Review

Bacteriophages as enteric viral indicators in bivalve mollusc management

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A B S T R A C T

Human enteric viruses, such as norovirus and hepatitis A virus, are spread by a variety of routes including faecal-oral transmission. Contaminated bivalve shellfish are regularly implicated in foodborne viral disease outbreaks internationally. Traditionally indicator bacteria, the coliforms and *Escherichia coli*, have been used to detect faecal pollution in growing waters and shellfish. However, studies have established that they are inadequate as indicators of the risk of human enteric viruses. Bacteriophages have been identified as potential indicators or surrogates for human enteric viruses due to their similarities in morphology, behaviour in water environments and resistance to disinfectant treatments. The somatic coliphages, male-specific RNA coliphages (FRNA coliphages) and the bacteriophages of *Bacteroides* are the groups recognised as most suitable for water and shellfish testing. In this review, we discuss the rationale and supporting evidence for the application of bacteriophages as surrogates for human enteric viruses in shellfish under a variety of conditions. There is some evidence to support the validity of using bacteriophage levels to indicate viral risk in shellfish in highly contaminated sites and following adverse sewage events.

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1. Introduction

The human health risks associated with consumption of raw or

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lightly cooked shellfish containing human enteric viruses are well recognised with numerous foodborne outbreaks documented. Internationally, there were 368 foodborne viral outbreaks associated with shellfish reported in the scientific literature between 1980 and 2012 (Bellou et al., 2013). The most common viral pathogens involved were norovirus (NoV) (83.7%) and hepatitis A virus (HAV) (12.8%) with oysters (58.4%) the most frequent shellfish implicated in outbreaks.

The major source of viruses in bivalve molluscs is contamination of growing waters with human sewage pre-harvest (FAO/WHO, 2012). Both NoV and HAV are shed at high levels in the faeces of infected individuals (10^4-10^{11} viral genomic copies/g) (Atmar et al., 2008; Chan et al., 2006; Fiore, 2004). If this faecal matter reaches the growing waters, enteric micro-organisms can be bio-accumulated in shellfish tissues through filter feeding, at rates dependent on shellfish species, environmental conditions, season and type of microorganism (Burkhardt and Calci, 2000; Polo et al., 2014; Ropert and Goulletquer, 2000). Oysters can selectively retain NoV strains through specific binding to carbohydrate ligands within their tissues (Le Guyader et al., 2012).

The median infectious dose (ID_{50}) for NoV is only 18 infectious particles (Baert et al., 2011; Teunis et al., 2008) and for HAV is 10−100 virus particles (Atmar, 2010; Yezli and Otter, 2011). The ability of bivalve shellfish to accumulate virus particles, combined with the low infectious dose, contributes to the high risk of illness if shellfish are harvested from contaminated waters. Furthermore, NoV and HAV have been recognised to remain infectious in shellfish for days to weeks following contamination (Lee et al., 2015; Maalouf et al., 2010a).

Currently there are no effective options to eliminate viral contamination of bivalve molluscs prior to consumption without changing the desired sensory characteristics of the shellfish. Therefore, effective risk management strategies need to focus on prevention of contamination. In the case of shellfish, prevention has to occur primarily at the pre-harvest level (FAO/WHO, 2008).

2. Indicator organisms and hydrodynamic modelling for water quality

The potential presence of faecal pathogenic micro-organisms in water and shellfish can be identified through the detection of appropriate indicator organisms. Bacteria have been used as indicators of water quality since the late 19th century (WHO, 2001). Indicator organisms should be readily detected, not present in the absence of contamination and present in relatively large numbers when pathogens from similar origins are present. They should also display similar survival times and sensitivities to disinfection and treatment processes as pathogens. No single organism has been identified that fulfils all these qualities, therefore multiple indicators are often preferred, with specific indicators being more suited for certain situations (NHMRC, 2011).

The validity of an indicator is affected by the relative rates of removal of the indicator versus the potential pathogen. Hence, differences in environmental resistance or even the ability to multiply in the environment influence their application. Thermotolerant coliforms, including *Escherichia coli* (*E. coli*), indicate the presence of recent faecal contamination and have been widely applied as useful indicators for this purpose, despite reports that some may multiply in tropical waters (Byappanahalli et al., 2012). Although on an individual sample basis *E. coli* is a poor predictor of NoV risk, on a site-specific basis average *E. coli* levels have been shown to correlate with average NoV levels in the United Kingdom (UK) during the winter season (Lowther et al., 2012). Faecal enterococci, from the *Streptococcus* and *Enterococcus* genera, exist in high numbers in the faeces of humans and other warm-blooded

animals, do not multiply in the environment, are absent from pristine waters and are found in faecally polluted water. Enterococci do not persist for long in water, although longer than *E. coli*, therefore are also useful for detection of recent faecal pollution (Leclerc et al., 1996; NHMRC, 2011). The coliforms are a heterogeneous group of bacteria, they are not exclusively faecal in origin with some occurring in the environment. Hence, they are unsuitable as absolute indicators of faecal pollution (Ashbolt et al., 2001).

Risk management for bivalve shellfish destined for human consumption relies on the use of enteric bacteria as indicators of faecal contamination. International regulations have been developed to specify the acceptable levels of enteric bacterial pathogens in shellfish tissues or in waters where shellfish are grown. These have led to the classification of production areas for shellfish harvest fit for human consumption. The United States (US) shellfish safety program utilises water based sampling developed around thermotolerant coliform indicators, including *E. coli*. In contrast, the European Union (EU) shellfish safety sampling program is based on *E. coli* levels in shellfish. Australia has flexibility to utilise either system. The Codex Alimentarius Commission has published guidelines to minimise the presence of human enteric viruses in foods (FAO/WHO, 2012). This document includes an annex on the control of HAV and NoV in bivalve molluscs with specific recommendations covering primary production with water or shellfish monitoring based on *E. coli* and/or coliform data.

Despite the universal use of coliforms and *E. coli* as indicators to predict the risk of exposure to pathogens of faecal origin in water and shellfish, bacteria have been shown to be poor indicators of human enteric viral contamination (Doré and Lees, 1995; Flannery et al., 2009). Structurally viruses are diverse and are quite distinct from bacterial cells. They also display significantly different resistance and susceptibility responses to environmental conditions such as desiccation, UV irradiation, and water and sewage treatment processes (Blatchley et al., 2007; Stewart et al., 2008). Bacteriophages (phages) have been proposed as indicators or surrogates for human enteric viruses due to similarities in morphology and survival dynamics (Havelaar et al., 1986).

Hydrodynamic modelling is an approach with potential for improved shellfish risk management through the prediction of water and shellfish contamination levels with associated flushing and depuration times after adverse events. Riou et al. (2007) developed a two-dimensional hydrodynamic model simulating the impact of rainfall events on water quality and shellfish in an area in Normandy, France (Riou et al., 2007.). The hydrodynamic model incorporated features specific for the study location, with inputs including water surface elevation, velocity, water column height and turbulent viscosity. The microbiological components included storm water input and fluxes for the selected indicator microorganisms, *E. coli*, Astrovirus (AstV) and FRNA phages, to model the subsequent decay rate of the microorganisms. This approach may contribute to the identification of periods of viral risk associated with shellfish.

3. Direct detection of human enteric viruses

Polymerase chain reaction (PCR) detection is currently the best methodology available for foodborne virus detection yet it is not conducive for routine end product monitoring in shellfish as it requires significant technical expertise, time and expense. Furthermore, whilst PCR methods are sensitive, the number of viral copies detected does not directly relate to infectivity (Liu et al., 2011; Stals et al., 2012). PCR will also detect naked non-encapsulated or degraded viral RNA and viruses with damaged capsids that cannot initiate infection but which contain a viral RNA genome (EFSA, 2011). This could lead to an over-estimate of the actual infective