Rapid identification of pathogenic Vibrio parahaemolyticus using human red blood cell and urea

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Introduction

Vibrio parahaemolyticus is a gram-negative pathogen associated with gastroenteritis after consuming raw or improperly cooked seafood (1). *Vibrio parahaemolyticus* (VP) usually lives in marine and estuarine environments and is part of the natural flora found in fish and shellfish. One unique characteristic of VP is it grows in salty alkaline environments which most bacteria cannot tolerate. In the food industry, detecting pathogenic strains of VP is difficult because current rapid-detection methods are not reliable for identifying pathogenic VP contamination. Osawa etl al. discuss how many rapid-detection kits such as API-20E use urease as a biomarker for pathogenic VP detection; however, current research suggests urease production is correlated with some virulence factors, but is not accurate enough as a pathogenic biomarker (1). Thermostable-direct hemolysin (TDH) is a heat-stable protein toxin that is a major virulence factor of VP-related food poisoning (2). TDH-related hemolysin (TRH) is similar to TDH but less virulent. The Kanagawa phenomenon is characterized by beta-hemolysis on Wagatsuma-blood agar and is indicative of TDH and TRH encoded genes (1,3). When expressed, TDH and TRH create pores in mammalian erythrocytes (red blood cells) resulting in cell lysis (hemolysis). In this study, the objective was to observe the influence of TDH, TRH, and urease expression on hemolytic activity in Wagatsuma broth formulated with human blood to develop a new rapid-detection method for identifying pathogenic *Vibrio parahaemolyticus.*

Materials and Methods

Initially, two VP selective media were used to observe VP growth and their hemolytic activity. The Wagastuma blood broth consisted of 1% beef extract, 0.3% yeast extract, 2% or 7% NaCl, 0.5% dipotassium phosphate, 1% mannitol, and 0.0001% crystal violet and heated to boiling. When the temperature dropped to 50 C 3mL of frech human blood was added to complete the media. VP selective arabinose broth contained 2% or 7% NaCl, 1% peptone, 1% arabinose, 1% sodium citrate, and 1.2% 10N NaOH and 0.5% bile salts were added after the media reached 70 C after boiling. Human blood (3 %) was added once the temperature reached 50 C. Sodium chloride concentration was altered to observe its effect on bacterial growth and hemolysis of red blood cell.. The prepared media (5 mL) was pipetted into 18mm glass tubes. 35 clinical samples of VP and non-VP were inoculated into media containing either 2% or 7% NaCl and incubated at 37 C for 24 hours. The growth and hemolytic activity was recorded. Samples with red pigments dispersed throughout were recorded as hemolysis positive while blood cells sinking to the bottom were negative. Agar plates were inoculated with samples using a 96 well plate prong and were used to observe overall hemolytic activity. Hemolysis shows light-colored halos around bacterial colonies on agar plates. Polymerase chain reaction (PCR) was performed to confirm the presence of TDH and TRH while urea tests confirmed urease production. After incubation,

samples were streaked onto Thiosulfate-citrate-bile salts-sucrose (TCBS) agar and incubated at 37 C for 24 hours to verify VP and non-VP growth.

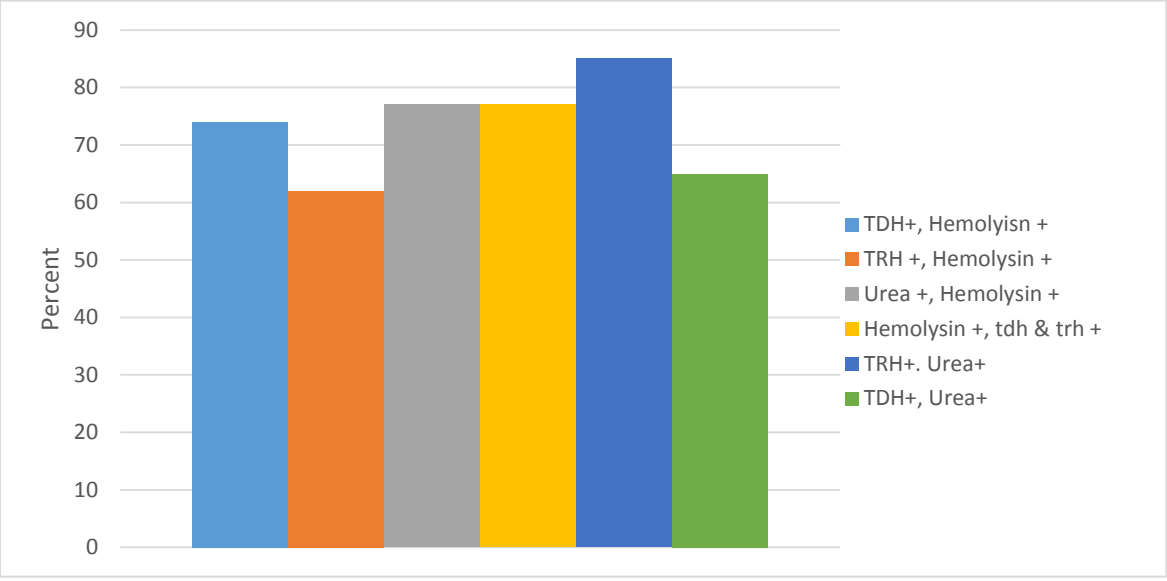
Results

In *V. parahaemolyticus* strains that tested positive for TDH and TRH, high sodium chloride concentration (>6%) stimulated the destruction of red blood cells but decreased the growth rate of the bacteria. A separate research study conducted by Chun et al. (year?) confirmed increased salt concentration enhanced hemolysin production (3). Non-VP strains were very sensitive to sodium chloride concentration and did not experience growth or hemolytic activity in 7% salt; therefore, NaCl concentration could be essential for isolating TDH and TRH positive strains.

Table 1: TDH, TRH, and urease production using PCR and urease tests on a 96 well plate

	1 tdh trh ure	2 tdh trh ure	3 tdh trh ure	4 tdh trh ure	5 tdh trh ure
A	+ - -	+ + +	- - +	+ + +	NA
B	+ - -	+ + +	- - +	NA	+ + +
C	- - -	+ + +	+ + +	+ + +	- + +
D	+ - -	+ + +	+ + +	+ + +	+ + +
E	+ + +	+ + +	+ + +	+ + +	- - +
F	+ + +	+ + +	+ + +	- + +	- - +
G	+ + +	+ - -	NA	+ + +	+ + +
H	NA	+ - -	NA	+ + +	+ - -

Table 2: Comparison of the prevalence of TDH, TRH, and urea from 35 clinical samples of *Vibrio parahaemolyticus* in Wagatsuma blood broth formulated with 7% NaCl



Out of 35 strains of *Vibrio parahaemolyticus*, urease production occurred more often with TRH (85%) than TDH (65%), indicating that urease tests were not as reliable for identifying TDH expression. However, TDH positive samples occurred in 74% of samples exhibiting hemolysis. This confirms that hemolysis activity is a strong indicator for TDH. Urease, TDH, and TRH were simultaneously detected in 65% of strains. Hemolytic activity matched 77% of samples containing TDH and TRH when compared to the PCR results. Urease was also present in 77% of hemolysin producing samples. However, the combination of TDH and TRH increased the total number of hemolysis positive samples with urease positive samples while TDH was directly linked to hemolysis 74% of the time. Using Wagastuma media formulated with human red blood cells to detect TDH and TRH increased the accuracy of identifying pathogenic strains of *V. parahaemolyticus* when used in combination with urease tests.

Conclusion

Vibrio parahaemolyticus is a major cause of food poisoning throughout the world. It is difficult to detect and easily slips into consumer markets. Pathogenic strains of VP are rarely found in the environment and little is known about what causes strains to develop pathogenicity. Research has found that the proteins TDH and TRH are almost always found in clinical samples taken from infected persons; therefore, are a major virulence factor. Current methods of identifying TDH and TRH include PCR, Most-probable number (MPN), and API-20E. However, these methods require skilled, educated technologists or do not adequately isolate pathogenic strains of VP. Using Wagatsuma broth formulated with human blood and 7% NaCl increases the accuracy of TDH and TRH identification. Although urease tests are strongly correlated with TDH expression, they are not dependable for detecting TDH. Urease tests can be useful as a supplemental test for pathogenic VP, but modified Wagatsuma broth is superior for detecting TDH and TRH. The food industry as well as governmental agencies will benefit from this method because it is low cost, does not require expensive equipment, and can be performed easily.

Works Cited

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