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Adherence of *Neisseria gonorrhoeae* to surface of SiHa cells (human cervical carcinoma cell line)

Preparation and imaging:

Joanna Białecka PhD, Centre of Microbiological Research and Autovaccines, Cracow, Poland,
Kamil Drożdż MSc, Department of Molecular Medical Microbiology;
Chair of Microbiology, Faculty of Medicine Jagiellonian University Medical College in Krakow;
Monika Gołda-Cępa PhD, Materials and Surface Chemistry Group,
Department of Inorganic Chemistry, Faculty of Chemistry, Jagiellonian University

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GONOCOCCI – PATHOGENS OF GROWING IMPORTANCE. PART 1. CURRENT DATA ON DIAGNOSTICS, GENOTYPING AND THERAPY.

Joanna Białecka[1, 2], Katarzyna Rak[1], Aneta Kiecka[1, 3]

[1] Centre of Microbiological Research and Autovaccines, Cracow, Poland
[2] Department of Pharmaceutical Microbiology, Jagiellonian University Medical College, Cracow, Poland
[3] Chair of Biomedical Sciences, Institute of Physiotherapy, Jagiellonian University Medical College, Cracow, Poland

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Abstract: *Neisseria gonorrhoeae* is an aetiological agent of gonorrhoea, which is a sexually transmitted disease (STD), a public health challenge worldwide. Gonorrhoea is undoubtedly a serious health risk, especially for women, due to its often asymptomatic course and the possibility of upper genital tract complications such as pelvic inflammatory disease (PID), which can result in infertility. The data show that the diagnostic method for *N. gonorrhoeae* should be specific, simple, sensitive, rapid and inexpensive. Currently, phenotypic identification methods have been dominated by NAAT methods, which allow detection and identification of gonococcus directly in the clinical specimen. However, up today molecular methods do not allow full determination of drug susceptibility.

Keywords: AMR, diagnosis, gonorrhoea, molecular typing, STD

1. Introduction

Neisseria gonorrhoeae is a Gram-negative diplococcus called gonococcus. It belongs to the genus *Neisseria* and the family *Neisseriaceae*. *N. gonorrhoeae* is an obligate human pathogen, the aetiological agent of gonorrhoea. Another human pathogenic species of this family is *N. meningitidis*. In contrast, eight commensal species are part of the human microbiome (*N. cinera*, *N. elongata*, *N. flavescens*, *N. lactamica*, *N. mucosa*, *N. polysaccharea*, *N. sicca*, *N. subflava*), colonizing mainly the mucous membranes of the upper respiratory tract, genitourinary tract, and anal area. Commensal *Neisseria* species can be opportunistic pathogens for humans (Humbert and Christodoulides 2019).

Gonorrhoea is a sexually transmitted disease that spreads through direct contact with secretions. Infection can also occur through vertical transmission from infected mother to child during childbirth. Gonorrhoea is an acute or chronic disease that develops mainly in the columnar and transitional epithelium of the lower genitourinary tract. It less commonly affects the anus, pharynx, conjunctiva, or cornea. The squamous epithelium is less susceptible to infection (Bignell *et al.* 2013).

In Poland, gonococcal infection has been subject to mandatory reporting since 1948. This time, the recorded incidence was about 180 cases per 100,000 people. Polish data on gonorrhoea epidemiology come from annual reports published by the National Institute of Public Health – National Institute of Hygiene (NIZP PZH, www.pzh.gov.pl). Since 2014, data on gonococci isolated in Poland are presented in ECDC reports of the Euro-GASP program (ECDC 2022). Since the 1980s, there has been a decline in reported incidence, from 87.6 per 100,000 in 1981 to 9.7 in 1992 and less than 2 per 100,000 in 2000 (Młynarczyk-Bonikowska *et al.* 2014). In 2014, only 458 cases were reported in Poland, with an incidence of 1.19 per 100,000, and in 2021, only 287 cases of gonorrhoea were reported, with an incidence of 0.75 per 100,000. After the Covid-19 pandemic

* Corresponding Author: J. Białecka, Centre of Microbiological Research and Autovaccines, Kraków, Poland, e-mail: joanna.bialecka@cbm.com.pl

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in 2022, 630 cases of gonorrhoea were reported, with an incidence of 1.67 per 100,000. In 2023, there was twice an increase in cases – 1372, indicating 3,64 per 100,000. But still, the number of registered gonorrhoea in Poland is low compared to the European average. However, the Polish data may be underestimated significantly (NIZP PZH – PIB 2022, ECDC 2022). Globally, the number of cases is increasing. According to WHO, more than 82.4 million cases of gonorrhoea were reported in 2020, which ranks it as the second most common bacterial sexually transmitted disease after *Chlamydia trachomatis* infection (WHO 2021). According to epidemiological reports from the E.U. area, incidence has increased from 6.6 per 100,000 in 2009 to 31.6 per 100,000 in 2019 (ECDC 2023). In the U.S., an even higher incidence rate was found in 2020: 206.5 per 100,000, and there was a further increase of 4.6% in 2021. This may be due to better epidemiological surveillance (Rowley *et al.* 2019; WHO 2021, ECDC 2023).

The clinical picture of *N. gonorrhoeae* infection in men and women differs fundamentally. The first symptoms of infection usually appear within a week after sexual intercourse (usually between 3–7 days). In men, acute urethritis develops that is difficult to overlook, with the presence of a mucopurulent discharge (> 80% of cases), as well as dysuria (> 50%). The asymptomatic infections in men are rare (< 10%). In untreated patients, a complication of prolonged infection can be testicular inflammation, epididymitis, and prostatitis (Bignell *et al.* 2013). In women, *N. gonorrhoeae* infection mainly affects the cervical canal or urethra, causing gonococcal cervicitis or urethritis, respectively. The disease can present as a change in the type of vaginal discharge (about 50%), lower abdominal pain (< 20%), or dysuria (10–15%). In women, the symptoms of infection are much milder than in men. In more than 50% of cases, the infection in women is asymptomatic, resulting in a lack of recognition and treatment and the development of different complications. The main sequel of gonorrhoea infection in women worth to be listed is Bartholin's gland inflammation, pelvic inflammatory disease (PID) involving the endometrium, fallopian tubes and ovaries, perianal abscesses, ovarian abscess, tubal factor infertility (TFI), ectopic pregnancy, Fitz-Hugh-Curtis syndrome (FHCS), perihepatitis. An unrecognized infection also facilitates the transmission of the pathogen to sexual partners. An ascending gonococcal infection involving the upper urogenital tract develops in 10–20% of cases. Consequently, it can be threatened with disseminated gonococcal infection (DGI) and gonococcal arthritis in both sexes (Mroczkowski 1998; Bignell *et al*. 2013; Workowski *et al.* 2021). Gonococcal infection may be the reason for infertility globally (Chemaitelly *et al*. 2021).

Noteworthy is the significant difference in the number of strains isolated from women and men, possibly due to differences in the clinical picture of this infection in both sexes and its epidemiology. According to ECDC's 2020 reports (ECDC 2022), between 2014 and 2020, among all strains analyzed for resistance, isolates from women ranged from 14.7% to 18.2%. In 2019, the reporting of gonorrhoea cases in men and women in Europe was 48 per 100,000 population and 16 per 100,000 population, respectively, resulting the male-to-female ratio of gonorrhoea reported cases 3 to 1. In Poland, the male-to-female ratio of reported gonorrhoea was almost ten times higher, as high as 29 to 1 (ECDC 2023). It is noted that gonococcal strains isolated from men come from two groups of patients – heterosexual men and men who have sex with men (Xiridou *et al.* 2015; Sánchez-Busó *et al.* 2022). As mentioned above, there has been an increase in gonococcal infections between 2010 and 2019 among both men and women, but the highest increase has been in the group of men who have sex with men (ECDC 2023). The observed significant differences in the detection of gonococcal infections in men and women in Poland compared to European data may, therefore, be the result of an underestimation of the prevalence of Polish infections among heterosexual partners and the more frequent performance of diagnostic tests in men who have sex with men due to increased awareness of such risks in these individuals.

In both sexes, the picture of gonococcal infection is different. The often asymptomatic or nonspecific course of infection in the vagina and cervix can be easily overlooked by female patients. Gonococcal infections are more easily detected, diagnosed, and treated correctly in men who report to specialists with severe symptoms of infection. The question arises as to what other factors may be associated with the lower rates of infection recorded in women. The issue requires further research based on epidemiological and clinical patient data.

2. Diagnosis of gonococcal infection

The diagnosis of gonorrhoea is based on the detection of gonococci or their genetic material in swabs taken from the genitourinary tract, anus, pharynx, and conjunctival sac, using culture or molecular methods based on nucleic acid amplification NAATs (*Nucleic Acid Amplification Tests*) (Fig. 1). The gold standard for diagnosis of gonococcal infection for many years was a culture of *N. gonorrhoeae*, which has now been replaced mainly by molecular methods (CDC 2014). However, it should be noted that at the current stage of diagnostic development, only classical microbiological culture methods allow the complete determination of drug susceptibility of clinical strains of *N. gonorrhoeae*.

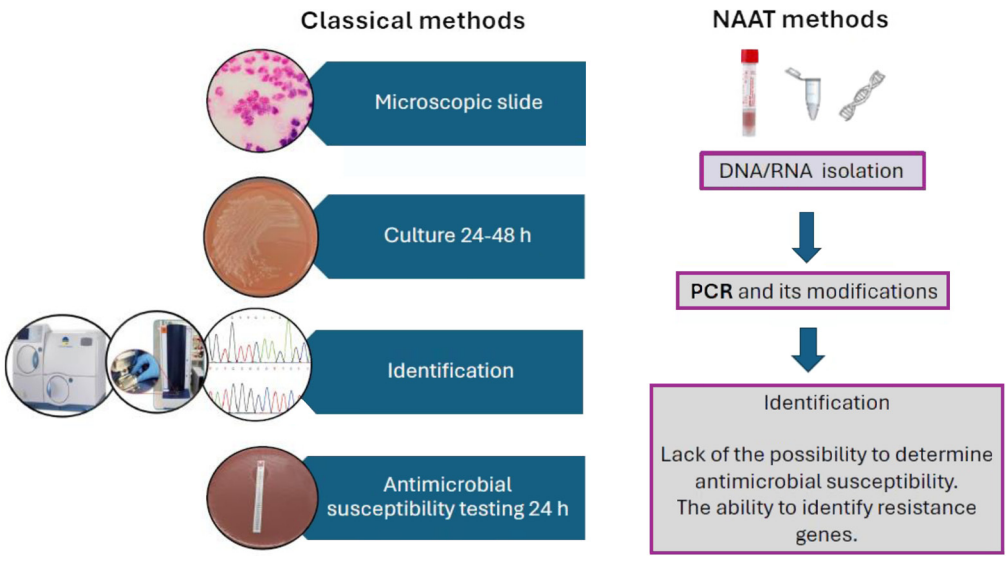

Fig. 1. Methods used in the diagnosis of *N. gonorrhoeae*.

2.1. Microscopy

Microscopically, *N. gonorrhoeae* is a Gram-negative diplococcus with a diameter of 0.6 to 1 μm, arranged in characteristic pairs resembling coffee beans. It does not have a capsule and does not produce spores.

Direct Gram-stained microscopic preparation should always be performed for purulent or mucopurulent urethral discharge in men, a symptom of gonococcal urethritis. Observing numerous polymorphonuclear leucocytes (PMNs) and Gram-negative gonococci within or closely associated with some PNMs in the microscopic image is diagnostically significant and allows us to identify gonococcal infection in clinically symptomatic men. In this case, if the preparation is appropriately performed and evaluated by an experienced microbiologist, the microscopic examination has high sensitivity (90–95%) and specificity (95–100%) (Janda and Gaydos 2007). However, direct microscopic examination has shown low sensitivity (< 55%) in diagnosing asymptomatic men. It is also the case for the diagnosis of cervicitis (sensitivity < 50%) and rectal inflammation (sensitivity < 40%). Hence, direct examination of Gram-stained preparation of smears from the vagina, cervical canal, pharynx, or rectum has no diagnostic significance (Bignell *et al.* 2013). Methylene blue (M.B.) stain may also establish the intracellular location of gonococci in leucocytes (PMNs) of male urethral exudate accompanying gonococcal urethritis (Workowski 2021).

2.2. Culture methods

N. gonorrhoeae is an aerobic bacterium with high nutritional requirements, is oxidase- and catalase-positive and oxidizes only glucose to acid. It requires an energy source of glucose, pyruvate or lactate and the presence of cysteine to grow on culture media. Because of changes in metabolic pathways, some isolates show special growth requirements for amino acids, purines, and pyrimidines (Ng and Martin 2005; Quillin *et al.* 2018).

The culture of gonococci requires special nutrient-rich growth media and selective media that inhibit the growth of other microorganisms on human mucous membranes. The pathogen grows at a temperature of 35–37°C in an atmosphere with up to 5–10% CO_2 and increased humidity. Gonococci outside the host are sensitive to environmental factors. They die within hours at temperatures above 40°C. The bacteria are sensitive to drying, light and low concentrations of disinfectants. Collected clinical materials should be inoculated into culture media as soon as possible. Culture media with added animal protein are used, primarily chocolate agar, modified Thayer-Martin (MTM) medium, Roiron medium, Martin-Lewis (ML) medium, GC-Lect agar and New York City (NYC) agar. MTM, ML, and GC-Lect media based on chocolate agar are supplemented with growth factors (e.g., IsoVitalex, PolyVitex, and Kellog supplement). NYC medium is a transparent medium based on peptone, corn starch with yeast extract, horse plasma and lyophilized horse erythrocytes. Roiron medium contains, among others, enzymatic casein hydrolysate, horse plasma and glucose. Antibiotics and antimycotics are added to selective media to inhibit the growth of commensal microorganisms present in clinical materials. Vancomycin and colimycin inhibit Gram-positive and Gram-negative microorganisms, respectively, trimethoprim *Proteus* species, while nystatin, amphotericin B or anisomycin have antifungal effects (Janda and Gaydos 2007).

For urogenital tract specimens, cultures for *N. gonorrhoeae* are usually conducted in parallel on Columbia

agar with the addition of 5% sheep blood, chocolate agar without any selection factors, and selective medium with the selection factors mentioned above. An example of a selective medium is chocolate agar with polyvitex with the addition of vancomycin, colimycin, amphotericin and trimethoprim (Chocolate + PVS + VCAT). Basal media without the addition of antibiotics are necessary to ensure the growth of those few *N. gonorrhoeae* strains that show sensitivity to antibiotics present in selective media. On chocolate agar, gonococci grow after a 24- to 48-hour culture as small, round, smooth colonies of greyish-beige colour or clear and shiny, 0.5 to 2 mm in diameter. Their morphology depends on the number of *in vitro* passages. Fine, elevated colonies are associated with the production of pili.

Further morphotypes appear during passages on solid media, variants of larger diameter, flat colonies, often with irregular edges. Under the influence of phase changes of Opa surface proteins, dull and iridescent variants appear. Due to the different types of colonies, cultures on chocolate agar can give the impression of a mixed culture (Janda and Gaydos 2007).

2.3. Species identification

After a 24–48 hour incubation, the characteristic small grey-beige or transparent colonies of *N. gonorrhoeae* grown on chocolate agar or selective medium require isolation to identify the species level and determine drug susceptibility. Identification by classical microbiological methods is based on the evaluation of colony morphology on chocolate medium, finding of Gram-negative diplococci in a Gram-stained slide, confirmation of the ability to produce oxidase, catalase, and the results of biochemical tests, considering carbohydrate degradation and enzymatic activity. The bacterium oxidizes glucose and does not metabolize maltose, sucrose, fructose or lactose. It shows proliiminopeptidase (PIP) activity, while it does not have β-galactosidase nor γ-glutamylaminopeptidase activity, unlike *N. lactamica* and *N. meningitidis*. The specific enzymatic activity is used to identify *N. gonorrhoeae* in manual enzyme chromogenic assays such as API NH – bioMerieux, Gonochek II – TCS Biosciences, *Neisseria* PET – BioConnections (Alexander *et al.* 2005; Meyer and Buder 2020). The automated system VITEK 2 (bioMerieux) also uses biochemical traits for species identification of *N. gonorrhoeae*. A modern identification method by which gonococci can be reliably identified is MALDI-TOF (*Matrix-Assisted Laser Desorption/Ionization – Time Of Flight*) mass spectrometry (Ilina *et al.* 2009; Cassagne *et al.* 2011; Carannante *et al.* 2015; van Belkum *et al.* 2013; Morrel *et al.* 2018), which analyses the protein profile, mainly of the microbe's ribosomal proteins. 16S rRNA gene sequencing can also be used for species identification of *N. gonorrhoeae*. A fragment of the *rplF* gene (413 pz), encoding the L6 50S ribosomal protein, is a suitable genetic target for differentiating this species (Bennett *et al.* 2014).

2.3.1. VITEK 2

VITEK 2 Compact (bioMerieux, France) is an automated system commonly used by clinical laboratories for routine microbiological diagnosis. Using disposable NH ID cards with 30 different substrates for biochemical and enzymatic reactions, identification of *N. gonorrhoeae* is possible. NH ID cards provide reliable identification results for six clinically relevant species of the *Neisseria* genus: *N. gonorrhoeae*, *N. meningitidis*, *N. cinerea*, *N. elongata*, *N. lactamica* and *N. sicca*. The panel of biochemical reactions assays the distribution of sugars and the activity of 7 peptidases: arginine, lysine, leucine, tyrosine, L-proline, phenylalanine and alanine-phenylalanine-proline. The assay requires multiplying the strain and making a suspension of bacteria with a density equivalent to 2.7–3.3 of the McFarland scale from fresh, preferably from an 18–24 hour culture on chocolate medium. The identification process takes about 6 hours. Results are generated through the system's database, with specific confidence levels and percentage probability ranges reflecting the compliance level with species-typical responses. Ranges ≥ 96%, 93–95%, 89–92%, and 85–88% correspond to species identification at excellent, very good, good, and acceptable levels. The "low distinction" results when 2 or 3 species show an identical numerical pattern. In such cases, the percentage probability of species identification is subjected to the manual selection of one of the options. Inconclusive results, when a given numerical pattern corresponds to more than three taxonomic groups or is atypical, require repetition after verifying the purity of the sample. Biochemical methods have been widely used for years in identifying *N. gonorrhoeae* (Alexander *et al.* 2005; Meyer *and* Buder 2020). It was important for microbiological diagnostics that in 2000, mutants with a deletion in the *pip* gene appeared. These strains did not express proline aminopeptidase (PIP), an enzyme the presence of which is a differentiating feature in biochemical tests. The spread of gonococci not producing PIP was reported in England, Denmark, Australia, and New Zealand after 2000. Such strains showed high genetic relationships (Unemo *et al.* 2007).

2.3.2. MALDI-TOF

*Matrix-Assisted Laser Desorption/Ionization – Time Of Flight (*MALDI-TOF) mass spectrometry for analyzing the protein profile, mainly of the microbial ribosomal proteins, is increasingly used in routine microbial

diagnostics (Ilina *et al.* 2009; Cassagne *et al.* 2011; van Belkum *et al.* 2013; Carannante *et al.* 2015; Morrel *et al.* 2018). This system uses laser energy to desorb biomolecules that co-crystallize with the matrix and ionize them. The matrix plays a key role in this process. Positively charged ions are accelerated in the electric field of the device's through-tube at a speed proportional to their mass and reach the detector at different time intervals. According to the *Time Of Flight* (TOF) measured in nanoseconds, the signal value obtained, converted to the ion's molecular mass ratio to its electric charge (m/z), creates a specific pattern of spectral peaks.

The value of the identification index can take point values from 0 to 3, which are logarithms of the spectra's consistency values. Scores above 2, consistent with "A" that excludes a mixed bacterial population, indicate high confidence in species identification. A score of 2,300 to 3,000 is a reliable identification of microorganism to the species level, a score of 2,000–2,299 is a reliable identification to the genus level and a highly probable species identification result, values of 1,700–1,999 indicate a probable identification result to the genus level, and below 1,699 indicate an unreliable identification result. The usefulness of mass spectrometry in the identification of *N. gonorrhoeae* has been described in papers by other authors (Schweitzer *et al.* 2016; Ilina *et al.* 2009; Carannante *et al.* 2015; Buchanan *et al.* 2016; Morrel *et al.* 2018). Buchanan *et al.* (2016) conducted a retrospective analysis of the identification results of 1,090 gonococci by two methods: manual, biochemical using API NH assays (bioMerieux) and spectrometric using the SepsiTyper® system (Bruker) or VITEK® MS (bioMerieux), estimated the positive predictive value for the MALDI TOF method at 99.3%. Morel *et al.* noted the need for cautious interpretation of gonococcal identification results for strains isolated from non-genital infections due to the presence of commensal species of the genus *Neisseria* (Morrel *et al.* 2018). Strains that do not produce PIP and the arylamidases TyrA and APPA and do not oxidase glucose were incorrectly identified by the VITEK 2 biochemical method but correctly by the MALDI TOF method (Bruker) (Plakhova *et al.* 2020; Nosov *et al.* 2022).

2.4. Molecular methods

According to the Centers for Disease Control and Prevention (CDC), NAAT methods are currently recommended for diagnosing and screening gonococcal infections among men and women (Table I). They have high sensitivity and specificity and the added advantage of simultaneously detecting other sexually transmitted diseases (*Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*). Examples of NAATs that detect *N. gonorrhoeae* approved by the Food and Drug Administration (FDA) for clinical use are shown in Table I (CDC 2014; Low and Unemo 2016). Recommendations for transporting protected swabs for gonorrhoea diagnosis by molecular methods are less restrictive compared to culture methods. Any delay in inoculation of materials into appropriate microbiological media can adversely affect the result of *N. gonorrhoeae* culture.

In contrast, this is not the case with NAAT methods. Longer sample storage time does not impair the method sensitivity. The very high sensitivity of molecular methods, associated with the ability to multiplicate

Table I
FDA-approved molecular diagnostic tests for the clinical diagnosis of gonorrhea, based on CDC Recommendations (2014)

NAAT test detected pathogens	Producer	Method	Molecular targets *N. gonorrhoeae*
Aptima NG assay *N. gonorrhoeae*	(Hologic/Gen-Probe, SanDiego, California)	TMA	Specific region of 16S rRNA, different than in the Aptima Combo 2 assay
Aptima Combo 2 assay *C. trachomatis*, *N. gonorrhoeae*	(Hologic/Gen-Probe, SanDiego, California)	TMA	Specific region of 16S rRNA
Abbott Real Time 2000 CT/NG *C. trachomatis*, *N. gonorrhoeae*	Abbott Molecular Inc. Des Plaines, Illinois	qPCR	48 bp sequence within the *Opa* gene
Cobas CT/NG *C. trachomatis*, *N. gonorrhoeae*	Roche Molecular Diagnostics, Branchburg, New Jersey	PCR	two sequences within the fragment DR 9A and DR 9B (DR – *direct repeat*)
BD ProbeTec ET CT/GC Ampliefied DNA assay *C. trachomatis*, *N. gonorrhoeae*	Becton Dickinson, Sparks, Maryland	SDA	Chromosomal pilin gene-inverting protein homolog
BD ProbeTec NG Q *N. gonorrhoeae*	Becton Dickinson, Sparks, Maryland	SDA	Chromosomal pilin gene-inverting protein homolog
Xpert CT/NG Assay *C. trachomatis*, *N. gonorrhoeaes*	Cepheid, Sunnyvale, California	qPCR	two specific chromosomal DNA sequence

TMA – *Transcription-mediated amplification*, SDA – *Strand displacement amplification*, qPCR – *Real-Time Polimerase chain reaction*

even single copies of target DNA or RNA, allows diagnostic use of samples with low bacterial loads, such as the initial urine stream or vaginal swabs. On the other hand, due to the high sensitivity, there is the possibility of false-positive results, such as in cases of cross-reactions with genetic material from other species of the genus *Neisseria* or patients just after treatment, due to the persistence of the pathogen's DNA in the body. With molecular techniques, it is essential to strictly control the course of laboratory procedures to ensure the reliability of test results.

Most importantly, all safeguards should be used to prevent contamination of the laboratory environment with pathogen amplicons, the presence of which can be a source of false positives. Control of the efficiency of isolation, amplification and detection process of the pathogen's DNA is ensured by using so-called positive controls, and each time, the inclusion of negative control, i.e. sterile water, verifies the possibility of contamination of, for example, reagents or the environment. To date, the development and good standardization of molecular methods have improved the diagnosis of gonococcal infections but have not enabled routine determination of drug susceptibility.

2.5. The importance of drug susceptibility assessment

Antimicrobial resistance (AMR) of *N. gonorrhoeae* has a multifactorial basis, both chromosomal and plasmid-mediated (Unemo *et al.* 2016; Unemo and Shafer 2014). The drug susceptibility profiles of gonococci and plasmid and chromosomal resistance prevalence vary by geographic region and change over time. The increasing antimicrobial resistance of the pathogen is a global problem and requires constant monitoring (Tapsall *et al.* 2009; Town *et al.* 2020). With the introduction of sulfonamides and then penicillin for the treatment of gonorrhoea in the 1930s, *N. gonorrhoeae* has acquired resistance to successive groups of antibiotics formerly used in therapeutic regimens: sulfonamides, penicillin, tetracyclines, fluoroquinolones (Ohnishi *et al.* 2011; Unemo and Shafer 2014; Grad *et al.* 2016). Third-generation cephalosporins, along with azithromycin or tetracycline, appeared in treatment recommendations, but the use of these antibiotics may soon prove less effective or even ineffective (Grad *et al.* 2016; Martin *et al.* 2016; Martin *et al.* 2019). Strains with a wild-type phenotype regarding drug susceptibility, which do not show resistance or reduced sensitivity to antibiotics, have become increasingly rare. Multidrug-resistant strains emerge worldwide, such as those with Multi Drug Resistance (MDR) or Extensively Drug-Resistant (XDR) phenotypes. *N. gonorrhoeae strains with the MDR phenotype* are defined as resistant or with reduced sensitivity to one of the antibiotics recommended for current empiric therapy (i.e., cefixime, ceftriaxone or azithromycin) with resistance to two other therapeutic groups (e.g., fluoroquinolones or tetracyclines). In contrast, strains with extensive drug resistance, XDR *N. gonorrhoeae,* are understood to be resistant or with reduced sensitivity to both recommended drugs, with concurrent resistance to two other antibiotic groups (Tapsall *et al.* 2009). In 2011, the first case of treatment failure of gonorrhoeal pharyngitis caused by a strain with the XDR phenotype was described in Japan (Ohnishi *et al.* 2011). Europe's first ceftriaxone-resistant gonococcal strains were isolated in France in 2010 (Unemo *et al.* 2012) and Spain in 2011 (Carnicer-Pont *et al.* 2012). They showed phenotypic and genotypic similarity (NG-MAST 1407, MLST ST1901). In 2018, XDR strains emerged in the U.K. and Australia, resistant to ceftriaxone and with high azithromycin resistance (≥ 256 mg/l) (Eyre *et al.* 2017; Whiley *et al.* 2018; Jennison *et al.* 2019). Due to the inreasing resistance of gonococci, the WHO presented the "Global Action Plan to Control the Spread and Prevent Antibiotic Resistance of *N. gonorrhoeae*" (WHO 2012), and in 2017, *N. gonorrhoeae* was included in the list of bacterial pathogens for which there is a particular need for new drugs (WHO 2021). The drug susceptibility of gonococci is monitored mainly through widely conducted national and international surveillance programs: WHO-Global GASP (*Gonococcal Antimicrobial Surveillance Programme*) (Unemo *et al.* 2019), Euro GASP (Cole *et al.* 2013), US-GISP (Gonococcal *Isolate Surveillance Project*) (Grad *et al.* 2016), Canadian GASP (Martin *et al.* 2019), Australian AGSP (*Australian Gonococcal Surveillance Programme*) (Lahra *et al.* 2015), UK GRASP (*Gonococcal Resistance to Antimicrobials Surveillance Programme*) (Unemo *et al.* 2020).

2.5.1. Antimicrobial susceptibility determination methods

Drug susceptibility testing of *N. gonorrhoeae* isolates, according to current recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as well as Clinical and Laboratory Standards Institute (CLSI), requires the determination of values of minimum inhibitory concentrations – MIC (mg/l). Two determination methods are recommended: the WHO's preferred method of serial antibiotic dilutions in agar (Unemo *et al.* 2013) or gradient strips. The antibiotic gradient strip method combines the diffusion method with the serial dilution method, requiring the use of media recommended by the strip manufacturer (Unemo *et al.* 2013; Liu *et al.* 2015; Papp *et al.* 2018). The range of MIC limits applicable in U.S. and European recommendations differs slightly (Tab. II). A reference strain of *N. gonorrhoeae* ATCC 49226 is recommended for quality control of

Table II.
MIC breakpoint range for *N. gonorrhoeae* according to EUCAST recommendations v 12.0 2023.01.01 and CLSI 2020

Antibiotic (abbreviation)	MIC limit values (mg/l)			
	EUCAST *v* 12.0 2023		CLSI 2020	
	≤ W	> O	≤ W	> O
Penicillin (P)	0,06	1,0	0,06	2,0
Cefixime (CFM)	0,125	0,125	0,25	–
Ceftriaxone (CRO)	0,125	0,125	0,25	–
Cefotaxime (CTX)	0,125	0,125	0,5	–
Azithromycin (AZM)*	1,0 (ECOFF)		1,0	–
Ciprofloxacin (CIP)	0,03	0,06	0,06	1,0
Tetracycline (T)	0,5	0,5	0,25	2,0
Spectinomycin (SPT)	64,0	64,0	32,0	128,0

Azitromycin* – According to EUCAST v.12.0 recommendations for azithromycin, it is possible to provide MIC values (mg/l) without a sensitive/resistant interpretation. The ECOFF value (epidemiological cut-off value) is 1.0 mg/l, in the case of MIC value ≤ 1 mg/l, azithromycin can be used in combination with another active antibiotic. MIC value > 1 mg/l indicates the presence of acquired resistance mechanisms.

the procedure. Determination of MIC values for penicillin-resistant strains, according to EUCAST recommendations, should be extended by testing the ability to produce penicillinase using the cephinase assay. Nitrocefin (a chromogenic cephalosporin) paper discs allow the detection of penicillinase activity in live *N. gonorrhoeae* isolates, thanks to the disc's colour change from yellow to red. The colour reaction results from the hydrolysis of the amide bond in the β-lactam ring of cephalosporin by bacterial β-lactamase.

Routinely used in microbiological diagnostics to determine drug susceptibility, the disk-diffusion method is not recommended for gonococci but can be used due to limited resources. Interpretation of drug susceptibility results based on zones of inhibition around antibiotic discs is presented by CLSI (https://clsi.org) (Singh *et al*. 2012, Mal *et al*. 2016).

2.6. Genotypic analysis methods

Over the past 30 years, several methods have been developed to analyze the genetic diversity of *N. gonorrhoeae* strains. They aim to identify polymorphisms at a single locus, several loci, or even the entire genome. The variability of gonococcal strains was assessed based on patterns of electrophoretically separated strands of amplified DNA, which allowed the demonstration of, among others, different plasmid profiles (PPA – *Plasmid Profile Analysis*), restriction fragment length polymorphism (RFLP/PFGE pulse electrophoresis method), ribotyping and Opa protein typing (Unemo and Dillon 2011). The use of sequencing techniques allowed the differentiation of gonococci initially for the *porB* gene (Bash *et al*. 2005; Liao *et al*. 2009), and then, after expanding the scope, gave the possibility to type several loci. Nowadays the most popular molecular typing schemes are MLST (Maiden *et al.* 1998), NG-MAST (Martin *et al.* 2004), NG-STAR (Demczuk *et al.* 2017). The whole genome sequencing method (WGS) is now playing an increasingly important role in epidemiological and phylogenetic studies, as well as in monitoring antibiotic resistance of gonococci. This method certainly facilitates surveillance of circulating *N. gonorrhoeae* strains in an epidemiological context (Eyre *et al*. 2018; Harris *et al.* 2018; Sánchez-Busó *et al*. 2019; Sánchez-Busó *et al.* 2022). The Multi-Locus Sequence Typing (MLST) method is based on the sequencing of 7 housekeeping genes: *abcZ* (putative ABC transporter), adenylate kinase *(adk), aroE* (shikimate dehydrogenase), *fumC* (fumarate hydratase), *gdh* (glucose-6-phosphate dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit), *pgm* (phosphoglucomutase) (Maiden 2008). Sequence type relatedness analysis allowed us to define MLST CC clonal complexes grouping related clonal lines. With the same scheme being used to type other species in the human genus *Neisseriaceae*, the pathogenic *N. meningitidis* and the commensal *N. lactamica,* the MLST database is a valuable source of data for genetic analyses within the genus and broad studies in an evolutionary context over the long term (Maiden 2008; Harrison *et al.* 2020). As of June 2022, the PubMLST database contained 16700 ST types for the genus *Neisseria*. The PubMLST website is maintained by the Department of Zoology at Oxford University (Jolley *et al*. 2018). *N.gonorrhoeae* Multi-Antigen Sequence Typing (NG-MAST) method is based on the sequencing of two highly variable loci within the *porB* (*490 bp*) and *tbpB* (390 bp) genes, which encode two *N. gonorrhoeae* membrane proteins: porin B (PorB) and transferrin binding protein (tbpB), respectively. The combination of both loci allows the determination of the Sequence Type (ST). NG-MAST STs are grouped into genogroups for phylogenetic and epidemiological analysis (Chisholm *et al.* 2013). The NG-MAST method is widely used worldwide. The website https://pubmlst.org contains the current database for typing with this method. As of May 2023, 12735 *porB* and 3147 *tbpB* alleles and 21490 ST profiles of *N. gonorrhoeae* have been published on the site (Jolley *et al.* 2018). *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR) is the typing scheme that considers seven chromosomal determinants of *N. gonorrhoeae* resistance to β-lactam antibiotics, macrolides, fluoroquinolones and tetracyclines. The method requires sequencing of the following loci: *penA, mtrR, porB, ponA, gyrA, parC* and *23S rRNA* and is consistent with classical Sanger sequencing and WGS (Demczuk *et al.* 2017). The unique sequence of

each gene is assigned an allele number, while the combination of alleles of seven genes allows to determine the sequence type of NG-STAR. Sequences can be analyzed and compared through an online application in a publicly available database (https://ngstar.canada.ca/) maintained by the Public Health Agency of Canada and the National Microbiology Laboratory. As of May 2023, 5259 ST types of *N. gonorrhoeae* have been published on the NG-STAR database website. NG-STAR typing allows analysis of strain relatedness, tracking of clonal lineage development and monitoring of antibiotic resistance (Golparian *et al.* 2021).

2.7. Rapid diagnostic tests

Today's challenge is to develop a quicker, more straightforward, and more sensitive diagnostic method to detect *N. gonorrhoeae* infection (Bignell *et al.* 2013; Li *et al.* 2019). Both standard methods for identifying *N. gonorrhoeae* – NAAT or culturing of the pathogen prove expensive and require specialized laboratory equipment, making its use in low- and middle-income countries much limited or impossible.

In 2004, the WHO drew attention to the urgent need for rapid diagnostic tests for bacterial sexually transmitted diseases. The ASSURED criteria were established to determine the characteristics that such a diagnostic test should have. First and foremost, it is to be affordable, sensitive and specific, avoiding false results. In addition, it is to be user-friendly, i.e., simple to perform, with a minimum number of steps and requiring no advanced training. Moreover, the test must be fast and durable to survive various transportation conditions. It should also require no additional equipment (Land *et al.* 2019).

Rapid gonococcal diagnostic tests are emerging on the market. Such tests allow bypassing the multi-step and lengthy identification of the pathogen. Most often, they consist of a carrier such as a cassette, frequently containing all the reagents necessary for analysis, in which the collected sample is placed directly and inserted into the appropriate analyzer. These tests allow rapid identification of the pathogen for up to 30 minutes. However, they have limitations. First, not every test will prove suitable for every type of sample.

One rapid diagnostic test is Xpert CT/NG, which uses the GeneXpert platform (Cepheid). It is a real-time *in vitro* PCR performed directly from the collected sample. The FDA approves the test for use in urine, vaginal, cervical, rectal, and pharynx samples. It shows a sensitivity and specificity between 86–99% (Herbst De Cortina *et al.* 2016; Doernberg *et al.* 2019). Another rapid test is binx health io CT/NG (binx health, INC), a PCR-based qualitative test consisting of a stationary device and easy-to-use coffers. The waiting time for the result is about 30 minutes. As of 2019, the FDA has authorized the use of this test in vaginal swab samples. In vaginal samples, its high sensitivity and specificity of 96–97.7% was stated (Van Der Pol and Gaydos 2021; Gaydos *et al.* 2013). The Truelab Preal Time micro PCR system (Molbio Diagnostics Pvt Ltd) is a rapid semi-quantitative PCR-based test. The entire process involves sample collection and an automated extraction system. Then, the nucleic acid is transferred to an analyzer chip using a fluorophore-capturing optical sensor for determination. Analysis results are available after about an hour. The test is not FDA-certified (Nair *et al.* 2016). The STI Array test (Randox Biosciences) requires the Vivalytic Analyzer (Bosch Healthcare Solutions), a device that enables quantitative PCR reaction, to perform the assay. It comes with a coffer containing all the necessary reagents for the analysis (Adamson *et al.* 2020). A different type of assay is loop-mediated isothermal amplification (LAMP). It is a reliable, low-cost, sensitive, rapid technique for amplifying nucleic acids. It has been widely used to identify pathogens, including SARS-CoV-2, *Mycobacterium tuberculosis* and *Brucella* (Shete *et al.* 2019). DNA polymerase in the LAMP method can effectively amplify target genes at 58–69°C (Notomi *et al.* 2000). The *N. gonorrhoeae* – LAMP-PNB assay combines isothermal amplification with a polymer nanoparticle-based biosensor. The entire identification process takes about 60 minutes (Wong *et al.* 2018; Chen *et al.* 2021). The documented development of rapid diagnostic methods for *N. gonorrhoeae* infection is insufficient. There is still a need for specific, sensitive assays that are simple and inexpensive.

2.8. Therapy of uncomplicated gonorrhoea

According to current European recommendations for treating uncomplicated gonorrhoea in adults, ceftriaxone is administered intramuscularly in a single dose of 1 g (Unemo *et al.* 2021). In CDC recommendations for anogenital and pharyngeal infections, the dose of ceftriaxone depends on the patient's body weight (500 mg for individuals < 150 kg and 1 g for individuals > 150 kg). If chlamydial infection has not been excluded, chlamydia should be treated with doxycycline 100 mg orally twice daily for seven days, except for pregnant patients for whom a single dose of azithromycin 1 g is recommended. An alternative treatment for uncomplicated gonococcal infections of the urethra or anus is intramuscular gentamicin in a single dose of 240 mg with azithromycin (2 g) or a single oral dose of cefixime (800 mg). In cases of gonococcal throat infections with an allergy to ceftriaxone, CDC recommends consultation with an infectious disease specialist to choose alternative therapy. The treatment regimen is the same for HIV-positive patients. Abstaining from sexual activity is necessary during treatment and for

seven days afterwards. According to recommendations, all patients diagnosed with gonorrhoea should also be tested for other STIs, including chlamydia, syphilis, and HIV. Recent sex partners of diagnosed persons should be referred for testing and presumptive treatment (Workowski *et al*. 2021).

3. Conclusion

Gonococcal infection, a sexually transmitted disease caused by *N. gonorrhoeae*, is a public health challenge worldwide. Initially, the primary method of identifying this pathogen was using microscopy. Microscopy is a sensitive enough diagnostic method only in men with clinical urethritis symptoms. Unfortunately, in the case of swabs taken from the vagina, cervical canal, pharyngeal or rectum, the microscopic slide has no diagnostic significance. Currently, culture and phenotypic identification methods have been dominated by NAAT methods, which allow the detection and identification of the species directly in the clinical specimen. However, today's molecular methods do not allow for the complete determination of drug susceptibility. This makes empirical therapy predominate over targeted therapy in the treatment of gonorrhoea. The reason for deviating from classical culture methods in diagnosing gonorrhoea is the biology of the pathogen, its high growth requirements, and the sensitivity of gonococci to the conditions of sample transport and incubation of clinical materials.

The increasing antimicrobial resistance of *N. gonorrhoeae* is a global problem and requires constant monitoring. Although determining MIC values by serial dilutions or using antibiotic-gradient strips is costly and time-consuming, taking a minimum of 3 days, it is still irreplaceable despite attempts to construct an assay detecting gonococcal resistance at the molecular level. Difficulties in developing a suitable molecular method for antimicrobial susceptibility determination of *N. gonorrhoeae* relate to the nature of this pathogen, its variability, and precisely the multifactorial, complex genetic basis of resistance. An additional problem is that clinical specimens such as swabs taken from the genitourinary tract, pharynx or rectum are microbe-rich material, which creates the possibility of cross-reactions in molecular tests.

A steadily increasing number of gonococcal infections are being reported globally, also in low- and middle-income regions. The problem in poor areas is often the lack of proper diagnosis of gonorrhoea due to the shortage of medical equipment and even electricity. Hence, considering the difficulties of developing countries, a specific, sensitive, rapid, and inexpensive method of gonorrhoea diagnosis is highly needed.

Gonorrhoea is undoubtedly a severe health risk, especially for women, due to its often asymptomatic course and the possibility of upper genital tract complications such as PID, which can result in infertility. Gonorrhoea infection prevalence in infertile populations was several folds higher than that for the general population globally, according to the meta-analysis by Chemaitelly et al. (2021). Prophylaxis using immunization is unavailable, as attempts to develop an effective vaccine have failed. Screening is not routinely performed in the population. Because of these facts, the epidemiology of *N. gonorrhoeae* infection and drug resistance should be monitored continuously, as infection surveillance and antibiotic therapy are the only means of controlling gonorrhoea in the current situation. It is necessary to use the approved, sensitive diagnostic methods and rational treatment of gonococcal infections based on recommendations and reliable knowledge of medical microbiology and pharmacology.

ORCID
Joanna Białecka https://orcid.org/0000-0002-0820-3174
Katarzyna Rak https://orcid.org/0000-0003-6434-619X
Aneta Kiecka https://orcid.org/0000-0002-3818-8972

Conflict of interest

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PATHOGENIC FEATURES OF *PORPHYROMONAS GINGIVALIS* INFLUENCE PROGRESSION OF RHEUMATOID ARTHRITIS

Wiktoria Krakowiak[1]*, Halina Lisowska[1], Wieslaw Roman Kaca[2]*

[1] Department of Medical Biology, Institute of Biology, Jan Kochanowski University, Kielce, Poland
[2] Department of Microbiology, Institute of Biology, Jan Kochanowski University, Kielce, Poland

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Abstract: Autoimmune diseases, such as rheumatoid arthritis (RA), are examples of yet not entirely understood etiology. They are linked to immune system dysfunction, which becomes immunologically overactive, damaging the body's tissues and organs. At least three major factors underlie the development of autoimmune disorders: environmental factors, including the oral and intestinal microbiomes, genetic predisposition, and aberrant autoimmune response. The dysbiosis of the oral microbiota, in particular, exerts a significant effect on RA, clinically manifested by damage of the joints. RA is significantly associated with periodontitis, which is caused by an increased abundance of *Porphyromonas gingivalis* in the subgingival niche, which disturbs the homeostasis of the oral microbial community. *P. gingivalis* is considered to contribute to the development and progression of RA. Although this bacterium may escape detection by the host immune system, it still induces an immune imbalance. RA and periodontitis also share similar pathological and clinical features. The progression of both chronic periodontitis and RA is linked to the dysregulation of the immune system and the damage caused by the immune response. Previous detailed studies have indicated that a specific enzyme of *P. gingivalis*, peptidyl-arginine deiminase, which catalyzes the citrullination of proteins, may trigger the autoimmune response resulting in the development of RA.

Keywords: autoimmune disease, citrullination process, dysbiosis, PPAD enzyme, rheumatoid arthritis

1. Introduction

The posttranslational modification of proteins is an important determinant of protein function. One type of posttranslational modification recently attracting attention is citrullination of peptidyl-arginine residues in protein and peptides catalyzed in mammals by five peptidyl arginine deiminases (PADs) isotypes play an essential role in many physiological processes (Tilvawala *et al*. 2018). Unfortunately, excessive citrullination associated with inflammatory responses can lead to generation of autoantibodies recognizing citrullinated epitopes. The *Porphyromonas gingivalis* enzyme peptidyl-arginine deiminase (PPAD) is also responsible for citrullination (Pyrc *et al.* 2012), and citrullination by enzymes of both human and bacterial origin promotes autoimmune diseases (Koziel *et al*. 2014; Ciaston *et al.* 2022; Krutyhołowa *et al.* 2022; Wielento *et al*. 2022). The molecular mechanism of arginine modification and its role in rheumatoid arthritis (RA) are the subjects of our presentation.

2. Characteristics of the oral microbiome

The oral microbiome is a highly diverse and dynamic environment, and bacteria in the buccal cavity participate in the nutrient metabolism of the host. The first bacterial colonies appear immediately after birth, originating from the mother (Strużycka 2014). The biological characteristics of the oral cavity determine which microorganisms will successfully colonize and predominate the buccal microbiome. Each area of the mouth supports an individual microbiota com-

* Corresponding Author: W. Krakowiak, W.R. Kaca, Department of Medical Biology, Institute of Biology, Jan Kochanowski University, Kielce, Polska, wiktoria.krakowiak9@gmail.com wieslaw.kaca@ujk.edu.pl

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munity with specific characteristics. Moreover, there is a dynamic connection between the activity and composition of the oral microflora and the host environment (Marsh *et al*. 2016). Initially, the oral microbiome of the neonate primarily contains *Streptococcus* bacteria, such as *S. salivarius*, *S. mitis*, and *S. oralis* (Strużycka 2014). These species are called the "pioneer community". Other populations colonize the oral cavity after the metabolic activity of the pioneer community modifies it. After tooth eruption, the diversity of the bacterial species in the oral cavity increases, predominantly around the gingival margins of the newly erupted teeth (Marsh *et al*. 2016). The oral microbiome becomes more diverse with time, with mainly anaerobic and Gram-negative bacteria, including *Fusobacterium nucleatum*, *Prevotella melaninogenica*, and *Veilonella*. The most stable oral microbiome is generally achieved in the young adult. It is represented by the genera *Streptococcus*, *Veilonella*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Treponema*, *Neisseria*, *Eubacteria*, *Haemophilus*, *Propionibacterium*, *Lactobacillus*, and *Leptotrichia* (Strużycka 2014).

Both autogenic and allogenic succession are involved in its development to achieve such a diverse microbial community. In autogenic succession, microbial factors are responsible for microbiome development, whereas in allogenic succession, nonmicrobial factors influence the community (Marsh *et al*. 2016). The developmental process of the climax community in the human oral cavity is quite complex. Over time, the microbiota remains relatively stable. Still, unexpected and substantial disturbances in the local environment can cause severe disorders of the microbial community, which may lead to several diseases. According to an oral microbiome database (www.homd.org), 688 species of bacteria have been identified in the oral microbiome. Moreover, up to 50% of these species are uncultivated. Factors that significantly influence the oral microbiome include temperature, pH, oxidoreductive potential, and saliva's nutrients, enzymes, and metabolites (Nelson 2011, Malinowska *et al.* 2017). During the lifetime of an individual, the oral microbiota changes in response to modifications to the oral habitat, the loss or eruption of teeth, lifestyle changes, or changes in the immune system (Marsh *et al.* 2016). 16S ribosomal RNA (rRNA) sequencing has shown that the oral microbiome was altered, for example, in patients with stomach cancer (Hu *et al*. 2015).

Bacteria mainly exist in the mouth in the form of biofilms. Most oral biofilms are composed of Gram-positive and Gram-negative bacteria, including aerotolerant and anaerobic bacteria (Salyers and Whitt 2003). An oral biofilm is very complex, and its structure is determined by its location in the mouth (e.g., subgingival, supragingival). Gram-positive bacteria are present on most oral surfaces, with *Streptococcus* and *Actinomyces* dominant. These species are located at both healthy and diseased sites, and the only difference between them is their prevalence in the oral microbiome of the host. Gram-negative bacteria vary greatly and include obligative, facultatively anaerobic, or even microaerophilic species (Marsh *et al*. 2016). Some bacterial species are responsible for plaque formation on the surfaces of teeth. The prevalence and diversity of different species change over time, and new species, such as the spirochete *Treponema denticola*, are now found with *Veillonella*, *Prevotella*, or *Propionibacterium* (Salyers and Whitt 2003) are incorporated into oral biofilms. Gram-positive bacteria, such as *Streptococcus*, are dominant in the supragingival regions, whereas Gram-negative bacteria, such as *P. gingivalis*, are more common in the subgingival space (Chałas *et al*. 2015), He and Shi 2009).

P. gingivalis is one of the main pathogens responsible for periodontal disease (PD). Periodontitis has not been linked directly to the appearance of certain types of bacteria. Still, it is initiated by a dysregulated immune response, which changes the proportions of various bacterial species in the subgingival dental plaque. This leads to the mixed activities of different bacteria, predominantly anaerobic and proteolytic Gram-negative bacteria, causing an inflammatory response in the deeper periodontal sockets, in which species such as *Tannerella forsythia*, *P. gingivalis*, and *T. denticola* are found (Marsh *et al*. 2016). Meyle and his colleague have provided a list of critical changes that occur during the development of PD. In general, the host provides all the nutrients to the oral microbiota in the gingival crevicular fluid, and the release of various proteins by microbes can trigger the host's immune response. The growth of species such as *Fusobacterium nucleatum* is promoted, influencing its environment via its "quorum sensing" ability. This elicits a stronger response from the host immune system, causing gingival inflammation and stimulating the proliferation of *P. gingivalis*. In susceptible hosts, the dysbiosis of the oral microbiota can trigger an excessive immune response, with the subsequent overproduction of reactive oxygen species, cytokines, and matrix metalloproteinases, which cause severe tissue damage. These are the first steps in the development of PD. The disease's further progression involves angiogenesis, which, rather than healing the damaged tissue, leads to chronic inflammation (Meyle and Chapple 2015). The detailed mechanism underlying the progression of periodontitis is described in Meyle and Chapple article (2015), and its mechanism is highly complex. Periodontal disease irreversibly destroys the tissue surrounding the teeth, with the simultaneous loss of attachment between the bone and the teeth. Why PD develops predominantly in older adults is still unclear. Like increased levels of *P. gingivalis* in the oral micro-

biota, PD can precipitate autoimmune disorders, such as RA (Koziel *et al*. 2014).

Periodontal disease is the chronic inflammation of the tissues surrounding the teeth, and *P. gingivalis* is one of the major pathogens recorded in periodontitis. The colonization of the teeth by pathogenic bacteria, such as *T. forsythia*, *T. denticola*, and *P. gingivalis*, causes the subsequent development of the pathogenic dental plaque. Therefore, these species are called the "red complex" because they are strongly associated with severe periodontitis and are thought to initiate the disease. They have a broad array of virulence factors, which may influence systemic severe diseases, such as the autoimmune disorders that develop in patients suffering from periodontitis (Berthelot and Le Goff 2010; Dissick *et al*. 2010). Recent studies have suggested that *P. gingivalis* is the keystone pathogen of the red complex, which may cause an imbalance in the microbiota and promote dysbiosis. However, after the disruption of microbial homeostasis, the other two members of the red complex, *T. denticola* and *T. forsythia,* accelerate the progression of the disease in concert with other species because periodontitis is a polymicrobial disease (Lamont *et al*. 2018; Silva and Cascales 2021). Four different groups of bacteria are involved in the etiology of PD, as well as the red complex (Marsh *et al*. 2016). Therefore, the pathogenesis of periodontitis is highly complex, involving oral bacteria and environmental factors, the host's genetic predisposition, and the host's lifestyle. The initiation of PD is still under investigation.

3. Selected pathogenic features of *P. gingivalis*

P. gingivalis is known as the "keystone pathogen" that disturbs the homeostatic system of the human host through its virulence factors, including lipopolysaccharides (LPSs), hemagglutinins, and fimbriae, which allow *P. gingivalis* to colonize the periodontal pockets. One of the most critical factors leading to PD is the production of extracellular cysteine proteases, such as gingipains. *P. gingivalis* uses the immune response of its human host for its benefit, i.e. through the activation of complementary pathways. The gingipains of *P. gingivalis* affect the host proinflammatory signaling pathways by activating proteinase-activated receptor 2 (PAR2) in human neutrophils. This induces connective tissue damage, including the resorption of the alveolar bone (Wegner *et al*. 2010; Koziel *et al*. 2014). The primary role of gingipains is the degradation of host proteins, including cytokines and chemokines, causing the deterioration of the host's immune response and contributing to the transition to dysbiosis. Gingipains are transported through bacterial cell membrane via the general secretory (Sec) pathway. They are then recruited and transported by the type IX secretion system (T9SS) throughout the cell and outer membrane. Gingipains are considered the major virulence factors of *P. gingivalis* (Silva and Cascales 2021).

P. gingivalis differs from other periodontal microbes in several unique features, including its ability to synthesize peptidyl-arginine deiminase (PPAD). The enzymatic properties of PPAD differ from those of human deiminases in that its activity is optimal at a higher pH and does not require calcium ions. The primary function of PPAD is the citrullination of the C-terminal arginine residues of human proteins (Pyrc *et al*. 2012). PPAD occurs on the bacterial surface with arginine-specific gingipains (cysteine proteases), which cleaves host proteins, exposing C-terminal Arg residues that are then citrullinated by PPAD. The citrullination of surface proteins generally depends on the activity of gingipain proteases. The degradation of α-enolases and fibrinogen by *P. gingivalis* gingipains generates PPAD-citrullinated peptides. Moreover, the deimination of the C-terminal arginine in epidermal growth factor (EGF) by PPAD can disturb the biological activity of cytokines (Koziel *et al*. 2014, Olsen *et al*. 2018).

As the crucial virulence factors of *P. gingivalis*, gingipains are necessary to assimilate nutrients, such as heme, a growth-limiting agent for bacterial cells. Among pathogenic bacteria, the accumulation of cell-surface heme is unique to *P. gingivalis* when cultured on a blood-containing medium (Silva and Cascales (2021)). The *hus, hmu* and *iht* operons encode several proteins, including HmuY, which is responsible for transporting heme molecules through the bacterial membrane of *P. gingivalis* (Fig. 1). Proteins that bind heme molecules advantage *P. gingivalis* during the initial stages of infection. These proteins enhance the pathogenic capacity of the bacterium, leading to dental periodontitis and the subsequent development of RA (Sato *et al*. 2013; Śmiga *et al*. 2020).

The pathogenicity of *P. gingivalis* is associated with its low abundance and reduced inflammatory potential. One possible explanation for its association with pathogenicity is the structure of lipid A of LPS, which has only one phosphate residue of 4-acyl lipid A moieties, which confers low proinflammatory potential (Olsen and Singhrao 2018). The inhibition of host interleukin 8 (IL8) in the presence of LPS type A and local chemokine paralysis facilitates the colonization of the host mouth tissue by *P. gingivalis* (Hajishengallis and Lamont 2012).

Apart from the virulence factors mentioned above, *P. gingivalis* also produces capsular polysaccharides, fimbriae, and outer-membrane vesicles to avoid the immune system of its host. After it is established within the cell, *P. gingivalis* produces an ATP-hydrolase that contributes to its survival by inactivating the host's ATP-dependent apoptosis pathway (Silva and Cascales 2021).

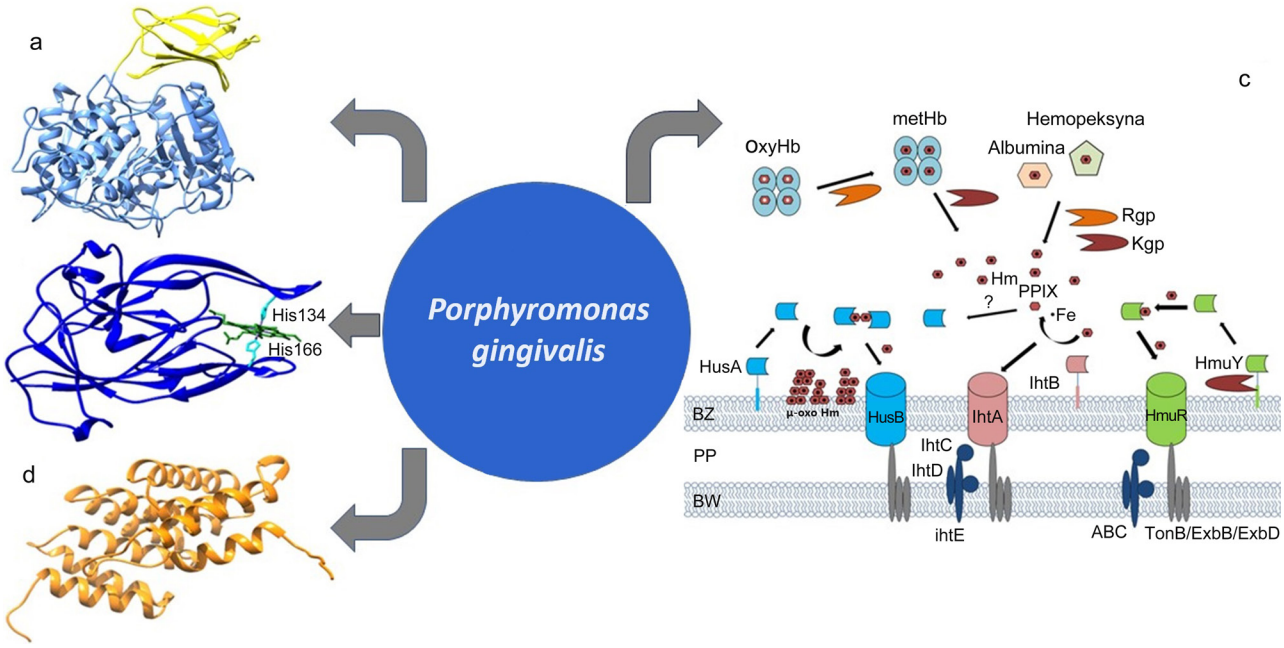

Fig. 1. Virulence factors of *Porphyromonas gingivalis*.

(a) Scheme of the catalytic domain of gingipain; (b) structure of HmuY protein, (c) operons in the bacterial membrane and its heme-binding mechanism, and (d) structure of HusA protein (modified from Śmiga *et al. 2020,* "Virulence mechanisms used in the pathogenesis of periodontal diseases caused by *Porphyromonas gingivalis*").

PPAD localizes to different sites: as a component of the outer membrane, anchored to LPS-A; on outer-membrane vesicles (OMVs); and secreted as a 47-kDa soluble form (type II). It has been reported that 93 clinical *P. gingivalis* isolates produced OMVs carrying 75–85-kDa type I PPAD modified with LPS-A. It has been suggested that replacing glutamine with lysine at position 373 of PPAD is crucial for intracellular sorting. The delivery of PPAD type I to phagocytic cells by OMVs results in the presentation of citrullinated peptides and the production of anti-citrullinated-protein antibodies (ACPAs). The role of soluble PPAD type II seems to be less critical (Gabarrini *et al.* 2018, Stobernack *et al.* 2018).

4. Citrullination of amino-acid residues by *Porphyromonas* PPAD

Citrullination is a complex posttranslational modification that occurs in higher organisms and leads to the deimination of arginine in proteins and peptides in both physiological processes and pathological diseases, including Alzheimer's disease, RA, and multiple sclerosis. This process is catalyzed by peptidyl arginine deiminases (PADs) found in vertebrates. As mentioned above, *P. gingivalis* also secretes PAD, designated PPAD (*Porphyromonas* peptidyl arginine deiminase), which differs from the eukaryotic enzyme. PPAD consists of a planar, cylindrical catalytic domain with a quintuple α/β-propeller architecture and a C-terminal immunoglobulin-like domain (Olsen *et al.* 2018). The reaction side of PPAD is on one of the cylinder's bases, which allows it to accommodate arginine from peptide substrates after rearranging the so-called "Michaelis loop, " which closes the cleft. The close relationship between the guanidinium and carboxylate groups of the substrates explains the activity of PPAD on the arginine at the C-terminus, although not in the case of peptides. The entire catalytic process is based on the cysteine (C)-histidine (H)-asparagine (R) triad and is similar to those of human PAD1-PAD4, with a guanidino group used to modify enzymes (Goulas *et al.* 2015).

L-Citrulline is a nonprotein amino-acid derivative and one of the animal urea cycle intermediates. Citrullination causes the transition of free arginine into citrulline through its deamination, which involves the replacement of the guanidino group with a ureido group. This causes the removal of the positively charged arginine side chain with the liberation of ammonia. This reaction increases the functional and structural diversity of the proteome. Citrullination is essential in the immune response because PADs are involved in autophagy, apoptosis, and NETosis. In some specific genetic backgrounds, citrullinated proteins behave as autoantigens, inducing antibodies against citrullinated proteins, leading to autoimmune responses and prolonged inflammation, which make them hallmarks of both PD and RA (Koziel *et al.* 2014, Goulas *et al.* 2015). A comparison of human-origin PAD and bac-

terial PPAD is described in detail in several references (Jonsson *et al*. 2020; Ciesielski *et al*. 2022; Matuz-Flores *et al*. 2022; Curran *et al*. 2023).

PPAD includes a catalytic triad (C^{351}-H^{236}-N^{297}) and a seven-stage citrullination process. The Michaelis loop containing tyrosine (Y^{233}) is in the substrate-free state and has an open conformation, which accommodates the reaction side peptides with C-terminal arginine. Electrostatic interactions between the guanidinium group and the side chain stabilize arginine. These groups are arranged in an extended conformation and then oriented appropriately for the catalysis reaction (de Diego *et al*. 2014). Arginine at residue 152 (R^{152}) and R^{154} bind to the C-terminal carboxylate of arginine and the carbonyl of the previous peptide bond. The formation of the Michaelis complex also involves a significant regrouping of the Michaelis loop, which blocks the active site. This binds the C-terminal carboxylate to the substrate (de Diego *et al*. 2013). Further changes in the structure indicate that the H^{236} side chain is rotated here. As a result of this process, the plane of the guanidinium group is compressed between H^{236}Nδ1, C^{351}Sγ, and H^{236}Nε2. This geometry is a determinant of the identification of H^{236} as the general acid/base of the mechanism and the Nη1 guanidinium atom as the nitrogen atom, leaving the ammonia product (Shirai *et al*. 2006).

Similarly, C^{351}Sγ hydrogen bonds to N^{297}Oδ1, which probably extends the nucleophilicity of the catalyst of the sulfur residues. At the beginning of the reaction, C^{351}Sγ executes a nucleophilic attack on the flat like the sp2 conformation Cζ atom of the guanidine substrate. This process gives rise to the first tetrahedral reaction intermediate and yields a Cζ atom with an sp3 conformation. Overall, H^{236}, which first reacts as a general base, strips a proton from Nη1. The last substrate then captures protons from the catalytic thiol group, after which histidine remains without a proton. The tetrahedral intermediate breaks down into a positively charged flat covalent thiouronium compound. This causes ammonia cations to adopt a function after receiving a proton from H^{236}Nδ1. Ammonia omits the active site through the NH_3-exit/H_2O-entry channel to reach the enzyme surface. The diluent molecule fills the former position of ammonia, leading to the polarization of the aspartic acid (D)^{238} and H^{236}Nδ1 side chains (Goulas *et al*. 2015). The latter acts as a base and takes a proton from the water molecule, which then executes a nucleophilic attack on the central carbon atom of thiouronium. This produces another neutral intermediate centered on the sp3-like tetrahedral Cζ and diprotonated H^{236}. The middle substrate then breaks down into a citrullinated product and an intact catalytic mercaptocysteine group, forming hydrogen bonds with N^{297}Oδ1. The repulsion between the carbonyl oxygen of the impartial reaction product and D^{238} provides the motive force for the clearance of the substrate from the active-site gap. At the end of the process, a hydroxide formed by the reaction of ammonia with water may provide entrance to the active site via the hydroxide entry channel. This process ultimately leads to the exchange of one of two solvent molecules bound to H^{236}Nε1. A proton is transferred to hydroxide with the subsequent transfer of histidine and the shift of a proton from Nδ1 to Nε2, which restores the functional monoprotonated condition of H^{236}. Therefore, the active site is left available to repeat the reactions described above (Goulas *et al*. 2015).

5. Effects of citrullinated proteins on the immune system cause rheumatoid arthritis progression

Data have shown that *P. gingivalis* infection precedes RA. Moreover, this microbe is one of the most significant factors in maintaining and prolonging the autoimmune inflammatory response that occurs during infection. Therefore, the PPAD enzyme produced by *P. gingivalis* may have a profound effect on the appearance and progression of RA through protein citrullination, generating neo-epitopes and thus breaching the immunological tolerance of citrullinated proteins (Bielecka *et al*. 2014). Citrullination also leads to changes in the inter- and intramolecular interactions of proteins containing arginine residues, which are essential for their structure. Therefore, it potentially changes the three-dimensional structures of the modified proteins and their water solubility. Ultimately, this process can trigger a cascade of events leading to RA (Maresz *et al*. 2013).

There may also be an association between RA and PD based on the similarities in their environmental and genetic risk factors, including smoking and the expression of MHC class II HLA-DRB1 alleles (Berthelot and Le Goff 2010; Koziel *et al.* 2014). There are also similarities in their initiating mechanisms, with evidence emerging from various studies of an association between RA and PD. A comparison of the whole population and individuals with PD demonstrated an increased risk of developing RA among subjects with PD. Moreover, the course of PD in patients with RA is more severe than in patients without RA, independently of age, sex, smoking history, and ethnicity. Moreover, RA and PD use similar mechanisms insofar as proinflammatory cytokines and inflammatory cells cause the chronic erosion of bones in RA and chronic gum destruction in PD similarly. A recent study suggested that PD is one of the main factors in the induction and maintenance of the inflammatory response by the autoimmune system that occurs in RA (Maresz *et al*. 2013).

Many studies have shown that RA is caused by the dysregulated response of the immune system by cit-

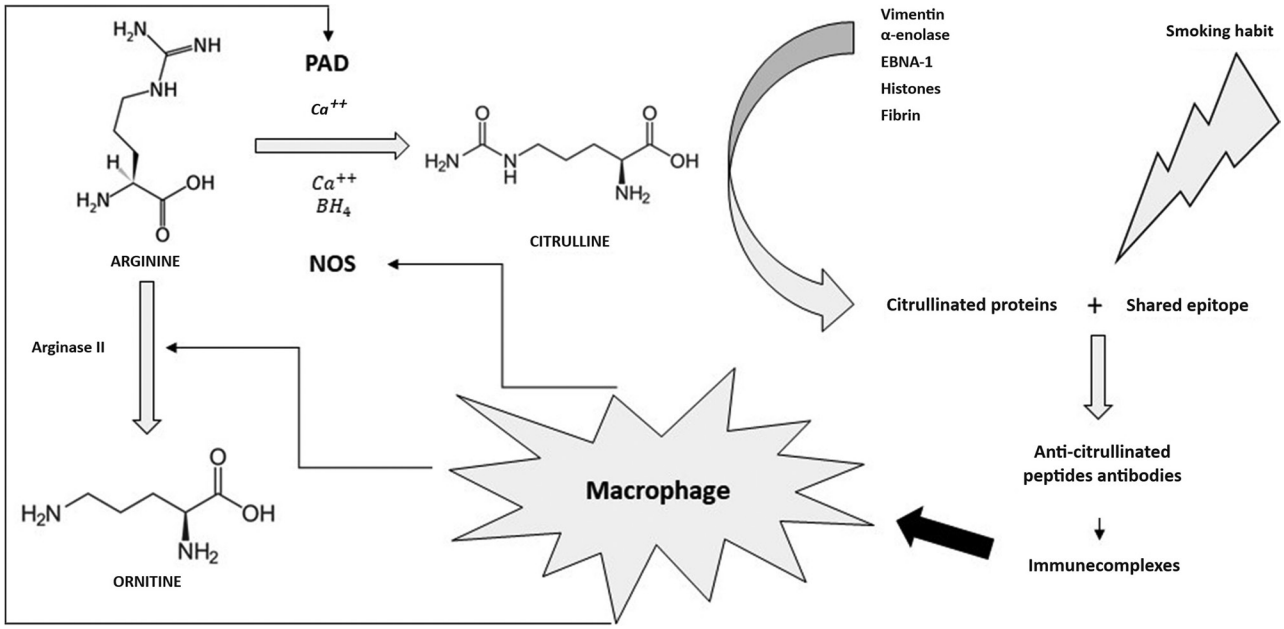

Figure 2. Changing tolerance to citrullinated proteins in rheumatoid arthritis diseases.
Putative biological pathways that may be responsible for the loss of tolerance for citrullinated proteins (modified from Alivernini *et al.,* 2008*, "Citrullination: the loss of tolerance and development of autoimmunity in rheumatoid arthritis". Reumatismo*).

rullinated proteins. Such proteins are produced under physiological conditions, but the loss of immunotolerance for citrullinated proteins in genetically susceptible individuals initiates the production of autoantibodies against citrullinated proteins (ACPAs), ultimately leading to RA (Smit *et al*. 2012).

Our studies have indicated that molecular mimicry and autoantibodies were important factors in the etiology of RA. We observed a positive correlation between ACPAs and antibodies directed against *Proteus mirabilis* LPS O3 or high levels of anti-urease antibodies, which recognized synthetic ureases epitopes (Durlik-Popińska *et al*. 2020; Konieczna *et al*. 2020).

The citrullination of proteins through the deimination of the guanidino group on the side chain of arginine is a posttranslational modification that converts positively charged peptidyl arginine to neutral peptidyl citrulline (Fig. 2). The citrullination reaction is essential in various physiological processes, including the differentiation of the epidermis (citrullination of profilaggrin and keratin), brain development (citrullination of myelin basic protein [MBP]), and the regulation of gene expression (Smit *et al*. 2012; Maresz *et al*. 2013).

PPAD-induced citrullination reduces the activity of the immune system by inactivating EGF or activating the prostaglandin E2 signaling pathways in fibroblasts, which causes alveolar bone loss. The citrullination of the surface proteins of *P. gingivalis* is also involved in its invasion of host cells and adherence to other bacteria. PPAD also significantly affects the host cell cytokine response to *P. gingivalis* infection. This enzyme is vital for the expression of IL36 in the epithelial cells of the human gingiva and increases the expression of other cytokines, such as IL8, IL13, CCL20, and CXCL8. Previous studies have shown that IL36 regulates dendritic and T-cell responses and is essential for inflammatory diseases (Goulas *et al*. 2015).

The bone destruction associated with RA and periodontitis involves similar inflammatory responses. In both cases, an immune system imbalance is linked to the dysregulation of immune cells, including Treg and Th17 cells (Zhou *et al.* 2021).

The association between PPAD, ACPAs, and the development of RA has been discussed in several comprehensive reviews and experimental studies (Koziel *et al*. 2014; Tilvawala *et al*. 2018; Jonsson *et al*. 2020; Gomez-Banuelos *et al.* 2022; Matuz-Flores *et al*. 2022; Zoubi and Gordon 2022). Recent progress in microbiome studies has correlated the oral and gut microbiomes with the progression of RA (Stobernack *et al.* 2018; Du Teil Espina *et al*. 2019).

6. Conclusion

Porphyromonas gingivalis strongly affects the human immune system. There is evidence from many studies that *P. gingivalis* infections correlate with the development of PD and, subsequently, with the progression of RA. Citrullination, which converts arginine to citrulline, initiates the immune system's inflammatory response, and the whole process is triggered by the expression of

P. gingivalis peptidylarginine deiminase (PPAD). Citrullination occurs under the pathological inflammatory conditions caused by *P. gingivalis* invasion. It is a natural process involved in cellular apoptosis, necrosis, and NETosis. For instance, histone hypercitrullination is necessary to create neutrophil extracellular traps. These traps are components of the immune system that respond to the disturbance of the homeostasis of the oral microbiota caused by an increase in *P. gingivalis*, leading to the imbalanced autoimmune responses that result in PD, RA, and other diseases of the immune system (Wang *et al*. 2009). Therefore, further detailed study of the mechanisms and relationships between the citrullination caused by *P. gingivalis* and the development of RA and PD is required.

Other proinflammatory factors that must be considered in both RA and PD are lipopolysaccharides of *P. gingivalis* types of O-, A- and K. Their binding to and activation of toll-like receptors (TLRs 2 and 4) via the NF-κB ligand contribute to peripheral polyarthritis. It has been suggested that the inhibition of the inflammatory potential of oral LPSs reduces the progression of RA, and fluoride is proposed as an inactivator of bacterial LPS (Marcano *et al*. 2021). The short-chain fatty acids (SCFAs) produced during the anaerobic metabolism of *P. gingivalis* reportedly attract neutrophils to the gingival pocket through the interaction between SCFAs and free fatty acid receptor 2 (FFAR2) (Dahlstrand *et al.* 2021). It is suggested that monitoring the level of SCFAs might reduce the proinflammatory potential of the oral microbiome and, consequently, the likelihood of RA. A meta-analysis of 28 of 2050 studies with human subjects revealed a high odds ratio (1.86, 95% confidence interval) for the risk of RA in individuals infected with *P. gingivalis* (Li *et al.* 2022). Periodontal disease and RA are multifactorial and interrelated, and *P. gingivalis* is undisputedly an essential player in both.

ORCID
Wiesław Kaca https://orcid.org/0000-0002-8734-7191

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Author Contributions Statement
WRK contributed to the conception and design of the study. WK wrote the first draft of the manuscript. All authors contributed to the manuscript revision and read and approved the submitted version.

Conflict of interest
The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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FUNGAL PATHOGEN IN DIGITAL AGE: REVIEW ON CURRENT STATE AND TREND OF COMPARATIVE GENOMICS STUDIES OF PATHOGENIC FUNGI

Kenneth L.S. Tan[1, 2], Saharuddin B. Mohamad[1, 3]

[1] Bioinformatics Program, Institute of Biological Sciences, University of Malaya Kuala Lumpur, 50603, Malaysia
[2] LeapOmics Services, D-10-08, Jadite Suites, Persiaran Jade Hills, 43000 Kajang, Malaysia
[3] Centre of Research for Computational Sciences & Informatics for Biology, Environment, Agriculture and Healthcare (CRYSTAL), University of Malaya, 50603, Bioindustry, Kuala Lumpur, Malaysia

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Abstract: Fungal pathogenicity to plants, animals, and humans leads to several detrimental effects in our society by causing diseases that impact livelihood and food security. While the recent pandemic shifted focus to viral pathogens, fungal pathogens are still impacting the world that we live in. It is important to study fungal pathogenicity with the latest scientific advancement. One way to do that is to understand the conservation of pathogenicity in the fungus kingdom which will further elucidate the underlying mechanisms behind fungal pathogenicity across all species of fungi. This review provides an outlook on the various bioinformatics and genomics approaches and currently available resources in understanding fungal pathogenicity. It also discusses the current state of affairs and emerging trends in the study of fungal pathogenicity. Finally, this review also provide suggestions different approaches for the study of fungal pathogenicity to see further improve our understanding in this field.

Keywords: Bioinformatics, Comparative Genomics, Data Mining, InterPhyla, Pathogenic Fungi

1. Introduction

Pathogenic fungi continue to be a problem to humankind to this date. Fungal diseases create severe issues in public health and leave a catastrophic impact on agriculturally important crops. This review intends to highlight the importance of the continuous effort in studying fungal diseases amid more recently popular areas of study, such as virology and bacteriology while suggesting essential bioinformatics resources and techniques that can accelerate new knowledge discovery in this area of interest.

1.1. Overview of Pathogenic Fungi

Pathogenic fungi continue to impact global public health and food security as they impact human and commercially essential food crops. Plant pathogenic fungi are mainly constituted of members from the Ascomycota and Basidiomycota phylum (Heitman 2011). The statement was supported by the list of plant pathogenic fungi surveyed by Dean *et al.* (2012) as listed in Table I, where out of the top ten entries, three of the fungi in the list were from the Basidiomycota phylum, and the rest were members of the Ascomycota phylum. Most of these fungi have had devastating impacts on agriculturally important plants by affecting yield, thus causing ripple effects on the economy and food security issues.

Pathogenic fungi kill approximately 1.5 million people annually (Brown *et al.* 2012). This alarming statistic often goes unnoticed compared to other pathogens, such as viruses and bacteria. This raises the question of whether the scientific community should conduct more comprehensive comparative studies on fungi using bioinformatics databases and tools. The World

* Corresponding Author: Saharuddin Bin Mohamad, Bioinformatics Program, Institute of Biological Sciences, University of Malaya Kuala Lumpur, 50603, Malaysia, e-mail: saharuddin@um.edu.my

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TABLE I
Top 10 fungal plant pathogens (Dean *et al.* 2012).

Rank	Fungal pathogen	Author of fungal description
1	*Magnaporthe oryzae*	Ralph Dean
2	*Botrytis cinerea*	Jan A.L. van Kan
3	*Puccinia spp.*	Zacharias A. Pretorius
4	*Fusarium graminearum*	Kim Hammond-Kosack
5	*Fusarium oxysporum*	Antonio Di Pietro
6	*Blumeria graminis*	Pietro Spanu
7	*Mycoshaerella graminicola*	Jason J. Rudd
8	*Colletotrichum spp.*	Marty Dickman
9	*Usitlago maydis*	Regine Kahmann
10	*Melampsora lini*	Jeff Ellis

Health Organization published a ranking of human fungal pathogens (World Health Organization 2022). That list, as seen in Table II, includes members from the genera of *Cryptococcus*, *Candida*, *Aspergillus*, and more. These fungi cause a wide variety of diseases in humans and animals. For example, members from the genus *Aspergillus* can cause health issues in humans, animals, and birds, which could result in localized infections and fatal diseases (Seyedmousavi *et al*. 2015). Hence, it is essential to be aware of available resources to help further understand fungal pathogens.

1.2. Advancement of DNA Sequencing Technology and Bioinformatics Tools and its Impact

Fungal, pathogens-inflicted diseases continue to affect humans, animals, and plants, causing public health and food security repercussions. This requires a deeper understanding of genomics and genetics of fungal pathogens to find ways to manage fungal diseases. The rapid advancements in genome and proteome sequencing technologies, coupled with a wide range of bioinformatics tools and applications available, provide an opportunity for the scientific community to perform comparative genomics studies. These will explore and answer research questions at a speed that could not have been achieved in previous years. This has proven to be a gateway for new research initiatives to blossom. The Genome 10K Project (Koepfli *et al*. 2015) aims to sequence genomes from at least one individual from every vertebrate genus, which accounts for approximately 10,000 genomes, and that is only one example of many such studies. Worldwide genomics studies now produce more genome sequences at a higher rate and lower cost, focusing critically on comparative genomics studies. For example, the fishes of Genome 10K (Bernardi *et al*. 2012) and Fungal Genome Initiative (Broad Institute 2008) aim to accelerate research on microbial metabolism, physiology, and functional genomics.

TABLE II
Ranking of Human Fungal Priority Pathogen according to World Health Organization (World Health Organization 2022).

Grouping	Fungal pathogen
Critical Group	*Cryptococcus neoformans*
	Candida auris
	Aspergillus fumigatus
	Candida albicans
High Group	*Nakaseomyces glabrata*
	Histoplasma spp.
	Eumycetoma causative agents
	Mucorales
	Fusarium spp.
	Candida tropicalis
	Candida parapsilosis
Medium Group	*Scedosporium* spp.
	Lomentospora prolificans
	Coccidioides spp.
	Pichia kudriavzeveii (*Candida krusei*)
	Cryptococcus gattii
	Talaromyces marneffei
	Pneumocystis jirovecii
	Paracoccidioides spp.

This massive amount of data generated from projects allows researchers to leverage the raw and curated genomics datasets for secondary research, and comparative genomics study is at the forefront of this. Comparative genomics is a critical technique applied to understand homology and phylogenetic relationships for any subject of study, including fungal genomics.

2. First Things First: Publicly Available Pathogenic Fungi Resources

Several massive sequencing projects worldwide have produced many genomic resources for studying fungal pathogenicity. For instance, general genetic sequence resources such as GenBank (Benson *et al*. 2017), DDBJ (Fukuda *et al*. 2020), and EMBL (Hingamp *et al*. 1999) provide a plethora of genetic sequences for research. Specialized databases like the Fungal Genome Initiatives by Broad Institute (Fungal Genomics 2008), FungiDB (Basenko *et al*. 2018), and EnsemblFungi (Howe *et al*. 2020), to name a few, are fungi genome databases that serve as a repository for fungal genome and genetic sequences. NCBI, DDBJ, and EMBL are universal repositories for all sequence data types, such as raw sequencing data, whole genome assemblies, gene annotations, protein sequences, and variant calls. These data cover all organisms, including various species of fungi across the kingdom of fungi. Accordingly, bioinformatics analysis of pathogenic fungi is highly challenging because

enormous effort is needed for data clean-up to obtain the specific datasets of interest, which creates a gap to be filled by specialized databases or repositories.

FungiDB contains 220 fungus genome sequences for fungi species associated with infectious diseases with mammalian hosts and invertebrate disease vectors (Basenko *et al*. 2018). FungiDB is an integrated platform for data mining and functional genomics analysis besides containing fungi sequence data. FungiDB provides online bioinformatics tools to allow homology studies using BLAST tools (Camacho *et al*. 2009), enabling downstream analysis in comparative genomics efforts in various studies such as those performed on *Aspergillus fumigatus* (Abad *et al*. 2021) and *Cryptococcus* isolates (Yu *et al*. 2021). FungiDB Enrichment Analysis in FungiDB allows GO annotations of the studies and contains many other tools that enable convenient downstream analysis of fungi genomics study. Publicly available fungi genomics data can help accelerate *in silico* research for the bioinformatics community, and various findings can be discovered more quickly without performing genome or DNA sequencing projects. Launched in November 2000, the Fungal Genome Initiative by Broad Institute was anchored by a group of fungal geneticists and biologists who believed that the speed of discovery in biomedical research was limited by minimal publicly available fungal genome data (Fungal Genomics 2008). Since then, the initiative has focused on species of fungi that are important to human health and commercial activities (i.e., agriculture) and are valuable for fungal diversity and comparative genomics.

Publicly available fungal genomics data are a valuable starting point for downstream analysis for comparative genomics studies. Using annotation data such as genes, proteins, exons, and transcript sequences, researchers can create secondary databases based on data in primary databases. The Pathogenic Host Interaction Database, PHI-base, is a specialized database that catalogues experimentally verified pathogenicity, virulence, and effector genes from fungal, oomycete, and bacterial pathogens (Urban *et al*. 2020). The database is beneficial as it provides validated experimental data on genes that participate directly and impact the pathogenicity of fungus within host-pathogen interactions. The database is used in various genomics studies of pathogenic fungi in comparative genomics studies, pathogenic genes annotation, and homology searches. For example, the database has been employed to annotate pathogenic genes in *Ganoderma boninense* (Ramzi *et al*. 2019) and successfully identify candidate genes that participate in the virulence of *Ganoderma boninense* in oil palm. It has also been used to predict virulence determinants in draft genomes of *Apophysomyces variabilis,* where the species are prevalent causative agents of mucormycosis in India (Prakash *et al*. 2017). The most recent PHI-base release 4.16 contains 9,666 gene sequences in 21,676 interactions. These entries are available for public download for local usage of the data. This provides an opportunity to build a fungal pathogenic genes annotation pipeline that can quickly predict the presence of candidate pathogenic genes in new genome sequence projects.

Fungal pathogenicity in plants involves specific mechanisms to challenge the rigid plant cell wall during the proliferation of the host organism. Fungus secret enzymes that can break down the rigid plant cell wall, and these enzymes are also commonly known as carbohydrate-active enzymes. The Carbohydrate-Active enZymes Database (Lombard *et al*. 2013), known more popularly by its acronym CAZy, is a database that contains protein sequences of structurally related catalytic and carbohydrate-binding modules that are known to have different modes of interactions with glycosidic bonds, which is a significant linkage and type of covalent bond that joins carbohydrate molecules to other groups. Glycosidic bonds are fundamental linkages in cellular walls (Joseleau and Pérez 2016). Thus, they are the prime target of carbohydrate-active enzymes, which are considered candidate fungal pathogenic genes because of their capability to degrade the plant cell wall. As described in Table III, these enzyme classes and associated modules are involved in various biological pathways of the host organism.

Massive sequence data and literature published on fungal pathogenicity also allow the opportunity to create a database based on these experimental data published

TABLE III
Enzyme Classes and Associated Modules that are involved in Breakdown, Biosynthesis or Modification of Carbohydrates and Glycoconjugates

Family	Description
Glycoside Hydrolases (GHs)	Involves in hydrolysis and/or rearrangement of glycosidic bonds
Glycosyl Transferases (GTs)	Involves in the formation of glycosidic bonds
Polysaccharide Lyases (PLs)	Involves in non-hydrolytic cleavage of glycosidic bonds
Carbohydrate Esterases (CEs)	Involves in hydrolysis of carbohydrate esters
Auxiliary Activities (AAs)	Involves in redox enzymes that act in conjunction with CAZymes
Carbohydrate-Binding Modules (CBM)	Involves in adhesion to carbohydrates

in the literature. The Database of Virulence Factors in Fungal Pathogens (DFVF) (Lu et al. 2012) was a project aimed at filling the missing gaps in understanding fungal pathogenicity by aggregating all known virulence factors. This project also develops an algorithm that allows the prediction of potential candidate genes that will contribute to the development of fungal pathogenicity. The database was built by leveraging the text-mining technique used by PubMed and the Internet by looking for fungal disease virulence keywords. In-house tools were also developed to allow relevant supporting literature to be searched. With this methodology, the database currently contains 2058 protein sequences.

Other fungal pathogen-related databases provide information about antifungal genetics properties, and one such example is the AFRbase. AFRbase is a database that keeps information about protein mutations responsible for antifungal resistance (Jain *et al.* 2023), which will help the scientific community understand antifungal resistance as clinicians. Biologists are looking for better treatments and cures for fungal diseases. The database was created through text mining of publicly available research papers and further enhanced with information from publicly available databases such as NCBI. Other similar databases include FunResDB (Weber *et al.* 2018) and MARDy (Nash *et al.* 2018). FunResDB focuses on susceptibility testing of *Aspergillus fumigatus,* which has high public health importance. At the same time, MARDy includes data on existing antifungal resistance in humans, animals, and plants with its associated antifungal agents.

3. Current Bioinformatics Tools

Developing bioinformatics databases and tools that focus on different study paradigms is vital to increasing the spectrum of understanding and helping expand the perspective of biological research on pathogenic fungi. These bioinformatics databases and tools are developed to deal with data in various stages of readiness, ranging from tools like FastQC (de Sena Brandine and Smith 2021) that enable quality control of DNA/RNA sequences generated by sequencing machines to downstream through that deal with the more complex interpretation of data such as Cytoscape (Shannon 2003), VisANT (Hu 2014), Pathway Studio (Nikitin *et al.* 2003) and Patika (Demir *et al.* 2002) that enable scientists to explore biological networks as a mean to understand better integrative biology, system biology, and integrative bioinformatics.

Standalone tools include BLAST+ (Camacho *et al.* 2009), a universally common tool for comparing two or more DNA/RNA/protein sequences to understand the degree of similarity and identity between sequences, which implies the degree of conservation of sequences among subjects of studies. It is often utilized to understand the relationship between species of organisms. ClustalW (Thompson *et al.* 2002), MAFFT (Katoh 2002), and MUSCLE (Edgar 2004) are other examples of such standalone tools that incorporate statistical analyses of subject sequences, building multiple sequence alignments. Phylogenetics tools such as PHYLIP (Retief 2000) and MEGA (Hall 2013) generate relationship trees of input sequences that allow not only understanding but also visualize relative relationships between multiple sequences in the study. Recent trends in bioinformatics tool development indicate an increasing need within the scientific community to have integrated tools that serve as a "one-stop centre" for biological data analysis. An integrated bioinformatics platform will allow more biological scientists without high computing skill sets to perform bioinformatics analysis, such as executing sequence analysis via multiple bioinformatics tools and visualizing results. This is extremely important as it requires time to understand different bioinformatics tools, and as such, this requirement is a higher barrier to entry for most scientists. Given such unique demands, significantly more integrated bioinformatics analysis platforms are being developed for scientists to conduct integrated sequence data analysis.

Unipro UGENE (Okonechnikov *et al.* 2012) is an example of a bioinformatics tool that provides a platform for developing an integrated pipeline. UGENE delivers a user-friendly interface for scientists to build their desired bioinformatics pipeline and workflows for sequence data analysis. With many popular standalone bioinformatics tools available within UGENE, it also provides a user-friendly interface for scientists to easily build desired workflows with a drag-and--drop feature that requires minimum computer programming knowledge.

Comparative genomic analysis involves the comparative analysis of sequence data from multiple sources, some within species and some across numerous species. These analyses usually involve multi-stage data analysis and, therefore, require a combination of bioinformatics tools and applications to draw meaningful discussions and deductions while answering experimental hypotheses. Most comparative genomics platforms allow comparative analysis of DNA sequences and streamline the process from data analysis to visualization of results. EDGAR (Dieckmann *et al.* 2021) is an example of an integrated comparative genomics platform. It is one of the most popular platforms for gene-based comparative genomics and differential gene content analysis. Venn diagrams or synteny plots can be generated to provide a user-friendly and visually appealing interpretation of results. A list of all databases and tools and a link to the website can be found in Table IV.

Table IV
List of Current Databases and Bioinformatics Tools for Comparative Fungal Genomics Studies.

Tools	Type	Link to Website
FastQC	QC tool for high throughput sequence data	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Cytoscape	Network Visualization Tool	https://cytoscape.org/
VisANT	Biological Network Analysis Tool	http://www.visantnet.org
Pathway Studio	Navigation and Analysis of Biological Pathways, Gene Regulation Networks and Protein Interaction Maps	https://ariadnegenomics.com/products/pathway-studio
Patika	Integrated Visual Environment for Collaborative Construction and Analysis of Cellular Pathways	https://www.patika.org/
BLAST+	Sequence Homology Search Tool	https://blast.ncbi.nlm.nih.gov/blast/Blast.cgi
ClustalW	Multiple Sequence Alignment Tool	http://www.clustal.org/clustal2/
MAFFT	Multiple Sequence Alignment Tool	https://mafft.cbrc.jp/alignment/server/index.html
MUSCLE	Multiple Sequence Alignment Tool	https://www.drive5.com/muscle/
PHYLIP	Phylogenetics Tree Building Tool	https://phylipweb.github.io/phylip/
MEGA	Molecular Evolutionary Genetics Analysis Tool	https://www.megasoftware.net/
Unipro UGENE	Integrated Bioinformatics Tool	https://ugene.net/
EDGAR	Software Platform for Comparative Genomics	https://edgar3.computational.bio.uni-giessen.de/cgi-bin/edgar_login.cgi?cookie_test=1
PHI-base	Pathogen Host Interactions Database	http://www.phi-base.org/
CAZy	Carbohydrate-Active enZYmes Database	http://www.cazy.org/Home.html
DFVF	Database of Virulence Factors in Fungal Pathogens	http://sysbio.unl.edu/DFVF/
FungiDB	Database	https://fungidb.org/fungidb/app
GenBank	Genetic Sequence Database	https://www.ncbi.nlm.nih.gov/genbank/
DDBJ	Genetic Sequence Database	https://www.ddbj.nig.ac.jp/index-e.html
EMBL	Genetic Sequence Database	https://www.ebi.ac.uk/
Fungal Genome Initiatives	Fungal Genome Database	https://www.broadinstitute.org/scientific-community/science/projects/fungal-genome-initiative/status-fungal-genome-projects
EnsemblFungi	Genome Portal for Selected Fungal Species	https://fungi.ensembl.org/index.html
ARFbase	Database on protein mutations responsible for antifungal resistance	http://proteininformatics.org/mkumar/afrbase/
FunResDB	Web source for genotyping susceptibility testing of *Aspergillus fumigatus*	https://elbe.hki-jena.de/FunResDb/index.php
MARDy	Mycology Antifungal Resistance Database	http://mardy.net

4. Current Trend: Comparative Genomics Studies of Pathogenic Fungi

Studying fungal pathogenicity continuously centers around treatment and diagnosis, intending to identify methods for early diagnosis or eradication of diseases. Studies in this field are ongoing, and sequencing technologies serve as an enabling platform for various downstream research and development projects. They set the foundation for bioinformatics research and development. The discovery of different polymorphic markers, such as single nucleotide polymorphism, insertions and deletions, copy number variations, and the presence of genes, is essential. Each of these polymorphisms plays an important role in causing pathogenicity in fungi, and this could confer pathogenicity to pathogenic isolates, as shown in human research.

The emergence of sequencing technologies has increased the resolution of research into underlying molecular causative factors in molecular plant pathology. Through genome sequencing of plant pathogens like *Magnaporthe oryzae* (Dean *et al.* 2005), *Botrytis cinerea* (Amselem *et al.* 2011), *Ustilago maydis* (Kämper *et al.* 2006), and *Puccinia graminis* (Duplessis *et al.* 2011) coupled with improvements in bioinformatics methodology such as genome assembly, genome annotation, comparative genomics, pathologists can identify genomics features in fungal pathogens that play important roles in fungal pathogenicity. On top of that, further understanding of these genomics' features will allow scientists to pursue and develop faster and more accurate diagnostic tools for fungal-related diseases.

Whole genome sequencing of plant fungal pathogens allows high-quality genome assembly to identify

and reveal the underlying sequences of the fungus. The genome annotation of the assembled genome then predicts gene models based on *ab initio* prediction and homology searches (Yandell and Ence 2012) to known nucleotide or protein sequences. The availability of an annotated genome allows downstream bioinformatics analysis, such as polymorphic markers identification through genome mapping (Davey *et al.* 2011) and comparative genomics (Wei *et al.* 2002). A study on *Verticillium dahliae* proposed the possibility of horizontal gene transfer (HGT) from bacterial origins, which directly contributed to the pathogenicity of the fungus, which is known to be a plant pathogen that affects hundreds of plant species and causes substantial economic losses annually (Shi-Kunne *et al.* 2019).

The same effort was applied to the comparative genomics of human pathogenic fungi. *Candida* and *Aspergillus* are the most prevalent fungal genera that cause significant health implications in human (Moran *et al.* 2010). Hence, elucidating the sequences at a genomic level is extremely important to allow the development of effective antifungal therapy and understand the emergence of drug resistance. A study was done to understand the drug resistance of *Candida auris* using genomic data such as epidemiology and evolutionary information (Chybowska *et al.* 2020). A comparative genomics study was also done on *Aspergillus* to improve understanding of genome heterogeneity between *Aspergillus fumigatus*, *Aspergillus lentulus*, and *Aspergillus fumigatiaffinis* (dos Santos *et al.* 2020). These three species are highly similar in morphology, making it challenging to distinguish one species from another by phenotype observation (Izquierdo *et al.* 2014). Molecular markers can be developed into rapid serology-based test methods that can yield fast results, and one example of such assay is the (1,3)-β-D-glucan (BDG) based assay (Fang *et al.* 2023). This assay detects a polysaccharide fungal cell wall component from *Candida* spp. *Pneumocystis jivoveci*, *Aspergillus* spp., *Acremonium* spp., *Fusarium* spp (Tissot *et al.* 2013) in patients to determine disease causes. This demonstrated the importance of genomics studies as sequencing and downstream bioinformatics analysis can uncover unique genomics features of each species, allowing accurate diagnostic results.

Comparative genomics techniques were applied in studying not only genetic diversity but also in the discovery of critical genomic markers such as short sequence repeats (SSR), short tandem repeats (STR), long tandem repeats (LTR), and single nucleotide polymorphisms (SNP). A recent study on *Fusarium oxysporum* is an example of such application of comparative genomics in uncovering genomics markers for quicker detection of pathogenic isolates of the species (van Dam *et al.* 2018). The study includes candidate effector genes from 88 *Fusarium oxysporum* genomic assemblies for comparative genomics. It aimed to distinguish the isolates based on the traits where it could differentiate between cucurbit-affecting formae speciales from each other and differentiate between pathogenic and non-pathogenic isolates.

General identification of pathogenic and non-pathogenic fungi often investigates genetic features such as the presence of pathogenicity-related genes and proteins. The presence or absence of pathogenicity-related genes is important in understanding fungal pathogenicity and its viability. This was demonstrated in a study comparing *Fusarium graminearum* and *Fusarium venenatum,* non-pathogenic and pathogenic fungus species, respectively (King *et al.* 2018). The study presented helpful insights to support such a hypothesis. Through comparative genomics involving a comparison of the proteomes of each species, the scientists discovered 15 putative secondary metabolite gene clusters, 109 secreted proteins, and 38 candidate effectors that are not identified in the non-pathogenic subject. One recent study identified gene presence-absence variation (PAV) in *Magnaporthe oryzae,* which can help understand fungal pangenome evolution (Pierre and Ksenia 2024). This approach is helpful to understand and identify core genes of a specific that potentially supports fungal pathogenicity (Chen *et al.* 2023). As new methods and tools appear, new ways of studying fungal pathogenicity will continue to evolve and improve.

5. A Different Take: Inter-Phyla Comparison and Host-Independent Comparison

Unsurprisingly, there are similarities between pathogenic fungi that attack plants and animal hosts (Dickman and de Figueiredo 2011). Both groups of fungi are similar in the mechanisms of pathogenicity, which are all part of the fungi lifecycle from spore germination, invasion via physical openings, colonization and alteration of host, reproduction, and transmission. These similarities in the pathogenicity mechanism prompted the interest in studying fungi not as a separate group but as the same study group, which allows further understanding of pathogenic mechanisms in the kingdom of fungi.

By adopting a different perspective to compare pathogenic fungi that infect plant hosts and animal hosts, genomics identification provides a means of understanding the adaptation of these species of fungi based on host-specificity. Fungal species that infect plant hosts can have broad or narrow host ranges (Sexton and Howlett 2006). Specificity is defined by R genes known as resistance genes in the host and the virulence factors found in the pathogenic fungi (van der Does and Rep

2007). The range of hosts a fungus can infect is not limited to plants or animals. Some extreme examples, like within the genus *Fusarium*, cause disease across plant species and animals, including human (Sharon and Shlezinger 2013), which makes understanding the mechanism behind pathogenicity even more peculiar.

The same is true for bacterial pathogens; a study has found that *Pseudomonas aeruginosa,* which causes pneumonia and infections in the blood (CDC 2019) in humans, shows a high degree of conservation in the virulence mechanism used to infect both humans and plants. The pathogen also causes infection on *Arabidopsis* and sweet basil roots, forming a biofilm layer under specific physiological conditions (Walker *et al*. 2003). Evidence also showed that the bacterial pathogen used a common subset of virulence factors for pathogenesis in plants and animals (Walker *et al*. 2003), further demonstrating that pathogens that infect a range of hosts use a common pathogenesis mechanism. Understanding the common mechanism behind the range of potential hosts for infection can shed light and give rise to a better understanding of host specificity and the mechanism of pathogenicity in the kingdom of fungi. This is supported by studies from the past reporting commonalities of pathogenicity among mechanisms for plants and animals (Hamer and Holden 1997).

6. Application and Outcome of Comparative Genomics Studies

Comparative genomics studies will create a good foundation for using the identified pathogenicity-related genes and molecular markers for molecular diagnostic purposes. Fungal infections in human or animal hosts are easier to detect and identify compared to plant diseases caused by pathogenic fungi. For example, fungal nail infections known technically as "onychomycosis" can be diagnosed easily as the disease symptoms can be observed visually through the rotting of nails (Gupta *et al*. 2000). The same can be said for many fungal diseases, such as Aspergillosis, Candidiasis, Mucormycosis, *Pneumocystis pneumonia* and many more (CDC 2019). Furthermore, fungal infections in humans and animals jeopardize the health and livelihood of the subject; hence, early diagnosis is crucial. Early detection is easier with visible symptoms such as skin rashes or coughing. In addition, diagnosis can be done through direct microscopic examination of clinical samples, histopathology, culture, and serology of patient clinical samples (Kozel and Wickes 2014). Fungal diseases in plants, however, can be challenging to detect as the symptoms are not visible, and it could be too late when symptoms are finally observed. A classic example of this is the basal stem rot (BSR) and upper stem rot (USR) caused by *Ganoderma boninense* (Hushiarian *et al*. 2013). As the infection is not visually apparent during the early stages, it will be too late when its symptoms are visible. Once the symptoms are observable, palm trees die within 1 or 2 years to 3 or more years, depending on the age of the palm (Corley and Tinker 2003).

In the case of BSR or USR caused by *Ganoderma boninense,* traditional diagnostic methods are impractical as it will be too late when the disease symptom is observed. Molecular diagnostic methods using PCR amplifications are the way forward for early detection of fungal diseases that are not observable. This method requires the presence of a unique genome sequence of the target organism, which is usually a well-conserved region with polymorphic markers identifying different species. A specific primer (Hariharan and Prasannath 2021) will amplify the target region of interest. Example target regions of the fungal genome that have been identified for molecular diagnostics include the highly conserved internal transcribed spacer ITS-region in fungus, known for fungal diversity analysis and an important marker for fungal DNA barcoding (Bellemain *et al*. 2010), and alternative sequences such as cytochrome b gene which was used as a target region for Loop-Mediated Isothermal Amplification (LAMP) assay for detection of airborne *Uromyces betae* (Kaczmarek *et al*. 2019). Molecular diagnostic methods provide the possibility of early detection, and these methods can be applied to fungal diseases in plants, animals, and humans.

7. Conclusion

The negative impact of fungal disease on public health and the global economy requires continuous effort in research and development to expand our understanding of fungal pathogenicity with existing data repositories and bioinformatics tools. However, the rapid advancement of sequencing technology and computational biology calls for creating more fungal pathogen-focused bioinformatics databases and tools that identify causative factors of fungal pathogenicity and the underlying genetics. This will allow in-depth comparative genomics on fungal pathogens and help develop new fungal pathogen identification, diagnosis, and treatment methods.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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THE ROLE OF GUT MICROBIOTA IN OBESITY

Anna Celina Durma[1], Adam Daniel Durma[2], Adam Śmiałowski[3], Leszek Czupryniak[1]

[1]Department of Diabetology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland
[2]Department of Endocrinology and Radioisotope Therapy, Military Institute of Medicine – National Research Institute, Warsaw, Poland
[3]Department of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital, Research Institute; Medical University of Lodz, Poland

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Abstract: Obesity is a disease which is currently one of the most serious problems affecting approximately 650 million people worldwide. Improper lifestyle is considered the primary cause of the disease; however, many other factors contribute to the problem. In recent years, attention has been drawn to the role of gut microbiota in developing and controlling obesity and overweight. Microorganisms in the gastrointestinal tract are responsible for the fermentation of certain nutrients, causing efficient digestion, stimulation of intestinal transit, vitamin production, and modulation of the host's immune system. Numerous studies have demonstrated that gut microbiota composition differs between obese individuals and those with a normal body mass index (BMI). It has also been shown that altering gut microbiota can influence the phenotype of the host organism, promoting metabolic changes, including BMI reduction. Recent studies aimed at using probiotics to modify gut microbiota composition to reduce body weight are still inconclusive.

Keywords: gut microbiota, obesity, probiotics

1. Introduction

In recent years, the problem of being overweight and obese has been increasing and affecting even younger individuals. This disease is diagnosed in all ages and socioeconomic groups, mainly in developed and developing countries (NCD-RisC 2017). The most common cause of obesity is excessive energy intake compared to its expenditure. Lifestyle factors (such as improper diet and lack of physical activity) play a significant role in the pathogenesis of this phenomenon, along with genetic, socioeconomic, and mental factors. Recently, more attention has been given to the role of gut microbiota in the pathogenesis of obesity, both in diagnosis and treating the disease.

2. Obesity

Obesity is characterized by excessive accumulation of adipose tissue in the body. Due to its close location to internal organs and hormonal activity, visceral fat has a multifactorial and detrimental impact on the human body (Góralska *et al.* 2015). The body mass index (BMI) is the most common parameter for assessing obesity. It is calculated by dividing body mass (in kilograms) by the square of height (in meters). Diagnosis of overweight or distinct degrees of obesity are demarcated according to specified BMI thresholds – Stage I obesity is designated by a BMI of 30–34.9 kg/m^2, Stage II by 35–39.9 kg/m^2, and Stage III by a BMI surpassing 40 kg/m2. The extreme manifestation – class III obesity – is commonly referred to as "massive obesity" or "morbid obesity". Obesity can underlie or exacerbate many health problems and diseases, such as hypertension, dyslipidemia, carbohydrate metabolism disorders, obstructive sleep apnea, depression, fertility disorders, and many others (Zhang *et al.* 2023). The scale of the obesity problem is particularly significant in Western countries with particular feeding patterns. It is estimated that the current number of obese individuals worldwide has reached 650 million, and the prevalence of obesity continues to rise, according to

* Corresponding Author: Anna Celina Durma, Department of Diabetology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland, e-mail: a__owczarczyk@wp.pl

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the World Health Organization (WHO). The significant dynamics of this problem in recent decades are worth noticing. Since 1975, the number of obese patients has tripled, leading to frequent references to the contemporary "obesity epidemic". It is anticipated that by the end of 2030, one in five women and one in seven men will have obesity-related issues, equivalent to approximately 1 billion people (data from IASO) (Lobstein *et al*. 2022).

Currently, the treatment of obesity primarily focuses on lifestyle changes, followed and accompanied by pharmacological treatment. Recommended interventions include modifying dietary habits, particularly reducing calorie intake, and incorporating physical activity (Bischoff and Schweinlin 2020). Pharmacotherapy has also become available for obesity treatment in the last decades. In the Polish market, there are currently three registered drugs: a combination of bupropion and naltrexone, liraglutide, and orlistat. In the United States of America and some countries in Europe (United Kingdom, Denmark), there are also some other drugs used for obesity treatment, like phentermine or semaglutide (Table I). Nevertheless, the lack of registration of some agents on local markets limits the treatment options (Richelsen *et al*. 2007; Smith *et al*. 2013; Wadden *et al*. 2013; Bello 2019; Azuri *et al*. 2023). For patients with a BMI $>40\,kg/m^2$ or a BMI $>35\,kg/m^2$ with obesity-related comorbidities (for example, cardiovascular diseases, diabetes, osteoarthritis), bariatric surgery is also a recommended alternative. The most offered and considered safe bariatric surgery is sleeve gastrectomy (SLG) (Rosen *et al*. 2009). The other types of operation, like gastric bypass or ingastric balloon, are less effective or burdened with more possible complications (Lager *et al*. 2017; Singh *et al*. 2020). One of the newest hypotheses regarding obesity suggests the potential therapeutic use of probiotics, which will modify the composition of gut microbiota, improve obesity treatment, and increase the pace of the weight reduction process (Bischoff and Schweinlin 2020).

Table I. Obesity treatment options.

Non-pharmacological treatment of obesity	Pharmacological treatment of obesity
Reduction diet	GLP 1 analogs (liraglutyd, semaglutyd)
Physical activity	Bupropion and naltrexone
Bariatric surgery	Orlistat
	Phentermine

3. Gut microbiota

The gut microbiome is a complex ecosystem with numerous microorganisms inhabiting the human digestive tract. The number of bacteria and fungi in the gastrointestinal system is immense – it is estimated that the human gut is colonized by approximately 10 billion microorganisms (Karney 2017). Moreover, gut microbiota is also comprised of viruses – DNA and RNA. It is estimated that gut virome even outnumber bacterial cells, including eucaryotic viruses, endogenous retroviruses, bacteriophages, and archaeal viruses. Less than 1% of human gut virome is estimated to be sequenced (Mukhopadhya *et al*. 2019). Metagenomic studies of the human gut microbiota have identified over 1000 species of bacteria (Zhu *et al.* 2010). Until recently, it was believed that the gut microbiota outnumbered the body's cells by ten. However, current research suggests a more likely ratio of 1:1 (Sender *et al*. 2016). The majority of bacteria in the human intestines belong to four phyla of microorganisms: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (according to the International Committee on Systematics 2021: Bacillota, Bacteroidota, Psuedomonadota and Actinomycetota), with Firmicutes and Bacteroidetes being the most dominant bacteria (Pokrzywnicka and Gumprecht 2016; Frank *et al*. 2007, Oren and Garrity 2021)

The gut microbiome begins to form from birth, although some study indicates the presence of gut microbiota during the prenatal period. Bacterial presence has been detected in the placenta and amniotic fluid, characterized by low diversity and a predominance of Proteobacteria. A study noted common gut microbiota characteristics detected in the placenta, amniotic fluid, and infant meconium, suggesting microbial transfer even during the fetal period (Collado *et al*. 2016). The mechanism of this process is not fully explained. It is possible that the transition of bacteria results from the translocation of microbiota located near the mother's reproductive organs (Panasiuk and Kowalińska 2023). Various factors are believed to influence the composition of gut microbiota. These include breastfeeding/formula feeding, antibiotic therapy administered by the mother during pregnancy, and antibiotic therapy for the child after birth (Karney 2017; Petrov *et al.* 2021). One of the main factors significantly influencing the composition of gut microbiota is the mode of delivery – newborns delivered by cesarean section do not have contact with the maternal vaginal flora, resulting in a higher likelihood of their gastrointestinal tract being colonized by bacteria such as *Clostridium*, *Enterococcus*, *Staphylococcus*, as well as hospital-acquired bacteria present in the air or on the skin of individuals the baby had contact with during the labor procedure. In comparison, stool samples from infants born vaginally predominantly contain *Enterobacterales* and bacteria from the genera *Bifidobacterium* and *Bacteroides*, which naturally come from the mother's feces (Karney 2017; Panasiuk and Kowalińska 2023). In a study conducted by Mueller et al., 436 mothers and their children

were assessed to examine the influence of the mode of delivery and maternal antibiotic use during pregnancy on the development of obesity in offspring (Mueller *et al.* 2015). The results obtained indicate that children who were exposed to antibiotic treatment during the prenatal period had an 84% higher risk of obesity. Regardless of antibiotic use, it was also demonstrated that the mode of delivery, specifically cesarean section, was associated with a 46% increased risk of obesity in children (Mueller *et al.* 2015). Early colonization of the gastrointestinal tract may have implications for future gut microbiota differentiation and indirectly impact human health, possibly even influencing tendencies toward altered nutrient absorption profiles and, consequently – abnormal body weight.

So far, the dynamics of gut microbiota changes in toddlers are not fully known, mainly as high-interindividual variability is observed. At the age of 3 years, the gut microbiota pattern becomes more adult-like (D'Argenio and Salvatore 2015). The gut microbiota in adults also changes with age. An impact of this is a modification of lifestyle, diet, drugs, some diseases and even environmental factors, e.g. comparing older people living in their own houses with those living in nursing homes, more diversity is observed in the first group (Panasiuk and Kowalińska 2023).

4. Diet and gut microbiota

Diet is one of the fundamental factor influencing gut microbiota composition (Leeming *et al.* 2019). In a study conducted by Wu et al., changes in gut microbiota were examined before and after the introduction of two different diets by study participants. Patients were given a high-fat, low-fibre diet and a low-fat, high-fibre diet. Changes in gut microbiota composition were observed as early as 24 hours after starting the new diet. However, the study results showed significant individual variability in both subgroups despite having the same diet (Wu *et al.* 2011). Differences in metabolism and gut microbiota composition have also been observed between vegetarians and non-vegetarians, suggesting a significant impact of the diet on the gut microbiome (Wu *et al.* 2016). Xiao *et al.* conducted research on the modification of gut microbiota in humans – in 93 volunteers with central obesity, a specific pattern of whole grain and prebiotic-based diet was introduced. This dietary modification resulted in a change in the profile of gut microbiota, with a decrease in opportunistic bacteria (*Enterobacteriaceae* and *Desulfovibrionaceae*) accompanied by an increase in beneficial gut barrier-forming bacteria like *Bifidobacteriaceae* (Xiao *et al.* 2014). Thus, it can be concluded that the percentage of macronutrients, including carbohydrates, protein, fats, and fibre influences the individual variability of the gut microbiota. Changes in dietary habits towards the unification and westernization of diets are characterized by increased consumption of monosaccharides and fats and reduced fibre intake, which can negatively affect the gut microbiota. It appears that fibre, especially its insoluble fraction, could potentially influence the diversity of the gut microbiota because it undergoes fermentation after consumption, thus providing nutrition for the microorganisms living in the intestines (Chassaing *et al.* 2017). Other functions of gut microbiota include not only the fermentation of certain food components, which aids in efficient digestion and stimulates intestinal transit but also the production of vitamins and immunomodulatory effects that influence the host's immune system (Tokarz-Deptuła *et al.* 2016; DiBaise *et al.* 2008).

On the other hand, some of the gut microbiota and products of their metabolism may have carcinogenic potential (Mima *et al.* 2017; Honjo *et al.* 2023). There is no confirmed explanation of how the microbiome influences cancer development. Still, one of the most probable hypotheses is that microorganisms modify the immune response by producing substances that affect cell division rate and apoptosis (Al-Ishaq *et al.* 2022).

5. Dysbiosis

Dysbiosis disrupts the microbial community (De Gruttola *et al.* 2016). It can be caused by an improper diet, the use of xenobiotics, antibiotics, or groups of medications used in the treatment of chronic diseases (e.g., methotrexate, proton pump inhibitors, non-steroidal anti-inflammatory drugs). Furthermore, disorders of the gastrointestinal tract, intestinal infections (bacterial, viral, or fungal), as well as certain chronic diseases such as liver diseases, metabolic and endocrine disorders, and psychiatric conditions (chronic stress, anxiety disorders) can also influence the abnormal composition of gut microbial (Shanahan and Murphy 2011; Baohong *et al.* 2017). Dysbiosis leads to impaired intestinal barrier functioning, exacerbating local inflammation and increasing systemic inflammation risk (Lin *et al.* 2022). Moreover, repeated exposure to antibiotics during infancy is linked with a higher risk of obesity (Kesavelu and Jog 2023, Duong *et al.* 2022).

6. Microbiological assessment

The standard method for assessing gut microbiota is still the conventional bacterial culture (Karlsson *et al.* 2013, D'Argenio and Salvatore 2015). However, this method is somewhat limited because it may favor

the selection of some species. And in addition, some microbes are uncultivable (D'Argenio and Salvatore 2015). Moreover, genetic analysis methods based on PCR testing are more accurate and preferable due to the many species involved. Such tests allow for a collective identification of the gut microbiota genome based on extracted DNA samples (Sarangi *et al.* 2019). In recent years, a new technology has become preferred – Next Generation Sequencing (NGS). The method represents an innovative approach applied to the sequencing of DNA and RNA, as well as the identification of variants and mutations. NGS can rapidly sequence hundreds or thousands of genes or an entire genome. The identified sequence variations and mutations through NGS are extensively utilized in disease diagnosis, prognosis, therapeutic decision-making, and patient follow-up. Its extensive parallel sequencing capacity opens up new possibilities for personalized precision medicine, simultaneously reducing sequencing costs (D'Argenio and Salvatore 2015, Behjati and Tarpey 2013). It is important to note that the gut microbiome differs depending on the segment of the digestive tract. This is due to varying conditions present in each segment. The main factors determining these changes include the pH of the environment, the speed of peristalsis, and the types of digestive enzymes and intestinal hormones secreted. The most densely colonized segment by microorganisms is the terminal part of the digestive tract – the large intestine (Canny and McCormick 2008).

7. Gut microbiota in patients with obesity and normal body weight

Previous studies indicate that the microbiota initially differs between obese individuals and those with a normal body mass (Ley *et al.* 2006; Dominianni *et al.* 2015; Bervoets *et al.* 2013). These studies showed that individuals with a normal body mass have more Bacteroidetes bacteria than Firmicutes. Examples of these bacteria species can be found in Table II. Additionally, it has been observed that the gut microbiota of individuals with higher BMI is characterized by a lower abundance of *Rikenellaceae*, *Alistipes finegoldii*, and *Alistipes senegalensis* bacteria (Zhernakova *et al.* 2016). In another study, a 20% increase in the abundance of Firmicutes bacteria and a decrease in Bacteroidetes bacteria were

Table II
Examples of Firmicutes and Bacteroidetes affecting the body mass alteration.

Species of bacteria FIRMICUTES (examples)	Species of bacteria BACTEROIDETES (examples)
Faecalibacterium prausnitzii	Bacteroides fragilis
Clostridium spp.	Bacteroides vulgaris
Roseburia intestinalis	*Bacteroides uniformis*
Blautia obeum	*Prevotella* spp.
Lactobacillus reuteri	*Alistipes finegoldii*
Enterococcus faecium	*Parabacteroides distasonis*
Staphylococcus leei	

associated with an increased energy harvest from food by up to 150 kcal (Jumpertz *et al.* 2011). Furthermore, in individuals whose diet is fibre-rich, their gut microbiota can produce more short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate (Hur *et al.* 2015). The presence of those substances promotes lower weight gain by influencing metabolic pathways and intestinal hormone activity (Hur *et al.* 2015). SCFA, despite being an energy substrate, also influences the regulation of hunger and satiety by affecting hormones responsible for these sensations – mainly ghrelin and peptide YY (Fig. 1). The development of obesity may also be influenced by Pseudomonadota (Proteobacteria) *(e.g.* family *Enterobacteriaceae)*, which are responsible for producing pro-inflammatory substances and predispose to increased energy storage in the form of fat (Rinninella *et al.* 2019; Bai *et al.* 2019; Rizzatti *et al.* 2017).

A study by Kalliomäki et al. demonstrated that an abnormal composition of gut microbiota precedes the development of overweight and obesity. The study found that the number of *Bifidobacteria* in fecal samples from infancy was higher in 7-year-old patients with a normal body mass compared to children who are overweight. Additionally, abnormalities were observed in the increased presence of *Staphylococcus aureus* in fecal samples from infancy in patients who were overweight compared to patients with normal weight (Kalliomäki *et al*. 2008).

Turngbauh *et al.* demonstrated that differences in energy extraction from consumed food may depend on the type of gut microbiota. Mice that received transplants of bacteria from obese individuals gained weight

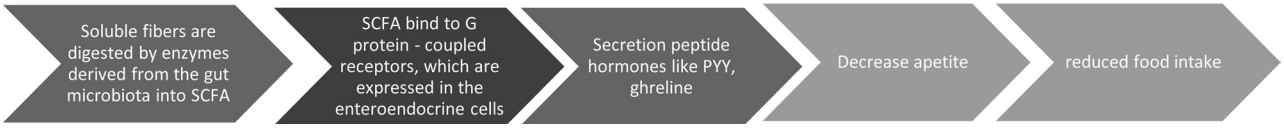

Fig. 1. Correlation of diet fibre intake and SCFA (short-chain fatty acids) production leading to food intake control.

Table III
Bacteria promoting obesity and normal Body mass.

Gut microbiota corresponding with obesity/overweight	Ref	Gut microbiota in normal BMI	Ref
Firmicutes/*Bacteroidetes* ratio increased	(Turnbaugh *et al.* 2006)	Supplementation with *Akkermansia muciniphila* reduced body weight	(Depommier *et al.* 2019)
Lower level of two species from the family Rikenellaceae, *Alistipes finegoldii* and *Alistipes senegalensis*	(Zhernakova *et al.* 2016)	Increased levels of *Bifidobacterium animals, Lactobacillus paracasei, Lactobacillus plantarum*	(Million *et al.* 2012)
Reduced level of *Methanobrevibacter smithii*	(Million *et al.* 2012)	*Lactobacillus* and *Bifidobacterium* showed results in reducing body weight	(Álvarez-Arraño *et al.* 2021)
Higher levels of *Lactobacillus reuteri*	(Million *et al.* 2012)	*Bifidobacterium infantis, L. acidofilus reducing body weight*	(Chang *et al.* 2011)

more quickly than mice that received gut microbiota transplants from individuals with a normal starting body mass (Turnbaugh *et al.* 2006). In another study on gut microbiota, twins with different body masses (obese vs. normal BMI) were examined. Fecal samples were taken from both female twins and were transplanted into mice. It was followed by introducing those animals to an identical diet containing low fat and high fibre content. The study showed that mice receiving fecal transplants from a woman with a normal BMI had a greater tendency towards a normal body mass than mice receiving gut microbiota from an obese patient (Ridaura *et al.* 2013). Therefore, it appears that gut microbiota may influence the phenotype of the host organism (Ridaura *et al.* 2013; Walker and Parkhill 2013).

In Table III, some examples of bacteria that promote obesity or normal body mass are presented. It is demonstrated that the perturbed ratio of Bacterioidetes/Firmicutes phylum is linked with increased intestinal permeability, triggering subsequent inflammation characteristic of diabetes (Iatcu *et al.* 2021). People who suffer from obesity and type 2 diabetes have lower level of *Faecalibacterium prausnitzii* (phylum Firmicutes) than individuals with obesity but without diabetes (Pokrzywnicka and Gumprecht 2016). Larsen *et al.* also showed that obesity patients with diabetes mellitus have lower level of Firmicutes compared to obesity patients without diabetes (Larsen *et al.* 2010).

8. Use of probiotics in obese patients

Based on the studies conducted so far regarding the correlation of modifying the gut microbiota with body mass reduction, it can be speculated that specific bacterial strains could potentially promote or treat obesity. Therefore, attempts have been made to implement new therapeutic strategies involving probiotics in obesity treatment (Cerdó *et al.* 2019; Abenavoli *et al.* 2019). According to The International Scientific Association for Probiotics and Prebiotics, probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill *et al.* 2014). Sources have mainly focused on the bacteria strains of *Lactobacillus* (primarily *L. casei*, *L. gasseri*, *L. rhamnosus*, *L. plantarum*) and *Bifidobacterium* (*B. infantis*, *B. longum*, *B. breve*) (Ejtahed *et al.* 2019). These strains are characterized by low pathogenicity and low antibiotic resistance, making them highly potential with a favorable safety profile (Cerdó *et al.* 2019). Cerdó et al. mentioned that in most studies, rodents (both mice and rats) supplemented with these bacterial strains exhibited less weight gain (Cerdó *et al.* 2019). In a survey conducted by Luoto *et al.*, the impact of a perinatal probiotic intervention on the later risk of overweight and obesity in children was evaluated over a 10-year observation period (Luoto *et al.* 2010). The study involved 159 women randomly given probiotics (colony-forming units of *Lactobacillus rhamnosus*) or a placebo for four weeks before the expected delivery date. Anthropometric measurements (height, weight, and BMI) were taken on the newborns at 3, 6, 12, 24 months, and 4, 7, and 10 years. It was found that probiotic administration resulted in a reduction in excessive weight gain until the 24–48-month period, particularly noticeable in children who were overweight. Thus, modifying the maternal gut microbiota during pregnancy may influence the child's growth pattern. In another study, the effects of *Lactobacillus acidophilus, Lactobacillus casei, and Bifidobacterium bifidum*, given in combination with isoflavones, on weight reduction were assessed (Zhao *et al.* 2012). After the supplementation, a decrease in body weight and a reduction in fat tissue accumulation were observed. However, a similar effect was not achieved with probiotic supplementation alone. The bacterium *Pediococcus pentosaceus LP28* can also have a beneficial impact on obesity treatment. In a study conducted on obese mice, administration of this strain reduced body weight gain and liver lipid content (Zhao *et al.* 2012). In Table IV,

Table IV
Current research regarding the use of probiotics in body mass reduction

Current research regarding the use of probiotics in body mass reduction	
Bifidobacterium infantis, L. acidofilus[H]	(Chang *et al.* 2011)
L. acidophilus in combination with *L. casei* and *Bifidobacterium*[H]	(Hadi *et al.* 2019)
Akkermansia muciniphila[H]	(Depommier *et al.* 2019)
L. curvatus HY7601, L. plantarum KY1032[H]	(Jung *et al.* 2015)
Pediococcus pentosaceus LP28	(Zhao *et al.* 2012)[M]
L. casei, L. gasseri, L. rhamnosus, L. plantarum[M]	(Ejtahed *et al.* 2019)
Lactobacillus acidophilus, *Lactobacillus casei*, and *Bifidobacterium bifidum* were given in combination with isoflavones[M]	(Ali *et al.* 2004)

M – Mice Studies, H – Human Studies

some examples of probiotics that were studied in body mass reduction are mentioned.

When considering gut microbiota in obesity, attention should also be paid to the bacterium *Akkermansia muciniphila* (Derrien *et al.* 2004). This microorganism primarily resides in the colon's mucus layer, and its leading role is the breakdown of mucus. It constitutes about 1–4% of the gut microbiota (Macchione *et al.* 2019). The presence of this bacterium is inversely correlated with the body weight of rodents and humans (Everard *et al.* 2013). In a study conducted on obese mice, a lower number of *A. muciniphila* bacteria was observed. Improvements in metabolic health were achieved in these individuals by modifying the gut microbiota and increasing the abundance of *A. muciniphila* (Everard *et al.* 2013). Other sources have shown that supplementation with this bacterium is associated with increased insulin sensitivity and higher levels of propionic acid (Panasiuk and Kowalińska 2023). An increase in the number of these bacteria in the intestines has also been observed during the use of metformin, which enhances insulin sensitivity (Panasiuk and Kowalińska 2023). Schneeberger et al. demonstrated that the number of *A. muciniphila* bacteria depends on the diet. A high-fat diet leads to a decrease in the number of this bacterium in mice. The study also proved that the level of *A. muciniphila* is inversely proportional to certain indicators of insulin resistance and inflammatory markers (Schneeberger *et al.* 2015).

Unfortunately, studies on the impact of probiotics on weight reduction have not always yielded the expected results. In a study on mice subjected to a high-fat diet (HFD), an attempt to use probiotics *Lactobacillus plantarum DSM 15313* even increased body weight (Cedró *et al.* 2019; Andersson *et al.* 2010). Table V summarizes some studies on the influence of probiotics on body weight.

One potential target of treatment obesity was Fecal Microbiota Transplantation (FMT). This treatment consists of attempting stool of a healthy lean donor and transplanting it into the patient's duodenum or large intestine using endoscopy methods (Pokrzywnicka and

Table 5
Summary of effects of probiotics on body weight

Source	No. of subjects	Study subject	Duration	Bacteria	Delta weight
1. Depommier *et al.* (2019)	32	Human	3 months	Akkermansia muciniphila	–2,27 kg +/– 0,92 kg
2. Jung *et al.* (2015)	120	Human	12 weeks	Lactobacillus curvatus HY7601, Lactobacillus plantarum KY1032	–0,65 kg +/– 0,23 kg
3. Andersson *et al.* (2010)	20	Mice	20 weeks	Lactobacillus plantarum DSM 15313	+21,5 g +/– 1,1 g
4. Chang *et al.* (2011)	101	Human	8 weeks	mixture of Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacterium infantis and extra-ingredients containing Bifidobacterium breve (CBG-C2), Enterococcus faecalis FK-23	–0,24 kg +/– 1,5 kg
5. Yin *et al.* (2010)	48	Rats	6 weeks	Bifidobasteria M13 –4	+51,72 g
6. Hadi *et al.* (2019)	60	Human	8 weeks	Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum + inulina	–4,01 +/– 4,05 kg

Gumprecht 2016). Sources mentioned that FTM results were slight improvements in insulin sensitivity, lipid metabolism and abdominal adiposity but haven't significantly impacted body weight (Lahtinen *et al.* 2022).

9. Conclusions

The obesity epidemic is a significant problem in modern society. Since the primary causes of obesity are primarily related to unhealthy dietary habits and associated disorders, the fundamental form of treatment involves modifying these factors. The differences in gut microbiota composition between obese and normal-weight individuals suggest that microbiome diagnostics and procedures aimed at altering it may serve as co-factors in obesity treatment. However, a definitive recommendation for this therapy should be withheld due to the inconclusive nature of studies on using specific bacterial strains for treating or assisting in weight management and the lack of high-quality data. Currently, based on the results of the available studies, the use of probiotics in the treatment of obesity can not be recommended. Nevertheless, the potential lying within the study of gut microbiota is substantial and promising, which may lead to a significant increase in interest in this field and a tremendous amount of data soon.

ORCID

Anna Celina Durma https://orcid.org/0000-0001-5021-446X
Adam Daniel Durma https://orcid.org/0000-0001-7103-2577
Adam Śmiałowski https://orcid.org/0009-0006-4394-1072
Leszek Czupryniak https://orcid.org/0000-0003-2396-8885

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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INWAZYJNA LISTERIOZA W EUROPIE – PRZEGLĄD PRZYPADKÓW

Anna Żurawik[1], Paulina Szczesiul-Paszkiewicz[1], Agnieszka Chmielarczyk[2]

[1] Wydział Lekarski, Uniwersytet Jagielloński Collegium Medicum, Kraków, Polska
[2] Katedra Mikrobiologii, Wydział Lekarski, Uniwersytet Jagielloński Collegium Medicum, Kraków, Polska

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Streszczenie: Pałeczki *Listeria monocytogenes* są znaną przyczyną nieinwazyjnej listeriozy przewodu pokarmowego przebiegającej z biegunką, rzadziej powodują one zakażenia inwazyjne: zapalenie opon mózgowo-rdzeniowych i mózgu, bakteriemię i jej powikłania: zapalenie wsierdzia, zapalenia wewnątrzgałkowe oka i inne. Ryzyko zakażenia człowieka jest głównie związane ze spożyciem zanieczyszczonej tymi pałeczkami żywności. Według danych Europejskiego Urzędu ds. Bezpieczeństwa Żywności (EFSA) zakażenia o etiologii *L. monocytogenes* powodowały najwyższy odsetek hospitalizacji i zgonów wśród chorób odzwierzęcych zgłoszonych w 2022 roku. W raporcie Europejskiego Centrum do spraw Zapobiegania i Kontroli Chorób (ECDC) w 2021 roku zanotowano 0,44 przypadków listeriozy na 100 tys. mieszkańców, a najwyższy wskaźnik zapadalności dotyczył osób powyżej 64 lat i wynosił 1,7.

Celem pracy był przegląd i opis przypadków inwazyjnej listeriozy, którą zdiagnozowano u dorosłych pacjentów w Europie w latach 2010–2023. Uwzględniono 52 przypadki choroby, z których 19 stanowiły zapalenia opon mózgowo-rdzeniowych, w 13 wykazano bakteriemię, dziewięć związanych było z zapaleniem wsierdzia, a 10 z zapaleniem wnętrza gałki ocznej. Jeden przypadek miał postać kliniczną zapalenia wątroby. Tylko w siedmiu przypadkach zakażenie *L. monocytogenes* powiązano ze spożyciem zanieczyszczonej nimi żywności. Jedenaście przypadków zakończyło się zgonem pacjenta.

Inwazyjna listerioza jest trudną w leczeniu infekcją ze znacznym wskaźnikiem śmiertelności i wymaga zdecydowanego działania poprzez monitorowanie rozpowszechnienia i stopnia zanieczyszczenia żywności tymi drobnoustrojami i niedopuszczania do powstawania ognisk epidemicznych. Badania dotyczące listeriozy powinny skupiać się na poprawie leczenia klinicznego ciężkich przypadków zakażeń oraz badaniu skomplikowanych mechanizmów ich patogenezy.

INVASIVE LISTERIOSIS IN EUROPE – A CASE REVIEW

Abstract: *Listeria monocytogenes* bacilli are a known cause of non-invasive gastrointestinal listeriosis with diarrhea, less frequently they cause invasive infections: meningitis and encephalitis, bacteremia and its complications: endocarditis, intraocular inflammation of the eye and others. The risk of human infection is mainly associated with consuming food contaminated with these bacteria. According to the European Food Safety Authority (EFSA), infections with *L. monocytogenes* etiology caused the highest hospitalizations and deaths among zoonotic diseases reported in 2022. A report by the European Center for Disease Prevention and Control (ECDC) reported 0.44 cases of listeriosis per 100,000 population in 2021, with the highest incidence rate for people over 64 years old at 1.7.

The study aimed to review and describe cases of invasive listeriosis diagnosed in adult patients in Europe between 2010 and 2023. Fifty-two cases of the disease were included, of which 19 were meningitis, 13 showed bacteremia, nine were associated with endocarditis and 10 with intraocular inflammation. One case had a clinical form of hepatitis. Only seven cases of *L. monocytogenes* infection were related to consuming contaminated food. Eleven cases ended in the death of patients.

Invasive listeriosis is a difficult-to-treat infection with a significant mortality rate. It requires decisive action by monitoring the prevalence and extent of food contamination with these microorganisms and preventing epidemic outbreaks. Research on listeriosis should focus on improving the clinical treatment of severe cases of infection and studying the complex mechanisms of their pathogenesis.

Słowa kluczowe: inwazyjna listerioza, *Listeria monocytogenes*, opis przypadku
Keywords: invasive listeriosis, *Listeria monocytogenes*, case report

* Corresponding Author: A. Chmielarczyk, Wydział Lekarski, Uniwersytet Jagielloński Collegium Medicum, Kraków, Polska, e-mail: agnieszka.chmielarczyk@uj.edu.pl

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1. Wprowadzenie

Gram-dodatnie pałeczki *Listeria monocytogenes* są powszechnie obecne w środowisku, przede wszystkim w wodzie, glebie i na gnijących roślinach. Są to bakterie psychrotolerancyjne, zdolne do wzrostu w szerokim zakresie temperatur (1–45°C), przy czym optymalna temperatura mieści się w przedziale 30–37°C. Głównym źródłem zakażenia jest dla człowieka żywność, między innymi miękkie sery, surowe mięso, warzywa i mrożonki oraz sałatki i inne dania przygotowane przemysłowo jako gotowe do spożycia. Bakterie te mogą się bowiem mnożyć w warunkach chłodniczych. Złe praktyki higieniczne i nieodpowiednie stosowanie procedur w zakresie higieny w przemyśle spożywczym mogą doprowadzać do zakażeń, w tym także epidemicznych. Pałeczki te mogą być przyczyną nieinwazyjnej listeriozy przewodu pokarmowego, przebiegającej bez znaczących objawów lub z biegunką i bólem brzucha. Objawy zakażenia są często niespecyficzne, z reguły obejmują gorączkę i ogólne złe samopoczucie. Rzadziej *L. monocytogenes* powoduje zakażenia inwazyjne: zapalenie opon mózgowo-rdzeniowych i mózgu, bakteriemię i jej powikłania takie jak zapalenie wsierdzia czy zapalenie kości i stawów (Lorber 2010). Zakażenia inwazyjne dotykają głównie pacjentów z obniżoną odpornością, nowotworami i chorobami przewlekłymi. Listerioza może doprowadzić do poronień u kobiet w ciąży i stanowić groźne zakażenie noworodków. Zagrożone mogą być osoby przyjmujące leki neutralizujące kwas żołądkowy, a także pacjenci z przewlekłą niewydolnością nerek i marskością wątroby (Ramaswamy *et al*. 2007).

L. monocytogenes nie znajduje się w czołówce listy patogenów przenoszonych przez żywność, ale, według danych Europejskiego Urzędu ds. Bezpieczeństwa Żywności (EFSA), zakażenia tymi pałeczkami były chorobami odzwierzęcymi z najwyższym odsetkiem zarówno hospitalizacji pacjentów, jak i ich zgonów wśród zgłoszonych w 2022 roku. Choroba ta stanowi poważne zagrożenie dla zdrowia publicznego także dlatego, że może występować w postaci ognisk epidemicznych. Śmiertelność w przypadku zakażeń ogólnoustrojowych wynosi 20–30% (WHO 2023). W wyniku działania subletalnych stężeń środków przeciwdrobnoustrojowych pałeczki *L. monocytogenes* mogą rozwinąć oporność i stanowić problem terapeutyczny (Olaimat *et al.* 2018).

Według raportu Europejskiego Centrum do spraw Zapobiegania i Kontroli Chorób (ECDC) w Europie w 2021 roku odnotowano 0,44 przypadku listeriozy na 100 000 mieszkańców, przy czym najwyższy wskaźnik zapadalności (1,7) dotyczył osób powyżej 64 roku życia. W latach 2010–2014 w Europie zauważalny był stały wzrost liczby potwierdzonych przypadków listeriozy co było wiązane ze zwiększonym korzystaniem z przetworzonej przemysłowo żywności gotowej do spożycia i względnym wzrostem liczebności populacji szczególnie podatnej – osób z obniżoną odpornością i osób starszych (Koopmans *et al.* 2023). Obecnie liczba przypadków listeriozy zgłaszanych rocznie w Unii Europejskiej jest stabilna, a najwyższa częstość jej występowania notowana jest w Finlandii, Islandii i Danii. W Polsce zauważalna jest tendencja rosnąca – w 2023 roku zanotowano 242 przypadki, podczas gdy w 2022 było ich 142, a w 2015 – 70 (dane epidemiologiczne NIZP – PZH 2023).

Celem tej publikacji jest przedstawienie przeglądu przypadków inwazyjnej listeriozy zdiagnozowanej u dorosłych pacjentów ostatnich latach, jej objawów klinicznych oraz sposobów leczenia, a także przedstawienie mechanizmu rozwoju tych infekcji.

2. Metoda i kryteria poszukiwania i wyboru danych

Publikację przygotowano wykorzystując internetową bazę danych PubMed. Wyszukiwaniem zostały objęte artykuły typu „case report" opublikowane w latach 2010–2023. W wyszukiwaniu wykorzystano następujące słowa kluczowe: „*Listeria monocytogenes* AND jedno z następujących: bacteriemia, sepsis, myocarditis, endocarditis, hepatitis, endopthalmitis" oraz „meningitis AND case", a także „invasive listeriosis AND case".

Na podstawie treści abstraktów do analizy w tej pracy wybrano tylko artykuły opisujące przypadki zakażeń dotyczące osób dorosłych – powyżej 18 roku życia, które wystąpiły na terenie Europy. Wykluczono artykuły opisujące zakażenia *L. monocytogenes* kobiet ciężarnych związane z poronieniem, a także zakażenia noworodków i dzieci. Nie włączono też do analizy prac dotyczących zakażeń zwierząt, a także dużych przeglądów stanowiących wieloletnie obserwacje epidemiologiczne poszczególnych państw.

Ostatecznie do przeglądu zakwalifikowano 44 a odrzucono około 780 publikacji. Opisane przypadki zostały przeanalizowane pod kątem cech i przebiegu klinicznego zakażenia a także wieku pacjentów i ich chorób współistniejących oraz identyfikacji źródeł zakażenia.

3. Mechanizm rozwoju inwazyjnych zakażeń *Listeria monocytogenes*

Pałeczki *L. monocytogenes* są oporne na kwaśne środowisko w żołądku co pozwala im przedostać się do jelit, tam zaś wykazują oporność na działanie enzymów proteolitycznych i kwasów żółciowych (Lewańska *et al*. 2018). Chorobotwórczość tych pałeczek jest przede wszystkim związana ze zdolnością namnażania wewnątrzkomórkowego i tym samym unikania mechanizmów obronnych organizmu gospodarza. Bakterie

mogą wnikać nie tylko do nabłonka jelitowego, ale także do innych komórek w tym do śródbłonka naczyń czy wątroby. Mnożenie w tych warunkach jest możliwe dzięki białku PrfA (dodatni czynnik regulacyjny A). Jest to czynnik transkrypcyjny, który umożliwia przejście patogenu ze stadium saprofitycznego (mnożenia w środowisku) do stadium infekcyjnego. Ekspresja PrfA podlega termoregulacji i uaktywnia się w temperaturze 37°C (Bagatella *et al*. 2022).

Pierwszym etapem zakażenia jest indukcja fagocytozy, która rozpoczyna wnikanie bakterii do enterocytów. Opisano szereg białek powierzchniowych (InI – internalin) biorących udział na różnych etapach tego procesu. Najpierw dochodzi do interakcji białek powierzchniowych patogenu InIA oraz InIB ze specyficznymi receptorami powierzchniowymi komórek gospodarza. Białko InIA wiąże się z E-kadheryną, natomiast białko InIB korzysta z receptorów takich jak gC1qR/p32, Met/HGFR (receptor czynnika wzrostu hepatocytów) oraz GAGs (glikozoaminoglikany). Bakterie po wniknięciu do komórek lokują się w fagosomie, w którym wydzielają listeriolizynę O i fosfolipazę A – enzymy będące ważnymi czynnikami ich wirulencji. Enzymy te umożliwiają bakteriom degradację błony pierwotnego fagosomu, a następnie wniknięcie do cytoplazmy i intensywne w niej namnażanie. Jednocześnie białko bakteryjne ActA indukuje polimeryzację aktyny cytoszkieletu komórek gospodarza. Kolejne cząsteczki aktyny przyłączają się do jednego bieguna bakterii w wyniku czego powstają kurczliwe „ogony aktynowe" wypychające je do kolejnych komórek. Dzięki tej strukturze mają one możliwość przemieszczania się w kierunku błony komórkowej, w której następnie tworzą się uwypuklenia, wypustki, wewnątrz których patogen przenosi się do komórek sąsiadujących. Rozpuszczalne białko patogenu InIC stymuluje tworzenie tych wypustek błonowych, poprzez oddziaływanie z białkami rusztowania komórki gospodarza. Mechanizm ten pośredniczy w rozprzestrzenianiu się *L. monocytogenes* między komórkami. Wewnątrz powstałego kolejnego fagosomu z podwójną membraną, pałeczki uwalniają, obok fosfolipazy A oraz listeriolizyny O, fosfolipazę B – enzymy doprowadzające do jego zniszczenia. Po ucieczce pałeczek do cytoplazmy komórki gospodarza dochodzi do ponowienia całego cyklu, dzięki czemu patogen może się intensywnie namnażać i przemieszczać między komórkami. Po przedostaniu się przez barierę jelitową dochodzi do zakażenia rozsianego i drobnoustrój wraz krwią dociera do kolejnych tkanek. Jest to kluczowy moment w rozwoju choroby, ponieważ jeśli patogen przetrwa i namnoży się wystarczająco, przemieści się do kolejnych narządów organizmu człowieka. Pałeczki *L. monocytogenes* charakteryzują się tropizmem do tkanek płodu co pozwala mu u kobiet ciężarnych pokonać barierę łożyskową (Sołtysiuk *et al*. 2019). Bakterie te mają także powinowactwo do tkanek układu nerwowego (Ireton *et al.* 2021). W ostatnich latach wiele badań poświęcono rozstrzygnięciu w jaki sposób bakterie są w stanie pokonać barierę krew–mózg, powodując zakażenie centralnego układu nerwowego. Według Ghosh i wsp. do penetracji tej bariery przyczynia się bezpośrednio białko InIF, celujące dokładnie w komórki śródbłonka mózgu (Ghosh *et al*. 2018). Należy podkreślić, iż oprócz wyżej wymienionych białek z rodziny internalin pałeczki *L. monocytogenes* syntetyzują jeszcze około 20 innych białek, które jednak są słabiej scharakteryzowane. Według Ireton i wsp. dla dokładniejszego określenia poszczególnych etapów patogenezy listeriozy niezbędna jest dokładna identyfikacja poszczególnych receptorów organizmu gospodarza dla tych mniej poznanych białek (Ireton *et al*. 2021).

W rozwoju zakażeń *L. monocytogenes* ważnym elementem jest również interakcja mitochondrium-fagosom. Zainfekowanie komórek gospodarza, pobudza sygnalizację receptora TLR (ang. toll-like receptor) fagosomu, co sprzyja mobilizacji mitochondriów. W efekcie powinno to ułatwić zabicie bakterii. Mitochondria wykazują również zdolność uwalniania pęcherzyków zawierających reaktywne formy tlenu (ROS, ang. reactive oxygen species) do fagosomów w celu destrukcji patogenu. Tymczasem jak pokazały badania Li i wsp. *L. monocytogenes* ma możliwość takiej modulacji sygnalizacji mitochondrialnej związanej z Ca^{2+}, która pozwala na ich przeżycie wewnątrz komórek. Podstawowym mechanizmem regulującym metabolizm mitochondriów jest bowiem oddziaływanie na niezbędne enzymy uczestniczące w cyklu kwasu trikarboksylowego poprzez sygnalizację mitochondrialną ($mtCa^{2+}$). Pałeczki *L. monocytogenes* umieją przejąć ten system promując swoje przetrwanie (Li *et al.* 2021).

4. Zapalenia opon mózgowo-rdzeniowych (łac. meningitis) powodowane przez *Listeria monocytogenes*

U pacjentów o obniżonej odporności zapalenie opon mózgowo-rdzeniowych wywołane zakażeniem *L. monocytogenes* jest jednym z najbardziej niebezpiecznych obok powodowanego przez *Streptococcus pneumoniae* czy *Neisseria meningitidis*. Do grupy ryzyka należą osoby po przeszczepach, czy leczone kortykosteroidami, ale także osoby starsze po 65 roku życia. Objawy wskazujące na zapalenie opon to m.in. silne bóle głowy, sztywnienie karku, napady padaczkowe, podwyższona temperatura ciała, drżenie mięśni, ataksja oraz drgawki. Obecność patogenu stwierdza się najczęściej we krwi, rzadziej natomiast jest on znajdowany w preparatach z płynu mózgowo-rdzeniowego (Lewańska *et al.* 2018). Leczeniem z wyboru zapalenia opon mózgowo-rdzeniowych

wywołanego przez *L. monocytogenes* jest benzylopenicylina lub trimetoprim/sulfametoksazol u osób z nadwrażliwością na penicylinę. Young i Thomas sugerują, że włączenie do terapii gentamycyny może zmniejszać śmiertelność, ale nie zostało to potwierdzone w badaniach randomizowanych (Young and Thomas 2018).

Na podstawie zgromadzonej literatury opisano 19 przypadków zapalenia opon mózgowo-rdzeniowych wywołanego szczepami *L. monocytogenes* (Tabela I).

We Włoszech w latach 2010–2023 opisano trzy przypadki zapalenia opon mózgowo-rdzeniowych wywołane przez *L. monocytogenes*: najmłodsza pacjentka to 28-letnia kobieta, która po zabiegu cięcia cesarskiego była hospitalizowana ze względu na silne bóle głowy, wysoką gorączkę oraz złe samopoczucie. W posiewie potwierdzono obecność pałeczek listerii (Colomba *et al.* 2020). U kolejnego pacjenta z licznymi chorobami współistniejącymi zdiagnozowano bakteriemię i zapalenie opon mózgowo-rdzeniowych. Pomimo szybkiego wdrożenia leczenia, nastąpiło pogorszenie funkcji życiowych oraz powikłanie z rozwojem sepsy wywołanej *Candida glabrata,* co przyczyniło się do jego zgonu (Grima *et al.* 2022). Barocci i wsp. opisali przypadek 59-letniej kobiety z przebytą chorobą nowotworową oraz z nadciśnieniem tętniczym, która została przyjęta na oddział szpitalny z powodu zaburzeń świadomości, bólu głowy oraz wysokiej gorączki. W próbce płynu mózgowo-rdzeniowego, zidentyfikowano *L. monocytogenes.* Przypuszcza się, że przyjmowanie przez pacjentkę omeprazolu mogło być czynnikiem sprzyjającym zakażeniu, ze względu na jego działanie alkalizujące w żołądku (Barocci *et al.* 2015). Trzy opisane portugalskie przypadki to pacjenci powyżej 50 roku życia z licznymi chorobami współistniejącymi, w trakcie leczenia szpitalnego. U dwojga występowało wrzodziejące zapalenie jelita grubego (Abreu *et al.* 2013, Silva *et al.* 2021). W Hiszpanii Pérez-Pereda i wsp. opisali listeriozę u dwóch mężczyzn z prawidłową odpornością, u których pierwszym objawem był silny ból głowy. U jednego z nich doszło do niedowładu połowiczego lewostronnego. Wykonano nakłucie lędźwiowe, a pałeczki *L. monocytogenes* wykazano w posiewie płynu mózgowo-rdzeniowego. Na tej podstawie autorzy wskazują, że listeriowe zapalenie opon mózgowo-rdzeniowych może się objawiać jako jedynym objawem izolowanym bólem głowy (Pérez-Pereda *et al.* 2020). U jednego z niemieckich pacjentów z niedowładem połowicznym prawostronnym zdiagnozowano ropień mózgu. Mężczyzna w wywiadzie przyznał, że często spożywał niepasteryzowane produkty mleczne, co mogło przyczynić się do rozwoju listeriozy (Steinbrecher *et al.* 2023). Druga opisana w Niemczech pacjentka była 47-letnią kobietą chorującą na stwardnienie rozsiane, która w badaniu zgłosiła ból głowy, gorączkę oraz sztywność karku, a posiew płynu mózgowo-rdzeniowego wykazał obecność *L. monocytogenes* (Rau *et al.* 2015). Dwa przypadki listeriowego zapalenia opon mózgowo-rdzeniowych opisano na Wyspach Owczych. Pierwszy to mężczyzna 74-letni hobbystycznie zajmujący się rolnictwem, ze zdiagnozowanym nadciśnieniem tętniczym, który zgłosił się do szpitala na skutek dezorientacji, bólu głowy oraz gorączki. U pacjenta stwierdzono zapalenie opon mózgowo-rdzeniowych powikłane wodogłowiem, w posiewie płynu wyhodowano *L. monocytogenes*, zakażenie zakończyło się zgonem pacjenta (Gaini 2015). Drugi przypadek to 51-letnia kobieta z autoimmunologicznym zapaleniem wątroby, która została przyjęta do szpitala w związku z silnym bólem głowy, wymiotami oraz gorączką. W płynie mózgowo-rdzeniowym obecne były pałeczki *L. monocytogenes.* Autorzy podkreślają, że jest to pierwszy opisany przypadek współistniejącego zapalenia opon mózgowo-rdzeniowych oraz autoimmunologicznego zapalenia wątroby (Gaini *et al.* 2015). W Polsce w okresie 2010–2023 opisano w piśmiennictwie dwa przypadki inwazyjnej listeriozy związanej z zapaleniem opon mózgowo-rdzeniowych. 55-letnia pacjentka, bez chorób współistniejących, prowadząca zdrowy tryb życia, została przyjęta na oddział szpitalny ze względu na utrzymującą się wysoką temperaturę, silne bóle głowy oraz wymioty. U pacjentki zdiagnozowano ostrą niewydolność oddechową oraz śpiączkę mózgową w wyniku zapalenia opon-mózgowych i towarzyszącej mu sepsy (Godziszewska *et al.* 2015). Drugi przypadek w Polsce to 47-letni mężczyzna hospitalizowany ze względu na silne zaostrzenie przebiegu wrzodziejącego zapalenia jelita grubego. Badanie płynu mózgowo-rdzeniowego wykazało zakażenie *L. monocytogenes*. U pacjenta wykryto ropnie śródmózgowe (Żak-Gołąb *et al.* 2017). Pojedyncze przypadki listeriowego zapalenia opon mózgowo-rdzeniowych opisano także w kilku innych krajach. Na Węgrzech zakażenie dotyczyło pacjenta z nadciśnieniem tętniczym i marskością wątroby (Kocsis *et al.* 2023) a w Rumunii 45-letniego mężczyzny, bez współistniejących chorób. Magiar i wsp. podkreślają, że był to przypadek niezwiązany z występującymi w tym okresie ogniskami listeriozy (Magiar *et al.* 2022). Zapalenie opon mózgowo-rdzeniowych spowodowane przez *L. monocytogenes* zdiagnozowano też u pacjentki z Wielkiej Brytanii bez innych chorób, która w wywiadzie podała, że zajmowała się ogrodem i spożywała warzywa ze swojej działki (Harrington *et al.* 2023). W Serbii zapalenie opon stwierdzono u mężczyzny immunokompetentnego będącego z zawodu rolnikiem (Delić *et al.* 2014). W Grecji zdiagnozowano takie zakażenie współwystępujące z zapaleniem stawów u 65-letniej kobiety. Wyniki dodatnie posiewów otrzymano zarówno z krwi jak i płynu mózgowo-rdzeniowego (Anyfantakis *et al.* 2014). W literaturze znajdziemy także przeglądy przypadków zapalenia opon mózgowo-rdzeniowych w ostatnich latach w różnych krajach np. 27 przypadków opisanych w Holandii, gdzie autorzy

Tabela I
Przypadki zapalenia opon mózgowo-rdzeniowych spowodowanego przez *Listeria monocytogenes*.

Nr	Państwo	Wiek pacjenta	Płeć	Choroby współistniejące	Objawy	Źródło zakażenia/ powiązanie z żywnością	Wynik leczenia	Piśmiennictwo
1	Włochy	28	K	brak	silne bóle głowy, wysoka gorączka, złe samopoczucie, sztywność karku, wycofana pozycja leżąca	nie	pozytywny	Colomba *et al.* (2020)
2	Włochy	bd	M	cukrzyca typu II, niedoczynność tarczycy, nadciśnienie tętnicze, przewlekła niedokrwienna encefalopatia naczyniowa, przewlekła niewydolność nerek, migotanie przedsionków	brak danych	nie	zgon	Grima *et al.* (2022)
3	Włochy	59	K	nadciśnienie tętnicze	zaburzenia świadomości, ból głowy, wysoka gorączka	nie	pozytywny	Barocci *et al.* (2015)
4	Portugalia	69	K	cukrzyca typu II, otyłość, niedoczynność tarczycy, migotanie przedsionków, małopłytkowość	silny ból głowy ze światłowstrętem, gorączka	nie	pozytywny	Silva *et al.* (2021)
5	Portugalia	51	K	wrzodziejące zapalenie jelita grubego	ból głowy, nudności, ból brzucha, gorączka	nie	pozytywny	Abreu *et al.* (2013)
6	Portugalia	69	M	wrzodziejące zapalenie jelita grubego	brak danych	nie	pozytywny	Abreu *et al.* (2013)
7	Hiszpania	40	M	wrzodziejące zapalenie jelita grubego	silny ból głowy	nie	pozytywny	Pérez-Pereda *et al.* (2020)
8	Hiszpania	66	M	cukrzyca typu II, alkoholizm, wymiana zastawki aortalnej	silny ból głowy, niedowład połowiczy lewostronny	nie	zgon	Pérez-Pereda *et al.* (2020)
9	Niemcy	51	M	cukrzyca typu II	niedowład połowiczy prawostronny, porażenie twarzy	spożywanie niepasteryzowanych produktów mlecznych,	pozytywny	Steinbrecher *et al.* (2023)
10	Niemcy	47	K	stwardnienie rozsiane	ból głowy, gorączka, sztywność karku	nie	pozytywny	Rau *et al.* (2015)
11	Wyspy Owcze	51	K	autoimmunologiczne zapalenie wątroby	silny ból głowy, wymioty, gorączka	nie	pozytywny	Gaini (2015)
12	Wyspy Owcze	74	M	nadciśnienie tętnicze	dezorientacja, ból głowy, gorączka	zawód rolnika	zgon	Gaini *et al.* (2015)
13	Polska	55	K	brak	wysoka temperatura, silne bóle głowy, wymioty	nie	pozytywny	Godziszewska *et al.* (2015)
14	Polska	47	M	wrzodziejące zapalenie jelita grubego	silne zaostrzenie choroby przewlekłej	nie	pozytywny	Żak-Gołąb *et al.* (2017)
15	Węgry	72	M	nadciśnienie tętnicze, marskość wątroby	nietrzymanie moczu, wysoka gorączka, osłabienie	nie	pozytywny	Kocsis *et al.* (2023)
16	Rumunia	45	M	brak	zaburzenia widzenia, opadanie lewej powieki, silny ból głowy	nie	pozytywny	Magiar *et al.* (2022)
17	Wielka Brytania	62	K	brak	brak danych	spożywanie warzyw ze swojej działki	brak danych	Harrington *et al.* (2023)
18	Serbia	79	M	brak	silny ból głowy, nudności, wymioty, gorączka	zawód rolnika	pozytywny	Delić *et al.* (2014)
19	Grecja	65	K	brak	wymioty, biegunka, ból głowy, podwyższona temperatura	nie	pozytywny	Anyfantakis *et al.* (2014)

przypuszczają, że główną przyczyną zakażenia mogło być spożycie miękkich serów, parówek, surowych i ugotowanych wędlin, kurczaków lub ryb (van der Voort *et al*. 2019). W wieloośrodkowym badaniu w Hiszpanii wszyscy pacjenci, u których zdiagnozowano pozaszpitalne listeriowe zapalenie opon mózgowo-rdzeniowych mieli powyżej 50 lat oraz cechowali się obniżoną odpornością. Odnotowano u nich triadę objawów: gorączka, sztywność karku a także zaburzenia psychiczne (Amaya-Villar *et al.* 2010).

W opisie ocenianych w tym przeglądzie przypadków średnia wieku pacjentów z zapaleniem opon mózgowo-rdzeniowych spowodowanych przez *L. monocytogenes* wynosiła 57 lat, mediana 57, a mężczyźni stanowili 52,6%. Nieco ponad 68% pacjentów charakteryzowało się wiekiem powyżej 50 lat oraz tyle samo miało inne współistniejące schorzenia. Większość opisanych zdiagnozowanych pacjentów (70%) wykazało podobne dolegliwości, tj.: ból głowy, gorączka, sztywność karku. Powyższa triada objawów jest wskazaniem do wykonania badań posiewu krwi oraz płynu mózgowo-rdzeniowego. Inne objawy były związane z niedowładami oraz światłowstrętem. Z chorób współistniejących najczęściej stwierdzano cukrzycę typu II, nadciśnienie tętnicze i wrzodziejące zapalenie jelita grubego. W większości przypadków nie udało się ustalić źródła zakażenia pałeczkami listerii. U jednego z pacjentów były nim niepasteryzowane produkty mleczne (Steinbrecher *et al*. 2003), a u innej pacjentki warzywa (Harrington *et al.* 2023). Co ciekawe w czterech przypadkach możliwość zakażenia poprzez spożycie zainfekowanej żywności zostało wykluczone (Abreu *et al*. 2013, Gaini *et al.* 2015, Magiar *et al*. 2022). W dwóch przypadkach zakażenie mogło jednak być związane z pracami rolniczymi wykonywanymi zawodowo (Delić *et al*. 2014; Gaini 2015). U trzech z opisanych w cytowanych pracach pacjentów (15,7%) stwierdzono zgon będący skutkiem listeriozy. Podsumowując, u pacjentów z obniżoną odpornością i licznymi chorobami współistniejącymi należy przy charakterystycznych objawach zapalenia opon mózgowo-rdzeniowych należy rozważać zakażenie o etiologii *L. monocytogenes*.

5. Zakażenia łożyska krwi

Bakteriemia i następująca infekcja ogólnoustrojowa jest najczęstszą inwazyjną formą listeriozy, ale nie zawsze jest ona potwierdzana w posiewach krwi (Charlier *et al*. 2017). Objawy obejmują od gorączki, biegunki, dreszczy, bólu mięśni/stawów, tachykardii, aż po objawy septyczne i wstrząs prowadzący do niewydolności wielonarządowej i śmierci. Najbardziej narażone są osoby starsze i osoby będące w immunosupresji z powodu chorób nowotworowych, ale także osoby stosujące leki kortykosteroidowe i pacjenci z cukrzycą (Koopmans *et al*. 2023). Listerioza ogólnoustrojowa wiąże się z wysokim wskaźnikiem śmiertelności wynoszącym od 21 do 46% (Scobie *et al*. 2019; Huang *et al.* 2023). Należy jednak zauważyć, że krótkotrwała obecność bakterii we krwi, może towarzyszyć też innym postaciom klinicznym listeriozy i nie zawsze prowadzi ona do sepsy.

W przestawianym przeglądzie uwzględniono 13 przypadków zdiagnozowanej bakteriemii wywołanej przez *L. monocytogenes* (Tabela II).

De Francesco i wsp. opisali pięć przypadków bakteriemii u pacjentów hospitalizowanych we Włoszech z różnych przyczyn. U pacjenta 73-letniego, cierpiącego na niewydolność nerek, szybko zdiagnozowano bakteriemię a leczenie ampicyliną przyniosło pozytywny wynik. Powiodła się także terapia wankomycyną u pacjenta przyjętego z powodu niewydolności serca, u którego zdiagnozowano bakteriemię *L. monocytogenes* oraz problemy jelitowe wraz z niedrożnością przewodu pokarmowego. Z kolei u 61-letniego mężczyzny z marskością wątroby i zgłaszanymi jednocześnie bólami brzucha i biegunką, bakteriemię *L. monocytogenes* zdiagnozowano dopiero po 25 dniach hospitalizacji. Wdrożone leczenie amoksycyliną nie pozwoliło na opanowanie infekcji, która zakończyła się zgonem pacjenta. Terapia skojarzona (wankomycyna, piperacylina i meropenem) nie przyniosła też efektu u 35-letniego pacjenta z HIV, u którego bakteriemię *L. monocytogenes* zdiagnozowano po 10 dniach pobytu w szpitalu. Powodem hospitalizacji było pogorszenie stanu zdrowia związane z mięsakiem Kaposiego. Z kolei bakteriemia u pacjenta ze szpiczakiem, hospitalizowanego na oddziale hematologicznym została zdiagnozowana dopiero po 15 dniach, ale w tym przypadku zastosowane leczenie ampicyliną i lewofloksacyną doprowadziło do wyleczenia (De Francesco *et al.* 2015). W Hiszpanii cierpiąca na cukrzycę 76-letnia kobieta była hospitalizowana na oddziale hematologicznym z powodu ostrej białaczki szpikowej. Nie zgłaszała innych dolegliwości, także ze strony układu pokarmowego. Zakażenie *L. monocytogenes* stwierdzono w posiewie krwi, jednak pomimo wdrożenia empirycznego skojarzonego leczenia antybiotykowego – podano tobramycynę, imipenem i lewofloksacynę – pacjentka zmarła. U pacjentki z rakiem gruczołowym, która także zmarła, również nie wykazano dolegliwości ze strony przewodu pokarmowego ani podwyższonej temperatury. U pacjentki tej była stosowana chemioterapia z powodu nowotworu a leczenie zakażenia rozpoczęto stosując empirycznie ceftazydym i amikacynę, które po uzyskaniu wyniku badania mikrobiologicznego zmieniono na ampicylinę i gentamycynę (Martín-Fortea *et al*. 2020). U innych pacjentów z Hiszpanii opisywano objawy ze strony przewodu pokarmowego i choroby współistniejące, wśród których była miastenia, rak prostaty czy cukrzyca (Martín-Fortea

et al. 2020). U 86-letniego pacjenta z chorobą naczyń obwodowych, u którego wykonano przeszczep udowo-podkolanowy, pałeczki *L. monocytogenes* wyizolowano z posiewu krwi oraz z płynu okołoprotezowego. Z czynników ryzyka typowych dla listeriozy, jedynym jaki brano pod uwagę było spożywanie mięsa z lokalnego sklepu mięsnego (Chavada *et al*. 2014). Przypadek opisany w Niemczech to 62-letni mężczyzna hospitalizowany z powodu migotania przedsionków. Rozpoznano u niego również biegunkę, której towarzyszyły gorączka, dreszcze oraz zły stan ogólny. W wywiadzie pacjent zgłosił chorobę wieńcową i wrzodziejące zapalenie jelita grubego. Wykonane posiewy krwi potwierdziły bakteriemię wywołaną *L. monocytogenes*. W leczeniu zastosowano terapię ampicyliną a po dwóch tygodniach pacjent został wypisany ze szpitala w stanie ogólnym dobrym. Autorzy podają, że przypadek ten był jednym z niewielu opisujących taką bakteriemię w powiązaniu z wrzodziejącym zapaleniem jelita grubego. Podczas badania endoskopowego w jelicie grubym zauważono liczne, uniesione nadżerki z wysiękiem żółtym, nietypowym dla tej choroby. Autorzy zwracają uwagę na potrzebę wykonywania posiewów krwi i kału, które w takich przypadkach mogą wykazać zakażenie *L. monocytogenes* w przebiegu wrzodziejącego zapalenia jelita grubego (Kassalik *et al*. 2012). W Portugalii opisano złożony i rzadki przypadek 40-letniej pacjentki, u której bakteriemia towarzyszyła zapaleniu jelita grubego. Pacjentka była przyjęta do szpitala z powodu nasilających się krwawych biegunek. W posiewach z krwi wykryto *L. monocytogenes*, ale jednocześnie w tkance okrężnicy stwierdzono obecność wirusa cytomegalii. Stan pacjentki poprawił się po 21 dniach antybiotykoterapii ampicyliną i gentamycyną oraz 15 dniach terapii walgancyklowirem (Santos-Antunes *et al.* 2014). Bakteriemię *L. monocytogenes* oraz jednocześnie zapalenie opon mózgowo-rdzeniowych zdiagnozowano u pacjenta z Rumunii. Przyjęto go na oddział chorób zakaźnych z gorączką, bólem głowy, ataksją oraz podwójnym widzeniem, bradykardią oraz objawami podrażnienia opon mózgowo-rdzeniowych. Posiewy z krwi oraz płynu mózgowo rdzeniowego w kierunku obecności *L. monocytogenes* były dodatnie. Pacjent leczony był ampicyliną oraz ciprofloksacyną, ale po 12 dniach zmarł.

Analizując wszystkie opisane tu przypadki widać, że dotyczyły osób powyżej 60 roku życia. Średnia wieku pacjentów wynosiła 65,3 a mediana 65. Wśród chorych 69,2% stanowili mężczyźni. Wśród 13 pacjentów z bakteriemią czworo chorowało na nowotwór. Pięciu pacjentów (38,5%) zmarło. Za czynniki ryzyka zgonu z powodu bakteriemii *L. monocytogenes* są uważane zaawansowany wiek, aktywne nowotwory złośliwe, płeć żeńska, utrata masy ciała, niewyrównane choroby współistniejące, niewydolność wielonarządowa oraz nieodpowiednia, początkowa empiryczna antybiotykoterapia, stosowanie kortykosteroidów i wstrząs jako forma manifestacji u pacjentów z guzami narządów litych (Charlier *et al*. 2017). Jeśli chodzi o źródło zakażenia, tylko w jednym przypadku powiązano je ze spożywaniem mięsa (Chavada *et al.* 2014), chociaż w przypadku czworga pacjentów wystąpiły objawy ze strony przewodu pokarmowego (Tabela II). Powikłaniami bakteriemii u pacjentów mogą być omówione w dalszej części publikacji infekcyjne zapalenie wsierdzia, zapalenie wątroby czy endogenne zakażenie wnętrza gałki ocznej.

6. Infekcyjne zapalenie wsierdzia (łac. endocarditis)

Infekcyjne zapalenie wsierdzia definiuje się chorobę zapalną rozwijającą się w związku z obecnością drobnoustrojów (bakterii lub rzadko grzybów) na wewnętrznej wyściółce komór serca i na zastawkach, ale także na śródbłonku dużych naczyń klatki piersiowej (np. zwężonej cieśni aorty) lub w obrębie połączeń naczyniowych. W przebiegu zapalenia dochodzi do agregacji płytek krwi oraz komórek zapalnych, fibryny oraz przerwania płatków zastawek serca. Do najczęściej wykrywanych patogenów należą *Streptococcus „viridans"* (paciorkowce zieleniejące) i *Staphylococcus aureus* lub *Staphylococcus epidermidis* (Khan and Hollenberg 2017). Zapalenie wsierdzia powodowane przez *L. monocytogenes* jest rzadkim, poważnym powikłaniem dotyczącym zastawek naturalnych lub protezowych, występującym u około 8% pacjentów z listeriozą, z częstymi powikłaniami zatorowymi i związaną z nimi wysoką śmiertelnością na poziomie 31–41% (Summa and Walker 2010). Zapalenie wsierdzia wywołane przez *L. monocytogenes* zostało po raz pierwszy opisane w roku 1955 (Hoeprich and Chernoff 1955). Do roku 2008 liczba opisanych przypadków wyniosła 68 (Antolín *et al.* 2008). Zapalenie wsierdzia jest rzadką, ale bardzo poważną manifestacją zakażeń wywołanych przez *L. monocytogenes,* jednak optymalna strategia jego leczenia nie została jeszcze ustalona. Obecne strategie antybiotykowe w leczeniu listeriozy obejmują monoterapię penicyliną G lub ampicyliną lub terapię skojarzoną ampicyliną z gentamycyną (Kumaraswamy *et al*. 2018).

W przestawianym przeglądzie uwzględniono dziewięć przypadków zapalenia wsierdzia wywołanego przez *L. monocytogenes* (Tabela III).

W Hiszpanii w okresie 2010–2023 opisano trzy przypadki infekcyjnego zapalenia wsierdzia o tej etiologii. Pierwszy to 36-letnia kobieta, która 5 miesięcy wcześniej była w ciąży a zgłosiła się do szpitala z artretyzmem kostki, bólem wielostawowym i nawracającą gorączką. Badanie lekarskie poza zapaleniem stawów ujawniło szmer rozkurczowy w okolicy aorty i trzeszczenia w podstawach obu płuc. W badaniu echokardiograficznym ujawniono częściowo zwapnioną zastawkę

Tabela II
Przypadki zakażenia krwi spowodowanego przez *Listeria monocytogenes*

Nr	Państwo	Wiek pacjenta	Płeć	Choroby współistniejące	Objawy	Źródło zakażenia/ powiązanie z żywnością	Wynik leczenia	Piśmiennictwo
1	Włochy	73	M	przewlekła niewydolność nerek	gorączka, niewydolność nerek	nie	pozytywny	de Francesco *et al.* (2015)
2	Włochy	65	M	choroba serca	niewydolność serca, niedrożność przewodu pokarmowego	nie	pozytywny	de Francesco *et al.* (2015)
3	Włochy	61	M	marskość wątroby	bóle brzucha, biegunka	nie	zgon	de Francesco *et al.* (2015)
4	Włochy	35	M	HIV, mięsak Kaposiego	pogorszenie stanu ogólnego	nie	zgon	de Francesco *et al.* (2015)
5	Włochy	54	M	szpiczak	gorączka	nie	pozytywny	de Francesco *et al.* (2015)
6	Hiszpania	76	K	białaczka szpikowa, cukrzyca	pogorszenie stanu ogólnego	nie	zgon	Martín-Fortea *et al.* (2020)
7	Hiszpania	77	K	gruczolakorak płuc	pogorszenie stanu ogólnego	nie	zgon	Martín-Fortea *et al.* (2020)
8	Hiszpania	77	K	miastenia gravis	objawy ze strony p.pokarmowego	nie	pozytywny	Martín-Fortea *et al.* (2020)
9	Hiszpania	82	M	nowotwór prostaty	bd	nie	pozytywny	Martín-Fortea *et al.* (2020)
10	Hiszpania	86	M	zapalenie naczyń obwodowych	bd	spożywanie mięsa z lokalnego sklepu mięsnego	pozytywny	Chavada *et al.* (2014)
11	Niemcy	62	M	choroba wieńcowa, wrzodziejące zapalenie jelita grubego	migotanie przedsionków, biegunka, gorączka, zły stan ogólny	nie	pozytywny	Kassalik *et al.* (2012)
12	Portugalia	40	K	zapalenie jelita grubego,	krwawe biegunki	nie	pozytywny	Santos-Antunes *et al.* (2014)
13	Rumunia	62	M	brak	gorączka, ból głowy, ataksja, podwójne widzenie, bradykardia, podrażnienie opon mózgowo-rdzeniowych	nie	zgon	Teodor *et al.* (2012)

Tabela III
Przypadki zapalenia wsierdzia spowodowanego przez *Listeria monocytogenes*.

Nr	Państwo	Wiek pacjenta	Płeć	Choroby współistniejące	Objawy	Źródło zakażenia/ powiązanie z żywnością	Wynik leczenia	Piśmiennictwo
1	Hiszpania	36	K	dwupłatkowa zastawka aortalna	gorączka, ból stawów	brak	pozytywny	Gallego-Flores *et al.* (2016)
2	Hiszpania	74	M	przewlekłe zapalenie żołądka, reumatyczna chorobę serca z zajęciem aorty i zastawek, migotanie przedsionków	gorączka, pogorszenie stanu ogólnego	brak	zgon	Fuertes *et al.* (2012)
3	Hiszpania	58	K	protezowanie zastawek	gorączka, nagły ból głowy	brak	zgon	Fuertes *et al.* (2012)
4	Niemcy	74	M	wymianę zastawki mitralnej, operacja wszczepiania rozrusznika	wysoka gorączka, ogólne osłabienie, zaburzenia krzepnięcia	brak	brak danych	Marschner *et al.* (2023)
5	Niemcy	38	M	przeszczep wątroby oraz wstawienie zastawki mitralnej	objawy ze strony układu pokarmowego, dreszcze, problemy z oddawaniem moczu	częste spożywanie surowego mięsa	pozytywny	Dölle *et al.* (2022)
6	Dania	70	M	operacje zastawek, cukrzyca typu II, przebyty udar, reumatoidalne zapalenie stawów, przewlekła obturacyjną chorobę płuc, przewlekłe migotanie przedsionków, wszczepiony rozrusznik serca	gorączka, narastająca duszność, zmęczenie, zły stan ogólny	spożycie mięsa, powiązanie z epidemią infekcji pokarmowej	zgon	Frydland *et al.* (2014)
7	Francja	44	K	parastezje kończyn	tygodniowe zmęczenie, wysoka gorączka, łagodny ból w klatce piersiowej	brak	pozytywny	Berthelot *et al.* (2012)
8	Włochy	74	M	HIV	gorączka i kołatania serca	brak	pozytywny	Di Cori *et al.* (2012)
9	Holandia	74	M	dializy, operacja bajpasów wieńcowych, wszczepienie protezy aortalnej	gorączka, dreszcze, zapalenie płuc	brak	pozytywny	Valckx *et al.* (2017)

i ciężką niedomykalność zastawki aortalnej bez cech niewydolności serca. Z próbek krwi wyizolowano *L. monocytogenes*. Po 3 tygodniach antybiotykoterapii zastawkę aortalną zastąpiono mechaniczną zastawką protetyczną (Gallego-Flores *et al*. 2016). Fuertes i wsp. opisali dwa inne przypadki w Hiszpanii, oba zakończone zgonem. 74-letni mężczyzna przyjęty do szpitala z gorączką i pogorszeniem stanu ogólnego miał w wywiadzie przewlekłe zapalenie żołądka i reumatyczną chorobę serca z zajęciem aorty i zastawek oraz migotanie przedsionków podczas leczenia przeciwzakrzepowego. Z posiewów krwi wyhodowano *L. monocytogenes*, a w badaniu echokardiograficznym ujawniono brodawkę na zastawce aortalnej. Stan pacjenta pogarszał się, doszło do niewydolności nerek i zastoju w wątrobie, zdecydowano się na operację kardiochirurgiczną, jednak pacjent zmarł w ósmej dobie po interwencji. Kolejny to przypadek 58-letniej kobiety, którą przyjęto z powodu gorączki i nagłego bólu głowy. Pacjentka ta w wywiadzie zgłaszała zabieg protezowania zastawek. Nie stwierdzono bakterii w płynie mózgowo-rdzeniowym, ale posiewy krwi dały wynik dodatni. Echokardiogram przezprzełykowy wykazał dwie wegetacje na zastawce mitralnej. Wdrożono leczenie antybiotykowe i przeprowadzono operację w 19 dobie, ale pacjentka zmarła w wyniku powikłań pooperacyjnych (Fuertes *et al*. 2012). W Niemczech w 2022 roku opisano dwa przypadki infekcyjnego zapalenia wsierdzia. 74-letni mężczyzna zgłosił się na szpitalny oddział ratunkowy z powodu wysokiej gorączki, ogólnego osłabienia i z zaburzeniami krzepnięcia. W przeszłości przeszedł wymianę zastawki mitralnej oraz operacje wszczepiania rozrusznika. W badaniach u pacjenta wykazano zapalenie wsierdzia, zastawki mitralnej oraz obecność ropnia. Wykonano posiewy z krwi i wyhodowano *L. monocytogenes*. Konieczna była interwencja chirurgiczna, wdrożono także skuteczna antybiotykoterapię (Marschner *et al.* 2023). W drugim przypadku, 34-letni mężczyzna był hospitalizowany z powodu wstrząsu septycznego, któremu towarzyszyły niespecyficzne objawy ze strony układu pokarmowego, dreszcze oraz problemy z oddawaniem moczu. Pacjent w przeszłości przeszedł przeszczep wątroby oraz wstawienie zastawki mitralnej. Zdiagnozowano zapalenie wsierdzia, a w posiewie krwi – *L. monocytogenes*. Po terapii ampicyliną stan pacjenta uległ poprawie. W wywiadzie pacjent wskazał na regularne spożywanie surowego mięsa (Dölle *et al*. 2022). W Danii opisano przypadek 70-letniego pacjenta z chorobami towarzyszącymi (cukrzyca typu II, przebyty udar, reumatoidalne zapalenie stawów leczone metotreksatem, przewlekła obturacyjna choroba płuc, przewlekłe migotanie przedsionków, wszczepiony rozrusznik serca). Pacjenta przyjęto szpitala po czterech miesiącach od operacji zastawki z gorączką, narastającą dusznością, zmęczeniem i złym stanem ogólnym. Stwierdzono zapalenie płuc i zakażenie mostka, a w posiewie krwi wykryto *L. monocytogenes*. Rozpoczęto leczenie meropenemem i ciprofloksacyną, jednak ze względu na duże ogólne osłabienie, powikłane m.in. nawrotem krwawień z przewodu pokarmowego, infekcją grzybiczą oraz stopniowo pogłębiającą się degradacją czynności płuc, zrezygnowano z interwencji chirurgicznej. Pacjent zmarł po trzech tygodniach antybiotykoterapii. Szczep *L. monocytogenes,* który spowodował tę infekcję został zsekwencjonowany, dzięki czemu stwierdzono typ sekwencyjny ST224. Był to ten sam typ, który został zidentyfikowany w trakcie trwającej w Danii epidemii związanej ze spożyciem mięsnych produktów określonej firmy spożywczej (Frydland *et al*. 2014). Infekcyjne zapalenie wsierdzia stwierdzono także we Francji u 44-letniej kobiety z objawami przedłużonego zmęczenia, bólu w klatce piersiowej i wysokiej gorączki. Pierwsze posiewy krwi pobranej od pacjentki były ujemne, a dopiero w kolejnych wykazano obecność *L. monocytogenes*. Badanie kardiologiczne wykazało zespół węzła zatokowego z napadowym szybkim migotaniem przedsionków. Gdy leczenie nie przynosiło poprawy, pacjentka została poddana operacji – w badaniu śródoperacyjnym potwierdzono obecność guza rzekomego obejmującego ścianę prawego przedsionka. W badaniu histopatologicznym usuniętej tkanki potwierdzono obecność *L. monocytogenes* metodą molekularną (PCR). Terapia antybiotykowa w postaci dożylnej amoksycyliny z gentamycyną przyniosła poprawę, a pacjentka wyzdrowiała (Berthelot *et al.* 2013). We Włoszech opisano zapalenie wsierdzia nie związane z zastawkami spowodowane przez *L. monocytogenes* u pacjenta 74-letniego zakażonego HIV. W historii choroby podano okresową gorączkę i kołatanie serca. Posiew krwi dał wynik dodatni, a badania kardiologiczne wykazały przetokę w sercu. Po zabiegu i leczeniu antybiotykowym pacjent wrócił do zdrowia (di Cori *et al*. 2012). Infekcyjne zapalenie wsierdzia zdiagnozowano też w Holandii u 74-letniego pacjenta z zapaleniem płuc i przeciążeniem objętościowym. W wywiadzie opisywane były dializy, operacja bajpasów wieńcowych oraz wszczepienie protezy aortalnej. Tomografia komputerowa pomogła w zdiagnozowaniu zapalenia wsierdzia. Sześciotygodniowa terapia amoksycyliną przyniosła pozytywny skutek (Valckx *et al*. 2017). W Portugalii opisano bardzo rzadki przypadek zapalenia osierdzia. 60-letni pacjent z marskością wątroby w wyniku zakażenia wirusem zapalenia wątroby typu B został przyjęty na szpitalny oddział ratunkowy z powodu wyniszczenia, anoreksji, osłabienia z towarzyszącą dusznością. Podczas badania echokardiograficznego stwierdzono wysięk osierdziowy. Temperatura ciała była w normie. Wykonano perikardiocentezę, a w posiewie ewakuowanego płynu z worka osierdziowego wykryto *L. monocytogenes*. U pacjenta

zastosowano terapię ampicyliną oraz chirurgiczny drenaż osierdzia. W wyniku zastosowanej terapii pacjent powrócił do pełni zdrowia (Dias *et al*. 2011).

Średnia wieku analizowanych pacjentów, u których zdiagnozowano infekcyjne zapalenie wsierdzia o etiologii *L. monocytogenes* wynosiła 60 lat, mediana 70. W tej grupie mężczyźni stanowili 66,6%. Objawy występujące u większości pacjentów to gorączka i zły stan ogólny. Większość pacjentów (77,7%) przebyło wcześniej operacje kardiologiczne związane z wymianą zastawek lub wszczepieniem rozrusznika serca. Trzech pacjentów (33,3%) zmarło. Rozpoznanie zapalenia wsierdzia wywołanego przez *L. monocytogenes* wymaga zorganizowanej współpracy między kardiologami, diagnostyką obrazową i laboratorium mikrobiologicznym. Kardiolodzy powinni zdawać sobie sprawę z możliwości wystąpienia nietypowych mas rzekomo guzowych prawego przedsionka wywołanych przez *L. monocytogenes*. Zmiany te mogą powodować ciężkie zaburzenia rytmu przedsionków. Uważa się, że zapalenie wsierdzia wywołane przez *Listeria* należy leczyć ampicyliną lub penicyliną z aminoglikozydem przez co najmniej cztery tygodnie w przypadku infekcji naturalnej zastawki i przez 6–8 tygodni w przypadku infekcji protezy zastawki, a wskazania do zabiegu są takie same jak w przypadku innych form zapalenia wsierdzia (Kumaraswamy *et al.* 2018).

7. Zapalenie wnętrza gałki ocznej (łac.endophtalmitis)

Zapalenie wnętrza gałki ocznej jest jedną z najbardziej poważnych diagnoz jakie są stawiane w okulistyce. To wewnątrzgałkowe schorzenie zapalne może skutkować uszkodzeniem jamy ciała szklistego, a jego przyczyną jest egzogenne lub endogenne przedostanie się mikroorganizmów do oka (Kernt and Kampik 2010). Źródła endogenne powodują tylko 2–15% wszystkich przypadków zapalenia wnętrza gałki ocznej, większość ma charakter egzogenny. Endogenne zapalenie wnętrza gałki ocznej wynika z krwiopochodnego rozprzestrzeniania się bakterii lub jest powikłaniem zapalenia wsierdzia (Keynan *et al.* 2012). *L. monocytogenes* jest czynnikiem etiologicznym jedynie 4% przypadków zakażeń endogennych. Z gatunków Gram-dodatnich najczęściej spotykane jest zakażenie *S. aureus*, a z Gram-ujemnych *K. pneumoniae* (Keynan *et al*. 2012). Ryzyko zakażenia jest większe u pacjentów z obniżoną odpornością, cukrzycą, zakażonych wirusem HIV, z nowotworami, niewydolnością nerek czy chorobami serca (Tanaka *et al*. 2001).

W przestawianym przeglądzie uwzględniono 10 przypadków zapalenia wnętrza gałki ocznej wywołanego przez *L. monocytogenes* (Tabela IV).

Wśród opisanych przypadków trzy pochodziły z Włoch. U żadnego pacjenta nie dokumentowano wcześniejszych urazów ani operacji. 46-letnia immunokompetentna kobieta zgłosiła się na szpitalny oddział ratunkowy z powodu światłowstrętu, bólu lewego oka i nudności. Pacjentka podała, że wcześniej miała objawy zaczerwienienia i lekko niewyraźne widzenie. Drugi przypadek dotyczył 85-letniego mężczyzny. Zgłaszał on ból lewego oka, który pojawił się tydzień po epizodzie zapalenia żołądka i jelit przebiegającego bezgorączkowo. Cierpiał on na przewlekłą niewydolność nerek i inne choroby współistniejące. U obojga pacjentów posiew krwi w kierunku *L. monocytogenes* dał wynik dodatni, co wskazuje na rozprzestrzenienie się patogenu drogą krwiopochodną. W drugim przypadku powiązano zakażenie ze spożyciem skażonej żywności i epizodem zapalenia żołądka i jelit. Po wykonaniu sekwencjonowania genomu okazało się, że wyizolowany od tego pacjenta szczep *L. monocytogenes* należy do hyperwirulentnego klonu CC4, który często jest przyczyną inwazyjnej listeriozy. Szczep od pierwszej pacjentki nie był wcześniej sklasyfikowany jako wirulentny, ale wyizolowanie go od osoby z prawidłową odpornością, stanowi potwierdzenie wszechstronnego charakteru i nieprzewidywalności zakażeń *L. monocytogenes*. Mimo leczenia antybiotykami u obojga pacjentów pozostała słaba ostrość wzroku (Gori *et al*. 2022). Z kolei Chersich opisał przypadek 40-letniego pacjenta, który w wywiadzie zgłosił wystąpienie trzydzieści dni wcześniej krótkiego epizodu dreszczy i gorączki po zjedzeniu łososia. U pacjenta zaobserwowano ropostek w komorze przedniej oka i zdiagnozowano ciężkie zapalenie błony naczyniowej oka. Ropa była gęsta i niepigmentowana. Ciśnienie wewnątrzgałkowe było w normie, a badania nie wykazały drobnoustrojów ani białych krwinek. Uznano zatem, że etiologia infekcyjna jest mało prawdopodobna. Biorąc pod uwagę status HLA B27 dodatni u pacjenta oraz zapalenie stawów w wywiadzie, rozpoczęto leczenie sterydami i lekami przeciwzapalnymi. *L. monocytogenes* została wyizolowana i zidentyfikowana dopiero w cztery tygodnie po przyjęciu pacjenta. Mimo opóźnienia w diagnozowaniu oraz późnego włączenia antybiotykoterapii pacjent zachował wzrok a infekcja nie rozprzestrzeniła się mimo intensywnej terapii immunosupresyjnej (Chersich *et al.* 2018).

W Niemczech opisano przypadek 62-letniej kobiety skarżącej się na ból, zaczerwienienie i pogorszenie widzenia w lewym oku, a także nudności i wymioty. Trzy dni wcześniej wystąpiły u niej objawy grypopodobne. Nie informowała o wcześniejszych urazach lub operacjach, ale występowały u niej liczne choroby współistniejące: cukrzyca typu II, przewlekłe reumatoidalne zapalenie stawów, zesztywniające zapalenie stawów kręgosłupa, zespół jelita drażliwego, choroby tarczycy i otyłość. Wstępną diagnozą było nadciśnieniowe

Tabela IV
Przypadki zapalenia gałki ocznej spowodowanego przez *Listeria monocytogenes*

Nr	Państwo	Wiek pacjenta	Płeć	Choroby współistniejące	Objawy	Źródło zakażenia/ powiązanie z żywnością	Wynik leczenia	Piśmiennictwo
1	Włochy	46	K	brak	światłowstręt, ból, nudności, zaczerwienienie, pogorszenie widzenia	brak	pozytywny/słaba ostrość wzroku	Gori *et al.* (2022)
2	Włochy	85	M	nadciśnienie, cukrzyca typu II i jaskra prawego oka	ból, obrzęk powiek, pogorszenie widzenia, w wywiadzie zapalenie żołądka i jelit	powiązanie ze skażoną żywnością	pozytywny/słaba ostrość wzroku	Gori *et al.* (2022)
3	Włochy	40	M	zapalenie stawów	światłowstręt, rozdrażnienie i zaczerwienienie lewego oka	trzydzieści dni wcześniej krótki epizod dreszczy, gorączki i zapalenia żołądka i jelit po zjedzeniu łososia	pozytywny	Chersich *et al.* (2018)
4	Niemcy	62	K	cukrzyca typu II, jaskra, choroba reumatoidalna, lek immunosupresyjny	ból, zaczerwienienie oka, pogorszenie widzenia podwyższone ciśnienie wewnątrzgałkowe, ciemny ropień, reakcja włóknikowa w komorze przedniej oka	brak	pozytywny	Bajor *et al.* (2016)
5	Niemcy	53	M	choroba Leśniewskiego Crohna, choroba reumatyczna	ból, zaczerwienienie, pogorszenie widzenia, reakcja włóknikowa, ropostek, wcześniejsze objawy grypopodobne	brak	pozytywny	Bachmeier *et al.* (2021)
6	Niemcy	67	M	POChP, przewlekła niewydolność serca, niewydolność nerki steroidy ogólnoustrojowe	ból, pogarszające się widzenie, zapalenie błony śluzowej żołądka	brak	pozytywny	Hueber *et al.* (2010)
7	Hiszpania	55	M	marskością wątroby wywołana HCV i alkoholem	bolesne zaczerwienienie oka i zaburzenia widzenia, prawe oko przez 1 miesiąc.	brak	pozytywny/ słaba ostrość wzroku	Martin-Hita *et al.* (2020)
8	UK	50	K	brak	ostre jednostronna utrata widzenia, ból głowy i ból zaoczodołowy	ostre zapalenie żołądka i jelit	pozytywny/ słaba ostrość wzroku	Gaskell *et al.* (2017)
9	Belgia	72	K	brak	ból, zaczerwienienie i utrata wzroku lewego oka trwająca 8 dni	brak	pozytywny	Smeets *et al.* (2021)
10	Francja	33	K	brak	ból, zaczerwienienie, fotofobia, pogorszenie widzenia	zakażenie spowodowane uderzeniem ogonem krowy	pozytywny/ odwarstwienie siatkówki	Lécuyer *et al.* (2019)

zapalenie przedniego odcinka błony naczyniowej oka. Wykonano nakłucie komory przedniej oka i zbadano pod kątem patogenów. Po czterech dniach wyhodowano *L. monocytogenes*. Leczenie powiodło się. Źródło zakażenia nie zostało ustalone (Bajor *et al*. 2016). Identyczne objawy (ból, zaczerwienienie, pogorszenie widzenia) zgłaszał 53-letni pacjent bez wcześniejszych chorób oczu, ale z historią chorób zapalnych jelit i chorobą reumatyczną. Zgłosił on też wystąpienie wcześniej objawów grypopodobnych. Diagnoza okulistyczna wykazała ropostek. Pacjent początkowo był leczony lekami kortykosteroidowymi, później włączono leczenie antybiotykowe doszklistkowe i dożylne. Dopiero po dziewięciu dniach wykazano obecność *L. monocytogenes* w oku, szczep został wyhodowany także z wymazu z odbytu (Bachmeier *et al.* 2021). Inny przypadek w Niemczech dotyczył 67-letniego mężczyzny z wieloma chorobami współistniejącymi, u którego rozwinęło się piorunujące zapalenie przedniego odcinka błony naczyniowej. Metodą hodowli mikrobiologicznej i PCR potwierdzono obecność *L. monocytogenes,* ale dopiero po 23 dniach od przyjęcia pacjenta. Jednak agresywne, wczesne leczenie antybiotykowe zapobiegło zapaleniu wnętrza gałki ocznej, a pacjent odzyskał zadowalającą ostrość wzroku (Hueber *et al*. 2010).

W Hiszpanii opisano przypadek endogennego zapalenia wnętrza gałki ocznej spowodowany przez *L. monocytogenes* u 55-letniego pacjenta z marskością wątroby wywołaną wirusem HCV i chorobą alkoholową. Wystąpiło u niego bolesne zaczerwienienie oka i zaburzenia widzenia trwające przez miesiąc. Badanie na obecność wirusów w cieczy szklistej dało wynik negatywny. W barwieniu metodą Grama zaobserwowano natomiast Gram-dodatnie pałeczki, które w hodowli zidentyfikowano jako *L. monocytogenes*. Ponieważ ta diagnoza nastąpiła ponad miesiąc po wystąpieniu pierwszych objawów pacjent nie odzyskał ostrości wzroku pomimo odpowiedniej antybiotykoterapii (Martin-Hita *et al*. 2020).

Przypadek opisany w Anglii dotyczył endogennego zapalenia wnętrza gałki ocznej u 50-letniej immunokompetentnej pacjentki. Pierwsze objawy to ostra, bolesna, jednostronna utrata wzroku, która pojawiła się wkrótce po samoograniczającym się epizodzie nieżytu żołądka i jelit. Zdiagnozowano zapalenie błony naczyniowej oka, a biopsja ciała szklistego i hodowla pozwoliły zidentyfikować *L. monocytogenes* serotyp 1/2a. Posiew krwi dał wynik ujemny. Po leczeniu zapalenie wewnątrzgałkowe ustąpiło, ale konieczna była interwencja chirurgiczna w celu usunięcia resztek ciała szklistego, żeby poprawić ostrość wzroku (Gaskell *et al*. 2017).

W Belgii zapalenie gałki ocznej zdiagnozowano u 72-letniej kobiety. *L. monocytogenes* wyhodowano z posiewu cieczy wodnistej oka. Nie zidentyfikowano źródła zakażenia. Leczenie obejmowało miejscowe podanie wankomycyny a dożylne amoksycyliny, włączenie kortykosteroidów i leków obniżających ciśnienie wewnątrzgałkowe. Zapalenie ustąpiło i pacjentka wyzdrowiała ze skorygowaną ostrością wzroku (Smeets *et al.* 2021).

We Francji opisano wyjątkowy przypadek egzogennego zakażenia gałki ocznej u 33-letniej kobiety z prawidłową odpornością. Kobieta ta była hodowcą bydła. Do zakażenia doszło w wyniku zanieczyszczenia oka po uderzeniu krowim ogonem. Pacjentka zgłosiła się do okulisty z narastającym zaczerwienieniem, bólem, światłowstrętem i utratą ostrości wzroku w lewym oku. Pomimo leczenia objawy nasilały się i pacjentka była hospitalizowana z powodu zapalenia błony naczyniowej oka. W wywiadzie pacjentka zgłosiła incydent śmierci 24 spośród hodowanych cieląt z powodu biegunki oraz zaburzeń neurologicznych. Prawdopodobnie zwierzęta były zakażone *L. monocytogenes*. Miesiąc po ukończeniu antybiotykoterapii, pomimo lepszego widzenia z rozróżnianiem kształtów i kolorów, u pacjentki stwierdzono odwarstwienie siatkówki, które wymagało natychmiastowej interwencji chirurgicznej (Lécuye *et al*. 2019).

Zapalenie gałki ocznej spowodowane przez *L. monocytogenes* wystąpiło u pacjentów, których średnia wieku wynosiła 56,3, mediana 54. W tej grupie 50% stanowili mężczyźni. Objawy najczęściej zgłaszane przez pacjentów to ból, zaczerwienienie i pogorszenie widzenia. W badaniu okulistycznym infekcja najczęściej objawiała się ostrą reakcją włóknikową w komorze przedniej oka, wysiękiem ropnym i podwyższonym ciśnieniem wewnątrzgałkowym. Charakterystycznym, stosunkowo często opisywanym objawem była dyspersja pigmentu tęczówki oka. Zajęcie ciała szklistego następowało później niż objawy notowane w komorze przedniej oka. Czynnikami ryzyka są: immunosupresja, wiek, choroby ogólnoustrojowe, takie jak cukrzyca, zakażenie wirusem HIV, choroby serca, choroby nerek i nowotwory.

Żaden z omawianych przypadków nie zakończył się śmiercią, ale 50% pacjentów nie odzyskało dobrej ostrości widzenia i/lub wymagało dalszych interwencji chirurgicznych oka. Zapalenie wnętrza gałki ocznej wywołane przez *L. monocytogenes* ma w większości przypadków długotrwały przebieg, jednakże może być też piorunujące. Diagnostyka bakteriologiczna jest często z różnych przyczyn opóźniona, w przeglądach literatury zwykle jest mowa o ośmiu (Chersich *et al.* 2018) lub nawet 13 dniach potrzebnych na ustalenie tej diagnozy (Bajor *et al*. 2016) co wpływa na opóźnienie zastosowania celowanej antybiotykoterapii. Zapalenie wnętrza gałki ocznej najczęściej leczy się podając drogą doszklistkową injekcje wankomycyny i ceftazydymu. Jednakże w przypadku zakażeń *L. monocytogenes* najlepszą formą antybiotykoterapii pacjentów, u których nie ma uczulenia na penicyliny, jest ogólnoustrojowe leczenie ampicyliną i gentamycyną.

Wczesna diagnoza, również z wykorzystaniem technik molekularnych i wczesne rozpoczęcie leczenia są kluczowymi czynnikami wpływającymi na wynik leczenia endogennego zapalenia wnętrza gałki ocznej wywołane przez *L. monocytogenes*.

8. Ostre zapalenie wątroby (łac. hepatitis) w przebiegu listeriozy

L. monocytogenes jest patogenem, który rzadko przyczynia się do rozwoju ostrego zapalenia wątroby. Gwałtowna gorączka oraz żółtaczka to główne objawy zakażenia. Czynnikami predysponującymi do rozwoju tej infekcji są marskość wątroby lub przeszczep. *L. monocytogenes* może być odpowiedzialna za powstawanie pojedynczych lub mnogich ropni w wątrobie, które najczęściej występują wraz z podwyższoną temperaturą. Czynnikami sprzyjającymi rozwojowi ropni są przede wszystkim cukrzyca, przeczep, marskość wątroby oraz alkoholizm (Schlech 2019).

W literaturze lat niniejszego przeglądu znaleziono opisany jeden przypadek zapalenia wątroby wywołanego przez *L. monocytogenes*. Była to 73-letnia pacjentka w Holandii, która zgłosiła się do szpitala ze względu na silny ból głowy, gorączkę oraz senność.W wywiadzie stwierdzono cukrzycę oraz nadciśnienie tętnicze. Badania wykazały znacznie podwyższony poziom enzymów wątrobowych. Autorzy wskazują, że zapalenie wątroby oraz jej dysfunkcja w powyższym przypadku były wtórne do zakażenia *L. monocytogenes*. W leczeniu stosowano dożylnie amoksycylinę z kwasem klawulanowym cztery razy dziennie oraz gentamycynę dożylnie raz dziennie. Terapia odniosła pozytywny skutek (van der Voort *et al*. 2019).

9. Leczenie i profilaktyka zakażeń powodowanych przez *Listeria monocytogenes*

Kluczem do prawidłowego leczenia listeriozy jest bardzo wczesna identyfikacja patogenu, odpowiednie leczenie oraz jego ustandaryzowany przebieg (Wei *et al*. 2021). Szczepy *L. monocytogenes* zwykle wykazują wrażliwość na antybiotyki β-laktamowe m.in. penicyliny, aminopenicyliny, karboksypenicyliny, karbapenemy, a także aminoglikozydów, ryfamycyny, i tetracykliny. Oporne są głównie na cefalosporyny (słabo wiążące się do białka PBP3 ściany komórkowej tych bakterii), co powoduje ograniczenia w leczeniu tą grupą leków (Sołtysiuk *et al*. 2019; Schlech 2019). Oporność tę tłumaczy się przejściem bakterii do formy L, która charakteryzuje się deficytem ściany komórkowej – punktu uchwytu antybiotyków beta-laktamowych. Komórki w formie L charakteryzuje różnorodna morfologia, której przyczyną jest znaczny niedobór peptydoglikanu. Formy L cechują się więc zmienną wielkością oraz wyraźnie zmienioną aktywnością metaboliczną (Grosboillot *et al*. 2022). Aktualnym lekiem z wyboru w leczeniu wszystkich postaci listeriozy jest połączenie gentamycyny i ampicyliny. Jest to obecnie uważane za złoty standard w tych przypadkach (Schlech 2019, Grosboillot *et al*. 2022). Są jednak dane wskazujące na to, że nie zawsze to połączenie antybiotyków jest skuteczne. Grosboillot i wsp. podkreślają, że także już podczas terapii ampicyliną w połączeniu z gentamycyną *L. monocytogenes* ma możliwość przejścia do formy L (Grosboillot *et al*. 2022). Mimo iż komórki te charakteryzują się brakiem dojrzałej struktury peptydoglikanu oraz białek biorących udział w podziale komórki, są zdolne do wzrostu, a także do namnażania. Formy L są przejściowe co oznacza, że po ustąpieniu działania inhibitora są w stanie powrócić do formy macierzystej. Wyniki badań sugerują, iż formy L *L. monocytogenes* odgrywają istotną rolę w nawrotach listeriozy oraz jej przewlekłym przebiegu. W przypadku większości postaci inwazyjnej listeriozy leczenie ampicyliną lub amoksycyliną w połączeniu z gentamycyną powinno trwać 2–3 tygodnie, a w przypadku leczenia zapalenia wsierdzia od 4 do 8 tygodni w zależności od rodzaju zainfekowanej zastawki (Kumaraswamy *et al.* 2018). Zgodnie z wytycznymi ESCMID (European Society for Clinical Microbiology and Infectious Diseases) dotyczącymi leczenia bakteryjnego zapalenia opon mózgowo-rdzeniowych, neurolisteriozę dorosłych należy leczyć dawką 12 g antybiotyków β-laktamowych na dobę przez co najmniej 21 dni (van de Beek *et al*. 2016). Należy wziąć pod uwagę, że ampicylina i amoksycylina stosunkowo słabo przenikają przez barierę krew-mózg. U pacjentów z ropniami mózgu wywołanymi przez *Listeria* lub zapaleniem rdzenia kręgowego zaleca się przedłużenie stosowania antybiotyku przez co najmniej 6 tygodni z monitorowaniem radiologicznym (Koopmans *et al.* 2023) W przypadku wystąpienia reakcji alergicznej na penicylinę lub braku odpowiedzi ze strony organizmu na jej podanie, jako działanie alternatywne można podać wankomycynę lub sulfametoksazol z trimetoprimem (Wu et al. 2022). Należy zauważyć ciekawy paradoks – *L. monocytogenes* wykazuje oporność na fosfomycynę *in vitro*, podczas, gdy *in vivo*, w przebiegu infekcji, jest w pełni wrażliwa na ten antybiotyk. Powodem tego paradoksu jest fakt, że ekspresja transportera fosfomycyny jest ściśle kontrolowana przez regulator wirulencji PrfA. W rezultacie wychwyt fosfomycyny za pośrednictwem transportera Hpt jest w pełni aktywowany *in vivo*, gdy *L. monocytogenes* znajduje się wewnątrz zakażonych komórek gospodarza, podczas gdy w hodowli bakteryjnej antybiotyk jest usuwany z bakterii (Scortti *et al.* 2006). Stopień oporności na antybiotyki izolatów *L. monocytogenes*

izolowanych z przypadków listeriozy ludzi jest stosunkowo niski. We francuskim badaniu z lat 1926–2007 wykazano jedynie 1,27% opornych szczepów (Morvan *et al*. 2010). Chociaż oporność na środki przeciwdrobnoustrojowe nie jest problemem klinicznym, należy monitorować lekooporność oraz wzrost MIC antybiotyków β-laktamowych w stosunku do tych bakterii (Morvan *et al.* 2010). Istotna jest też szybka diagnostyka mikrobiologiczna listeriozy przede wszystkim oparta o posiewy krwi (Lecuit 2020).

W profilaktyce listeriozy najważniejszym aspektem jest przerwanie epidemiologicznego łańcucha zakażenia. Ważnym elementem są ustawowo wprowadzone programy badań i nadzoru w kontroli żywności np. rozporządzenia Komisji Unii Europejskiej oraz programy o charakterze edukacyjnym. W rozporządzeniu WE 2073/2005 dotyczącym *L. monocytogenes* zalecane jest badanie w tym kierunku żywności gotowej do spożycia przeznaczonej dla niemowląt oraz gotowej do spożycia żywności specjalnego medycznego przeznaczenia, a także każdej żywności gotowej do spożycia (Rozporządzenie z Dziennika Urzędowego Unii Europejskiej WE 2073/2005). Kluczowe jest również wprowadzanie i przestrzeganie przepisów dotyczących przechowywania oraz transportu żywności, która jest zagrożona namnażaniem *L. monocytogenes*. W przemyśle spożywczym stosowane są specjalne metody kontroli żywności oparte na algorytmie oceniającym ryzyko skażenia produktów *L. monocytogenes*. Bardzo ważną częścią profilaktyki są też szkolenia pracowników, którzy mają kontakt z produktami żywnościowymi na różnych etapach produkcji. Ukierunkowane działania edukacyjne powinny koncentrować się również na osobach starszych, ponieważ mają one skłonności do przechowywania w lodówkach i spożywania żywności po upływie terminu przydatności do spożycia (Koopmans *et al.* 2023).

Inwazyjna listerioza pozostaje trudnym do leczenia zakażeniem ze znacznym wskaźnikiem śmiertelności. Jej klasyfikacja jako choroby przenoszonej przez żywność nastąpiła stosunkowo niedawno, podobnie jak świadomość, że potrzebne są zdecydowane, ciągłe działania, aby ograniczyć te zakażenia (de Noordhout *et al.* 2014). W 2002 roku Unia Europejska (UE) powołała Europejski Urząd ds. Bezpieczeństwa Żywności (EFSA), który m.in. ocenia ryzyko stwarzane przez *L. monocytogenes* poprzez monitorowanie patogenu w żywności oraz doradza w sprawie podejmowania metod kontrolnych (EFSA 2023). Wielu opisanych dotąd inwazyjnych zakażeń *L. monocytogenes* nie udawało się powiązać z określonym źródłem żywności stąd, poza szybką diagnostyką mikrobiologiczną, istotne wydaje się przeprowadzanie szczegółowego wywiadu. Metody genotypowania wyhodowanych szczepów mogą być skuteczne w powiązaniu konkretnych zakażeń z występowaniem ognisk epidemicznych związanych z żywnością. Biorąc pod uwagę kluczową rolę systemów nadzoru w zwalczaniu zakażeń *L. monocytogenes* i potrzeb wynikających z rozwoju przemysłu i rynku spożywczego, możemy spodziewać się rozwoju badań w tym kierunku. Konieczne jest wykrywanie ognisk epidemicznych i identyfikacja źródeł zanieczyszczeń metodami sekwencjonowania całego genomu. Dalsze badania dotyczące *L. monocytogenes* powinny skupiać się na poprawie schematów diagnozowania i leczenia ciężkich przypadków zakażeń oraz badaniu skomplikowanych mechanizmów patogenezy tych bakterii (Koopmans *et al.* 2023).

ORCID
Agnieszka Chmielarczyk https://orcid.org/0000-0002-0814-863

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