they are no longer recommended as first-line treatment for urinary tract infections. Second-line options include oral cephalosporins such as cephalexin or cefixime, fluoroquinolones, and beta-lactams such as amoxicillin-clavulanate.[5]

In antimicrobial stewardship, microbiology laboratories have an important role in both determining empiric treatment and monitoring resistance profiles over the years by reporting cumulative antibiogram results regularly.[6] The aim of this study is to evaluate the ten-year change in the antibiotic resistance profiles of microorganisms grown in urine cultures.

MATERIALS and METHODS

A total of 35.132 urine culture samples sent to Gaziantep Dr. Ersin Arslan Training and Research Hospital Microbiology Laboratory between 01.01.2013 and 31.12.2022 were retrospectively evaluated. Urine samples were inoculated on sheep blood agar and Eosin Methylene Blue (EMB) agar by quantitative method using 0.01 ml loops. Urine culture plates inoculated for routine purposes were evaluated after incubation at 35-37 °C for 16-24 hours in accordance with the guide recommendations. Additional procedures were performed for yeasts.[7] Identification of microorganisms and their antimicrobial susceptibility were performed with two separate systems, between 2015-2016 with the BD Phoenix 100 (Becton Dickinson, Maryland, USA) device, and between 2013-2014 and 2017-2022 with the VITEK-2 compact system (BioMérieux, France). Antibiotic susceptibility results of the isolates were interpreted using the clinical breakpoints defined by the Clinical Laboratory and Standards Institute (CLSI) between 2013 and 2015, and were evaluated according to European Antimicrobial Susceptibility Testing Committee (EUCAST) criteria after 2015.[8-9]

Descriptive statistical analysis: Resistance rates of isolates in the study were determined numerically using Microsoft Excel 2013 (Microsoft Corp. Redmond, WA, USA) and calculated as percentages.

RESULTS

A total of 35.132 urine culture results, who were admitted to outpatient clinics with UTI symptoms and sent to the laboratory from intensive care units and wards with the preliminary diagnosis of UTI, were retrospectively examined. During the 10-year period of our study, growth was observed in 28.5%(n=10.000) of the samples sent to our laboratory, while 63.8% (n=22422) did not show growth, and 7.7%(n=2.710) was evaluated as contamination. A total of 19.365 (55.1%) samples were sent from outpatient clinics, while 15.767 (44.9%) samples were sent from inpatients. Of the samples in which microbial growth was detected, 64.4%(n=6440) were female and 35.6%(n=3560) were male. Growth was most frequently detected in samples sent from outpatient clinics with 56.5%(n=5652), followed by samples sent from intensive care with 27.6%(n=2756) and samples from patients sent from services with 15.9%(n=1592), respectively. When the distribution according to clinics is examined, 27.6%(n=2756) of the samples in which growth was detected were from intensive care, 18.6%(n=1859) from urology, 13%(n=1297) from nephrology, 10.3%(n=1030) from internal medicine, 9%(n=909) were sent from infectious diseases, 8.2%(n=819) were sent from pediatrics, and 2.1%(n=211) were sent from gynecology and obstetrics clinics.

The most common isolated microorganism was *E. coli* with 49%(n=4898), while the second most common agent was gram-negative microorganisms such as Klebsiella spp. with 13.8%(n=1.380). The third most common agent was *Enterecoccus spp*. with 7.8%(n=775) followed by coagulase negative staphylococci (CNS) with 6.6%(n=662), *Pseudomonas spp* with 4.7%(n=474), Candida spp. with 4.4%(n=440), *Proteus spp*. with 3.8%(n=379) and *Acinetobacter spp*. with 2.5%(254). The distribution percentage of causative microorganisms is shown in Figure 1.

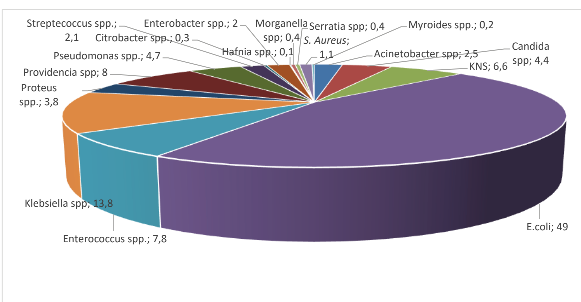

Figure 1. Distribution percentage of causative microorganisms

While the most sensitive antibiotics in *E. coli* and *Klebsiella spp*. were carbapenem and aminoglycoside groups, the resistance to ampicillin, ciprofloxacin and cephalosporins in *E. coli* was over 60%, while this rate was found to be around 80% in *Klebsiella spp*. (Figure 2-3).

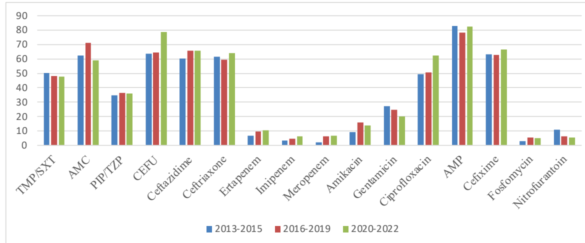

Figure 2. Antimicrobial resistance rates of E. coli

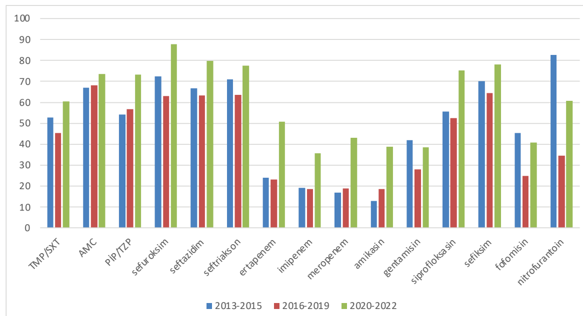

Figure 3. Antimicrobial resistance rates of Klebsiella spp.

It was observed that methicillin resistance increased from 50% in the 2013-2015 period for *S. aureus* to 60% in the 2020-2022 period, and from 70% to 80% for CNS (Figure 4).

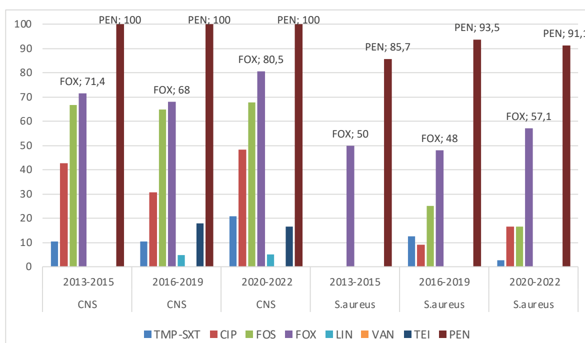

Figure 4. Antimicrobial resistance rates of Staphlococcus spp.

Vancomycin and teicoplanin resistance in *Enterecoccus spp*. increased from 5.8%, 4.3%, respectively, in the 2011-2013 period to 29.3% and 14.1% in the 2020-2022 period. The most sensitive antibiotic in *Acinetobacter spp*. was found to be TMP-SXT, while in *Pseudomonas spp*. it was observed to be aminoglycosides, meropenem and ceftazidime (Figure 5).

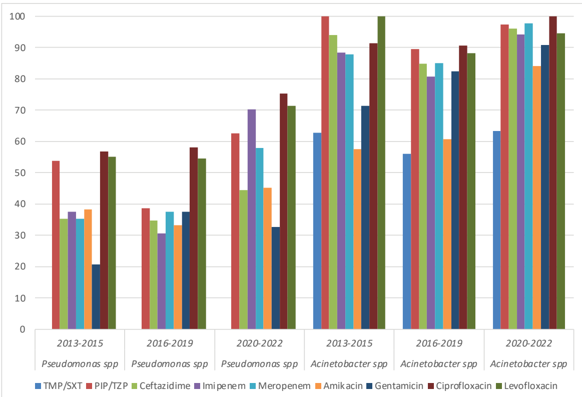

Figure 5. Antimicrobial resistance rates of Pseudomonas spp. ve Acinetobacter spp.

DISCUSSION

UTIs are among the most common bacterial infections occurring in both the community and health care settings worldwide, and most UTIs are usually treated empirically.[5]

In both community and healthcare settings, the Enterobacterales family predominates in UTIs, and the main pathogen isolated, *E. coli*, is the most common causative agent for both complicated and uncomplicated UTIs and this is followed by other pathogenic microorganisms such as *K. pneumoniae, P. mirabilis, Enterococcus spp*. and *Staphylococcus spp., P. mirabilis, P. aeruginosa, S. aureus*, group B *Streptococcus*, and *Candida species*.[10] In our study, *E. coli* was the most frequently detected agent with 49%, while Enterobacterales species ranked first with 69.6%.

Men and women of all ages can experience UTI, but due to female anatomy, the incidence of UTI was higher than in men.[11] The fact that 64.4%(n=6440) of the samples in which growth was detected in our study were female and 35.6%(n=3560) were male supports this information. When the National Healthcare Associated Infections Surveillance Network (USHIESA) agent distribution and antibiotic resistance report 2022 data is examined, amikacin (15%), amoxicillin-clavulanate (AMC) (59.2%), ampicillin (75.9%), gentamicin (24.8%), PIP-TZP (34.7%), ceftazidime (63.6%), ceftriaxone (60%), and ciprofloxacin (57.9%) resistance rates in *E. coli* were compatible with the resistance rates in our study.[12] Etiological agents such as *E. coli, K. pneumoniae* common cause of UTIs and poses the risk of nosocomial outbreaks.[13] The resistance rate of *Klebsiella spp*. was found to be more serious compared to E. coli. When the antibiogram data of the USHIESA 2022 report (for healthcare-associated UTI diagnoses caused by K. pneumoniae) were examined, amikacin (54.7%), AMC (82.7%), ceftazidime (85.2%), ceftriaxone (83%)) and ciprofloxacin (78.1%) resistance rates were similar to the resistance rates in our study.[12] When the antibiotic resistance of the isolates in Avcıoğlu et al.'s study was examined, resistance to ampicillin was found in 81%, gentamicin in 18%, TMP-SXT in 40%, nitrofurantoin in 4%, and fosfomycin in 4% and these rates were found to be compatible with our study. In the same study, AMC resistance was detected in 46% of the isolates, cefixime in 42%, ciprofloxacin in 41%, amikacin in 5%, and imipenem in 2%, and these rates were found to be lower than the resistance rates in our study.[14]

In our study, resistance rates to carbapenems, fosfomycin, and nitrofurantoin were generally <5% in *E. coli*; therefore, these drugs can be used for empiric treatment of UTI.
P. aeruginosa is a common cause of infection in hospitalized patients, especially those with compromised immune system. It is inherently resistant to many antimicrobial agents and has been difficult to control in the presence of healthcare-associated infections.[13] In our study, the resistance rates of PIP-TZP, ceftazidime, carbapenems, aminoglycosides and quinolones against *Acinetobacter spp*. were higher than against *Pseudomonas spp*. When the USHIESA 2022 report's antibiogram result for healthcare-associated UTI caused by *P. aeruginosa* and *Acineotobacter spp*. was examined, amikacin (24.4%, 81.8%), gentamicin (44.3%, 86.5%), meropenem (47.7%, 94.8%), PIP-TZP (72.7%, -), cefepime (70.3%, -) and ciprofloxacin (72.6%,

98.6%) resistance rates, were consistent with our study (between 2020-2022 period).[12] Tanriverdi et al. examined the urine culture results of a total of 4257 pediatric patients between 2015-2020. In their studies, resistance rates to imipenem, meropenem, amikacin, gentamicin and ciprofloxacin for *Pseudomonas spp*. and *Acinetobacter spp*. were found 15.9%-69.5%, 8.6%-69.5%, 7.44%-48.65%, 10.23%-66.72%, 7.3%-69.5%, respectively. These results were more sensitive compared to the results of our study.[15] In the study of Çuha et al., when the antibiotic resistance profiles were examined, the highest resistance rates were found in *Acinetobacter spp*., and over 60% resistance was observed in all antibiotics tested. Resistance to carbapenems was detected in 77.2%, PIP-TZP in 78.1% and ciprofloxacin in 78.6%. In *P. aeruginosa* isolates, resistance rates to aminoglycoside, carbapenem, PIP-TZP and antipseudomonal cephalosporins were determined to be below 20%, and ciprofloxacin resistance was detected to be 25.8%. These rates are much lower than the resistance rates of our study for both groups of microorganisms.[16]

In the study of Kalyoncu et al., AMC, nitrofurantoin, imipenem, levofloxacin, meropenem, cefepime, ceftazidime, ceftriaxone, ciprofloxacin and TMP-SXT antibiotic resistance rates for *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* isolates between 2017 and 2022, were found to be lower than the antibiotic resistance rates of our study covering the same years.[17]

Recently *Enterococcus spp*. has developed high levels of resistance to glycopeptides, including vancomycin, which is considered one of the last line of defense against multidrug-resistant organisms.[2] The USHIESA 2022 report's ampicillin, linezolid, teicoplanin and vancomycin resistance rates for *E. faecium* and *E. faecalis* are 96.4%-10%, 2.7%-1.7%, 26.7%-4.1% and 26%-2.9%, respectively. Since we gave total *Enterococcus spp*. resistance rates in our study, no comparison could be made.[12] Keskin et al.'s study, they did not detect vancomycin, teicoplanin and linezolid resistance in *Enterococcus spp*., while ampicillin (28%) and ciprofloxacin (42%) resistance rates were lower than our study.[18]

In this study, methicillin resistance rates in *S. aureus* and CNS ranged from 48% to 57.1% and 68% to 80.5%, respectively. In the study that included urine samples from a total of 2791 patients between November 2019 and November 2020 in our country, resistance to vancomycin, teicoplanin and linezolid was not detected in *S. aureus*, similar to our study, while resistance rates for TMP-SXT and ciprofloxacin were detected as 12% and 21%, respectively. Unlike our study, methicillin resistance (44%) was found to be lower.[18]

In general, in our study, resistance was more serious in *Acinetobacter spp*. and *Klebsiella spp*., and there was a tendency for the resistance rates of all microorganisms to increase over the years.

Our study has certain limitations. First of all, due to its single-center and retrospective nature, detailed clinical features such as underlying disease and detailed clinical diagnosis were not available. Secondly, although a total of 35.132 urine culture samples were examined in a 10-year period, a distinction between community-acquired UTI and hospital-acquired UTI could not be made and prospective studies are needed. Finally, since the isolates could not be stored, more detailed studies to determine antibiotic resistance genes could not be carried out.

Implementing good antimicrobial stewardship is critical to preventing the development of resistance and improving patient outcomes. The goal of antimicrobial stewardship is threefold and involves the implementation of specific strategies. The primary goal is to prevent treatment of asymptomatic bacteriuria; The second goal is to avoid the use of broad-spectrum fluoroquinolones; The third goal is to minimize the development of resistance by adhering to recommended drug courses and dosages.[6]

CONCLUSION

Creating more detailed cumulative antibiogram reports, sharing these reports with relevant clinics within specified periods, establishing antimicrobial management teams that will work in harmony with infection control committees in hospitals, structuring a nationwide surveillance program; It will contribute to the determination of empirical treatment, prevention of the development of antimicrobial resistance and control of hospital-acquired infections.

Ethical Approval

Gaziantep University Clinical Research Ethics Committee and following the Declaration of Helsinki (decision no: No 2023/267).

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: O.S.C., Design: O.S.C., Data collection or Processing: O.S.C., Analysis or interpretation: O.S.C., Literature Search: O.S.C., Writing: O.S.C.

Conflict of Interest

The authors declare that they have no conflict of interest.

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