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Articles

THE EXISTENCE OF VIBRIO CHOLERAE IN INDONESIA: FROM ENVIRONMENTAL TO CLINICAL ASPECTS (A CONCISE REVIEW)

Rian Ka Praja, Anggita Ratri Pusporini, Reny Rosalina, I Wayan Muda Suta Arta, I Dewa Made Sukrama, Ni Nengah Dwi Fatmawati

1-8

PDF

GOVERNMENT POLICIES STRATEGY IN DEALING WITH THE COVID-19 OUTBREAK: LESSONS FROM TAIWAN'S EXPERIENCE

Fridayani Helen Dian

9-20

PDF

PERUBAHAN SOSIAL BUDAYA MASYARAKAT KOREA SELATAN AKIBAT PANDEMI COVID-19

Margareth Theresia

21-27

PDF

INDONESIAN COVID-19 PREVENTION POLICIES ANALYSIS USING CUMULATIVE CASES DATA REGRESSION

Bhustomy Hakim, Akhmat Fauzi

28-33

PDF

RISK MITIGATION FOR SMALL AND MEDIUM-SIZED ENTERPRISES (SMES) IN THE MIDDLE OF VOLATILITY IN THE WORLD'S ECONOMY CONDITION

Gresika Bunga Sylvana

34-39

PDF

About OISAA Journal of Indonesia Emas

Author Fees

Information for Author

Author Guidelines

Template

Online Submissions

Online Submissions Guidelines

Online Revissions Guidelines

Privacy Statement

Tools

Mendeley User Guide

Insert Citation using Mendeley

Index

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THE EXISTENCE OF *VIBRIO CHOLERAE* IN INDONESIA: FROM ENVIRONMENTAL TO CLINICAL ASPECTS (A CONCISE REVIEW)

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ABSTRACT

Vibrio cholerae is an infectious agent causing cholera disease with a high prevalence in various developing countries. *V. cholerae* is a pathogen with broad spectrum host that can infect humans and animals, especially aquaculture. The existence of this disease in Indonesia has long been identified in several outbreaks. Various reports in Indonesia have succeeded in finding the existence of *V. cholerae* in the environment, aquaculture, food and beverage, as well as in clinical cases of *V. cholerae* infection. The presence of *V. cholerae* in environment such as water source is commonly related with contamination. However, *V. cholerae* can be found in aquatic environment as this environment is natural habitat for *V. cholerae*. Thus, aquaculture is prone to be infected with *V. cholerae* because the presence of this pathogen is abundant in aquatic environment. Contaminated food and beverage are associated with hygiene and sanitation and human is commonly infected after consuming contaminated food or beverage. This brief review has the main focus to discuss the existence of *V. cholerae* from environmental to clinical aspects found in Indonesia.

Keywords: *Vibrio cholerae*, environment, aquaculture, clinical cases, Indonesia

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INTRODUCTION

Vibrio cholerae causes cholerae disease with a fairly high prevalence in various developing countries including Indonesia and its transmission is closely related to poor hygiene and sanitation [1]. The ability of *V. cholerae* to express several virulence factors is the cause of disease in humans. Outer membrane protein W (*ompW), ompU,* zonula occludens toxin *(Zot),* cholera toxin *(ctx),* and hemolysin A *(hlyA)* are virulence factors produced by *V. cholerae* playing pivotal role in cholera disease [2–4]. Several studies related to the presence of *V. cholerae* in Indonesia have been conducted. About 22 papers were discovered using specific keywords "Vibrio cholerae" and "Indonesia" in PubMed, Science Direct and neliti.com (offical Indonesia's research repository) in November, 2019. In some studies, *V. cholerae* has been found in environments such as water sources, mangrove sediments and hospital cutlery [5,6]. Other studies revealed that *V. cholerae* is one of the pathogenic bacteria that cause vibriosis in aquaculture and results in economic losses [7]. In term of food contamination, several researchers in Indonesia have found *V. cholerae* contamination in various types of

food and beverages that can threaten health [1,4,8]. Furthermore, clinically, reports of diarrhea caused by *V. cholerae* were found in various big cities in Indonesia with different characteristics [9–11]. Herein, this perspective aims to explain the existence of *V. cholerae* in Indonesia through environmental, aquaculture, food safety, and clinical aspects. These four aspects have been selected because they are important areas where *V. cholerae* can be mainly isolated [12]. Unfortunately, some reports exerted *V. cholerae* isolated from edible ice, beverages, and clinical samples were resistant to several antibiotics. This perspective should be useful not only for microbiologists but also for some elements related.

THE PRESENCE OF *VIBRIO CHOLERAE* IN ENVIRONMENT

Outbreaks of *V. cholerae* infection can occur anywhere sporadically, especially in areas with poor water supply, sanitation, food hygiene. A dense settlement with a poor supply of drinking water is one of important factors in the transmission of this pathogen. Investigation of *V. cholerae* contamination in residents' drinking water sources in Indonesia has been carried out by Kharirie (2013) in Cisarua District, Bogor, which was a cholera outbreak area. The results showed that the drinking water source of residents was contaminated by pathogenic *V. cholerae* serogroup O1 [5]. In addition to water sources, the presence of *V. cholerae* has also been reported from sediment samples in the mangrove forest area of marine station belonged to University of Riau, Dumai [6]. However, the presence of *V. cholerae* in marine environment is normal because it is natural habitat for this species [13–15].

Another report related to the presence of *V. cholerae* in hospital has been done in the period of November 2014 - January 2015. Samples were cutlery and food at the Nutrition Installation of Prof. RSUP DR. R. D. Kandou, Manado as many as 24 samples of cutlery. The results found that one out of 24 (4.17%) samples derived from cutlery was *V. cholerae* positive. Providing food that does not meet health requirements, in addition to prolonging the treatment process can also cause cross-infection or nosocomial infections which can be through food [16].

The presence of *V. cholerae* in water source is related with water contamination. Water source contamination in bogor was also found in various countries such as Bangladesh, Uganda, Nepal, and India [17–21]. Programs and interventions related to hygiene education in water processing must be done to control *V. cholera* infection from contaminated water [18].

VIBRIO CHOLERAE INFECTION IN AQUACULTURE

Aquaculture disease caused by bacteria, especially *Vibrio sp*. is a major concern due to causing economic loss. Vibriosis often attacks fish and invertebrates and results in death in aquaculture with a high prevalence. There are a few reports related to the existence of this pathogen in aquacultur in in Indonesia. Research in Pemalang, Indonesia showed that agents causing vibriosis in mangrove crabs were *V. harveyi, V. cholerae, V. parahaemolyticus, V. alginolyticus* and *V. fischeri* after isolation of the causative agent and confirmation using Koch postulate test [7]. In Indonesia, reports related to *V. cholerae* infection in aquaculture is not specific only in crabs. Saputra and coworkers in 2016 found *V. cholerae* from shellfish *(Pharella acutidens)* in the mangrove forest area of University of Riau [6]. These findings suggest that *V. cholerae* is a pathogen for aquaculture and control of infection is needed due to economic loss.

As mentioned previously, marine aquatic environment is the natural habitat of *V. cholerae*. That is why the presence of abundant *V. cholerae* is usually found in this environtment [13–15]. Fish has been reported to be the reservoir of *V. cholerae* and may spread *V. cholerae* in aquatic environment. Mostly this species can be isolated from fish intestine but in some cases it can be found in liver, kidney, skin, brain, and gills tissue. In most reports, the fish were healthy but in several they were diseased [22].

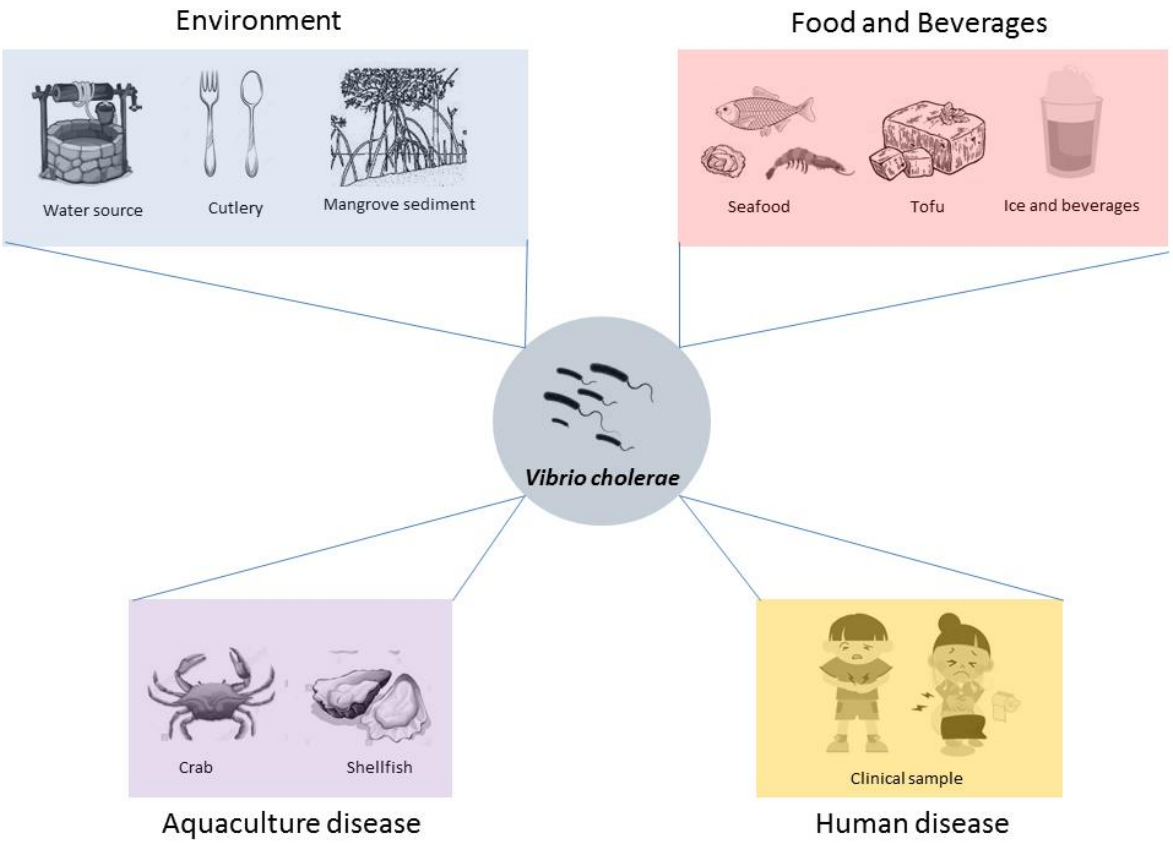

Figure 1. The presence of *V. cholerae* in Indonesia. *V. cholerae* can be isolated from environment, food and beverages, aquaculture, and humans. Aquaculture can get infection through contaminated environment and human can get cholera disease from contaminated environmental, food and beverages, and even human-to-human contact. However, there were no reports about infection from *V. cholerae*-infected aquaculture to human.

THE CONTAMINATION OF *VIBRIO CHOLERAE* IN FOOD AND BEVERAGE

Safe food is food that is free of harmful components or organisms that can cause poisoning or cause disease (food-borne disease). However, in reality not all food circulating is safe food. Several studies investigating *V. cholerae* contamination in Indonesia have found that some foods and drinks were contaminated with *V. cholerae*. It has been revealed that *petis*, a traditional Javanese sauce made from shrimp, sold in several places in Semarang was contaminated with *V. cholerae*. Contamination of *V. cholerae* in *petis* was considered to be caused by poor processing, serving, and sanitation of the processing station [1].

Indonesia is a country rich in seafood (fishery products) and it is common for Indonesians to consider seafood as a favorite food. Contamination findings in marine products such as shrimp, shellfish and fish have been reported. Praja and coworkers in 2019 revealed that all suspected isolates of *V. cholerae* isolated from shrimp and shellfish at the wet fish market in Bali carried *ompW* gene, a *V. cholerae* specific marker gene, but none of them that carried *ctx* gene [2]. Pheno-genotypic studies showed isolates from shrimps and shellfish carried *hlyA* gene that can lyse red blood cells [3]. Moreover, in another province, *V. cholerae* contamination in mackerel has also been reported in Duppa market which is a traditional market in Pekanbaru City [23]. Ice used as marine product preservative was also contaminated. Five of six samples (83.33%) from modern market were contaminated with *V. cholerae* O1 Inaba and samples from the traditional market showed four (66.67%) and one (16.67%) of six samples were contaminated with *V. cholerae* O1 Inaba and Hikojima, respectively [24].

A study conducted in Jakarta surprisingly found the existence of the cholera toxin gene which was isolated from ice and beverages. The findings showed that from 110 isolated

samples, 33 (30%) were positive of *V. cholerae* contamination. 21 (64%) were ice samples and the remaining 12 (36%) were beverage samples. From this study, Waturangi and coworkers also found that isolates of *V. cholerae* were pathogenic strains due to the presence of pathogenic genes *hlyA* (59%), *ctxA* (19%) and *ompU* (16%) [8]. Furthermore, in 2011, the antibiotic-resistance profile of *V. cholerae* isolated from edible ice exerted 65, 60, 52, 39, 37, 19 and 3% of the isolates were resistant to some antibiotics including ampicillin, streptomycin, kanamycin, sulfamethoxazole-trimethoprim, erythromycin, tetracycline and ciprofloxacin, streptomycin, kanamycin, sulfamethoxazole-trimethoprim, erythromycin, tetracycline and ciprofloxacin, respectively [4]. This result suggested that *V. cholerae* isolated from edible Ice in Jakarta were multi-drug resistance and this condition was unfavorable.

VIBRIO CHOLERAE INFECTION IN HUMAN

Although the mechanism of the spread of cholera is well known but this is not fully understood why until now cholera epidemics often occur. Weak treatment of human feces and water handling are considered as one of the factors causing the spread of cholera. Moreover, seafood and water have been identified to serve as a reservoir of *V. cholerae* transmission. Several reports of *V. cholerae* in clinical setting have been reported in Aceh, Semarang, Surabaya, Irian Jaya, Jakarta, Bali, Makassar, and Medan [9–11,25,26].

The laboratory verified 63 out of 138 suspected cases of cholera in the epidemiological study in Aceh between 12 July and 15 August 1982. From this report, 53 of these patients were seen and followed up. Nonetheless, the disease was associated with a number of factors, including water exposure from the Tiro-Sigli River and ice consumption. Other results call into question the importance of existing chlorinating methods to dug wells and disinfect homes with Lysol during cholera outbreaks. The case-control approach to investigating the mode of cholera transmission has distinct weaknesses when used in endemic environments where there may not be a single primary transmission vehicle, or where the vehicle, such as river water, is used by all and is only frequently contaminated [9].

The phenol-genotypic characteristics of sic clinical isolates of *V. cholerae* isolated from Surabaya in 2009 were identified. The obtained DNA fingerprints indicated that those isolates were not from a single clone. In addition, all isolates produced cholera toxin and exhibited the classical toxin B subunit gene, suggesting that this is the first report of the occurrence of V. cholerae El Tor in Indonesia. Although all isolates were susceptible to nearly all the antibiotics studied, including ampicillin, chloramphenicol, ciprofloxacin, gentamicin, levofloxacin, kanamycin, nalidixic acid, norfloxacin, streptomycin, trimethoprim-sulfamethoxazole, and tetracycline, and had no mutations in the genes gyrA and parC, they nevertheless had the class 1 integron, which is a molecular carrier for the acquisition of drug resistance genes, suggesting they are able to get genetic element for antibiotic resistance [25].

An El Tor biotype cholera outbreak that occurred in a rural village in Irian Jaya, Indonesia was tested for risk factors associated with cholera disease. A strong correlation with membership in one of the five tribal groups in the village complex was associated with an elevated risk of cholera mortality (odds ratio=5.9) among those who died in the village during the epidemics. Interviews with members of the deceased's family showed a very strong association (odds ratio=11.6) between the likelihood of cholera mortality and attending a woman's two-day funeral a few days before a cholera-like disease outbreak in the village area. Previous flooding might provide suitable environment to the transmission of cholera [26].

However, the case of *V. cholerae* infection is underreported in Southeast Asia, underestimating the global burden of this disease. The World Health Organization claimed that, due to inadequate laboratory and epidemiological monitoring, officially reported cases of cholera account for only 5-10 percent of the total number of cases occurring annually worldwide [27]. Cases of cholera officially confirmed to the WHO in Southeast Asia do not include excess cases of 500,000–700,000, identified as acute watery diarrhea [28].

Farmed fish may be infected with *V. cholerae*, due mainly to poor hygiene during post-harvest handling. Saravanan et al. (2007) observed that in India's shrimps handled under HACCP (Hazard Analysis Critical Control Point) conditions, *V. cholerae* was not present but found in a single domestic shrimp sample [29]. Chen et al. (2004) isolated *V. cholerae* O1 and O139 from domestic shrimps in Malaysia, possibly due to contamination after harvest. Nevertheless, it is important to note that only those strains which produce cholera toxins which belong to the O1 and O139 serotypes are causative agents of cholera [30].

It is important to note, however, that only those strains developing cholera toxin that belong to serotypes O1 and O139 are causative agents of cholera. High levels of contamination (100%) were observed in a small number of raw seafood samples from street vendors during the Peruvian cholera epidemic but only one of the 1011 seafood samples tested for export under HACCP was positive [31]. Shrimp processors' adherence to GHP/GMP/HACCP standards has been proven as adequately effective on the exporting management to minimize the risk of cross-contamination. Additionally, processing steps in terms of freezing, washing, cooking or frying effect on choleragenic *V. cholerae* was also significant, resulting in up to 6 logs reduction [32].

The prevalence of choleragenic *V. cholerae* in water and shrimp ranged from 0 to 2 per cent based on existing FAO exposure reports. Reports have never shown the exact number of cells found in *V. cholerae*. Just two positive samples from about 22,000 tested hot-water shrimp samples showed a frequency of 0.01% for exported hot-water shrimp. While the qualitative risk assessment poses a situation where there seems to be little possibility of obtaining cholera from hot-water shrimps, quantitative approaches confirm this by estimating low disease risk levels for imported warm-water shrimps [32]. Though, global warming is now becoming a matter of concern. As the air warms up, the sea gets warmer and *Vibrio* is on the rise. Temperature monitoring is essential to ensure that food distributed throughout the industry is both safe and consumable. Failure to track temperature fluctuations can minimize consumer protection, leading to deadly foodborne illnesses and major recalls. The proper application of the HACCP shall at least cover this issue [33].

CONCLUSION

Investigations related to the presence of *V. cholerae* have revealed that this pathogenic agent can be found in environment, food and beverages, aquaculture, and clinical aspect. The presence of *V. cholerae* in environment such as water source and cutlery in hospital is related with contamination of *V. cholerae*. However, *V. cholerae* can be found in aquatic environment as this environment is natural habitat for *V. cholerae*. Thus, aquaculture is prone to be infected with *V. cholera* because the presence of this pathogen is abundant in aquatic environment. Contaminated food and beverage are associated with hygiene and sanitation and human is commonly infected after consuming contaminated food or beverage.

CONFLICT OF INTEREST

Authors declared there is no conflict of interests.

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