# Different Insights into Infectious Disease: Ebselen's Antimicrobial Activity, Cat-Scratch Disease, and HPV Genotype Distribution


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## Content

# PREFACE

The field of microbiology is rapidly evolving, providing critical insights into the complex relationships between microorganisms and human health. The importance of this field was starkly highlighted during the COVID-19 pandemic, which continues to affect the world and cause significant mortality. The pandemic has underscored the necessity of the One Health approach towards zoonotic agents, demonstrating the crucial importance of advancements in the rapid diagnosis of bacterial and viral pathogens, the development of new treatment options, and the identification of genotypic variations in infectious agents.

This book is a testament to the profound impact that advanced research can have on our understanding and management of infectious diseases. Within these pages, we delve into important topics that represent the forefront of microbiological investigation.

Each chapter reflects the dedication and meticulous research of experts in their respective fields. Their contributions not only enhance our understanding of these complex microbial interactions but also pave the way for innovative approaches to diagnosis, treatment, and prevention.

It is my hope that this book will serve as a valuable resource for researchers, clinicians, and students alike, inspiring further exploration and discovery in the dynamic field of microbiology.

**Editors**

Dr. Öğr. Üyesi Cihat ÖZTÜRK
Doç. Dr. Memiş BOLACALI

# CHAPTER I

# In Vitro Antimicrobial Efficiency of Ebselen (2-Phenyl-1,2-Benzisoselenazol-3(2h)-One) in Enterococcus Species

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## Introduction

*Enterococci* are Gram-positive bacteria that are facultatively anaerobic and non-spore forming. They are frequently present in the gastrointestinal tract of both humans and animals. Among the vast

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genus Enterococcus, consisting of over fifty species, *E. faecalis* and *E. faecium* are the predominant species (Lebreton, Willems, & Gilmore, 2014). Studies in ecology and epidemiology have indicated that *E. faecalis* and *E. faecium* are frequently detected in food products derived from animals and in the surrounding environment (Torres & al., 2018). They are also recognized as prevalent nosocomial pathogens (Khan, Ahmad & Mehboob 2015)

*Enterococcus* species harbor a variety of virulence genes and typically exhibit elevated levels of resistance to commonly prescribed antibiotics (Giraffa, 2002). The part of intestinal habitat of Enterococci, their transmission into the food chain, their resistance to antibiotics, and possession of virulence factors have raised concerns in recent years regarding their potential contribution to foodborne illnesses (Franz & al., 1999). These bacteria possess both innate and acquired resistance to the majority of antibiotics utilized in human medicine. The development of antibiotic resistance is facilitated by the horizontal transfer of resistance genes among different species and genera through conjugative plasmids and transposons (Clewell, 1990). The rise in multidrug resistance among bacteria to antibiotics represents a significant public health challenge, compelling the pharmaceutical industry to explore novel classes of antibiotics for combating infections (Giraffa, 2002).

Due to their important role in all cells, including bacteria and fungi, thioredoxin reductases (TrxRs) have recently become a target site for new antimicrobial drug design (Felix, Mylonakis & Fuchs, 2021). TrxRs, together with thioredoxin (Trx) and NADPH, form the thioredoxin system (TS), an antioxidant system that protects the cell against oxidative stress. TrxRs are responsible for the reduction and

recycling of oxidized thioredoxin. They are also electron donors to other oxidoreductive enzymes such as ribonucleotide reductase and thioredoxin peroxidase. Therefore, it plays an important role in DNA synthesis and cell protection against reactive oxygen species such as $H_2O_2$ (Arner & Holmgren, 2000, Lu & Holmgren, 2014, Maslanka & Mucha, 2023).

The thiol-dependent glutathione/glutathione reductase (GSH/GR) as well as the Trx/TrxR systems, play an important role in maintaining redox balance in a cell. In mammalian cells, the GSH and Trx systems can act as a secondary mechanism for each other, providing cross electrons for each other. The GSH system is absent in many bacteria, especially in most Gram-positive bacteria, making the Trx system essential for bacterial survival under conditions of oxidative stress (Silva & Rio-Tinto, 2024).

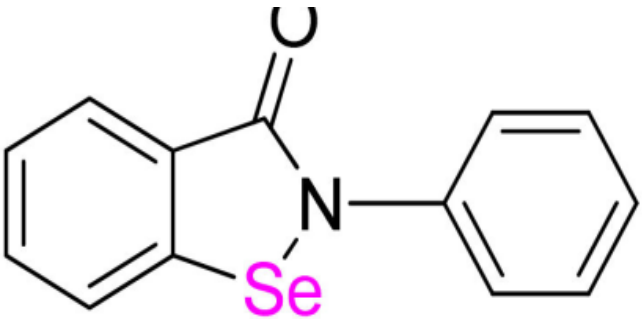


*Figure 1: The chemical structure of Ebselen.*

Ebselen, (2-phenyl-1,2-benzisoselenazole-3(2H) one) an organic selenium compound (Figure 1), exhibits anti-inflammatory and anti-atherosclerotic properties as well as antimicrobial effects by

inhibiting TrxR, especially in gram-positive bacteria lacking the GSH/GR system (Lu & al., 2013, Nozawa & al. 1996; Schewe, 1995; Nozawa & al, 1989; Thangamani, Younis & Seleem, 2015a, Thangamani, Younis & Seleem, 2015b). The aim of this study was to evaluate the efficacy of Ebselen against *Enterococci* strains isolated from various sources (including food and human samples).

## Material and Methods

### Sample Collection

In this study, 38 Enterococcus strains which antibiotic resistance profiles were predetermined from the microbiology culture collection of the Biology Department of Kütahya Dumlupınar University were used; 8 of them were obtained from cheese, 10 from minced meat and 10 from chicken samples (Table 1). Ten strains of human-derived *Enterococcus* spp. which antibiotic resistance profiles were predetermined were included in this study, originated from urine samples provided by Evliya Çelebi Training and Research Hospital. The bacterial cultures were maintained on Nutrient Agar slants at 4°C, respectively, and were subcultured in petri dishes prior to use.

### Determine the Antimicrobial Activity of Ebselen

Ebselen (Sigma) was diluted with dimethyl sulfoxide (DMSO) to prepare the solution 500, 125 and 31.2 μg/disc. Kirby-Bauer disc diffusion method was used to determine the antimicrobial activity of Ebselen extract on food-borne and human pathogens. Enterococcus faecalis ATCC 29212, was used as control strains. In short, Mueller Hinton Agar (MHA) was prepared by sterilizing a mixture of the dehydrated culture medium (Oxoid, UK) and distilled

water in an autoclave at 121°C for 15 minutes and cooling to 45 °C, then pouring 25 milliliters into sterile Petri dishes (Ø 90 mm). The cell suspensions containing $10^8$ CFU/ml cells of bacteria evenly spread onto the surface of Mueller-Hinton agar plates, using sterile swab sticks. Once the plates were dried aseptically, 6 mm discs were inserted. After 1 h pre-incubation at 4°C, the media were at 37°C for 24 h. The experiments were replicate three times . The assessment of antimicrobial efficacy was performed by quantifying the inhibition zone

**Istatistical analysis**

The software utilized for evaluating antibacterial activity is SPSS (Statistical Package for the Social Sciences Version 20.00). Statistical comparison of zone diameters against Ebselen between two independent groups was performed by using Mann-Whitney U test. P value < 0,05 was accepted as statistically significant.

**Results**

Our study tested the antimicrobial activity of ebselen (at 3 different concentrations) against enterococci, as shown in Table 1. The observed zone diameter interval for *E. faeci*um isolated from human at 500 µg Ebselen was 38-19 mm, 29 mm for cheese isolate, 28-15 mm for minced meat isolates and 29 and 27 mm for chicken isolates. The zone diameter interval observed for *E. faecalis* at the same concentrations of Ebselen was 23-19 mm for human isolates, 26-17 mm for cheese isolates, 25-19 mm for minced meat isolates and 25-15 mm for chicken isolates.

The change in zone diameter observed at 125 µg Ebselen for E. faecium and *E. feacalis* is shown in Table 1. For E. faecium, the

observed intervals were 22-14 mm for human isolates, 21 mm for a cheese isolate, 21-13 mm for minced meat isolates and 25-19 mm for chicken isolates. For *E. faecalis*, the observed intervals were 18-13 mm for human isolates, 16-10 mm for cheese isolates, and 25-11 mm for minced meat and chicken isolates.

The zone diameter intervals observed for *E. faecium* at 31.2 μg/disc of Ebselen were 18-10 mm for human isolates, 19 mm for cheese isolates, 20-10 mm for minced meat isolates and 20 and 18 mm for chicken isolates. Similarly, the zone diameter intervals observed for *E. faecalis* at the same concentrations of ebselen were 16-10 mm for human isolates, 17-8 mm for cheese isolates, 20-8 mm for minced meat isolates and 17-8 mm for chicken isolates.

Ebselen zone diameters against enterococcal strains resistant to ampicillin, chloramphenicol, ciprofloxacin, tetracycline and vancomycin (at the lowest concentration used in the study, 31.2 μg/disc) were 10-18 mm, 11-17 mm, 9-20 mm, 8-20 mm and 19-20 mm, respectively. In strains with single, double and multiple drug resistance, ebselen zone diameters were 8-20 mm, 10-20 mm, 17 mm, respectively.

In Figures 2 and 3, changes in zone diameter with increasing drug concentration are shown in a line graph separately for *E. faecium* and *E. faecalis*. For the *E.faecalis* ATCC 29212 strain, the zone diameter also increased proportionally at all 3 concentrations. While an increase in zone diameter with concentration was observed in all E. faecium strains, this proportional change was not observed in all E. faecalis strains; for example, the same zone diameters were obtained in 3 (E49, E11, E44) out of 24 *E. faecalis* strains at two

different concentrations. In general, the zone diameters were found to be close to each other in the two lower concentrations.

*Table 1: Ebselen susceptibility of E. faecium and E. faecalis*

| | No | Origin | Resistance profile* | Ebselen (mm) | | |
|---|---|---|---|---|---|---|
| | | | | 500 ug | 125µg | 31.2 µg |
| ***E.faecium*** (n: 14) | **E1** | Human | AM, CIP | 38 | 17 | 14 |
| | **E8** | Human | AM, CIP | 27 | 20 | 18 |
| | **E4** | Human | AM, CIP | 26 | 17 | 11 |
| | **E9** | Human | - | 29 | 22 | 18 |
| | **E3** | Human | AM, CIP | 19 | 14 | 10 |
| | **E16** | Cheese | V | 29 | 21 | 19 |
| | **E22** | Meat | - | 28 | 20 | 15 |
| | **E42** | Meat | - | 18 | 13 | 10 |
| | **E10** | Meat | - | 25 | 21 | 20 |
| | **E32** | Meat | - | 15 | 12 | 10 |
| | **E36** | Meat | - | 18 | 10 | 6 |
| | **E17** | Meat | - | 15 | 13 | 10 |
| | **E29** | Chicken | - | 27 | 19 | 18 |
| | **E12** | Chicken | CIP, V | 29 | 25 | 20 |
| ***E.faecalis*** (n: 24) | **E7** | Human | TE | 19 | 13 | 10 |
| | **E2** | Human | - | 23 | 14 | 10 |
| | **E34** | Human | - | 21 | 15 | 13 |
| | **E6** | Human | AM CIP | 20 | 18 | 14 |
| | **E38** | Human | TE | 22 | 18 | 16 |
| | **E15** | Cheese | - | 19 | 11 | 10 |
| | **E26** | Cheese | - | 20 | 15 | 11 |
| | **E44** | Cheese | - | 26 | 16 | 17 |
| | **E46** | Cheese | - | 20 | 10 | 8 |
| | **E43** | Cheese | CIP | 20 | 15 | 9 |
| | **E18** | Cheese | TE | 19 | 11 | 9 |
| | **E27** | Cheese | - | 17 | 17 | 11 |
| | **E23** | Meat | - | 22 | 18 | 18 |
| | **E49** | Meat | TE | 25 | 25 | 20 |
| | **E5** | Meat | TE | 19 | 14 | 10 |
| | **E33** | Meat | - | 19 | 11 | 8 |
| | **E47** | Chicken | TE | 16 | 11 | 8 |
| | **E37** | Chicken | TE, C | 24 | 18 | 11 |
| | **E11** | Chicken | TE | 25 | 25 | 14 |
| | **E14** | Chicken | - | 21 | 18 | 12 |
| | **E19** | Chicken | TE, C, CIP | 24 | 19 | 17 |
| | **E28** | Chicken | - | 20 | 16 | 10 |
| | **E41** | Chicken | - | 15 | 11 | 10 |
| | **E48** | Chicken | - | 19 | 13 | 11 |
| *E. faecalis* | ATCC 29212 | | | 25 | 20 | 12 |
| * AM: Ampicillin, TE: Tetracycline, C: Chloramphenicol, CIP: Ciprofloxacin, V:Vancomycin | | | | | | |

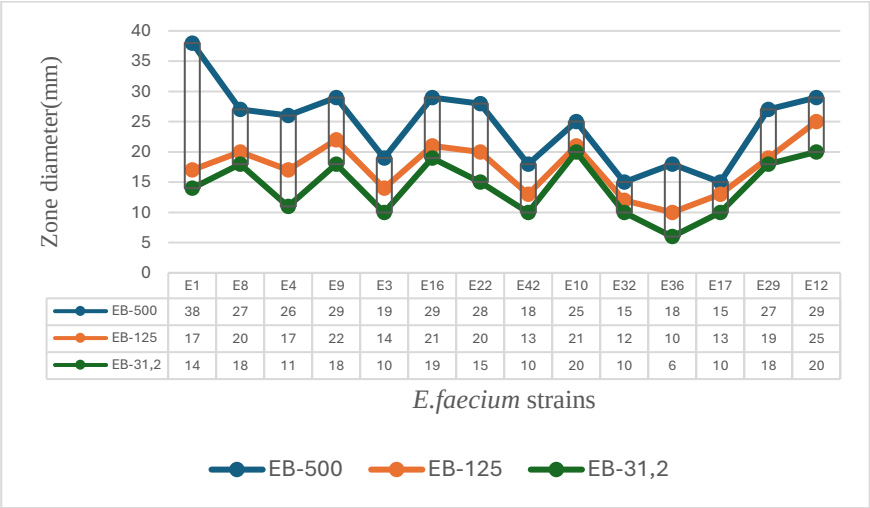


*Figure 2: Variation in the zone diameters against three consantrations of Ebselen for E. faecium strains*

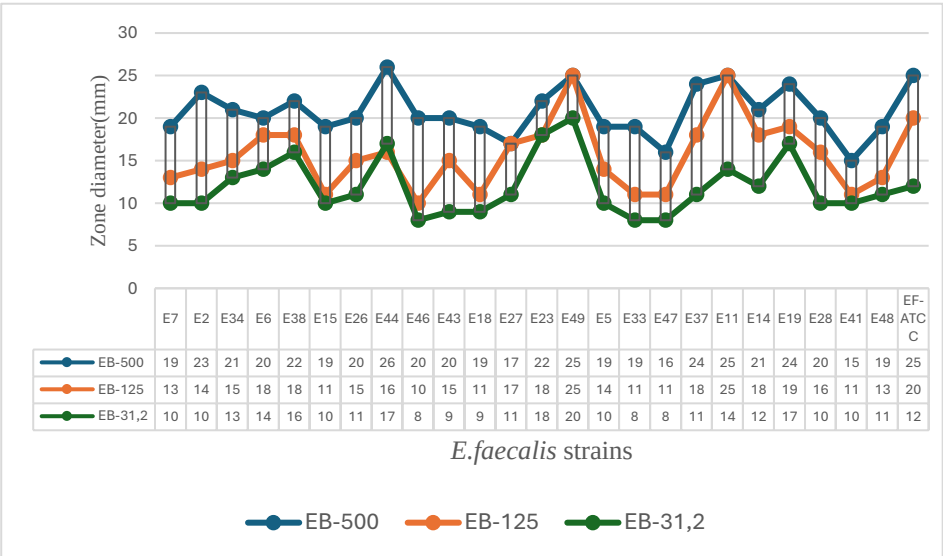


*Figure 3: Variation in the zone diameters against three concentrations of Ebselen for E. faecalis strain*

**Discussion**

The discovery of antibiotics is undoubtedly the most significant achievement in the history of pharmacology. Over the past eight decades, since the introduction of penicillin, several classes of antibiotics have become available. These include β-lactam antibiotics, aminoglycosides, tetracyclines, macrolides, lincomycin, vancomycin, bacitracin and others that act through mechanisms affecting the bacterial cell wall, cell membrane, nucleic acid and protein synthesis. Appropriate use of antibiotics could avert over 160 million cases of dysentery and one million deaths caused by pathogenic strains each year worldwide. However, a variety of mechanisms, including hotspot mutations driven by positive selection, allow many bacteria to develop antibiotic resistance (Urban-Chmiel & al., 2022). The overuse and misuse of antibiotics has increased the threat of resistance in pathogenic bacteria. The increasing prevalence of antibiotic resistance is a major global challenge for both human and animal health. It reduces the therapeutic efficacy of current treatments and narrows the range of available treatment options, potentially endangering human health and life (Depta, & Niedźwiedzka-Rystwej, 2023).

The World Health Organisation (WHO) has identified the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginos*a and *Enterobacter* spp.) as priority pathogens because they have developed resistance to certain antibiotics (Aggarwal & al. 2024). It is known that bacteria can acquire resistance to antimicrobials through mutations in genes or through the acquisition of exogenous DNA. In the case of mutations, it is clear that the

acquisition of resistance is more limited. However, the acquisition of exogenous DNA has a much greater impact because it involves the transfer of chromosomal genes or mobile transferable genetic elements, such as R plasmids, which can spread intra- and interspecies (Silva & Rio-Tinto, 2024). To combat such a global problem, it is of utmost importance to identify novel therapeutic strategies/agents as an alternative to such antibiotics (Aggarwal & al., 2024).

Ebselen, an organoselenium compound, has been discovered as a potential antimicrobial agent against some important Gram-positive pathogens and targets TrxR in the bacterial cell (Nozawa & al., 1989; Thangaman & al. 2015a, Lu & Holmgren, 2014; Ren & al., 2018; Ren, Zou & Holmgren, 2020; Chen & al., 2022). The presence of the selenium atom is essential for antibacterial activity (Nozawa & al., 1989; Maslanka & Mucha, 2023). The antibacterial properties of ebselen were originally tested by Nozawa & al. (1996) on a range of Gram-negative and Gram-positive clinical isolates resistant to methicillin, a narrow-spectrum β-lactam antibiotic of the penicillin class. Overall, Ebselen exhibited activity against Gram-positive bacteria, displaying either significant or moderate levels of effectiveness, while demonstrating lower efficacy against Gram-negative strains.

Ebselen showed promising results in clinical trials, with antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) strains (Younis, Thangamani & Seleem, 2015) Further investigations demonstrated that it exhibits strong bactericidal efficacy towards various clinical strains of methicillin-, vancomycin-

and other Gram-positive pathogenic bacteria that are resistant to multiple drugs. The corresponding sulfur analog lost the potency of the organoselenium counterpart. The authors correlated the Ebselen/Ebsulfur potency ratio with their antioxidant properties, which are principally relevant to the chemical reactivity with sulfur nucleophiles (Nozawa & al.,1989; Maslanka & Mucha, 2023). The potential antibacterial effect of Ebselen was demonstrated against clinical isolates of *Streptococcus pyogenes* and *Streptococcus agalactiae* with an MIC of 0.5 μg/ml. Thangamani, Younis & Seleem, (2015a) have found that the Minimum Inhibitory Concentrations of Ebselen at which 90% of clinical isolates of *Enterococcus* and *Staphylococcus* were inhibited (MIC90) were found to be 0.5 and 0.25 mg/L, respectively. A further investigation conducted by AbdelKhalek & al. (2018) demonstrated the antibacterial activity of Ebselen against Enterococci strains obtained from clinical specimens. The research revealed that Ebselen exhibited growth inhibitory effects on clinical VRE isolates even at concentrations as low as 2 μg/ml, with a similar impact observed in vancomycin-sensitive isolates. Moreover, the study indicated the absence of a gradual emergence of resistance to Ebselen among the tested isolates, while also highlighting the antibiofilm properties of Ebselen on the enterococcal isolate under examination. In this study, the inhibitory activity of Ebselen against antibiotic sensitive and resistant (ampicillin, chloramphenicol, ciprofloxacin, tetracycline and vancomycin) enterococcal strains was screened using the disc diffusion method, which is widely used in antimicrobial activity studies, and antibacterial activity was observed different zone diameters in almost all strains tested. In this in vitro study, Ebselen

activity was also evaluated against vancomycin-resistant enterococci strains, which pose a public health problem and it was found that the zone diameter width was larger compared to susceptible strains ($p<0.05$).

There are a number of data that indicate that ebselen has a synergistic effect with certain antibiotics or molecules (Xu & al., 2008, Chen & al., 2022). Zou & al. (2017) demonstrated that a combination of silver and Ebselen can target the redox system and exhibit a significant synergistic antimicrobial effect against clinically important Gram-negative bacteria. According to Tangamani (2015b), Ebselen shows synergistic activity with topical antibiotics against various resistant strains of Staphylococcus aureus. The authors suggest that combining Ebselen with topical antibiotics may be a promising strategy to reduce the likelihood of staphylococcal skin infections and the development of resistant strains. Ebselen also shows synergistic activity with conventional antimicrobials. However, no synergistic activity was observed when ebselen was combined with linezolid, vancomycin, chloramphenicol or gentamicin (Tangamani & al. 2015b; Silva & Rio-Tinto, 2024). According to the data obtained in this study in which the efficacy of ebselen on enterococci strains was evaluated, the efficacy of ebselen varies between strains. Strains with lower ebselen activity compared to others can be further analyzed. For this purpose, in future studies, it would be useful to evaluate the efficacy of ebselen together with other antibiotics to reveal the synergistic activity of ebselen.

In the present investigation, the effectiveness of Ebselen against enterococcus isolates originated from various food samples and clinical human samples whose resistance profiles to antibiotics

had been pre-established was explored. For this purpose, disc diffusion technique was used. In the disc diffusion method, we used to determine the antibacterial activity of Ebselen, 3 different concentrations of Ebselen (500, 125 and 31.2 μg/disc) were impregnated on discs and the inhibitory activity of each concentration on enterococcal strains was evaluated by measuring the zone diameters formed around the strains. This method we used is an alternative method to MIC because it includes diluted Ebselen concentrations (Çolak & al., 2010). Varied degrees of inhibitory effectiveness were observed across all isolates in response to the three doses administered during the investigation. The inhibition zone diameters of *E. faecium* strains were observed to be very proximate to each other in certain strains in the assessments conducted with 3 distinct concentrations (500, 125, 31.2 μg/disc) regarding their susceptibility to Ebselen (Figure 2). It was found that E32, E17 strain, the zone diameter observed against the three different concentrations were significantly close to each other. In two strains (E29 and E10), the same zone diameters were determined at 125 and 31.2 ug/disc. Again, when the effect of the same dose rates was evaluated on a species basis, it was observed that the zone diameters were close to each other for almost all strains. In 21 (87.5%) of the twenty-four *E. faecalis* strains, the inhibition zone diameter widened as the Ebselen concentration increased, whereas the same zone diameter was detected in E27, E49, E11 strains at 500 and 125 μg/disk. In addition, there was a consistent result between concentration and zone diameter in all other *E. faecelis* strains except E43, E11 strains exhibited similar zone diameters at 125 and 31.2 ug/disc (Figure 3). From the results of the experiments, it appears

that an MIC test is needed to determine the minimal inhibitory concentration of Ebselen against the tested strains.

When the results of this study were evaluated, antibacterial activity was detected in all isolates (except one isolate) at a concentration of 31.2 μg/disc of Ebselen. The discrepancy in sensitivity observed between this study and other studies (source) is believed to be attributable to differences in methodology, including disc diffusion and MIC. Although the preliminary data provided by the disc diffusion method on Ebselen are valuable, it is recommended that these findings be backed by further investigation using Minimal Inhibition Concentration and Minimal Bactericidal Concentration in more comprehensive studies.

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# CHAPTER II

# Cat-Scratch Disease

**Taylan ONDER**[1]

## I-Introduction

Cat scratch disease (CSD) is a febrile illness characterized by lymphadenopathy. The disease was first described in 1931 (Carithers, 1970), and it was recognized as being transmitted by cats and its diagnostic criteria were established in the 1950s (DEBRE et al., 1950). The causative microorganism was identified in the 1990s (Wear et al., 1983). CSD remains a rare infection that continues to harbor unresolved mysteries.

## II-Etiology

*Bartonella henselae* is responsible for nearly all cases of CSD (Zangwill et al., 1993). A few cases have implicated *Bartonella*

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*clarridgeiae* and *Bartonella grahamii* in the etiology (Kordick et al., 1997; Oksi et al., 2013). In the past, *Afipia felis* was thought to be the causative agent of CSD, but this notion has since been dismissed (Chomel et al., 2014).

*Bartonella henselae* is a gram-negative pleomorphic bacterium from the *Alphaproteobacteria* class of the *Bartonellaceae* family. It does not stain well with Gram stain but appears black when stained with silver stains like Warthin-Starry. The bacterium ranges in size from 0.2-0.6 μm by 0.5-1.0 μm. It grows well on freshly prepared rabbit-heart infusion agar. It also grows on blood agar and chocolate agar, but it requires specific conditions including a temperature of 35-37ºC, humidity above 40%, and 5-10% CO2, making its growth slow and difficult even under optimal conditions. *Bartonella henselae* has a doubling time of approximately 24 hours and typically takes 7-10 days to form colonies on culture media. Colonies on agar plates are rough, dry and white. They do not trigger CO2 detection sensors in automated blood culture systems, which complicates their detection. The bacterium is catalase-negative and oxidase-negative. It is heme-dependent for growth and does not ferment carbohydrates to produce acid (Gutiérrez et al., 2017; Larson et al., 1994; Welch et al., 1993). *Bartonella henselae* primarily causes infection by invading vascular endothelial cells and erythrocytes, leading to intracellular infection and stimulating angiogenesis (Kempf et al., 2005; Minnick et al., 1996). Pathogen utilizes various virulence factors such as low-potency lipopolysaccharide (LPS), type IV secretion systems (T4SS), heme acquisition/utilization mechanisms, and adhesins. These factors enable *Bartonella henselae* to invade erythrocytes and endothelial

cells, evade immune responses, and establish persistent intracellular infections (Minnick & Battisti, 2009).

## III-Epidemiology and Transmission

CSD is a zoonotic illness transmitted to humans from cats with *B. henselae* bacteremia. Bacteremia can persist for up to 454 days and at high levels $\geq 10^6$ colony-forming units (CFU)/mL, yet cats are usually asymptomatic (Kordick et al., 1999). Bacteremia is commonly observed in cats younger than 1 year old and in stray cats (Chomel et al., 1995). The bacterium primarily spreads among cats through the cat flea, *Ctenocephalides felis*. Direct transmission to humans via fleas has not been demonstrated; transmission from cats to humans occurs mainly through inoculation of flea feces during scratching (Boulouis et al., 2005). The infection is global but more prevalent in warm and humid climates where fleas are abundant (Jameson et al., 1995).

In a study conducted in the United States between 2005 and 2013, reported an incidence of 4.5 cases per 100,000 persons among outpatient visits and 0.19 cases per 100,000 persons among hospitalized patients for CSD. The disease was more frequently observed in children aged 5-9 years and in females. CSD exhibits a seasonal pattern, with higher frequencies noted between August and January, peaking in January with the highest number of cases reported during this month (Nelson et al., 2016). The mortality rate of the disease was reported to be 1.3% (Rodríguez Alonso et al., 2021).

## IV-Clinical Manifestations

### 1-Typical CSD

The clinical presentation of CSD typically begins 3-10 days after bacterial inoculation with the formation of a skin lesion at the site of the scratch. The lesion usually starts as an erythematous papule resembling an insect bite, commonly appearing on the hand or forearm, and progresses to form a vesicle and then a crusted ulcer. It typically heals within 1-3 weeks (Chian et al., 2002). In some cases, patients may also experience transient rash and skin manifestations such as erythema nodosum (Schattner et al., 2018). Systemic symptoms may accompany the skin lesion and lymphadenopathy, including fever, fatigue, loss of appetite, nausea, abdominal pain, headache, and sore throat, persisting for several days in some patients (Carithers, 1985).

CSD is characterized by its most prominent symptom, lymphadenopathy, which is observed in all patients. It typically begins 1-7 weeks after inoculation and often resolves spontaneously within 2-4 months. Rarely, lymphadenopathy can persist for several years. In approximately 85% of cases, only one ipsilateral lymph node is affected, and involvement of multiple lymph nodes in a single region or generalized lymphadenopathy is rare. The distribution of lymphadenopathy by frequency is as follows: epitrochlear and axillary regions (46%), head and neck (26%), and inguinal (17.5%) (Carithers, 1985; Zangwill, 2021). Ultrasonography is important for evaluating the characteristics of lymphadenopathy and detecting the presence of suppuration. Ultrasonographic findings often include highly vascularised, hypoechoic lymph nodes with increased echogenicity in the

surrounding soft tissues (García et al., 2000). Suppuration of lymph nodes occurs in about 10% of cases, necessitating intermittent drainage to alleviate associated symptoms (Massei et al., 2005).

CSD cases show musculoskeletal involvement in approximately 10.5% of patients (Maman et al., 2007). Severe myalgia is observed in 5.8% of cases and can persist for up to 4 weeks. Moderate to severe arthralgia is seen in 5.5% of cases and may continue for up to 13 weeks (Giladi et al., 2005; Maman et al., 2007). Cases of weakening arthritis lasting over 1 year have also been reported. Arthritis primarily affecting the knee, ankle, wrist, hand joints, and elbow. Complications such as osteomyelitis, neuralgia, and tendonitis are rare but can occur (Hajjaji et al., 2007). Female gender, age over 20 years, and the presence of erythema nodosum are considered risk factors for musculoskeletal involvement in CSD (Giladi et al., 2005).

**2-Atypical Manifestations of CSD**

**Ocular Involvement**

The most common ocular manifestations of CSD include Parinaud oculoglandular syndrome and neuroretinitis, with less frequent occurrences of papillitis, optic neuritis, optic nerve granuloma, and choroiditis (C. Kalogeropoulos et al., 2011; D. Kalogeropoulos et al., 2019). Parinaud oculoglandular syndrome is observed in 2-8% of CSD cases. It is characterized by granulomatous conjunctivitis and lymphadenopathy typically in the ipsilateral preauricular region. Symptoms may include foreign body sensation in the eye, redness, and occasionally serous or rarely purulent discharge, as well as increased tearing. This condition typically

resolves spontaneously without causing serious ocular complications (Cunningham & Koehler, 2000).

Neuroretinitis is observed in approximately 1-2% of CSD cases. It is characterized by optic nerve edema due to macular exudates, resulting in acute vision loss. It is considered the most common infectious cause of neuroretinitis (Bhatti et al., 2001). A hallmark feature is the 'macular star' appearance, which is formed by stellate macular exudates. Unlike other forms of neuroretinitis, this condition typically resolves spontaneously without leaving significant visual sequelae or with mild visual impairment.

**Neurological Involvement**

Approximately 2% of CSD cases exhibit neurological involvement, with 90% of these cases attributed to encephalopathy. Other neurological manifestations include transverse myelitis, radiculitis, and cerebellar ataxia (Baylor et al., 2007; Marra, 1995; Selby & Walker, 1979). Encephalopathy typically presents in adolescents and adults about 2-3 weeks after the development of lymphadenopathy. Generalized and persistent headache is the most common symptom, and fever is not present in every case. A wide range of seizures can occur in about half of the cases, from focal to generalized, and from mild and brief seizures to status epilepticus. Additional findings may include focal neurological deficits, pupillary dilation, neck stiffness, and pathological reflexes. Cerebrospinal fluid (CSF) analysis and brain MRI are usually normal. Electroencephalography (EEG) may show nonspecific findings like diffuse slowing that improve after recovery. The prognosis is generally favorable, with symptoms typically self-limiting within weeks to months (rarely up to 1 year). However,

persistent seizures and cognitive impairments can rarely occur (Hahn et al., 1994; Revol et al., 1992).

**Hepatosplenic Involvement**

Hepatosplenic involvement occurs in approximately 2.3% of CSD cases (Tirotta et al., 2021). It characterized by prolonged fever and microabscesses in the liver and/or spleen. Splenic rupture due to granulomatous disease can occur (Daybell et al., 2004). It often causes severe dull pain, particularly in the periumbilical region and upper quadrants, accompanied by weight loss. Hepatosplenomegaly is commonly observed without peripheral lymphadenopathy (Arisoy et al., 1999). Elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels are typical, while liver function tests are generally normal. Ultrasonography shows hypoechoic lesions, and CT reveals multiple hypodense lesions. Except for rare cases with sequelae calcifications, symptoms and lesions typically regress within 6 months (Ventura et al., 1999).

**Other Atypical Manifestations**

In CSD cases, rare atypical manifestations such fever of unknown origin (FUO) and prolonged fever (Landes et al., 2020), hemolytic anemia, thrombotic thrombocytopenic purpura (TTP) and coagulation disorders (Florin et al., 2008), hypercalcemia (Bosch, 1998), endocarditis (Brouqui & Raoult, 2001), atypical pneumonia (Abbasi & Chesney, 1995), glomerulonephritis (D'Agati et al., 1990) and pseudomalignancy (Florin et al., 2008) can be observed.

**3-Differential Diagnosis**

In the differential diagnosis of CSD, diseases characterized by lymphadenopathy such as brucellosis, mycobacterial infections,

sporotrichosis, tularemia, plague, histoplasmosis, syphilis, coccidioidomycosis, toxoplasmosis, infectious mononucleosis, lymphoma and other malignancies, and lymphogranuloma venereum should be considered (Koehler & Duncan, 2005).

## V-Diagnosis

The history, physical examination findings, and epidemiological risk factors such as history of cat exposure primarily guide the diagnosis of CSD. In laboratory diagnosis, molecular methods such as culture, serology, polymerase chain reaction (PCR), and histopathological examination are employed. Due to the difficulty in culturing the bacteria in vitro, culture methods are not commonly used in practice. Serological methods are the most frequently used diagnostic tools. The use of PCR is also increasingly common. Histopathological examination is utilized to demonstrate granulomatous infection associated with the bacterium, and to visualize the organism using special stains during the investigation of lymphadenopathy etiology.

### 1-Serology

Indirect fluorescence assay (IFA) and enzyme immunoassay (EIA) are the most commonly used serological methods for detecting *B. henselae* infection. Among these, IFA tests standardized by the Centers for Disease Control and Prevention (CDC) and commercially available tests used in laboratories are predominant (Dalton et al., 1995; Giladi et al., 2001). The sensitivity of IFA tests ranges from 84% to 95%, with a specificity of 94% to 98% (Zangwill et al., 1993). An IFA IgG titer <1:64 suggests the absence of *Bartonella* infection, while titers between 1:64 and 1:256 indicate

possible infection. An IFA IgG titer >1:256 strongly suggests *Bartonella* infection. A fourfold rise in consecutive IgG measurements taken between acute and convalescent phases 3-4 weeks apart is indicative of a definitive diagnosis (Dalton et al., 1995; Margileth, 2000). IFA IgG titers peak within 4-5 months and can remain positive for up to 3 years (Metzkor-Cotter et al., 2003; Zangwill et al., 1993). Due to potential false negatives early in the disease course, especially in the acute phase, false negatives can occur. EIA IgM testing can be positive within the first 3 months but is not recommended for diagnosing *Bartonella* infection due to its short-term positivity (Florin et al., 2008). Because IFA IgG can remain positive long-term, it cannot differentiate between recent and past infections. Cross-reactions in IFA tests among *Bartonella* species mean that positivity does not conclusively prove infection with *B. henselae* (Sander et al., 2001). Despite these limitations, IFA testing is non-invasive and practical, making it useful in aiding diagnosis.

**2-PCR**

PCR analysis is a method used for detecting *B. henselae* in tissues, blood, and other body fluids. Amplified genetic material typically targets genes such as *16S rRNA*, *gltA*, *htrA*, and *groEL*, although the sequences may vary (Fenollar & Raoult, 2004; Florin et al., 2008). The test has been reported to have a specificity of 100%, but its sensitivity ranges from 43% to 76%. Particularly in blood samples, the sensitivity is lower compared to tissue samples (Hansmann et al., 2005; Sander et al., 1999). If PCR analysis is to be performed on tissue samples, it has been shown that obtaining the

tissue sample within the first 6 weeks of the disease increases the likelihood of a positive result (Ridder et al., 2002).

### 3-Histopathology

In the diagnosis of CSD, histopathological examination is particularly useful for demonstrating the inflammatory reaction in tissues and identifying the bacteria. Biopsy samples reveal granulomas surrounded by lymphocytes in the outermost layer, containing palisading epithelioid cells, histiocytes, and occasionally multinucleated giant cells, surrounding stellate abscesses with central necrosis and neutrophilic infiltration. Bacteria, which stain black with silver stains such as Warthin-Starry, can be detected in biopsy samples (Florin et al., 2008).

## VI-Treatment and Prevention

In typical CSD cases, because the disease is self-limiting and the prognosis is generally good, monitoring without antibiotic therapy can be performed. However, azithromycin treatment has been shown to accelerate symptom resolution and is recommended. For typical CSD patients, the use of azithromycin involves an initial dose of 500 mg on the first day, followed by 250 mg once daily for the next 4 days, for a total of 5 days (Bass et al., 1998; Rolain et al., 2004). Alternative treatments may include ciprofloxacin, trimethoprim-sulfamethoxazole, and rifampin (Margileth, 2000). In patients with suppurative lymphadenopathy, intermittent aspirations can alleviate symptoms and signs. In severe cases, corticosteroid therapy may be considered. For patients whose symptoms and signs do not resolve despite current treatments, surgical drainage and excision of the lymph node can be considered as a last resort.

In patients with ocular and neurological involvement, although the disease can be self-limiting with follow-up without antibiotic therapy, the use of antibiotics is almost always recommended to accelerate symptom resolution. The recommended treatment is doxycycline 100 mg every 12 hours plus rifampin 300 mg every 12 hours for 4-6 weeks (Rolain et al., 2004). As an alternative treatment, azithromycin or trimethoprim-sulfamethoxazole can be considered in addition to rifampin instead of doxycycline. In patients with neuroretinitis, corticosteroid therapy under the supervision of an ophthalmologist may also be administered. For hepatosplenic involvement, the recommended treatment is azithromycin 500 mg on the first day, followed by 250 mg daily, plus rifampin 300 mg every 12 hours for a total of 14 days. As an alternative treatment, gentamicin can be considered in addition to rifampin instead of azithromycin. Corticosteroid therapy may be considered in severe cases (Arisoy et al., 1999; Dunn et al., 1997).

Prevention of the disease primarily involves avoiding the pathogen. Therefore, it is recommended to avoid stray cats, especially those under 1 year of age and those with fleas. For pet cats kept indoors, treating the cat to eliminate flea infestation is recommended. Antibiotic therapy aimed at *B. henselae* is not recommended for prevention, as it does not effectively treat bacteremia in cats. Although there is currently no routine vaccine available against *B. henselae*, efforts toward vaccine development are ongoing (Huwyler et al., 2017; Rahman et al., 2024).

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# CHAPTER III

# Distribution of HPV Genotypes in Men with Genital Warts

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## Introduction:

Human papillomavirus (HPV) is the most prevalent sexually transmitted pathogen globally (Sarıer, 2021). Beyond its mode of sexual transmission, HPV poses a significant public health risk as an oncogenic virus. For diagnosis, amplification tests like the nucleic acid polymerase chain reaction (PCR) are considered the gold

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standard for identifying and genotyping HPV (Abreu et al., 2012:9). This study aims to evaluate the HPV genotyping results in male patients who visit the urology clinic with genital warts caused by HPV Others may cause abnormal cells to develop and these may turn into cancer (De Sanjose et al., 2010:11). Cervical cancer caused by HPV; It is the most common type of cancer compared to other types that cause anal, vulvar, vaginal, mouth/throat and penile cancer. (WHO). Generally, the −16 and −18 types of HPV infection are responsible for almost 70% of cervical cancer cases (Khan et al., 2007:11) but it is known as −31, −33, −35, −39, −45, −51, −52, −56, −58 , −59, −68, −73 with HPV types in high risk and −82, −6, −11, −40, −42, −43, −44, −54, −61, −70, − 72, −81 with HPV types in low risk (Munoz et al, 2003:348).

It is estimated that 620,000 new cancer cases in women and 70,000 new cancer cases in men worldwide in 2019 were caused by HPV (https://gco.iarc.fr/tomorrow).

HPV threatens public health not only with its sexual transmission feature, but also as an oncogenic virus. (Sarıer, 2021). HPV diagnosis is based on molecular biology techniques and allows accurate detection and typing. Nucleic acid hybridization assays, signal amplification assays, and nucleic acid amplification are currently used techniques. (Abreu et al., 2012:9). PCR-based techniques are highly sensitive, specific and widely used. HPV-PCR protocols perform the amplification of multiple HPV genotypes in a single reaction using consensus primers such as PGMY09/PGMY1 and GP5+/GP6+. Real Time PCR is a reliable, sensitive and specific diagnostic method for the detection and genotyping of targeted HPV genotypes in tissue and cellular samples. Nucleic acids can be

detected even at very small concentrations using the dynamic range 7-log to estimate viral load/concentration via the standard curve. It is also a highly reproducible, rapid and applicable technique to clinical samples (Abreu et al., 2012:9).

**Method:**

This study included male patients aged 18 to 65 who visited the Antalya Medical Park Hospital Urology Polyclinic with complaints of genital warts between January 2021 and March 2024. Before condyloma cauterization, swab samples were collected from the patients' genital areas for HPV genotyping using a nylon-flocked swab moistened with isotonic solution and a multi-site sampling method. The samples were analyzed for 21 genotypes, comprising low-risk types (6, 11, 44) and high-risk types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82).

**Results:**

The study included 93 patients with a mean age of 33.54 ± 8.9 years. In total, 207 types of HPV-DNA were identified among these patients. Single type HPV-DNA was found in 32,2% of the patients, while multiple types were detected in 65,6%. No HPV-DNA was detected in two patients using the multi-site sampling method (Figure 1). At least one low-risk HPV type was identified in 50.24% of the patients, and at least one high-risk HPV type was found in 48.30% of the patients (Figure 2).

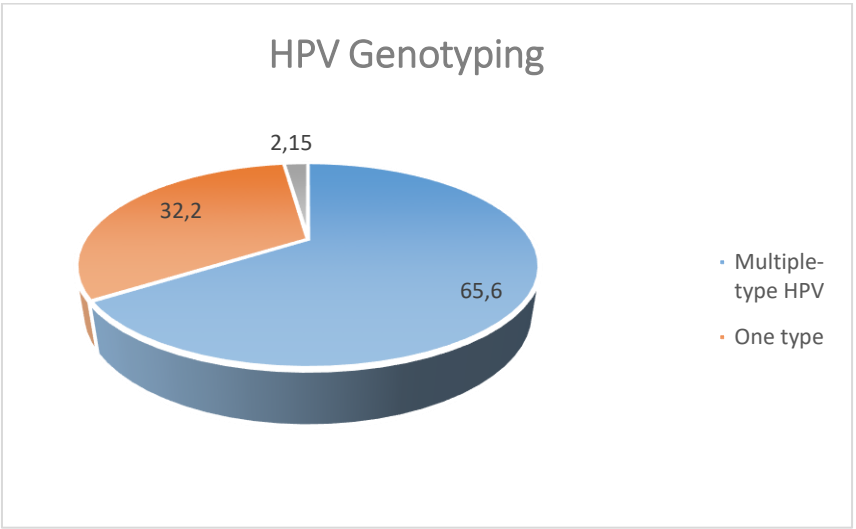


***Figure 1.*** *Distribution of HPV Genotypes according to their coexistence*

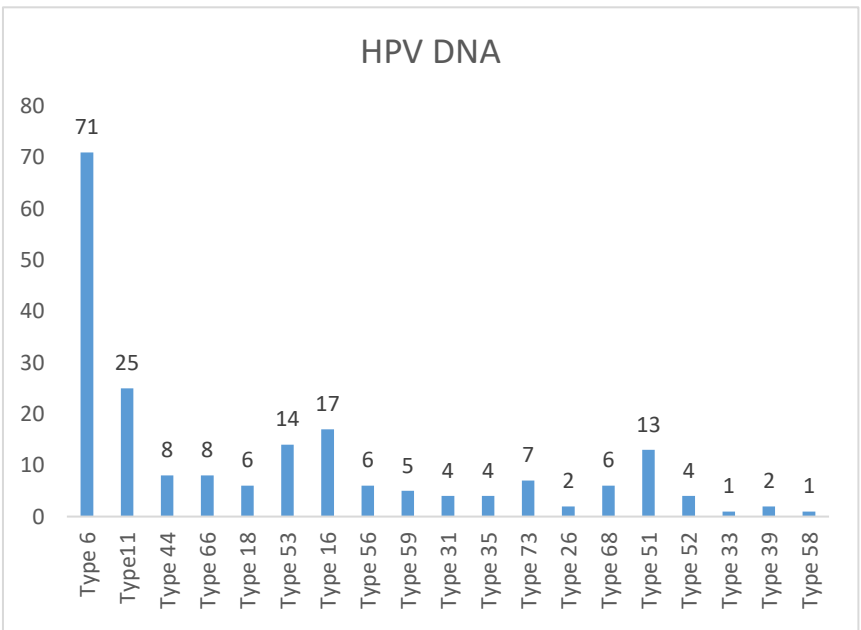


*Figure 2. HPV DNA distribution*

**Conclusion:**

HPV genotyping is necessary for men presenting with genital warts, as there is often a high rate of high-risk HPV types in these patients. Multi-site sampling proves to be a highly effective method for HPV genotyping in men.

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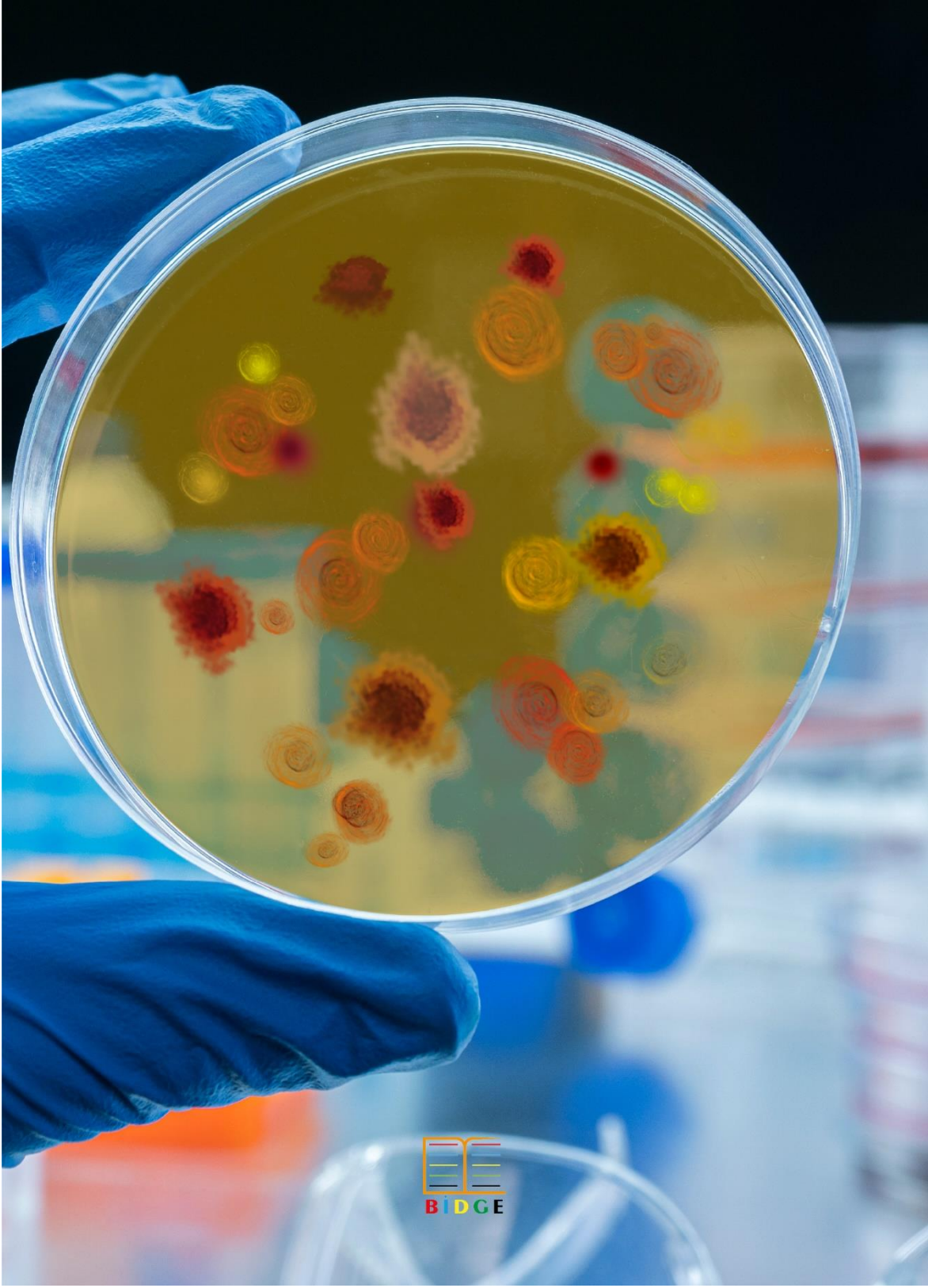
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