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Letter to Editor

Wastewater-Based Epidemiology: *Helicobacter pylori's* Gene in Wastewater and Its Prevalence Rate in Community

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To Editor,

Wastewater-based epidemiology (WBE) is an emerging field that analyzes wastewater for markers of infectious diseases, chemicals, and other indicators of population health (1). This letter focuses on the detection and surveillance of pathogen DNA/RNA, specifically for *Helicobacter pylori*'s virulence in wastewater. Wastewater epidemiology leverages the fact that humans excrete pathogens, drugs, and other substances in their waste. By analyzing wastewater, public health officials can gain insights into the health of a community. Recently, this approach has gained attraction for tracking the spread of the COVID-19 and drug usage. The ability to monitor the spread of pathogens such as *H. pylori*, a bacterium associated with gastric ulcers and cancer, is a promising application of WBE (2, 3).

It is estimated that around 4.4 billion individuals worldwide or about 58% of the global population are infected with *H. pylori*. However, prevalence varies greatly between regions and countries. Studies estimate the prevalence of *H. pylori* in Iran to be quite high, ranging from around 30%-90% of the population based on various studies. The overall prevalence in Iran is estimated to be around 50%-60%. One large study in the northern province of Guilan found an overall prevalence of over 80%. The humid climate and lower socioeconomic status in northern regions may contribute to higher rates. A few studies indicate high rates in western provinces such as Kurdistan (76%) and Kermanshah (60%-70%). One study in Bandar Abbas in southern Iran found an overall prevalence of around 60%. Furthermore, higher temperatures and unsanitary drinking water may promote transmission. Studies in central provinces such as Esfahan, Yazd, and Tehran tend to show moderate rates around 50-55%. Socioeconomic conditions are generally better in central urban areas. One study in Mashhad found a prevalence of 30% which is lower than that in other regions. Multiple studies revealed that rural areas have significantly higher infection rates (60%-90%) compared to urban areas (40%-50%), which is likely due to crowding and poor sanitation (4). *H. pylori* infection remains common throughout Iran, but rural western and northern provinces stand out as having particularly high rates in most studies. Ongoing improvements in sanitation and living conditions may help equalize regional differences in the future (4,5).

Factors Affecting Prevalence Rate

In general, *H. pylori* prevalence tends to be higher in developing countries compared to developed nations. Prevalence also increases with age and lower socioeconomic status. Environmental factors such as population density and sanitation also impact transmission.

Several factors that can affect the prevalence and transmission rates of the bacteria *H. pylori* in a population include (6):

- Sanitation and hygiene: Poor sanitation and hygiene practices, including the lack of clean water and proper sewage treatment, facilitate the transmission of *H. pylori*, and areas with poor sanitation tend to have a higher prevalence.
- Crowding: Higher population densities and crowded living conditions promote the person-to-person transmission of *H. pylori*, and prevalence is higher in crowded urban slums.
- Socioeconomic status: Low socioeconomic status is linked to higher *H. pylori* prevalence, likely due to crowded living conditions and poor sanitation.
- Age: The prevalence of *H. pylori* increases with age, with the highest rates seen in older adults who have

had decades of exposure to *H. pylori*

- Water source: The consumption of contaminated water can spread *H. pylori*. Moreover, the use of well water versus treated municipal water impacts the prevalence.
- Ethnicity and geography: Differences in prevalence exist between ethnic groups and geographic regions within a country. Genetic and environmental factors may also play a role.
- Antibiotic use: Widespread antibiotic use reduces *H. pylori* prevalence by eradicating infections, while the lack of access to antibiotics increases prevalence.
- Infection pressure: High infection rates within a population lead to repeated exposure and higher reinfection rates, propagating transmission.
- Living conditions: Factors such as household size, number of children, pets, toilet access, and water treatment affect the risk of *H. pylori* spread within families.
- Public health programs: Access to medical care, health education, and screening/treatment initiatives help lower the prevalence.

Contributing factors may include crowding, poor sanitation in some areas, the lack of clean water access, and low socioeconomic status among parts of the population. Studies showed that both rural areas and urban slums in Iran have very high infection rates (4, 5).

While still high in many developing nations, *H. pylori* prevalence has been slowly declining in most parts of the world likely due to improved living conditions and eradication therapy. However, prevalence remains stubbornly high in many lower-income regions. Improving sanitation infrastructure and living conditions may help reduce transmission in the future.

Detection of *Helicobacter pylori* in Water and Wastewater

There are several methods for detecting *H. pylori* in wastewater, some of which include (7, 8):

- Polymerase chain reaction (PCR): This method detects *H. pylori* DNA in wastewater samples. PCR can be very sensitive and specific for detecting low levels of *H. pylori*.
- Culture: Wastewater samples can be cultured on selective media to try to grow viable *H. pylori* bacteria. However, *H. pylori* is fastidious, so this method may underestimate its presence.
- Immunoassays: Antibody-based tests such as ELISAs can detect *H. pylori* antigens in wastewater if levels are high enough. This requires *H. pylori*-specific antibodies.
- Microscopy: Wastewater samples can be stained and examined under a microscope for the presence of *H. pylori* bacteria, and this method requires expertise and may not detect low levels.
- Adenosine triphosphate (ATP) bioluminescence: This method measures ATP, an indicator of microbial biomass, in wastewater samples. This can give an overall sense of microbial contamination but is not specific to *H. pylori*.
- Gene probes: Fluorescently-labeled DNA probes can bind and detect *H. pylori* rRNA in wastewater samples. They provide specificity but require intact rRNA.
- Biosensors: Novel biosensors are being developed with *H. pylori*-specific aptamers or antibodies that can provide rapid detection in wastewater, but it is still an emerging technology.

In general, PCR is considered the most sensitive and specific method for *H. pylori* detection in wastewater. Culture, microscopy, and immunoassays are also used. The method choice depends on the resources and capabilities of the laboratory.

Weaknesses of *Helicobacter pylori* Detection in Wastewater

- Low concentration: *H. pylori* is present in low concentrations in wastewater, making it difficult to detect *H. pylori* using traditional methods.
- Contamination: Wastewater samples may be contaminated by other bacteria and organic matter, making it challenging to specifically detect *H. pylori*.
- Inhibition: Some components in wastewater may inhibit the detection of *H. pylori*, leading to false negative results.
- Variability: Wastewater composition can vary widely depending on the source, which can affect the accuracy and reliability of *H. pylori* detection.
- Sensitivity: Some detection methods may lack the sensitivity required to accurately detect low levels of *H. pylori* in wastewater.
- Cost: Some detection methods for *H. pylori* in wastewater may be expensive and not feasible for routine monitoring.

The detection of vacuolating cytotoxin A (vacA) and cytotoxin-associated gene A (cagA) gens in wastewater can provide valuable information regarding the prevalence of *H. pylori* in a community. PCR and quantitative PCR (qPCR) are common methods used to detect these genes in wastewater. Studies have demonstrated that the prevalence of vacA and cagA in wastewater mirrors the prevalence of *H. pylori* infection in the population. This suggests that wastewater analysis can be a reliable tool for monitoring the spread of *H. pylori*. Despite its potential, there are several challenges in implementing WBE for the detection of vacA and cagA. These include variations in shedding rates among individuals, survival of DNA in wastewater, and the need for highly sensitive and specific detection methods. Another challenge is the differentiation between live and dead bacteria in wastewater. Moreover, current detection methods do not distinguish between DNA from live and dead bacteria, which can lead to the overestimation of the prevalence of *H. pylori* (7). In addition, since the monitoring of Helicobacter in wastewater can provide useful information on the prevalence of the bacteria in a given population, it cannot be used as a definitive measure of the number of infected individuals. Other methods such as clinical testing and epidemiological studies are needed to accurately determine the prevalence of Helicobacter infection in a population.

Future research needs to focus on improving detection methods and understanding the survival of *H. pylori* and its DNA in wastewater. Furthermore, the development of standardized protocols for sample collection, processing, and analysis is crucial for the wider application of WBE.

Wastewater-based epidemiology offers a promising tool for monitoring the spread of *H. pylori* and its pathogenicity factors, vacA and cagA. Although there are challenges, ongoing research and technological advances are expected to overcome these and make WBE a reliable tool for public health surveillance.

Competing Interests
None.

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