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PART I. DISEASES AND PROBLEMS DISTINGUISHED BY WHO AND FAO
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PROPERTIES AND PATHOGENICITY OF THE *VIBRIO* BACILLI

WŁAŚCIWOŚCI ORAZ CHOROBOTWÓRCZOŚĆ PAŁECZEK NALEŻĄCYCH DO RODZAJU *VIBRIO*

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Wkład autorów:
A. Study design/planning
zaplanowanie badań
B. Data collection/entry
zebranie danych
C. Data analysis/statistics
dane – analiza i statystyki
D. Data interpretation
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E. Preparation of manuscript
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G. Funds collection
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Summary

Currently, among 36 species of the *Vibrio* genus, 12 are potentially pathogenic to humans. The purpose of this study is to present the properties and pathogenicity of the *Vibrio* bacilli. The *Vibrio* family are pathogenic to humans causing dangerous infectious diseases that may often be fatal in the absence of effective therapy, but are also an infectious agent among animals. The most characteristic representative of the *Vibrionaceae* family is the species *Vibrio cholerae*. There are two strains that disrupt the water and electrolyte balance in humans: *V. cholerae* O1 and *V. cholerae* O139. The *V. cholerae* O1 serotype has two biotypes: classical and El Tor. Three serotypes are distinguished in each biotype – Inaba, Ogawa and Hikojima. The El Tor biotype shows the ability to survive and colonize the human body, causing chronic carrier state. Infections caused by species other than *V. cholerae* are referred to by the prefix "non" or NAG (Non-AgglutinatinG-Vibrio) – *V. cholerae* non-O1 and *V. cholerae* non-O139. They are found in natural bodies of water, lakes, estuaries and marine waters. In Poland, *V. cholerae* non-O1 and *V. cholerae* non-O139 were confirmed in previous years in the Bug River, near the border with Ukraine, during the summer-autumn period.

Keywords: *Vibrio cholerae,* diarrhea, Cholera, epidemic, water

Streszczenie

Obecnie znanych jest 36 gatunków należących do rodzaju *Vibrio*, z których potencjalnie chorobotwórcze dla człowieka jest aż 12 gatunków. Celem pracy jest przedstawienie właściwości oraz chorobotwórczość pałeczek należących do rodzaju *Vibrio*. Poszczególne gatunki przecinkowców są w dużej części chorobotwórcze dla człowieka, mogą powodować groźne choroby zakaźne, które często w przypadku braku skutecznej terapii mogą zakończyć się śmiercią, ale także stanowią czynnik zakaźny wśród zwierząt. Najpopularniejszym i najbardziej charakterystycznym przedstawicielem rodziny *Vibrionaceae* jest gatunek *Vibrio cholerae*. Wyróżnia się dwa szczepy tego patogenu, które u człowieka zaburzają gospodarkę wodno-elektrolitową, porażając jego układ pokarmowy: *V. cholerae* O1 i *V. cholerae* O139. Serotyp *V. cholerae* O1 posiada dwa biotypy: klasyczny i El Tor. U każdego biotypu wyróżnia się trzy serotypy – Inaba, Ogawa i Hikojima. Biotyp El Tor wykazuje zdolność do przetrwania i kolonizacji organizmu człowieka, wywołując u niego przewlekłe nosicielstwo. Zakażenia należące do rodzaju *Vibrio* wywoływane przez inne gatunki niż *V. cholerae*, określa się przedrostkiem „non" lub NAG (ang. *Non-AgglutinatinG-Vibrio*) – *V. cholerae* non-O1 i *V. cholerae* non-O139. Są bakteriami występującymi w naturalnych zbiornikach wodnych, jeziorach, w ujściach rzek oraz w wodach morskich. W Polsce w latach poprzednich w okresie letnio-jesiennym *V. cholerae* non-O1 oraz *V. cholerae* non-O139, potwierdzono w Bugu, przy granicy z Ukrainą.

Słowa kluczowe: *Vibrio cholerae,* biegunka, Cholera, epidemia, woda

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Introduction

In Bergey's systematics, cholera Vibrio is included in the order *Vibrionales*, family *Vibrionaceae* and genus *Vibrio* [1]. The genus *Vibrio* has a wide range of species, wherein those pathogenic to humans include: *V. furnissi* and *V. mimicus* (transmission through feces), *V. metschnikovii* biotype proteus (transmission through blood), *V. cholerae* and *V. fluvialis* (transmission through feces and blood), *V. hollisae* (transmission through feces and wounds), *V. damsela* and *V. parahaemolyticus* (transmission through feces, blood, wounds), *V. carchariae* (transmission through wounds – shark bite), *V. cincinnatiens* (transmission through blood and cerebrospinal fluid), *V. vulnificus* (transmission through blood, wounds, respiratory secretions and cerebrospinal fluid). The literature reports that there are 36 species belonging to the genus *Vibrio*, of which as many as 12 species are potentially pathogenic to humans, and these species were isolated from infected patients with symptoms of wound infection, diarrhea, food poisoning, or septicemia. Undoubtedly, however, *V. cholerae*, is the most widely studied and described by researchers in terms of causing pandemics and epidemics worldwide, an etiological agent of the cholera disease [1,2].

Aim of the work

The purpose of this study is to present the properties and pathogenicity of the *Vibrio* bacilli.

Vibrio cholerae

The most common and characteristic representative of the family *Vibrionaceae* is the species *Vibrio cholerae*. It is a Gram negative bacillus, ranging in size from 0.3-1.3x1.4-5.0 μm, showing movement thanks to a single, flagellum located at its pole. The microorganism grows under both aerobic and anaerobic conditions and, unlike *Enterobacteriaceae*, is characterized by the production of indophenol oxidase. There are two strains of this pathogen that disrupt the water and electrolyte balance in humans, paralyzing their digestive systems: *V. cholerae* O1 and *V. cholerae* O139 [3-5].

Cholera is a disease affecting the gastrointestinal tract and it has been known since ancient times (as reported by Hippocrates) [2]. *Vibrio cholerae* was first described in 1883 by R. Koch, who observed the curved bacterium in a microscopic examination in section specimens made from the intestinal tissue of people with diarrheal symptoms. Interestingly, the discoverer failed to infect laboratory animals with the microorganism, thus one of the postulates he established, the so-called Koch postulate, was not fulfilled [2]. History shows that cholera, causing severe intestinal catarrh and leading to dehydration of the body, was often an incurable and fatal disease in previous centuries. In later centuries, it was accepted that cholera included only those cases in which an O1 or O139 pathogen was successfully isolated from an infected person. Nowadays, cholera infections should be obligatorily reported to sanitary services [2]. In Poland, the risk of cholera infection has so far been low, but the ongoing war between Ukraine and Russia and the ensuing migration crisis mean that its risk has increased significantly over the past year. On the other hand, during the 12 years of monitoring this disease (2010-2022) in Poland, only one laboratory-confirmed case of infection was reported [3].

Serotype *V. cholerae* O1 has two biotypes: classical and El Tor. Three serotypes are distinguished in each biotype – Inaba, Ogawa and Hikojima [2,6]. More importantly, the El Tor biotype shows the ability to survive and colonize the human body, inducing chronic carrier state.

The El Tor biotype was discovered in 1906 at the quarantine station of the same name, on the Arabian Peninsula, in a healthy pilgrim traveling to Mecca. The aforementioned Inaba serotype was the cause of the seventh cholera pandemic, which broke out in Indonesia on the island of Sulawesi, as opposed to the previous six pandemics, which started in India, in the Ganges Delta. Since their first appearance in Asia in 1961, within 10 years they had been transmitted to Europe and Africa, and in the 1990s, they reached South and Latin America countries [1].

The main factor in the pathogenicity of *V. cholerae* is an enterotoxin known as choleragen, discovered in the 1950s, which is found only in strains that cause diarrhea and is absent in *Vibrio* spp. isolated from environmental samples. Cholera toxin belongs to the group of AB toxins composed of a single A subunit and three B subunits. The mechanism of toxic action is similar to the LT toxin of *Escherichia coli*, and the Stx toxin of Shigatoxigenic strains, and involves the binding of the toxin to receptors present in the epithelium of the small intestine, and after penetrating into its interior, disrupts the flow of signals carried by the G protein. As a result of this disruption, there is a permanent activation of c-AMP and the phenomenon of water and electrolyte secretion into the lumen of the small intestine. Other markers of *V. cholerae* pathogenicity are four genes localized in the so-called "virulence cassette", i.e. *ctx* AB, the *zot* gene encoding the protein of ZOT toxin (zonula occludens toxin) which increases the permeability of the small intestine and is responsible for closing intercellular spaces, the *ace* gene, encoding the protein of accessory cholera toxin, and the *cep* gene encoding the material of *fimbriae* that facilitate colonization of the intestine [7,8].

V. cholerae bacteria transmit very easily. Infection occurs through contact with contaminated water (excretions, sewage) or food [9]. Inadequate food and nutrition hygiene and hand hygiene result in the transmission of bacteria and the occurrence of new infections. That is why it is called the "dirty hands" disease – the name refers to the source of its origin. Epidemic outbreaks are determined by limited access to clean drinking water and sanitation, as well as being associated with humanitarian crises and the occurrence of armed conflicts [9,10]. Direct contact with an infected person can also be a source of infection [2].

The incubation period of the disease is very short – up to 5 days. The course of the disease is determined by the specific biotype of the bacteria – it can be mild, severe or asymptomatic [2,4,9].

In the classic form of cholera, symptoms appear suddenly and escalate rapidly. Its characteristic symptom in infected people is watery diarrhea without abdominal pain or a feeling of urgency. The stool is shapeless, discolored and has a characteristic faint odor. It does not contain blood. The infected person may experience vomiting or spasmodic muscle pain. No increase in body temperature is noted [7].

Over the course of cholera with the El Tor biotype, the symptoms are atypical – liquid stools resemble rice-washing water in their appearance, are abundant and iridescent. An infected person can pass up to 30 liters of liquid stool in a day. In the later stages of the disease, vomiting, increased thirst, muscle tremors and anuria may occur. Body temperature of a person affected by the El Tor biotype is normal or slightly elevated. Dehydration becomes the cause of ketoacidosis and rapid deterioration of the infected person's condition. As a result of inadequate treatment, dehydration symptoms lead to hypovolemic shock and acute renal failure. Immunity after the disease is short-lived [6,11,12].

The highly contagious cholera bacteria have been the cause of numerous epidemics around the world over the years. The World Health Organization (WHO) estimates that there are up to 4 million *V. cholerae* infections worldwide each year, of which 21,000 to 143,000 are fatal [9].

For centuries, the Ganges delta in the Bay of Bengal has been considered an endemic area for cholera. It is believed that this is where the first pandemic caused by the *V. cholerae* bacterium originated. The epidemic then covered the Indian subcontinent, Asia and parts of eastern African countries, and lasted from the early to mid-19th century [9,13]. The latest cholera outbreak was reported from March 1 to April 10, 2022 in Bangladesh. This is one of the most massive cholera outbreaks known in the country's history. During this time, more than 1,300 patients and about 42,000 cases of diarrhea were treated [9].

On the other hand, the literature reports that epidemic outbreaks of cholera are currently developing intensively mainly on the African continent, where the disease is a major epidemiological problem [2,6,10]. Numerous cases of cholera infection have been already detected in 12 African countries. Recent reports have been confirmed in South Africa, Tanzania and Zimbabwe. It is reported that a dramatic situation is developing in Malawi, where hundreds of infections have been confirmed, and that favorable conditions for the spread of the epidemic also

exist in Madagascar, which is struggling with the effects of two devastating cyclones. In contrast, about 3.5 million vaccines have been shipped to Kenya, Mozambique and the Democratic Republic of Congo to date [14,15].

The latest information shows that Syria is starting to face a cholera epidemic as a result of the February 6, 2023 earthquake in this country, and the resulting damage to its water and sanitation infrastructure. There have been 568 cases of infection and 2 deaths to date [11].

According to the latest 2021 report from the European Center for Disease Prevention and Control, 26 cases of cholera were reported in seven European Union countries in 2019, with the highest number in the UK (61.5%). The vast majority, 24 cases, were related to travelling to countries such as India, Pakistan, Tunisia, Bangladesh, Dominican Republic, Indonesia, Kenya and Yemen [16,17].

Treatment of the infected is carried out through intensive hydration of the body (primary treatment), and administration of appropriate pharmaceuticals (supportive treatment). The amount of glucose-electrolyte fluids taken should be adjusted according to the amount of fluids excreted in the stool and checked at two-hour intervals. In mild cases of the disease, rehydration is done orally through the administration of rehydration fluids, while severe cases of the disease may require intravenous rehydration. For symptoms that do not qualify as either mild or severe, the rule of thumb is to give 5 liters of fluids per 50 kg of body weight. In 2001, the World Health Organization, together with UNICEF, recommended the introduction of a new oral rehydration agent formula with the following chemical composition: sodium chloride – 3.5 g, sodium citrate – 2.5 g, potassium chloride 1.5 g and glucose 20 g dissolved in 1,000 ml of water. Pharmacological treatment is based on the administration of drugs from the tetracycline group, which reduce the duration of diarrhea [18,19]. The recommended dose is 1,000 mg/day for 5 days, and its contraindications include pregnancy and less than 12 years of age, yet even a single 1000-2000 mg dose of tetracyclines is effective for bacteremia [20].

Infection prevention includes five basic measures: immunization, rules of hygiene, quarantine, patient isolation, and use of personal protective equipment [5,21].

Cholera vaccines recommended by the World Health Organization are available on the global market. These include Shanchol™ (India), Euvichol-Plus® (South Korea), and Dukoral® (Sweden). Receiving only one dose of the vaccine does not provide full protection because of the short-lived post-vaccination immunity. A complete vaccination course provides protection for only about 3 years [3,13]. Due to the fact that most people (especially tourists traveling to epidemic and endemic cholera countries), take only one dose of the vaccine before their planned trip, thus not undergoing a full vaccination course and having only short-term protection against the disease, a new single-dose, oral Vaxchora vaccine has been launched, providing 90% effective protection after 10 days of ingestion [13].

Maintaining basic food and nutrition hygiene – such as consuming boiled water, heat-treated foods, washing hands with soap or other disinfectant, and refraining from eating raw foods in tourist areas where the disease is endemic – is also key in preventing the disease [21,22].

In case of confirmed infections, patients should be isolated, while those in contact should be quarantined (taking 5 days from last contact). It is also important to have medical personnel staying with infected people use mandatory personal protective equipment.

***Vibrio* NAG**

Infections caused by *Vibrio* spp. other than *V. cholerae* are referred to by the prefix "non" or simply NAG (NAG. Non-AgglutinatinG-*Vibrio*) – *V. cholerae* non-O1 and *V. cholerae* non-O139 [2,18,19]. These species are *Vibrio*-like with typical *Vibrio spp.* characteristics, although they differ in antigenic structure. Their characteristic property is causing infection with diarrhea and other symptoms, analogous to the classic course of cholera, although these symptoms are of shorter duration and less severe [16]. *V. cholerae* non-01 are similar in biochemical properties to El Tor, virulence cassettes have been identified in them, but they also have the ability to produce

a number of other toxins, including CT toxin, LT toxin and Kanagawa hemolysin (TDH) [2,14]. *V. cholerae* non-O1 and non-O139 *vibrio* are bacteria found in natural water bodies, lakes, estuaries and marine waters (especially in coastal waters) characterized by low salinity levels. Infection with such cholera species can be caused by consumption of contaminated water or the organisms that inhabit it (e.g., shellfish), or even consumption of natural products (e.g., various types of vegetables) that have been watered with contaminated water or sprayed at markets or in transport. The development of a pruritic infection or standard wound infection cannot be ruled out as a result of contact with contaminated water among fishermen or those bathing in the affected water while having damaged skin. Non-O1 cholera can also cause septicemia. The literature indicates that this pathogen has been isolated both from feces, blood, infected wounds, but also from urine, sputum and ear lesions from immunocompromised or chronically ill patients [6,7].

In Poland, the pathogenicity of *V. cholerae* non-O1 and *V. cholerae* non-O139, the presence of which was confirmed – with varying degrees of severity – in the waters of the Bug River, near the border with Ukraine, has been studied in previous years during the summer and autumn. Their occurrence has also been confirmed in other bodies of water in that region [1]. Infections with these strains have not been recorded, as river water is used for drinking purposes after treatment. The presence of cholera vibrio has also been found in the coastal waters of the Baltic Sea [2].

Infections with this pathogen cause diarrhea with an uncharacteristic course, ranging from an asymptomatic or mild form to severe cases of gastroenteritis. With established cirrhosis or blood neoplastic disease, infections can lead to serious complications. Mortality rates range from 50-60% [6,8].

Other *Vibrio* species *(V. mimicus, V. parahaemolyticus, V. vulnificus, V. fluvialis, V. hollisae, V. alginolyticus, V. damsela)*

Biochemically, the *V. mimicus* species is most similar to the *V. cholerae*species. The pathogen does not exhibit sucrose fermentation properties, hence the colonies on TCBS medium are green, not yellow. The use of TCBS medium in the diagnosis of *Vibrio* species, provides the opportunity for their rapid colony growth and morphological differentiation [23]. *V. mimicus* infections (manifesting as diarrhea) have been reported in the United States and Bangladesh. The infection vector can include contaminated seafood, i.e. shellfish, oysters, clams. The pathogen often causes disease among aquatic organisms living in contaminated waters [16].

V. parahaemolyticus is most often isolated from infected patients with sudden symptoms of *gastroenteritis*. It is mainly found in estuaries, including those in Poland. 20 deaths from infection with this *Vibrio* species were reported in Japan in the middle of the last century, wherein consumption of inaccurately dried sardines was reported as the cause [16]. In general, the pathogen is the leading cause of most bacterial gastrointestinal infections and food poisoning in Japan – especially in places where seafood is served (such as cruise ships). Infections are manifested by short-course diarrhea (up to 3 days), sometimes with blood in the stool. In the United States, the presence of *V. parahaemolyticus* has been confirmed in laboratory tests from biological samples taken from infected persons consuming improperly cooked crabs, lobsters, oysters, shrimp, as well as from the food itself, as served for consumption [2,16].

V. vulnificus strains have been implicated as a cause of wound infections – particularly in fishermen and sea bathers [23,24]. They can be particularly dangerous for people with immunosuppression or post-alcoholic cirrhosis – in case of whom they can cause sepsis – as well as for those with elevated plasma iron levels. The source of infection may be contaminated seafood. The mortality rate is as high as 67% [25]. Recent scientific reports indicate that *V. vulnificus* is expanding its range of occurrence on the US east coast as a result of ongoing climate change and could become a serious epidemiological problem. Studies show that the number of infections caused by this pathogen has increased dramatically from 10 to 80 cases per year [15,26]. Rapidly progressive tissue necrosis can

be caused by infection with *V. damsela*, which was most often isolated from wounds in contact with contaminated, salty seawater. The *V. alginolyticus* strain has also been isolated from patients after contact with seawater, from biological specimens collected from ears, conjunctivae, wounds and also cerebrospinal fluid [25] .

Infection with other *Vibrio* species, such as *V. fluvialis* and *V. hollisae* may occur after consumption of contaminated food, especially improperly heat-treated food, i.e. seafood such as shellfish, shrimp, lobster, oysters, raw fish, etc. [25,27].

Vibrio infections in animals

Not only humans, but also animals accompanying humans – both farmed (cattle, poultry) and domestic (dogs), but also wild (mainly fish and crustaceans) – are exposed to infections with *Vibrio* spp. (Table 1.).

Table 1. Infections with particular *Vibrio* species in selected groups of animals

Sea fish	Vibriosis in marine fish caused by *V. anguillarum* [28], as well as *V. parahaemolyticus* [16].
Crabs	*Vibrio cholerae* was the most commonly isolated bacterial agent among crabs *(Pachycheles pubescens*) [29].
Cattle	Studies of the liver microbiome in beef and dairy cattle have found that liver infection or other pathological conditions can be caused by pathogens from the genus *Vibrio*, but also *Salmonella*, *Yersinia*, or *Legionella*, and many others [30]. Studies confirm that cross-reactions of *Vibrio cholerae* with other pathogens *(Brucella* spp., *Escherichia coli*, or *Escherichia hermannii*) should also be taken into account when assessing seropositivity [31]. A study published in 2021 showed the presence of *Vibrio cholerae* in as many as 34% of fecal samples collected from dairy cows [32]. Some other studies report the presence of *Vibrio* spp. pathogens isolated from pork, buffalo and goat meat [33].
Dogs	A study of the endometrial microbiome structure in dogs with pyometra showed that there were significant differences between the microbiome of diseased and healthy dogs, and showed that *Vibrio* spp. Was the most common pathogen among diseased dogs [34].
Poultry	*Vibrio cholerae* and *Vibrio parahaemolyticus* have been demonstrated in poultry (broilers and chickens) [35].

Conclusions

The various species of *Vibrio* are largely pathogenic to humans, and can cause dangerous infectious diseases that can often be fatal in the absence of effective therapy, but are also an infectious agent among animals. A sizable number of species are mostly found in marine ecosystems, although their presence in freshwater, such as *V. cholerae*, cannot be ruled out. Their diversity and range of occurrence are mainly influenced by temperature, salinity and acidity levels of water bodies. They can be an epidemic factor causing numerous illnesses in a short period of time. Infection usually occurs through contact with contaminated water or after eating contaminated food. Many *Vibrio* species can cause acute gastrointestinal poisoning in humans (e.g. *V. cholerae, V. parahaemolyticus, V. vulnificus*), while others cause mild infections (e.g. *V. fluviolis, V. hollisae, V. alginolyticus*).

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