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REVIEW ARTICLE

Vibrio parahaemolyticus: a seafood-borne pathogen

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ABSTRACT

Vibrio parahaemolyticus is an important and significant food-borne bacterial pathogen causing public health problems in Thailand and also worldwide. The bacterium becomes the pandemic food-borne pathogen and has been reported to be the most common bacterial pathogen isolated from diarrhea patients every year. It can lead to self-limiting watery diarrhea, bloody diarrhea and gastroenteritis with its many virulence factors such as adhesins, thermostable direct hemolysin (*TDH*) and TDH related hemolysin (*TRH*) as well as type III secretion systems, T3SS1 and T3SS2. This review aims to provide the information of *V. parahaemolyticus* including its classification, characteristics, epidemiology, transmission, pathogenesis, virulence factors, identification method, treatment and prevention which may be advantageous to the control and prevention of the infection.

Keywords: *Vibrio parahaemolyticus*, food borne disease, virulence factors, toxins

INTRODUCTION

Food-borne disease is a significant and important public health threat that causes major economic and social problems [1]. During the last decade, many food-borne diseases associated with the consumption of contaminated pathogen and improper cooked foods have risen in some developed and developing countries. The epidemiological contagious of the food-borne disease is affected by many factors such as poor sanitation, the level of personal hygiene when cooking and handling foods, agriculture and farming, food cultural and distribution of raw foodstuff consumption behavior, transmission of the pathogens, host immunity, etc [2].

In Thailand, the situation of food-borne disease is always significant and important. According to the latest annual epidemiological surveillance report of the year 2016 by Bureau of Epidemiology, Thailand, there were 34,335 cases of food poisoning in early year (1st January to 18th April). Morbidity rate is 52.48 per 100,000 populations but no death was reported. In addition, the diagnostic result of food-borne pathogens that has been reported in monitoring epidemiology system for a total of 147 patients (0.43 % of 34,335 cases) mentioned that mostly 69 patients (46.94%) were infected with *V. parahaemolyticus*, followed by 57 cases (38.78%) of *Staphylococcus aureus* and 14 cases (9.53%) of *Salmonella* spp. [3]. Thai National Institute of Health reported the serotype of *V. parahaemolyticus* in clinical isolates in Thailand. Among 82

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V. parahaemolyticus samples, serotype O3:K6 was the most dominant with 17 samples (20.73%), followed by O1:K68 (12 samples or 14.63%) and O3:KUT (8 samples or 9.75%) [4].

V. parahaemolyticus infection mainly occurs via raw food consumption especially when seafood such as oysters, shrimp, squid, sardines and crab are consumed. Infection is also possible by consuming food with poor hygiene. The presence of *V. parahaemolyticus* in an outbreak of food borne diseases seems to be increasing and important worldwide especially in Thailand where food poisoning is annually reported as a public health problem. The outbreak of this food borne pathogen in many food industrial or food supply countries such as Thailand not only affects the health of its population, but also poses the impact on economic lost and international trading. Food export can be rejected due to unacceptable food contamination. Therefore, it is necessary to understand the nature of *V. parahaemolyticus* including bacterial characteristics, transmission, pathogenesis and virulence factors with the aim to prevent, control and minimize economic loss from such infection.

Background of *Vibrio parahaemolyticus*

Vibrio is classified into the bacteria domain and kingdom. It is in the proteobacteria phylum due to its Gram-negative staining bacteria [2]. The bacteria are grouped into Class Gammaproteobacteria, Order Vibrionales (possessing two circular chromosomes) and Family Vibrionaceae along with the other 8 genera [5]. The amount of G+C content of the bacteria in genus *Vibrio* is 38–51% as compared to that of the *Aeromonas* (about 57–63%), *Photobacterium* (about 40–44%), and *Plesiomonas* (about 51%). The genus *Vibrio* presently has about 48 species. Among those species, 11 are clinically important agents including *V. parahaemolyticus* [2]. *V. parahaemolyticus* is 0.5 - 0.8 µm wide and 1.4 - 2.4 µm long [6]. This agent is facultative anaerobe bacteria that can ferment glucose without producing gas and has an oxidase-positive test [7]. It is halophilic bacterium which could be naturally detected in many costal countries throughout the world in sea water, estuarine or marine environment [2]. It has a polar flagellum which renders the high motility in liquid media; more than that it also has lateral flagella allowing the migration across semi-solid surfaces by swarming [8]. Under stress condition, *V. parahaemolyticus* is able to adapt itself for survival. For example, the organism can switch its flagellum character at different surrounding environments (liquid environments or semi-solid surfaces) [9]. In liquid surrounding environment, such as marine or estuarine, *V. parahaemolyticus* produced a single flagellum at their polar. This polar flagellum is also sheathed and could help the bacterium for attachment and support them for swimming in liquid environment. With the ability of this single flagellum, *V. parahaemolyticus* can move faster with the speeds up at 60 µm/sec. Energy used to rotate the flagellum is supplied by a sodium motive force [10]. Meanwhile, in the surrounding environment of semi-solid surfaces, the organism creates multiple shorter flagella on its lateral side. The lateral flagella support the bacteria for swarming mobility at semi-solid environment. The numerous peritrichous and non-sheathed flagella help the bacterium to swarm the substrates [9].

V. parahaemolyticus have different types of flagella, i.e., swimming flagella (polar flagellum) and swarm flagella (lateral flagellum). The swarm flagella are made from one flagellin protein, not sheathed and move by the proton motive force. The adaptable production from a swimmer cell of flagella into swarmer cell flagella is highly regulated by *laf* genes (lateral flagella) under some conditions of environment circumstances (Figure 1) [9]. Decreasing of the rotating speed of flagella is an outcome effect from the increasing viscosity or consistency of the growth environment surrounding the bacteria, or the growth of bacteria under iron-limiting conditions. These factors involve in the switching of the flagella type from swimming to a swarming type [10]. *V. parahaemolyticus* requires salt to survive and sodium ion to stimulate the growth. The bacteria

can grow in 1-8 % NaCl, with suitable condition growth occurring in the 2-4 % range. It will die if cultured in the condition without salt such as distilled water. Thus, it is naturally occurring and found worldwide especially in coastal areas. Moreover, it is found in higher numbers in areas of large seafood consumption [8]. Mudoh and his team demonstrated that *V. parahaemolyticus* can grow at temperatures between 5-10˚C in food sources [11]. The optimum temperature of the bacterial growth is between 30-35˚C and the highest temperature is 44˚C [12]. Jay and colleagues observed the effect of pH condition to the growth of the bacteria. They found that the optimum pH condition of this organism is 7.6-8.6; however, the agent can survive in the pH 4.8-11.0 [13]. The optimal growth conditions of *V. parahaemolyticus* are showed in Table 1.

In fact, not all strains of *V. parahaemolyticus* cause a disease. *V. parahaemolyticus* is divided into 2 groups as pathogenic (clinical) strains that can cause the illness and non-pathogenic strains which usually live in environment and cannot cause the disease. On the other hand, pathogenic *V. parahaemolyticus* strains are slightly found in seafood or the environment as compared to non-pathogenic strains [6]. Pathogenic strains usually produce important virulence factors such as thermostable direct hemolysin (TDH) and related toxin, thermostable related hemolysin (TRH), while non-pathogenic strains cannot produce TDH and TRH but produce only thermolabile hemolysin (TLH). The dominant ability of clinical *V. parahaemolyticus* strains isolated from a stool sample of food-borne patients with gastroenteritis is the TDH-positive (TDH+) [8].

Epidemiology

V. parahaemolyticus was first isolated in 1950 after a huge outbreak of food poisoning from

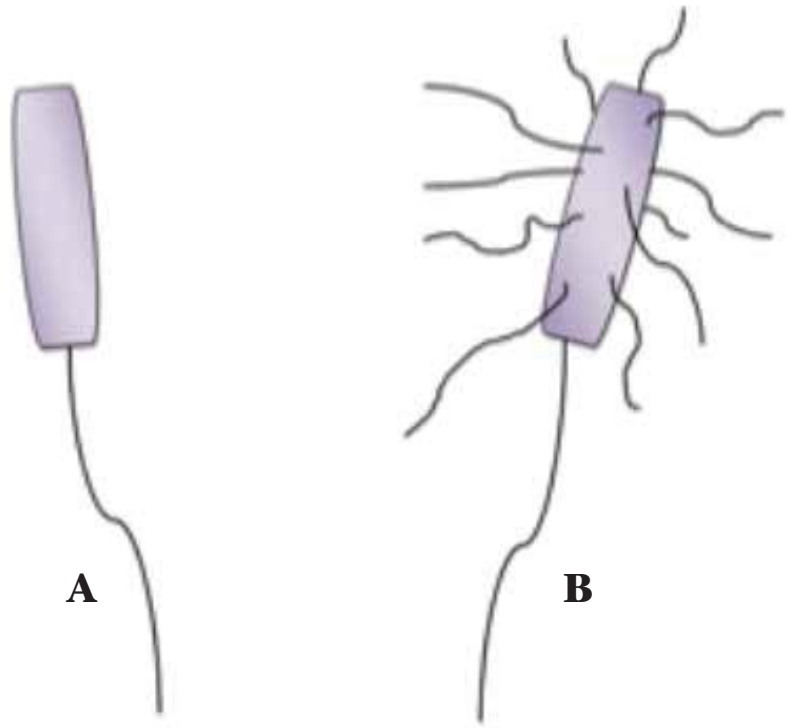

Fig 1: Differeent types of *Vibrio parahaemolyticus* flagella
A. Swimming flagella (polar flagellum)
B. Swarming flagella (lateral flagellum)

Table 1 Growth range and optimal range of *V. parahaemolyticus* growth conditions

Growth conditions	Growth range	Optimal range
Water activity (a_w)	0.940 - 0.996	0.992
Temperature (˚C)	5-44˚C	30-35˚C
pH	4.8-11.0	7.6-8.6
NaCl concentration	0.5-10%	3%
Atmosphere	Can grow in the presence or absence of oxygen	Aerobic conditions

Adopted from [13]

gastroenteritis patients in Japan [14]. After that, *V. parahaemolyticus* has been identified as one of the main pathogens that cause food-borne diseases or food poisoning in many countries where raw seafood are consumed such as Asian countries including Japan, Taiwan, India, Thailand and the others as the United States, France, Peru, Chile and Mexico [15]. In addition, these pathogenic infections have increased worldwide and become a significant pandemic infection.

The broad fundamental classification of *V. parahaemolyticus* employs the serotype classification scheme that relies on its antigenic properties of the somatic (O) and capsular (K) antigens, respectively. The serotype classification of *V. parahaemolyticus* is a combination of both O and K antigens that presently include 11 different O antigens and 71 different K types. It can be used to investigate the epidemiology of clinical isolates and the pandemic strains of *V. parahaemolyticus* which rapidly change their both O and K antigens, making it difficult to detect the outbreak in each pandemic strain and region [6]. In Thailand, *V. parahaemolyticus* was isolated for the first time in 1970 [16]. After that, the *V. parahaemolyticus* infections have been reported from various areas in Thailand [3]. The incidence rate of *V. parahaemolyticus* in Thailand was not significant except in 1996, when the infection occurred as a pandemic with a virulence gene call "tdh" and became an emerging disease by O3:K6 serotype in Southern part of Thailand [17]. In addition, other serovarients of O3:K6 had been identified from clinical isolates in Thailand such as O4:K68 in 1999, O1:K25 in 1999, O4:K12 in 1998-1999 and O1:K41 in 1998-1999 [4,6]. Since then, *V. parahaemolyticus* has become the most important food-borne pathogen and has been monitored as a public health warning [18].

Transmission

Generally, primary transmission route of *V. parahaemolyticus* is from the ingestion of raw seafood and raw contaminated or undercooked food such as shellfish (oysters, clams, and mussels). Cooked crustaceans (crab, lobster, and shrimp) can be contaminated with the bacteria if it has not been properly cooked by heat, or recontamination may occur by coming in contact with uncooked seafood at the period of storage [2,8]. Moreover, direct transmission is possible from the exposure of open wounds to *V. parahaemolyticus* contaminated seawater, shellfish, or finfish that can cause infections and septicemia [7,19]. The incubation period of *V. parahaemolyticus* is about 3 to 24 hours before patients have some symptoms, but the onset usually begins about 10-15 hours after infected with this pathogen [6].

Symptoms

The prominent symptom resulting from *V. parahaemolyticus* infection is self-limiting watery diarrhea but it occasionally causes bloody diarrhea and sudden cardiac arrhythmia [19]. The clinical

manifestations of patients are diarrhea, vomiting, abdominal cramps, nausea, head ache, fever and chills. Some cases may dehydrate, collapse and show abnormality on electrocardiograms [20].

Pathogenesis and Virulence Factors

Pathogenesis of *V. parahaemolyticus* can involve many virulence factors including adhesins, thermostable direct hemolysin (TDH) and TDH related hemolysin (TRH) as well as type III secretion systems, T3SS1 and T3SS2 [21-23].

Adhesin: Adhesion to host cells or Initial host cell binding step of *V. parahaemolyticus* is the most significant step for bacteria to develop pathogenesis. During the step of infection, adhesive factors of bacteria are exhibited at the bacterial surface, forming link with the host cell for secreting some bacterial effectors and bacterial toxin proteins to the host cells. An adhesion protein is called "MAM7" or Multivalent Adhesion Molecule 7 [24]. MAM7 is an adhesion protein which is preserved in Gram negative bacteria. It is essential for primary step for host binding during the infection [25]. *V. parahaemolyticus* using MAM7 in attaching to the host's fibronectin and phosphatidic acid; if these two substrates are blocked, the MAM7 adhesion to the host cells is prevented [9].

Furthermore, pandemic strains of *V.parahaemolyticus* which belongs to the O3:K6 serotype usually carry *orf8* gene. This *orf8* gene encodes an adherence protein which in turn, enhances the ability of this pathogenic strain in adhering to the host's intestinal cells. Therefore, this *orf8* gene can be used as the target gene in detecting pandemic *Vibrio parahaemolyticus* strain O3:K6 by molecular method [26].

Bacterial Toxin: The important toxins of *V. parahaemolyticus* called Themostable Direct Hemolysin (TDH) and TDH-Related Hemolysin (TRH) are two virulence factors that associated with *V. parahaemolyticus* hemolysis and cytotoxicity of the host cell [27]. Although *V. parahaemolyticus* is naturally occurring and found worldwide especially in sea water and coastal areas, there are only some pathogenic strains that can cause gastroenteritis in human. Most of *V. parahaemolyticus* strains (about 88-96%) isolated from clinical samples can produce β-hemolytic activity on blood agar which is attributed from TDH or TRH [21]. Pathogenic *V. parahaemolyticus* are able to completely lysed human or rabbit erythrocytes (β-hemolysis) by producing hemolysin when they were placed on a suitable high-salt selective media called Wagatsuma agar; this process is termed "Kanagawa Phenomenon (KP^+)" [28]. This Kanagawa Phenomenon test is commonly used to identify pathogenic *V. parahaemolyticus* in seafood as well as in patients' biological samples. In addition, it can be used in identifying the *tdh* gene in samples by molecular technique. Thus this bacterial hemolysin is the important virulence factor of the pathogenesis [29].

TDH was the first bacterial toxin recognized as virulence factor of *V. parahaemolyticus*. It was used as a biomarker to identify the pathogenic strains of these bacteria [30]. This toxin is produced from both *tdh1* and *tdh*2 genes but it was highly expressed from *tdh2* gene [23]. TDH produced from the *tdh* gene is translocates to the host cytoplasm by another virulence factor called "T3SS2" [31]. Nishibuchi and Kaper studied the relationship between TDH and secretory diarrhea and found that the TDH encoding gene is able to induce intestinal chloride secretion [23]. Moreover, Fabbri and team found that TDH also raises the cytosolic free calcium concentration [Ca^{2+}] in non-transformed rat intestinal IEC-6 cells [32]. When comparing the function of toxin activity produced by *V. parahaemolyticus* and *V. cholera*, TDH and Cholera Toxin (CT) stimulate Cl^- secretion from molecule signaling by dissimilar ways. CT will make the cAMP concentration increasing by ADP-ribosylating adenylate cyclase of intestinal epithelial cells whereas TDH will activate Ca^{2+}. When TDH stimulates $Ca^{2+,}$ it will cause a secretion of chloride ion from the calcium-activated chloride channels (CLCAs). In contrast, CT from *V. cholerae* stimulates cAMP from ADP-ribosylation of adenylate cyclase and after that

the stimulated cAMP will activate protein kinase A (PKA) that can phosphorylate the cystic fibrosis transmembrane receptor (CFTR) and lead to the secretion of Cl^- [33].

TRH has ability to lyse erythrocyte and it can induce chloride secretion in patient colonic epithelial cells similarly to TDH, so it was considered as an important virulence factor of *V. parahaemolyticus* [34]. Nucleotide sequence of *trh* gene has high similarity to *tdh* genes as 68.4% with the *tdh*1 gene copy and 68% with the *tdh*2 gene copy, respectively. However, the gene function is different from TDH by showing Kanagawa phenomenon negative (KP^-) appearance [35].

In addition, all *V. parahaemolyticus* strains usually carry *toxR and tlh* genes. *toxR* is a regulatory gene of the toxin operon and recognized to be a conserved gene among *V. parahaemolyticus* [36] while *tlh* is a gene that encode the thermolablile hemolysin (TLH). Similar to TDH, TLH has a phospholipase activity property that can lyse erythrocytes. Moreover, *tlh* are mentioned to be a specific target gene for *V. parahaemolyticus* detection [37]. Therefore, many molecular methods, such as PCR, can identify these bacteria by amplifying *tlh* genes.

Type III Secretion Systems (T3SS): The T3SS is a bacterial organelle protein accessory that delivers or transports proteins or some effectors directly from bacterial pathogens into cytoplasm of host cell [38]. T3SS1 and T3SS2 are the two T3SS generally found in Gram negative bacteria. T3SS generate a secretion apparatus which consists of a basal body that extends outer and inner membranes of bacteria, similarly to a needle that functions as a secreting duct or injector between the bacterial pathogen and host cells. There is also a translocon pore that is inserted into the host cell membrane to propose as a passing channel for bacterial toxin and effectors [39]. T3SS facilitate the transportation of some effectors or bacterial toxin to the host cell leading to pathogenesis and results in the alteration of homeostasis and host cell's integrity. T3SS1 can support *V. parahaemolyticus* strains to survive in circumstance [40]. The T3SS1 is one of virulence factor that has an ability to cause cellulysis, thus permit the release of significant nutrients [41]. *V. parahaemolyticus* has many effector proteins, which are translocated by different types of T3SS, responsible for pathogenesis. T3SS1, presented in all *V. parahaemolyticus* both pathogenic and non-pathogenic strains, has ability to translocate some effectors such as VopQ, VopR, VopS, and VPA0450 to cause cytotoxicity while T3SS2, found only in *V. parahaemolyticus* KP positive and *trh* positive strains, translocates some effectors such as VopA, VopC, VopL, and VopT to cause cytotoxicity of colon epithelial cells and enterotoxicity within the host [27]. The phylogenetic analysis from Okada and her team in 2009 also showed the two types of T3SS2; T3SS2α and T3SS2β which are also found in pathogenic *V. cholerae* serogroup non-O1 and non-O139 strains. The T3SS2α is only distributed in *V. parahaemolyticus* KP positive, *tdh* positive and *trh* negative strains, whereas the T3SS2β is only found in *V. parahaemolyticus* KP negative, *tdh* positive/negative and *trh* positive strains [42].

Type VI Secretion Systems (T6SS): T6SS is the protein secretion system widely spreading in many Gram-negative bacteria. It has two putative types and tightly regulation. The T6SS is a newly bacterial mechanism for protein transport into a recipient cell [43]. T6SS can transport some toxins or effectors into other nearby eukaryotic host cells or bacterial cells for a reason to mediate interbacterial competition or compete with other bacteria in various environments to gain resources. *V. parahaemolyticus* encodes two types of T6SS. T6SS1 is mainly found in pathogenic or clinical strains, while T6SS2 is generally found in all strains [44]. Presently, few T6SS secreting protein effectors have been studied and characterized [45]. T6SS is important in delivering toxic effectors protein into the cytoplasm of eukaryotic host cells along with permitting these effectors to interrupt innate immune system and then kill the host cells [46].

Vibrioferrin: Bacteria need some essential nutrients for their survival. Some iron storage proteins, such as heme-free ferritins and heme-

containing bacterioferritins, are important and widespread in bacteria [47]. Some bacteria produce iron chelator called siderophore to acquire iron from surroundings such as *E. coli* and *Klebsiella pneumoniae*, while *V. parahaemolyticus* can produce its siderophore called "vibrioferrin" when it grows under iron limiting conditions. The vibrioferrin is produced when the medium contains a limited amount of iron or when an iron chelator, EDDA, is added into iron rich medium. A study by Yamamoto and team [48] showed that clinical isolates of *V. parahaemolyticus* (n = 44) had higher levels of vibrioferrin than in food (n = 37) and environment (n = 26) when they were grown in medium consisting limited amount of $FeCl_3$. An increased vibrioferrin production may support *V. parahaemolyticus*'s survival in iron-limiting environments such as in the human host.

In addition, *V. parahaemolyticus* cultured under iron-limited conditions demonstrated an increasing hemolytic activity and adherence as well as a higher proliferation rate [49]. In consequence, the release of vibrioferrin may also lead to the pathogenesis of *V. parahaemolyticus* as mentioned above.

Bacterial Identification

Various methods are used to identify *V. parahaemolyticus* depending on the target results and type of the samples. These identification methods are different by their principles, sensitivity and specificity such as conventional, molecular and immunological technique.

Conventional method: Several selective media or enrichment media are chosen to isolate and detect *V. parahaemolyticus* from clinical samples of patient feces or seafood samples [50]. Because of its halophilicity, the selective media for the organism is usually prepared at pH about 8.6–9.4 by adding 1–7% NaCl. In appropriately condition, the selective media for this bacteria are composed of special agents such as bile salts, alkylbenzene sulphonate and sodium dodecyl sulphate (SDS), antibiotics such as polymyxin B or colistin and dye agents such as metachrome yellow II RD to inhibit other bacteria to grow [51]. In addition, *Vibrio* species need the enrichment broth such as salt colistin broth, alternative protein source broth (APS), salt polymyxin broth (SPB), bile salt sodium taurocholate (ST broth) and glucose salt teepol broth [52]. Several selective media for isolation and identification of *V. parahaemolyticus* are commercially available. The most usual selective medium is thiosulphate citrate bile salts sucrose (TCBS). TCBS is an effective selective medium and offer a high level differentiation to *V. cholera* and all other *Vibrio* pathogenic species except *V. hollisa* [53]. TCBS consists of 0.8% ox bile, 1% NaCl, and high concentration of sodium citrate and sodium thiosulfate to inhibit the growth of *Enterobacteriaceae* bacteria. It is adjusted to pH 8.6 in order to inhibit and suppress the growth of other Gram positive bacteria in the agar. Thymol blue and bromothymol blue are added to act as an indicator for pH changes. The advantage of TCBS agar is its differential diagnostic system; sucrose/bromothymol blue that can differentiate positive sucrose fermentation *Vibrios*, such as *V. cholera*, from the other *Vibrio* species. *V. cholerae* can grow on the TCBS agar and show the colony size and morphology about 2–3 mm diameter, and express yellow colonies on this agar [54]. On the other hand, *V. parahaemolyticus* colony size would be typical 2–3 mm diameter and has an opaque, round and green or bluish colony because it cannot ferment sucrose as shown in Figure 2. Another selective medium is "Wagatsuma agar" which is used to detect the Kanagawa Phenomenon of *V. parahaemolyticus*. This agar is produced from rabbit or human blood with mannitol, NaCl, K_2HPO_4 and crystal violet. The common preference of Wagatsuma agar is its usage in identifying and separating the TDH positive and TRH toxin negative producing strains of *V. parahaemolyticus*. The bacterial strains that can produce the *tdh* gene will result in hemolysis of red blood cell in Wagatsuma agar[23].

Molecular method: The conventional technique to identify *V. parahaemolyticus* is based on bacterial phenotypic analysis and the biochemical test

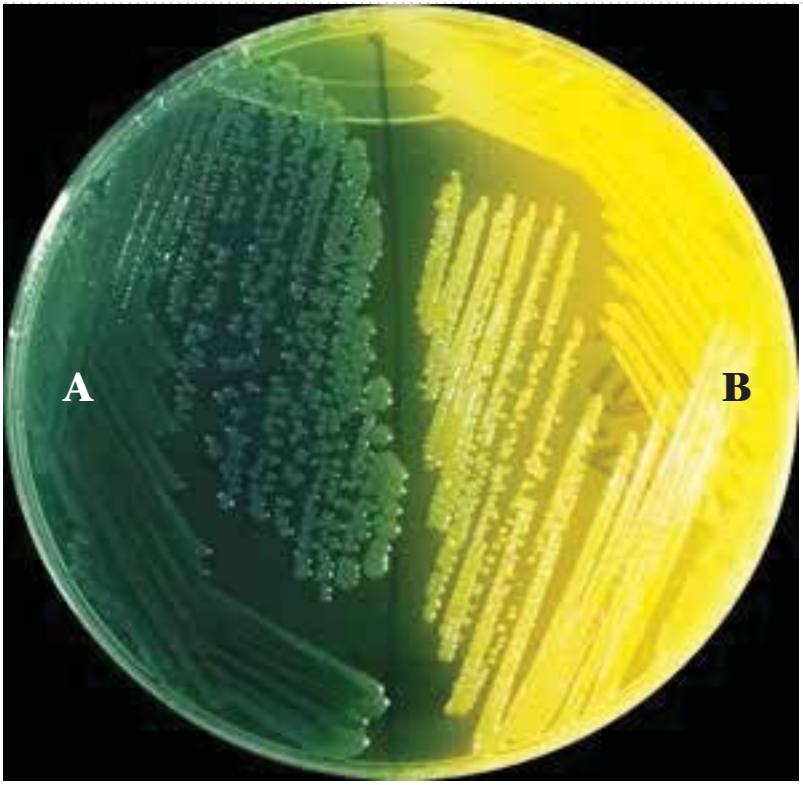

Fig 2 Vibrio species grown on TCBS agar (Photo by Nitaya Indrawattana)
A. *V. parahaemolyticus*, green colonies (non sucrose ferment) on TCBS agar.
B. *V. cholera*e, yellow colonies (sucrose ferment) on TCBS agar.

which is laboratories and time consuming [46]. Therefore, the polymerase chain reaction method (PCR) based assay is replacing the conventional one [55]. This molecular method will be used to differentiate *V. parahaemolyticus* strain. PCR technique, which is based on gene amplification, can be developed into multiplexed PCR or real time PCR to give better result of identification [56]. As the result, PCR has high specificity and sensitivity for identification and detection of pathogenic and non pathogenic strains from various samples such as clinical sample from patients, seafood and environmental samples [19]. Presently, PCR assays including realtime-PCR and multiplex PCR have been developed to effectively amplify *tdh*, *trh*, *tox*R genes of *V. parahaemolyticus*. Mainly, the amplification of *tlh* gene is applied for total *V. parahaemolyticus* dectection, meanwhile the amplification of *orf8* gene is applied for the detection of pandemic *V. parahaemolyticus* strain O3:K6 [53]. Virulence *tdh* and *trh* genes are frequently correlated with the clinical isolates or pathogenic *V. parahaemolyticus* strain, while, *tlh* gene is considered to be a specific molecular marker for both pathgonic and non pathogenic strains of this bacteria [57]. Thus, the PCR assays are generally used to identify, assess and inspect the presence of pathogenic and non pathogenic strains of *V. parahaemolyticus* in many outbreak areas as the diagnostic indicator [58]. The molecular method has high sensitivity and specificity for *V.parahaemolyticus* detection. It showed 100% for both sensitivity and specificity for *tlh* gene detection. Moreover, the detection of *tdh* gene had 100% sensitivity and 88% specificity, whereas the sensitivity and specificity for detecting *trh* gene are 100% and 96%, respectively [59]. Even though detection methods based on PCR are high

sensitivity and accuracy, the method are poorly controlled and the PCR technique optimization is needed for the satisfactorily results [53].

Immunological method: Enzyme linked immunosorbent assays (ELISA) can be used to detect the presence of TDH and TRH of *V. parahaemolyticus* [60]. ELISA is simple, rapid and sensitive identification method. The immunological techniques by using specific monoclonal antibodies are often used for rapid detection and quantification analysis of food-borne pathogens in seafood samples. Furthermore, other methods such as sandwich enzyme-linked immunosorbent assays developed from polyclonal antibodies or monoclonal antibodies directed specific to TDH and TRH toxins are usually used to identify pathogenic *V. parahaemolyticus* from clinical isolates [61]. However, this method cannot detect all of the clinical and environmental *V. parahaemolyticus* strains due to its cross reaction with other bacteria [62]. Currently, an immunochromatographic (ICT) assay has been improved for detecting the TDH toxin releasing from *V. parahaemolyticus* cultured from stool specimens of patients, also called as "TDH-ICA" [51]. By using an ICT technique with specific MAb to detect TDH, this developed method can detect levels of TDH in picograms per ml for very short time or within 10 minutes [63]. There are also limitations of these immunological methods such as availability of specific antibody. The procedure of ELISA requires trained or skilled staff and needs specific equipment (64).

Treatment and Prevention

V. parahaemolyticus is typically susceptible to antibiotic agents used to treat patients with enteric infections. However, most patients with gastroenteritis can effectively be treated with only oral rehydration [65]. Therefore, the antibiotic treatment may not necessary in most cases of *V. parahaemolyticus* infection. For patients who have wound infections and septicemia by this pathogen, the treatment is similar to the treatment for patients with *V. vulnificus* infection, i.e., intravenous delivery of antimicrobial agents such as Doxycycline, Cefotaxine or Ceftriaxone [66]. The guideline treatment focuses mostly on reducing symptoms of patients. Patients should drink abundance of water or liquid to replace fluids lost from diarrhea. In severe case or prolonged illnesses, antibiotic agents will be used to treat the patients such as tetracycline or ciprofloxicin. Tetracycline has been suggested as the antimicrobial drug of choice for treatment of severe patients with *Vibrio* infections [67], and alternative or combination drug treatments are the combination of expanded-spectrum cephalosporins (such as ceftazidime) and doxycycline or only fluoroquinolone. Sulfamethoxazole/trimethoprim plus an aminoglycoside is used to treat young age patients or children who have contraindication with doxycycline and fluoroquinolones [68]. The choice of antibiotic agents must be based on antimicrobial susceptibilities of the organism.

Since the infection of *V. parahaemolyticus* mostly occurs by consumption of contaminated aquatic foods such as shellfish, fish, shrimp, crab, and lobsters, the preventive methods then include proper or hygienic handling and cooking foods from the marine source and other aquatic foods before consumption as well as an avoidance of cross-contamination between raw and cooked products during storage and processing. Managing a proper environmental is important in controlling this infection. It can be managed by many methods such as no discharge of untreated sewage and untreated water in the sea, river or water source near coastal areas and not harvest or consume aquatic foods from heavily contaminated water sources [19].

CONCLUSION

The *V. parahaemolyticus* - borne disease is an important health problem concern worldwide. Since the bacterium inhabits in seawater or marine environment especially seafood such as shellfish, sea fish, and crustaceans, so these kinds of food can harbor this pathogen and cause some clinical diseases in human. Abundance of this organism

in marine environment or seawater depends on seasonal variation. More bacteria are found in the warmer period. Not all the bacterial strains isolated from seawater are of human pathogenic because some are KP- or Kanagawa Phenomenon negative of *V. parahaemolyticus*. The most pathogenic strain of *V. parahaemolyticus* can be detected by the presence of Kanagawa Phenomenon positive (KP+). Generally, *V. parahaemolyticus* can induce a self-limiting watery diarrhea. Patients relieve in a few days. Nevertheless, the swift onset of the disease which takes about 12 hours suggests that the symptoms of the disease are related to the virulence factor of *V. parahaemolyticus*; enterotoxin TDH and TRH. The important route of transmission of *V. parahaemolyticus* is by consuming some raw seafood such as raw mollusks or uncooked crustaceans and fish. These are the most common seafood sources of *V. parahaemolyticus*. The prevalence and emergence of *V. parahaemolyticus* O3:K6 serotype, which carries the virulence gene names *tdh* gene, is the cause of pandemic outbreaks worldwide. It is also the major pathogen affecting the seafood export trade nowadays. Therefore, an effective control and prevention is the key to diminish the risk of infection from this bacterium and thus ensures the food safety**.**

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