СОВРЕМЕННЫЙ ПОДХОД К ОПРЕДЕЛЕНИЮ МОЛЕКУЛЯРНЫХ МАРКЕРОВ МЕСТНЫХ ИЗОЛЯТОВ ОСТРЫХ КИШЕЧНЫХ ИНФЕКЦИЙ

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СОВРЕМЕННЫЙ ПОДХОД К ОПРЕДЕЛЕНИЮ МОЛЕКУЛЯРНЫХ МАРКЕРОВ МЕСТНЫХ ИЗОЛЯТОВ ОСТРЫХ КИШЕЧНЫХ ИНФЕКЦИЙ. ЖКМП.-2025.-Т.1.-№1.-С

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Аннотация: В ходе данного научного исследования были фенотипически изучены полные биологические свойства 496 изолятов Enterobacteriaceae spp., выделенных в бактериологической лаборатории РСНПМЦЭМПИЗ, а также с помощью классических и молекулярных методов оценена приобретенная резистентность 154 штаммов к антимикробным терапевтическим средствам. Вместе с тем, в рамках исследования с использованием индивидуальной ПЦР (полимеразной цепной реакции) были изучены гены резистентности (tem, ctx-M, shv, ndm и др.) у 154 штаммов, относящихся к патогенным (Shigella spp., Salmonella spp.) и условно-патогенным энтеробактериям.

Ключевые слова: *Enterobacteriaceae spp., изоляты, условно-патогенные энтеробактерии.*

O‘TKIR ICHAK INFEKSIYASI MAHALLIY IZOLYATLARI MOLEKULYAR MARKERLARINI ANIQLASHGA ZAMONAVIY YONDASHUV

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Annotatsiya: Mazkur ilmiy izlanish jarayonida RIEMYUPKIAMFF bakteriologik laboratoriyasida ajratib olingan 496 ta Enterobacteriaceae spp. izolyatlari to‘liq biologik xususiyatlari fenotipik o‘rganildi hamda 154 shtamlarning antimikrob davolash vositalariga orttirilgan turg‘unligi klassik/molekulyar usullar yordamida baholandi. Shu bilan birga, tadqiqot doirasida patogen (Shigella spp., Salmonella spp) hamda shartli-patogen enterobakteriyalarga mansub 154 ta hosilasi alohida PZR (polimeraza zanjirli reaksiyasi) yordamida turg‘unlik genlari (tem, ctx-M, shv, ndm va boshqalar) o‘rganildi.

Kalit so‘zlar: *Enterobacteriaceae spp, izolyatlar, shartli patogen enterobakteriyalar.*

A MODERN APPROACH TO DETERMINING MOLECULAR MARKERS OF LOCAL ACUTE INTESTINAL INFECTION ISOLATES

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Annotation: In the course of this scientific research, the complete biological properties of 496 Enterobacteriaceae spp. isolates obtained in the bacteriological laboratory of the RSNPMCEMPIZ were phenotypically studied. Furthermore, the acquired resistance of 154 strains to antimicrobial therapeutics was evaluated using classical and molecular methods. Concurrently, within the framework of the study, the resistance genes (tem, ctx-M, shv, ndm, etc.) of 154 isolates belonging to pathogenic (Shigella spp., Salmonella spp.) and opportunistic enterobacteria were analyzed using individual PCR (polymerase chain reaction).

Keywords: *Enterobacteriaceae spp., isolates, opportunistic enterobacteria.*

The widespread acquired resistance of the bacterial etiological agents of acute intestinal infections (AIIs) to antimicrobial therapeutic agents is recognized as one of the major problems of healthcare systems worldwide. [WHO. 2025 - Global GLASS System; CDC. National Antimicrobial Resistance Monitoring System (NARMS) Now: Human Data. Atlanta, Georgia: U.S. Department of Health and Human Services, CDC. Available at: https://www.cdc.gov/narmsnow; Antimicrobial resistance (AMR) reporting protocol 2023 European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 2022. 39 p.] Over recent decades, leading research institutions worldwide have conducted extensive studies on the molecular mechanisms that determine resistance of AII pathogens to antibacterial drugs. In particular, considerable attention has been paid to molecular-genetic and genomic analysis methods aimed at identifying resistance genes in pathogenic microorganisms, their transmission routes, and their genetic variability.

At the same time, comprehensive data on the susceptibility profiles of local pathogens in our country to drugs that are important for etiotropic therapy of AIIs, as well as data on their phenotypic heterogeneity, remain insufficiently studied. The emergence of atypical and nonconventional phenotypic characteristics among clinical strains may significantly complicate the differentiation of bacterial AII pathogens in laboratory practice, reduce the accuracy of laboratory diagnosis, and hinder the timely identification of infectious agents. Such variability negatively affects the effectiveness of selecting appropriate antibacterial therapy and creates conditions for the persistence and spread among the population of strains resistant to several medically important therapeutic agents.

Continuous monitoring of regional epidemiological and microbiological trends is also essential for improving laboratory diagnostic algorithms, optimizing empirical treatment approaches, and strengthening programs for the rational use of antimicrobial drugs. From this perspective, studying antimicrobial resistance characteristics and phenotypic variability among local AII isolates has high scientific and practical significance for strengthening the system of epidemiological surveillance of infectious diseases and public health protection in Uzbekistan. [Shadmanova N.A., Iskhakova K.I., Rasulmuhamedova M., Antibiotic resistance of hospital strains of Enterobacteriaceae and phenotypic methods for detecting beta-lactamases, European Science Review, 2016; Shadmanova N.A., Sayidmirzaeva N.G., Qodirova R.R., Epidemiological monitoring and etiological diagnosis of major bacterial pathogens of acute intestinal infections in Uzbekistan, Republican Scientific-Practical Conference Materials, Tashkent, May 2024, pp. 152-155.]

Research objective: To study the pheno-genotypic characteristics of acquired antimicrobial resistance among the main bacterial causative agents of acute intestinal infections in patients from Fergana Region, Republic of Uzbekistan.

Research methods: During 2024-2025, phenotypic identification (VITEK 2 Compact, France) of the main bacterial AII pathogens isolated in the bacteriological laboratory of the Fergana Branch of the Republican Specialized Scientific and Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases (RSNPMCEMPID-FB) was performed. Their susceptibility to clinically significant antimicrobial therapeutic agents and mechanisms of acquired resistance to these agents were also determined. A total of 496 representatives of the Enterobacteriaceae spp. family were isolated during the study, and the biological characteristics and antimicrobial resistance properties of 154 isolates were comprehensively studied using phenotypic and genotypic methods (DNA-Technology, BakResista GLA test kit, Russian Federation). Microbiological diagnosis of AII pathogens was performed in accordance with the methodological guidelines of the Ministry of Health of the Republic of Uzbekistan and EUCAST standards[1].

Research results: In this scientific study, the complete biological properties of 496 Enterobacteriaceae spp. isolates obtained in the bacteriological laboratory of the RSNPMCEMPID-FB were studied phenotypically, and the acquired resistance of 154 strains to antimicrobial therapeutic agents was evaluated using classical and molecular methods [2].

In addition, resistance genes (tem, ctx-M, shv, ndm, etc.) were investigated by individual PCR (polymerase chain reaction) in 154 isolates belonging to pathogenic bacteria (Shigella spp., Salmonella spp.) and opportunistic enterobacteria.

Using classical bacteriological methods, pathogenic enterobacteria were differentiated; 24 isolates were identified as Shigella flexneri, 10 as Shigella sonnei, and 35 as Salmonella enteritidis. During the study, 85 isolates classified as opportunistic pathogenic enterobacteria (OPE) were analyzed. Among them, the leading position was occupied by 43 Klebsiella spp. isolates (mostly Klebsiella pneumoniae), followed in frequency by 28 Proteus spp. isolates (Proteus mirabilis and Proteus vulgaris). In addition, 14 isolates belonging to the genus Enterobacter, including Enterobacter aerogenes, Enterobacter gergoviae, and Enterobacter cloacae, were included; these ranked third by detection frequency among OPEs.

All isolated strains were identified using modern bacteriological and biochemical methods, and their antibiotic susceptibility and phenotypic characteristics were studied at subsequent stages. Currently, in international practice, the diagnosis of AIIs primarily focuses on the detection of bacterial pathogens such as Salmonella spp., Shigella spp., pathogenic Escherichia coli, and Campylobacter spp. Modern laboratory diagnostic methods make it possible to identify causative agents early and accurately. In particular, the use of molecular-genetic diagnostic methods along with bacteriological methods increases the sensitivity and speed of AII diagnosis. This, in turn, is important for selecting effective treatment tactics and strengthening epidemiological surveillance [3].

The data obtained from our laboratory investigations showed that Shigella flexneri (n=24) predominated among local Shigella isolates, confirming earlier epidemiological data for this region. [Iskandarova, G. T. Epidemiological aspects of intestinal infections in Tashkent Region of the Republic of Uzbekistan / G. T. Iskandarova, O. N. Sharapov, D. Yu. Yusupova. Molodoy Ucheny. 2017; No. 1.2 (135.2): 57-59.] However, the fact that only two species of representatives of the Enterobacteriaceae family were identified during the study, and in particular that Salmonella enterica subsp. enterica serovar Typhimurium (n=35) was detected among Salmonella isolates, indicates the need for a systematic revision of microbiological diagnostic approaches for these AIIs. It should be emphasized that routine laboratory diagnosis is mainly directed toward officially recognized pathogenic enterobacteria (Shigella spp., Salmonella spp.), which may prevent a complete etiological analysis of acute intestinal infections. These limitations are associated with outdated regulatory documents, insufficient diagnostic resources, and the limited implementation of modern diagnostic methods [3,4].

In recent years, the rapid development of molecular-genetic methods has made it possible to perform infectious disease diagnostics with a higher level of precision. Taking these opportunities into account, etiological factors of AIIs were identified using the VITEK 2 Compact bacteriological analyzer located in the bacteriological laboratory of the RSNPMCEMPID-FB, while acquired resistance genes to antimicrobial therapeutic agents were detected using the DTprime PCR amplifier of DNA-Technology and the BakResista GLA test kit. At the primary stage of our research, all 154 isolated representatives of the Enterobacteriaceae family were examined by phenotypic methods (disk diffusion method) within the requirements of EUCAST (Version 15.0). The obtained results showed a marked decrease in the susceptibility of most isolates to cephalosporins (Figure 1).

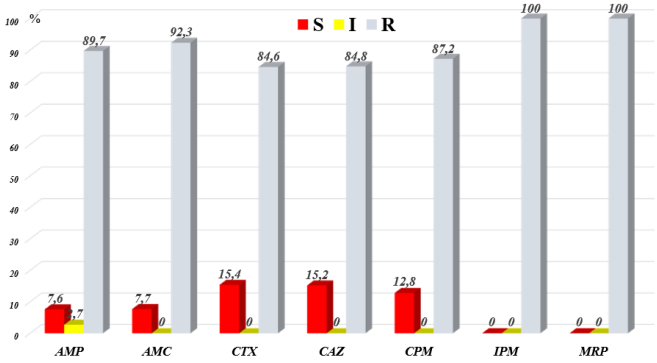

Figure 1. Resistance indicators of representatives of the Enterobacteriaceae family to β-lactam antimicrobial therapeutic agents.

Note: AMP - ampicillin; AMC - amoxicillin/clavulanic acid; CTX - cefotaxime; CAZ - ceftazidime; CPM - cefepime; IPM - imipenem; MRP - meropenem.

Acquired resistance of representatives of the Enterobacteriaceae family to fourth-generation cephalosporins, particularly cefepime, is a serious clinical problem. Cefepime has special clinical importance as a therapeutic agent and was developed as an antibiotic resistant to hydrolysis by many classical beta-lactamases. The emergence of resistance to such a drug in bacterial pathogens requires the presence of specific genes in enterobacteria.

In the scientific literature, this situation has been extensively studied mainly among pathogenic genera of the Enterobacteriaceae family, particularly Salmonella. Considering that 44.7% (n=69) of the 154 isolates obtained during the study were pathogenic enterobacteria, representatives of Salmonella spp. were studied separately as a major foodborne risk [5,6].

In particular, among Salmonella spp. isolates, resistance was detected in 8 of 35 isolates (22.9%) to ceftazidime, in 21 isolates (60%) to cefotaxime, and in 14 isolates (40%) to ceftriaxone. Microbiological analysis of the 35 Salmonella isolates showed that intermediate susceptibility to ceftazidime was 37.1% (n=13); at the same time, the highest susceptibility among cephalosporins was observed precisely to this drug (40%; 14 isolates).

At the next stage of our research, the susceptibility of S. Typhimurium and OPE isolates to five fluoroquinolone drugs was evaluated. In contrast to cephalosporins, a relatively high level of resistance to fluoroquinolones was observed. In particular, almost half of the isolates demonstrated resistance to drugs such as norfloxacin, ofloxacin, and moxifloxacin. This indicator was even more pronounced among local OPE strains: resistance to norfloxacin, ofloxacin, and moxifloxacin was around 49% and 48%, while susceptibility to ciprofloxa-

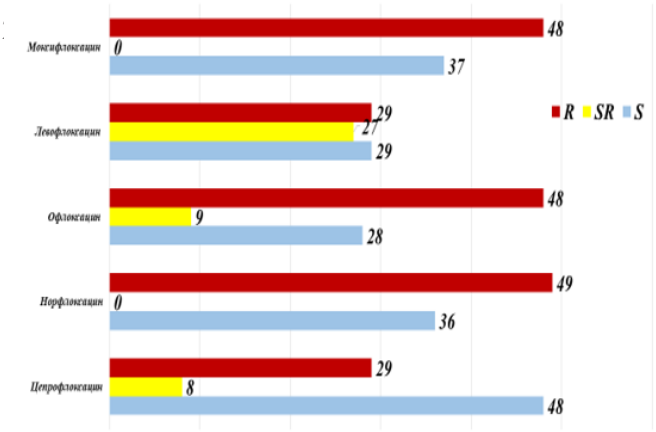

Figure 2. Results of acquired resistance of OPE isolates to fluoroquinolone drugs.

It should be noted that the EUCAST interpretation criteria for fluoroquinolone susceptibility in Salmonella strains have been improved over the years. Until 2011, nalidixic acid was widely used as a screening indicator, and resistance to this agent was assessed as a sign of resistance to all fluoroquinolones. Recent scientific studies recommend the combined use of pefloxacin and nalidixic acid disks for more accurate phenotypic assessment. In particular, the possibility of detecting both chromosomal and plasmid-mediated resistance mechanisms using pefloxacin determines its important diagnostic value. Although EUCAST phenotypic methods are widely used in international laboratory practice, a number of practical difficulties may lead to errors in the interpretation of results. Therefore, in foreign practice, PCR studies targeting qnrA, qnrB, qnrS, aac(6')-Ib-cr, qepA, and oqxAB genes have been performed to detect plasmid-mediated fluoroquinolone resistance genes.

A similar situation was observed among OPEs, with the highest resistance confirmed in Klebsiella spp. and Enterobacter spp. isolates to four generations of cephalosporins by both phenotypic and genotypic methods. In particular, two isolates belonging to the genus Klebsiella spp. were found to simultaneously carry blaNDM and blaTEM.[7]

An important aspect of such microbiological investigations is to confirm or refute data reported in scientific publications indicating that the blaNDM carbapenemase gene is more frequently encountered in Klebsiella pneumoniae isolates than in Salmonella spp., and to comparatively study the probability that this gene is detected at a high level in strains isolated from hospitalized patients.

As noted above, ceftriaxone, a third-generation cephalosporin, is widely used in our country for the treatment of infections caused by Gram-negative bacteria, including Salmonella. International guidelines also emphasize that cefepime, a fourth-generation cephalosporin, has high efficacy against representatives of the Enterobacteriaceae family that produce AmpC β-lactamase. [Pranita D. Tamma, Emily L. Heil, Julie Ann Justo, Amy J. Mathers, Michael J. Satlin, Robert A. Bonomo. Infectious Diseases Society of America 2024 Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections. Clinical Infectious Diseases. 2024; ciae403.] It should be noted that PCR test kits have a number of important advantages over the disk diffusion method for the rapid and high-quality detection of resistance genes. PCR methods, which are being rapidly introduced into microbiological practice, allow the direct detection of genes responsible for antibiotic resistance, thereby enabling highly accurate assessment of resistance mechanisms.

The simultaneous use of PCR along with classical methods in the bacteriological laboratory of the RSNPMCEMPID-FB made it possible to identify

a number of genes associated with resistance to β-lactam antibiotics in local enterobacterial isolates. In particular, the results obtained during our research showed that blaCTX-M family genes, as well as blaSHV and blaTEM series genes, are widespread among local Gram-negative AII pathogens and OPEs. At the same time, resistant Salmonella variants producing extended-spectrum β-lactamases (ESBLs) and AmpC cephalosporinases were also identified among the isolates. The obtained results showed that among Salmonella isolates recovered from patients with AIIs, the most common β-lactamase-encoding genes were blaTEM, blaCTX-M, blaSHV, and the plasmid-mediated AmpC gene blaC-MY-2 .

In Salmonella enterica subsp. enterica serovar Typhimurium isolates (n=35), blaTEM was detected in 34.3% (n=12), blaCTX-M in 42.8% (n=15), and blaSHV in 14.3% (n=5).

The fact that most blaCTX-M family genes and blaSHV and blaTEM series genes among enterobacteria are disseminated through plasmids (mobile genetic elements) once again confirms their widespread distribution in local AII isolates. At the same time, the scientific literature indicates that blaCTX-M-15 and blaCTX-M-14 genogroups are mainly observed among representatives of the Enterobacteriaceae family. PCR tests are currently highly useful for determining this condition; however, the ability of the test systems used in our study to detect the blaCTX-M-1 variant alone cannot substantiate that only this genogroup may be circulating. From the perspective of epidemiological surveillance, such data require separate investigation [8].

In conclusion, PCR diagnostics have high sensitivity and specificity and make it possible to obtain results within a short period of time. Our scientific investigations once again confirmed that while the disk diffusion method requires 18-24 hours or more for bacterial cultivation, PCR can provide preliminary results within several hours. PCR is important not only for the early detection of antibiotic resistance genes such as ESBL, AmpC, NDM, and qnr, but also for the rapid primary identification of the etiological agents of AIIs. This increases the capacity for rapid diagnosis of infections, selection of targeted antibacterial therapy, and control of the spread of resistant strains. In addition, PCR can also detect hidden or low-level resistance determinants that may not be expressed by phenotypic methods. Therefore, molecular-genetic methods occupy an important place in modern microbiological diagnostics.

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