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Gram-negative bacilli from the Enterobacteriaceae family. β-Lactamases with extended substrate spectrum (ESBL) – characteristics, selected molecular aspects of antibiotic resistance, diagnostics – short literature review

Abstract

The Enterobacteriaceae family includes various types of Gram-negative bacteria. Microorganisms treated with antibiotics modify "resistance mechanisms". An example are selected bacteria from the Enterobacteriaceae family, strains of which can produce extended-spectrum β-lactamases (ESBLs). β-lactamases are enzymes that can hydrolyse penicillins, cephalosporins (including third- and fourth-generation, C3G and C4G) and aztreonam, resulting in the development of infection, and fewer therapeutic options. Diagnosis is impeded by the presence of different phenotypes of ESBL resistance to β-lactamases. It leads to detailed substrate preferences of specific ESBL types, designated inhibitor sensitivity, and degree of enzymatic activity and expression, providing the basis for several identification steps. A single mutation in the active site of the enzyme led to the formation of known ESBLs (TEM-1, TEM-2 and SHV-1). Newer enzymes (CTX-M) are derived from cephalosporinases produced by certain plant bacterial strains (e.g. *Kluyvera ascorbata*), which are then inserted into mobile genetic elements. To date, more than 350 different ESBL enzymes have been identified.

Keywords: mechanisms of resistance, mutations, Gram-negative bacteria

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Introduction

For many years, β-lactam antibiotics have been among the most commonly chosen therapeutic drugs for bacterial infections (Livermore, 1995). The β-lactam ring in the antibiotic molecule has antimicrobial activity and the opening of the ring causes the loss of this activity. The choice of β-lactam antibiotics in antibacterial therapy is mainly influenced by good bactericidal results, low toxicity to humans and appropriate

pharmacokinetic properties (Dzierżanowska, 2018). The main, well-known mechanism of β-lactam antibiotics is based on blocking the synthesis of peptidoglycan (the main component of the bacterial cell wall) by inactivating the activity of transpeptidases, as well as carboxypeptidases and endopeptidases. The enzymes targeted by β-lactam antibiotics are called penicillin binding proteins (PBPs) (Bush, Bradford, 2016).

The Enterobacteriaceae Rahn, family includes non-fermenting, facultative Gram--negative anaerobic bacilli-shaped bacteria. Strains are mainly found in the intestines of humans as well as animals, and are also found in the environment, including water and soil (Cagnacci et al., 2008). Human bacilli of the *Klebsiella pneumoniae* (Schroeter) Trevisan and *Escherichia coli* (*E. coli*) (Migula) Castellani & Chalmers species stand out as one of the most viable agents of infection in both hospital and non-hospital settings. These microorganisms, whether entering the respiratory system or penetrating the urinary tract, the blood and even the nervous system, cause a range of alarming health symptoms (Nowakowska et al., 2004; Sękowska et al., 2008; Wang et al., 2023).

Enterobacteriaceae have acquired resistance to β-lactam antibiotics. This resistance involves the production of extended substrate spectrum β-lactamases (ESBLs), which are a key problem in antibiotic therapy and in the use of chemotherapeutics (Jabłoński, 2010). In recent years, abnormalities in the use of antibiotic therapies – overuse in treatment or prophylactic use – have been observed, leading to modification of resistance mechanisms in clinical strains, strains and to drug resistance (Livermore, 1995).

This paper discusses the molecular aspects of the production of extended-spectrum β-lactamases (ESBLs) by selected Enterobacteriaceae.

Extended-spectrum β-lactamases (ESBLs)

ESBL β-lactamases are extended spectrum enzymes that hydrolyse the β-lactam ring in antibiotics such as penicillins, cephalosporins and monobactams. They are secreted by alarm pathogens of the Enterobacteriaceae family (*Escherichia coli*, *Salmonella enterica* (ex Kauffmann & Edwards) Le Minor & Popoff, *Klebsiella pneumoniae*). The enzymes inactivate broad-spectrum oxyiminocephalosporins (third and fourth generation are distinguished), and monobactams (aztreonam) (Bush et al., 1995; Livermore, 1995; Breurec et al., 2012). Cefamycins (e.g. cefoxitins), carbapenems (e.g. meropenem, ertapenem, doripenem) and combinations of β-lactam antibiotics (penicillins and cephalosporins: e.g. ceftriaxone, ceftazidime) (Fig. 1) with β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam remain active against ESBL strains. Imipenem shows 100% susceptibility to ESBL according to the drug susceptibility studies of the authors of microbiological papers (Pfaller et al., 1998; Nowakowska et al., 2004; Radosz-Komoniewska et al., 2004; Quentin et al., 2004; Paterson, Bonomo, 2005; Rudnicka et al., 2005; Sacha et al., 2007; Zientara et al., 2008).

Genes encoding ESBLs are located in transposons but also found in the insertion sequences of large bacterial plasmids. The presence of plasmid genes is not the basis for bacterial survival, but their main function is to encode virulence, adaptation and antibiotic resistance genes which can even be transferred to other bacteria in the process of horizontal gene transfer (bacterial conjugation) (Hopkins et al., 2006; Coque et al., 2008; Marcade et al., 2009). ESBLs are most often encoded by large plasmids, which facilitate their rapid and uncontrolled spread among Gram-negative bacilli (Babini, Livermore, 2000).

ESBLs, which move within the same or different species classified as Gram-negative bacteria, lead to the selection of strains characterised by multidrug resistance. Resistance to many antibiotics commonly used in antimicrobial therapies, such as tetracyclines, chloramphenicol, fluoroquinolones, trimethoprim and even aminoglycosides, has been observed. (Pagani et al., 2003; Mammeri et al., 2005; Bush, Bradford, 2016).

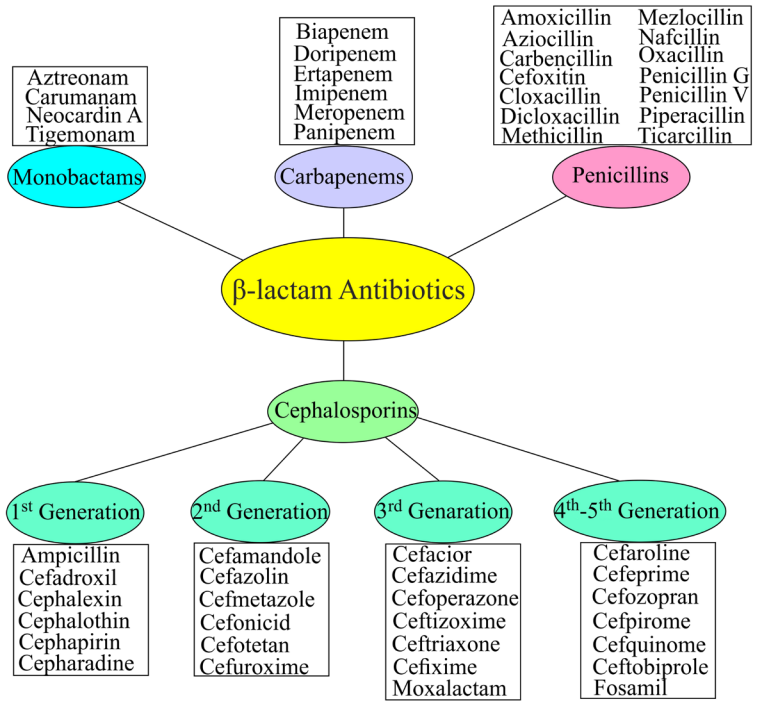

Fig. 1. The main groups of β-lactam antibiotics (Source: *CBMAR*– changed)

The ESBL β-lactamases most commonly found in hospitalised patients are species: *Klebsiella pneumoniae*, *E. coli* and *Enterobacter* spp. but *Acinetobacter baumannii* Bouvet & Grimont and *Pseudomonas aeruginosa* (Schröter) Migula are also encountered (Pagani et al., 2003). It has been assumed that the most common strain with the ability

to produce extended-spectrum ESBL β-lactamases (ESBL) is non-fermenting *E. coli*, followed by *Klebsiella pneumoniae* (Mulani et al., 2019; Castanheira et al., 2021).

There are ESBL variants belonging to families with following characteristics: TEM (most common in *E. coli*), SHV (most common in *Klebsiella* spp. and *E. coli*) and CTX-M (common among *E. coli*, *K. pneumoniae*) (Livermore, 1995; Achouak et al., 2001; Liu et al., 2012).

Characterisation and evolution of ESBLs

ESBLs are β-lactamases that can hydrolyse oxyimino- β-lactam compounds at a rate greater than 10% of the rate of benzylpenicillin hydrolysis (Livermore, 1995; Bonnet, 2004). It is necessary to distinguish between the schemes used to classify ESBL β-lactamases: the Bush-Jacoby scheme (describing the functional characteristics of the enzymes) and the Ambler scheme (based on molecular structure – sequence similarity). According to Ambler, β-lactamases are divided into four classes (A, B, C, D). Classes A, C and D have a localised serine in the active site, which is essential for the catalytic mechanism of hydrolysis, while class B β-lactamases are metalloenzymes that owe their activity to one or two zinc ions. These enzymes have been divided based on functional similarity, which is the substrate and inhibitor profile, which is the diagnostic essence (Bush et al., 1995; Gniadkowski, 2001; Bush, Jacoby, 2010).

ESBL β-lactamases were first identified in the 1980s (Knothe et al., 1983; Kliebe et al., 1985). The microorganisms were found to arise from point mutations of TEM and SHV enzymes that led to resistance to β-lactam antibiotics. It was also observed that the resulting mutations were also found to induce catalytic activity against β-lactam drugs indicating high affinity for these compounds. Currently, more than 300 ESBL variants have been described, dividing them into families with distinct features: TEM, SHV and CTX-M. The variants have largely been obtained through mutations in genes encoding β-lactamases with an extended substrate spectrum: TEM-1, TEM-2, SHV-1 (Gniadkowski, 2001).

Selected resistance mechanisms, resistance genes

There are several main mechanisms by which β-lactam antibiotics are inactivated by Gram-negative bacteria. The cell wall layer of Gram-negative bacteria, which is located between the outer membrane and the cell membrane, releases β-lactamase with high affinity for β-lactam antibiotics. The gene responsible for encoding β-lactamase is located (in the case of *Enterobacter* Hormaeche & Edwards) in extra-chromosomal mobile genetic elements (in plasmid, trans-membrane receptors, transposon). Resistance genes are acquired from bacteria of the same or different species (Gniadkowski, 2001).

Genes that encode ESBLs are located on plasmids, where resistance genes to tetracyclines, chloramphenicol, aminoglycosides, and cotrimoxazole, among others, are also often found (Mammeri et al., 2005; Bush, Jacoby, 2010). Proteins in the outer membrane of bacteria form porins, which are involved in the processes of adhesion, invasion and serum resistance. Removal of the porin proteins in ESBL-positive bacteria results in increased resistance to antibiotics and new mechanisms of resistance. The proteins LamB, OmpK26, PhoE, OmpK35, OmpK36 and KpnO stand out as sources of innate drug resistance (Buchanan, 1999; Achouak et al., 2001; Doménech-Sánchez et al., 2003; Pilonieta et al., 2009; Pulzova et al., 2017).

Many Gram-negative bacteria have genetic elements called integrons in their genomes. Integrons provide a vehicle for the transfer of resistance genes. The existence of intergrons enables the natural flow of genes located in transposons, chromosomal DNA, in conjugation plasmids in bacteria. Genes that are responsible for encoding β-lactamase TEM are transported by transposons such as Tn1, Tn2 or Tn3. The genes that encode the β-lactamases of the SHV version are derived from the chromosome and bacterial plasmids. CTX-M β-lactamases are transmitted during conjugation. The integrons intl1, intl2, intl3, intl4, and intl5 play a significant role in the recombination of bacterial genetic material and are critical for the spread of antibiotic and chemotherapeutic resistance genes (Knothe et al., 1983; Kliebe et al., 1985; Wolinowska et al., 2002; Castanheira et al., 2021).

Mechanisms of enzymatic treatment of β-lactam antibiotics lead to their hydrolysis. Antibiotic resistance in Gram-negative bacteria is due to the reduced permeability of cell membranes associated with reduced synthesis of porin proteins, and the presence of efflux pumps (AcrAB-TolC), which actively remove the applied antibiotic from the bacterial cell (Castanheira et al., 2021; Huy, 2024).

Biofilm production in Gram-negative bacteria (including *Klebsiella pneumoniae*) impedes the penetration of antibacterial substances. By producing an osmotic barrier, biofilm causes a decrease in the sensitivity of bacteria to many antibiotics, including gentamicin, ampicillin and even the popular ciprofloxacin. A correlation between biofilm production and colistin resistance has been demonstrated (Ong et al., 2008; Chung, 2016; Cepas et al., 2019; Desai et al., 2019; Huy, 2024).

Currently, new enzymes (CTX-M) are distinguished, which are formed from cephalosporinases produced by bacterial strains of plants such as *Kluyvera ascorbata* Farmer et al. from the Enterobacteriaceae family. These enzymes have the ability to migrate to genetic components. *K. ascorbata* produces β-lactamase, which leads to the development of a unique sensitivity pattern – low-level resistance to antibiotics: penicillins, cephalothin and cefuroxime, which is reversible by the use of clavulanate (Humeniuk et al., 2002).

Identification of ESBL β-lactamases

The World Health Organization (WHO) has determined that ESBL β-lactamases are among the most challenging pathogens present that is why it is so important to perform screening with an accurate interpretation of the bacteria present. (Castanheira et al., 2021). The Clinical and Laboratory Standards Institute (CLSI) indicates that the interpretation of strain detection should include two steps. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for the detection of ESBL bacilli are also distinguished (Novais et al., 2007). In the first step, it is recommended to use Kirby-Bauer discs and Vitek discs, which have a high sensitivity (even more than 90%). Subsequently, using the double-disk synergy test (DDST), confirmatory tests are made. It is also possible to use the disk combination method (sensitivity of 100% testing cefotaxime and cefepime) or E-test ESBL strips (sensitivity of 100% testing cefotaxime and cefepime tests). The above methods are widely used in diagnostic laboratories, however, there are growing problems in identifying individual ESBL types, so it is recommended to perform additional interpretations in the second stage. It includes, among others, genotypic confirmation tests to determine the enzymes present and their variants. Techniques by which identification of distinguished strains is performed include among others: polymerase chain reaction (PCR), sequencing, and real-time PCR.

The PCR reaction involves the use of primers that give rise to the multiplication of a DNA fragment that includes the sequence of the gene encoding ESBL. After the PCR reaction is completed in an apparatus-thermocycler, the obtained products are separated electrophoretically and visualised under a UV lamp. The test has several steps and includes controls – positive and negative samples. Multiplicity markers identify amplification products and determine the correct course of the PCR reaction. The result allows interpretation of ESBL-encoding genes (Hryniewicz et al., 2022).

The real- time PCR reaction is a sensitive technique that allows the determination of the gene present at a low initial concentration. It relies on measuring the fluorescence that is emitted by the amplified DNA fragments. The increase in fluorescence occurs as the concentration of amplification products increases in the cycles. The analysis of the reaction is observed and visualized on a monitor, which allows interpretation of the result during the reaction, this in turn allows shortening the testing process (Hryniewicz et al., 2022).

Different DNA markers can be used in the molecular diagnostics of ESBL prevalence, but the beta-lactamase (bla) genes: blaTEM, blaCTX-M and blaSHV are the most commonly ones (Hasman et al., 2005; Carvalho et al., 2016). What is important, there are numerous variants of these genes, i.e. TEM-4, TEM-29, TEM-85, TEM-86, TEM-93, and TEM-94 (Baraniak et al., 2005), CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-12,

CTX-M-15 (Bonnet, 2004) and SHV-2a, SHV-5, and SHV-12 (Liakopoulos et al., 2016). Table 1 summarises the most often amplified markers with the examples of their primer sequences.

Tab. 1. The examples of the most commonly used DNA markers in the molecular diagnostics of ESBL presence

Gene	Sequence	Size (bp)	Reference
blaSHV	5′-TTCGCCTGTGTATTATCTCCCTG-3′ 5′-TTAGCGTTGCCAGTGYTCG-3′	854	Hasman et al., 2005
blaTEM	5′-GCGGAACCCCTATTTG-3′ 5′-TCTAAAGTATATATGAGTAAACTTGGTCTGAC-3′	964	Mulvey et al., 2003
blaCTX-M	5′-ATGTGCAGYACCAGTAARGTKATGGC-3′ 5′-TGGGTRAARTARGTSACCAGAAYCAGCGG-3′	593	Miró et al., 2002

In the recent years, the whole genome sequencing (WGS) becomes more and more important method in the ESBL diagnostics (Founou et al., 2019; Kurittu et al., 2022). WGS can be performed using both short- and long-read technologies, allowing for the accurate identification of virulence and resistance gene sequence types (STs). It is also possible to perform a hybrid sequence analysis combining long- and short-read sequencing (Berbers et al., 2023). To identify the type of plasmid involved in the mechanism of resistance, sequences obtained for the tested strains can be compared to those previously published in the dedicated databases such as ResFinder (version 4.1) (Florensa et al., 2022).

Another method commonly used to detect of ESBL bacilli is an automated mass spectrometry-based microorganism identification system (Autof MS1000/MALDI--TOF). There are also immunochromatographic techniques that allow efficient and rapid detection of CTX-M enzymes (NG-Test CTX-M MULTI), and subsequent identification of Enterobacterales in tested materials using MALDI-TOF. Ongoing mutations, that lead to modifications in the patterns of ESBL subtypes, complicate bacterial identification and require the use of different pathogen detection techniques (Paterson et al., 2001; Novais et al., 2007; Zboromyrska et al., 2022).

Conclusions

Antibiotic resistance is now becoming a serious global problem. Organizations such as the World Health Organization (WHO), the European Parliament (EP) and the United States Centres for Disease Control and Prevention (CDC) have identified the fight against antibiotic resistance in bacteria as a priority (Amyes, Gemmell, 1992; Hopkins et al., 2006). The increase in infections caused by ESBL-producing strains of

the Enterobacteriaceae family poses a challenge to modern medicine. The occurrence of β-lactamases with an extended substrate spectrum in hospital settings brings a huge clinical problem. The complex and dynamic evolution and the diversity of producer strains that can express different combinations of β-lactamases, lead to an overestimation of the level and increase in the spectrum of resistance phenotypes in Enterobacteriaceae, resulting in the use of specialised antibiotic regimens that ultimately do not provide sufficient therapeutic options (Baraniak et al., 2005).

It is now stated that the epidemiology of ESBLs is evolving at a very rapid pace and in the future its consequences will require specialised pharmacological targeting, and the design of non-standard therapies that are not yet sufficiently defined (Paterson et al., 2001; Novais et al., 2007).

Conflict of interest

The authors declare no conflict of interest related to this article.

References

Achouak, W., Heulin, T., Pagès, J-M. (2001). Multiple facets of bacterial porins. *FEMS Microbiology Letters*, *199(1)*, 1–7. https://pubmed.ncbi.nlm.nih.gov/11356559/

Amyes, S.G.B., Gemmell, C.G. (1992). Antibiotic resistance in bacteria. *Medical Microbiology*, *36*, 4–29. https://doi.org/10.1099/00222615-36-1-4

Babini, G.S., Livermore, D.M. (2000). Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997–1998, *Journal of Antimicrobial Chemotherapy*, *45*, 183–189. https://doi.org/10.1093/jac/45.2.183

Baraniak, A., Fiett, J., Mrówka, A., Walory, J., Hrynkiewicz, W., Gniadkowski, M. (2005). Evolution of TEM-Type Extended-Spectrum β-Lactamases in Clinical Enterobacteriaceae Strains in Poland. *Antimicrobial Agents and Chemotherapy*, *49(5)*, 1872–1880. https://doi.org/10.1128/AAC.49.5.1872-1880.2005

Berbers, B., Vanneste, K., Roosens, N.H.C.J., Marchal, K., Ceyssens, P.-J., De Keersmaecker, S.C.J. (2023). Using a combination of short- and long-read sequencing to investigate the diversity in plasmid- and chromosomally encoded extended-spectrum beta-lactamases (ESBLs) in clinical *Shigella* and *Salmonella* isolates in Belgium. *Microbial Genomics*, *9(1)*, mgen000925. https://doi.org/10.1099/mgen.0.000925

Bonnet, R. (2004). Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrobial Agents Chemotherapy*, *48(1)*, 1–14. https://doi.org/10.1128/AAC.48.1.1-14.2004

Breurec, S., Guessennd, N., Timinouni, M., Le, T.T.H., Cao, V., Ngandjio, A., Randrianirina, F., Thiberge, J.M., Kinana, A., Dufougeray, A., Perrier-Gros-Claude, J.D., Boisier, P., Garin, B., Brisse, S. (2013). *Klebsiella pneumoniae* resistant to third- generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. *Clinical Microbiology Infection*, *19*, 349–355. https://doi.org/10.1111/j.1469-0691.2012.03805.x

Buchanan, S.K (1999). β-Barrel proteins from bacterial outer membranes: structure, function and refolding. *Current Opinion Structural Biology*, *9*, 455–461. https://doi.org/10.1016/S0959-440X(99)80064-5

Bush, K., Bradford, P.A. (2016). β-Lactams and β-lactamase inhibitors: an overview. *Cold Spring Harbor Perspectives Medicine*, *6(8)*, a 025347. https://doi.org/10.1101/cshperspect.a025247

Bush, K., Jacoby, G.A. (2010). Updated functional classification of beta-lactamases. *Antimicrobial Agents Chemotherapy*, *54*, 969–976. https://doi.org/10.1128/AAC.01009-09

Bush, K., Jacoby, G.A., Medeiros, A.A. (1995). A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrobial Agents Chemotherapy*, *39*, 1211–1233. https://doi.org/10.1128/AAC.39.6.1211

Cagnacci, S., Gualco, L., Roveta, S., Mannelli, S., Borgianni, L., Docquier, J.D. (2008). Bloodstream infections caused by multidrug-resistant *Klebsiella pneumonia* producing the carbapenem-hydrolysing VIM-1 metallo-beta-lactamase: first Italian outbreak. *Journal Antimicrobial Chemotherapy*, *61(2)*, 296–300. https://doi.org/10.1093/jac/dkm471

Carvalho, A.C., Barbosa, A.V., Arais, L.R., Ribeiro, P.F., Carneiro, V.C., Cerqueira, A.M.F. (2016). Resistance Patterns, ESBL Genes, and Genetic Relatedness of Escherichia Coli from Dogs and Owners. *Brazilian Journal of Microbiology*, *47(1)*, 150–158. https://doi.org/10.1016/j.bjm.2015.11.005

Castanheira, M., Simner, P.J., Bradford, P.A. (2021). Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. *JAC-Antimicrobal Resistance*, *3*, dlab092. https://doi.org/10.1093/jacamr/dlab092

CBMAR: Comprehensive Beta-lactamase Molecular Annotation Resource. β-lactamases. http://protein-informatics.org/mkumar/lactamasedb/lactamase.html (Access: 30 May 2024).

Cepas, V., López, Y., Muñoz, E., Rolo, D., Ardanuy, C., Martí, S., Xercavins, M., Horcajada, J.P., Bosch, J., Soto, S.M. (2019). Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. *Microbial Drug Resistance*, *25*, 72–79. https://doi.org/10.1089/mdr.2018.0027

Chung, P.Y. (2016). The emerging problems of *Klebsiella pneumoniae* infections: carbapenem resistance and biofilm formation. *FEMS Microbiology Letters*, *363(20)*, fnw219. http://doi.org/10.1093/FEMSLE/FNW219

Coque, T.M., Novais, A., Carattoli, A., Poirel, L., Pitout, J., Peixe, L., Baquero, F., Canton, R., Nordmann, P. (2008). Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. *Emerging Infectious Diseases*, *14*, 195–200. https://doi.org/10.3201/eid1402.070350

Desai, S., Sanghrajka, K., Gajjar, D. (2019). High adhesion and increased cell death contribute to strong biofilm formation in *Klebsiella pneumoniae*. *Pathogens*, *8(4)*, 277. https://doi.org/10.3390/pathogens8040277

Doménech-Sánchez, A., Martínez- Martínez, L., Hernández- Allés, S., del Carmen Conejo, M., Pascual, A, Tomás, J.M, Benedí, V.J. (2003). Rolle of *Klebsiella pneumoniae* OmpK35 Porin in antimicrobial resistance. *Antimicrobial Agents Chemotherapy*, *47(10)*, 3332–3335. https://doi.org/10.1128/AAC.47.10.3332-3335.2003

Dzierżanowska, D. (2018). Mechanizmy bakteryjnej oporności na antybiotyki i chemioterapeutyki. W: *Antybiotykoterapia praktyczna*. Wydawca: Alfa Medica Press. [In Polish]

Florensa, A., Kaas, R., Clausen, P., Aytan-Aktug, D., Aarestrup, F. (2022). ResFinder – an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. *Microbial Genomics*, *8(1)*, 000748. https://doi.org/10.1099/mgen.0.000748

Founou, R., Founou, L., Allam, M., Ismail, A., Essack, S. (2019). Whole genome sequencing of extended spectrum β-Lactamase (ESBL)-producing *Klebsiella pneumoniae* isolated from hospitalized patients in KwaZulu-Natal, South Africa. *Scientific Reports*, *9(1)*, 6266. https://doi.org/10.1038/s41598-019-42672-2

Gniadkowski, M. (2001). Evolution and epidemiology of extended spectrum β-lactamases (ESBLS) and producing microorganisms. *Clinical Microbiology and Infection*, *7(11)*, 597–608. https://doi.org/10.1046/j.1198-743x.2001.00330.x

Hasman, H., Mevius, D., Veldman, K., Olesen, I., Aarestrup, F.M. (2005). β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human

patients in the Netherlands. *Journal of Antimicrobial Chemotherapy*, *56(1)*, 115–121. https://doi.org/10.1093/jac/dki190

Hopkins, K.L., Liebana, E., Villa, L., Batchelor, M., Threlfall, E.J., Carattoli, A. (2006). Replicon typing of plasmids carrying CTX-M or CMY β-lactamases circulating among *Salmonella* and *Escherichia coli* isolates. *Antimicrobial Agents Chemotherapy*, *50*, 3203–3206. https://doi.org/10.1128/AAC.00149-06

Hryniewicz, W., Kuch, A., Wanke-Rytt, M., Żukowska, A. (red.) (2022). *Pałeczki Enterobacterales wytwarzające karbapenemazy (CPE)*. Epidemiologia, diagnostyka, leczenie i profilaktyka zakażeń. Wyd. Narodowy Instytut Leków, Warszawa. [In Polish].

Humeniuk, C., Arlet, G., Gautier V., Grimont, P., Labia, Roger., Philippon A. (2002). β-lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid- encoded CTX-M types. *Antimicrobial Agents and Chemotherapy – ASM Journals*, *46(9)*, 3045–3049. http://doi.org/10.1128/aac.46.9.3045-3049.2002

Huy, T.X.N. (2024). Overcoming *Klebsiella pneumoniae* antibiotic resistance: new insights into mechanisms and drug discovery. *Beni-Suef University Journal of Basic and Applied Sciences*, *13(13)*. https://doi.org/10.1186/s43088-024-00470-4

Jabłoński, A., Zębek, S., Mokrzycka, A. (2010). Selected resistance mechanisms of bacteria to chemotherapeutics. *Medycyna Weterynaryjna*, *66(7)*, 449–452. [In Polish]

Kliebe, C., Nies, B.A., Meyer, J.F., Tolxdorff-Neutzling, R.M., Wiedemann, B. (1985). Evolution of plasmid--coded resistance to broad-spectrum cephalosporins. *Antimicrobial Agents Chemotherapy*, *28*, 302–307. https://doi.org/10.1128/aac.28.2.302

Knothe, H., Shah, P., Krcmery, V., Antal, M., Mitsuhashi S. (1983). Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection*, *11*, 315–317. https://doi.org/10.1007/BF01641355

Kurittu, P., Khakipoor, B., Jalava, J., Karhukorpi, J., Heikinheimo, A. (2022). Whole-genome sequencing of extended-spectrum Beta-Lactamase-producing *Escherichia coli* from human infections in Finland revealed isolates belonging to internationally successful ST131-C1-M27 subclade but distinct from non-human sources. *Frontiers in Microbiology*, *4(12)*, 789280. https://doi.org/10.3389/fmicb.2021.789280.

Liakopoulos, A., Mevius, D., Ceccarelli, D. (2016). A review of SHV extended-spectrum β-Lactamases: neglected yet ubiquitous. *Frontiers in Microbiology*, *7*, 1374. https://doi.org/10.3389/fmicb.2016.01374

Liu, P., Li, P., Jiang, X., Bi. D., Xie, Y., Tai, C., Deng, Z., Rajakumar, K., Ou, H.Y. (2012). Complete genome sequence of *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286, a multidrug-resistant strain isolated from human sputum. *Journal of Bacteriology*, *194(7)*, 1841–1842. https://doi.org/10.1128/JB.00043-12

Livermore, D.M. (1995). Beta-Lactamases in laboratory and clinical resistance. *Clinical Microbiology Reviews*, *8*, 557–584. https://doi.org/10.1128/CMR.8.4.557

Mammeri, H., Van De Loo, M., Poirel, L., Martinez-Martinez, L., Nordmann, P. (2005). Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrobial Agents Chemotherapy*, *49*, 71–76. https://doi.org/10.1128/AAC.49.1.71-76.2005

Marcade, G., Deschamps, C., Boyd, A., Gautier, V., Picard, B., Branger, C., Denamur, E., Arlet, G. (2009). Replicon typing of plasmids in *Escherichia coli* producing extended-spectrum β-lactamases. *Journal of Antimicrobial Chemotherapy*, *63(1)*, 67–71. https://doi.org/10.1093/jac/dkn428

Miró., E., Navarro, F., Mirelis, B., Sabaté, M., Rivera, A., Coll, P., Prats, G. (2002). Prevalence of clinical isolates of *Escherichia coli* producing inhibitor-resistant β-Lactamases at a University Hospital in Barcelona, Spain, over a 3-year period. *Antimicrobial Agents and Chemotherapy*, *46(12)*, 3991–3994. https://doi.org/10.1128/AAC.46.12.3991-3994.2002

Mulani, M.S., Kamble, E.E., Kumkar, S.N., Tawre, M.S., Pardesi, K.R. (2019). Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Frontiers in Microbiology*, *10*, 539. https://doi.org/10.3389/fmicb.2019.00539

Mulvey, M., Soule, G., Boyd, D., Demczuk, W., Ahmed, R. (2003). Characterization of the first extended--spectrum Beta-Lactamase-producing *Salmonella* isolate identified in Canada. *Journal of Clinical Microbiology*, *41(1)*, 460–462. https://doi.org/10.1128/JCM.41.1.460-462.2003

Novais, A., Canton, R., Moreira, R., Peixe, L., Baquero, F., Coque, T.M.(2007). Emergence and dissemination of *Enterobacteriaceae* isolates producing CTX-M-1- like enzymes in Spain are associated with IncFII (CTX-M-15) and broadhostrange (CTX-M-1, -3, and -32) plasmids. *Antimicrobial Agents and Chemotherapy*, *51*, 796–799. https://doi.org/10.1128/AAC.01070-06

Nowakowska, M., Rogala-Zawada, D., Wiechuła, B. (2004). Czynniki etiologiczne zakażeń układu moczowego u dzieci i ich wrażliwość na antybiotyki. *Wiadomości Lekarskie*, *57*, 438–443. [In Polish]

Ong, C.-L.Y., Ulett, G.C., Mabbett, A.N., Beatson, S.A., Webb, R.I., Monaghan, W., Nimmo, G.R., Looke, D.F., Mcewan, A.G., Schembri, M.A. (2008). Identyfication of Type 3 fimbriae in uropathogenic *Escherichia coli* reveals a role in biofilm formation. *Journal of Bacteriology*, *190(3)*, 1054–1063. https://doi.org/10.1128/JB.01523-07

Pagani, L., Dell'Amico, E., Migliavacca, R., D'Andrea, M.M., Giacobone, E., Amicosante, G., Romero, E., Rossolini, G.M. (2003). Multiple CTX-M-type extended-spectrum β-lactamases in nosomical isolates of *Enterobacteriaceae* from a hospital in Nor- thern Italy. *Journal of Clinical Microbiology*, *41(9)*, 4264–4269. https://doi.org/10.1128/JCM.41.9.4264-4269.2003

Paterson, D.L., Bonomo, R.A. (2005). Extended-spectrum β-lactamases: a clinical update. *Clinical Microbiology*, *18*, 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

Paterson, D.L., Ko, W.C., Von, Gottberg, A., Casellas, J.M., Mulazimoglu, L., Klugman, K.P., Bonomo, R.A., Rice, L.B., McCormack, J.G., Yu, V.L. (2001). Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. *Journal of Clinical Microbiology*, *39*, 2206–2212. https://doi.org/10.1128/JCM.39.6.2206-2212.2001

Pfaller, M.A, Jones, R.N, Doern, G.V. (1998). Bacterial Pathogens Isolated from Patients with Bloodstream Infection: Frequencies of Occurrence and Antimicrobial Susceptibility Patterns from the SENTRY Antimicrobial Surveillance Program (United States and Canada, 1997). *Antimicrobial Agents and Chemotherapy*, *42*, 1762–1770. https://doi.org/10.1128/AAC.42.7.1762

Pilonieta, M.C., Erickson, K.D., Ernst, R.K., Detweiler, C.S. (2009). A protein important for antimicrobial peptide resistance, YdeI/OmdA, is in the periplasm and interacts with OmpD/NmpC. *Journal of Bacteriology*, *191*, 7243–7252. https://doi.org/10.1128/JB.00688-09

Pulzova, L., Navratilova, L., Comor, L. (2017). Alterations in outer membrane permeability favor drug-resistant phenotype of *Klebsiella pneumoniae. Microbial Drug Resistance*, *23(4)*, 413–420. https://doi.org/10.1089/mdr.2016.0017

Quentin, C., Arpin, C., Dubois, V., André, C., Lagrange, I., Fischer, I., Brochet, J.P., Grobost, F., Jullin, J., Dutilh, B., Larribet, G., Noury, P. (2004). Antibiotic resistance rates and phenotypes among isolates of *Enterobacteriaceae* in French extra-hospital practice. *European Journal of Clinical Microbiology Infectious Diseases*, *23*, 185–193. https://doi.org/10.1007/s10096-003-1081-5

Radosz-Komoniewska, H., Gniadkowski, M., Rogala-Zawada, D., Nowakowska, M., Rudy, M., Wiechuła, B., Martirosian, G. (2004). Incidence of extender spectrum β-lactamases in clinical isolates of the family *Enterobacteriaceae* in pediatric hospital. *Polish Journal of Microbiology*, *53(1)*, 27–34.

Rudnicka, J., Wróblewska, M., Marchel, H. (2005). Częstość występowania i lekooporność pałeczek z rodziny *Enterobacteriaceae* izolowanych od pacjentów hospitalizowanych na oddziałach intensywnej terapii. *Medycyna Doświadczalna i Mikrobiologia*, *57*, 185–191. [In Polish]

Sacha, P., Jakoniuk, P., Wieczorek, P. (2007). Mechanizmy oporności na antybiotyki β-laktamowe izolatów *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilit* i *Enterobacter cloacae* opornych na cefotaksym. *Wiadomości Lekarskie*, *76*, 314–321. [In Polish]

Sękowska, A., Wróblewska, J., Gospodarek., E. (2008). ESBL-dodatnie i ESBL-ujemne szczepy *Klebsiella pneumoniae* i *Klebsiella oxytoca* – występowanie w materiale klinicznym i wrażliwość na wybrane antybiotyki. *Medycyna Doświadczalna i Mikrobiologia*, *60*, 39–44 [In Polish]

Wang, S., Wang, S., Tang, Y., Peng, G., Hao, T., Wu, X., Wei, J., Qiu, X., Zhou, D., Zhu, S. (2023). Detection of *Klebsiella pneumonia* DNA and ESBL positive strains by PCR-based CRISPR-LbCas12a system. *Frontiers in Microbiology*, *14*, 1128261. https://doi.org/10.3389/fmicb.2023.1128261

Wolinowska, R., Masny, A., Płucienniczak, A. (2002). Integrony. *Kosmos*, 3, 353–364. [In Polish]

Zboromyrska, Y., Rico, V., Pitart, C., Fernández-Pittol, M.J., Soriano, Á., Bosch, J. (2022). Implementation of a New Protocol for Direct Identification from Urine in the Routine Microbiological Diagnosis. *Antibiotics (Basel)*, *11(5)*, 582. https://doi.org/10.3390/antibiotics11050582

Zientara, M., Rudy, M., Samulska, E. (2008). Ocena wyników posiewów krwi dzieci leczonych w Górnośląskim Centrum Zdrowia Dziecka i Matki. *Medycyna Doświadczalna i Mikrobiologia*, *60*, 65–69. [In Polish]

Pałeczki Gram-ujemne z rodziny Enterobacteriaceae. β-Laktamazy o rozszerzonym spektrum substratowym (ESBL) – charakterystyka, wybrane aspekty molekularne oporności na antybiotyki, diagnostyka – krótki przegląd bibliografii

Streszczenie

W niniejszej pracy omówiono aspekty molekularne wytwarzania β-laktamaz o poszerzonym spektrum działania (ESBL) przez wybrane pałeczki z rodziny Enterobacteriaceae. Rodzina Enterobacteriaceae obejmuje różne typy bakterii Gram-ujemnych. Drobnoustroje poddawane działaniu antybiotyków modyfikują „mechanizmy oporności". Przykładem są wybrane bakterie z rodziny Enterobacteriaceae, których szczepy posiadają zdolność do wytwarzania β-laktamaz o poszerzonym spektrum działania (ESBL). β-laktamazy to enzymy, które mogą hydrolizować penicyliny, cefalosporyny (również trzeciej i czwartej generacji, C3G i C4G) i aztreonam, czego skutkiem jest rozwinięcie się infekcji, oraz mniejsze możliwości terapeutyczne. Utrudniona diagnostyka przez występowanie zróżnicowanych fenotypów oporności ESBL na β-laktamazy prowadząca do szczegółowych preferencji substratowych konkretnych typów ESBL, wyznaczonej czułości na inhibitory, stopnia aktywności enzymatycznej oraz ekspresji, stanowi podstawę do wyznaczenia kilku etapów identyfikacyjnych. Pojedyncza mutacja miejsca aktywnego enzymu prowadziła do powstania znanych ESBL (TEM-1, TEM-2 i SHV-1). Nowsze enzymy (CTX-M) wywodzą się z cefalosporynaz wytwarzanych przez niektóre szczepy bakteryjne roślin (np. *Kluyvera ascorbata*), które następnie są wprowadzane do ruchomych elementów genetycznych. W chwili obecnej znanych jest ponad 350 różnych enzymów ESBL.

Słowa kluczowe: mechanizmy oporności, mutacje, gram-ujemne bakterie

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