CHAPTER 7

Bacterial Indicators of Viruses

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1.0. INTRODUCTION

Viruses that are transmitted by the fecal-oral route can cause disease in humans after the consumption of contaminated food or water. The diseases caused by many of these viruses are usually mild and self-limiting such as gastroenteritis caused by noroviruses (Nishida et al., 2003; Parshionikar et al., 2003). However, some viruses, such as hepatitis A virus, may cause more serious diseases that require hospitalization or that may be fatal (Halliday et al., 1991; Tang et al., 1991; Niu et al., 1992; Hutin et al., 1999). Prevention of contamination of food and water by pathogenic microorganisms is one method by which their transmission can be reduced/eliminated. However, water and wastewater treatment procedures do not always eliminate infectious viruses. In addition, food handlers who have mild or asymptomatic infection may be responsible for contaminating food at several stages during production and processing.

Detection of infectious viruses directly in food or water before they are released to consumers would be another way to prevent the transmission of these viruses. This is usually not done, mainly because methods are not available that can detect infectious units in foods with a certain degree of accuracy. Some viruses do not grow easily in cell cultures and may require inoculation of humans or other animals for detecting them as infectious viruses. Molecular methods such as RT-PCR (reverse transcription–polymerase chain reaction) are increasingly being used to detect these viruses (Le Guyader et al., 1993; Dore et al., 2000). However, only a limited number of laboratories are equipped to routinely conduct such tests. Also, the detection of viral genome in foods may or may not indicate the presence of infectious viruses. In fact, viral genomes have been reported to survive much longer in mineral water than infectious viruses (Gassilloud et al., 2003). One way to avoid this problem is to use integrated cell culture-PCR to determine if the genomes detected using molecular methods are infectious (Blackmer et al., 2000). Because of the above problems associated with the detection of pathogenic viruses in food and water, attempts have been made to find a suitable indicator that, when present, would indicate that viral contamination of food or water has occurred.

2.0. DESIRABLE CHARACTERISTICS OF INDICATORS

Because enteric viruses are usually transmitted by the fecal-oral route, components of feces have been used to detect the presence of fecal pollution in food and water. It was hoped that simple tests for components of sewage or feces could be used to indicate the presence of pathogenic microorganisms including viruses. Both chemical and microbiological indicators have been developed. Chemicals that may indicate the presence of fecal pollution include coprostanol (Dutka et al., 1974). However, tests for chemicals are of limited value because they require specialized equipment and are limited in their sensitivity. Because microbiological tests can detect one or a few living microorganisms, they are generally more sensitive than the chemical tests. Also, tests for bacteria usually require minimal equipment and are not too complicated to perform. Therefore, bacteria have been used as indicators of viruses in most studies.

For use as viral indicators, bacteria should possess certain characteristics as discussed by Berg and Metcalf (1978). The most important requirements are that the presence of bacteria should correlate well with the presence of enteric viruses in a given environment; viruses should not be found when the indicator is absent or is present in low numbers; the viruses should be frequently found when the number of indicator bacteria is high; and the indicators should not be pathogenic themselves, thus simplifying the procedures for their culture and identification and reducing the hazards to laboratory workers from accidental contamination. Indicator bacteria should survive in a given environment for approximately as long as viruses because if they are inactivated more rapidly than viruses, they may not be detected when viruses are still present. Conversely, if they survive much longer than viruses, they may indicate a threat long after the viruses have been inactivated. In summary, if the survival of bacteria in an environment differs greatly from the survival of viruses, their usefulness as indicators is diminished.

The procedures for detecting the indicators should be relatively simple. The need for special equipment and complex procedures would reduce the number of laboratories that are capable of doing the analyses. The availability of well-equipped laboratories in many different locations will reduce the time between sample collection and analysis. Also, simple and inexpensive procedures that do not rely on special operator skills are easier to standardize, and the results of standardized procedures are easier to compare from laboratory to laboratory.

3.0. BACTERIA USED AS INDICATORS FOR VIRUSES

It was recognized early in the study of microbiology that certain bacteria, such as *Escherichia coli*, were present in the intestines and feces of humans and other animals (Escherich, 1885). Initially, tests designed to detect *E. coli*

were based on its ability to ferment glucose. These tests were later modified to detect the fermentation of lactose. However, these tests were not specific for *E. coli* alone and detected a range of microorganisms. These microorganisms were collectively termed *coliform bacteria*. The latter group includes *Aerobacter aerogenes*, *Klebsiella pneumoniae*, *Enterobacter* spp., and *Serratia* spp. (Duncan and Razzell, 1972; Newton et al., 1977; Stiles and Ng, 1981), and all of these bacteria may not be associated with fecal pollution. Later, different media and incubation at higher temperatures were used to make the tests more specific for microorganisms associated with feces. The microorganisms detected in these tests were termed *fecal coliforms* or *thermotolerant coliforms* (Eijkman, 1904). Although *E. coli* is a significant component of the fecal coliform population, other thermotolerant bacteria, such as *Klebsiella pneumoniae*, may also be detected by the fecal coliform test (Bagley and Seidler, 1977; Hussong at al., 1981).

More recent modifications of the test used to detect *E. coli* and related microorganisms include the use of media that contain compounds that produce a colored or fluorescent compound when they are hydrolyzed by bacterial enzymes (Manafi et al., 1991). As efforts were being made to make these tests more specific for detecting *E. coli*, it was discovered that *E. coli* can also be found in pristine areas in tropical climates (Hazen and Toranzos, 1990). The ability of *E. coli* to exist and possibly grow in some tropical environments that are not fecally polluted should be considered when using it as an indicator organism in these environs (Hazen and Toranzos, 1990). Some of the developments in the use of *E. coli* and related bacteria as indicators of fecal pollution are given in Table 7.1.

Because the correlation between the detection of microorganisms using the above-mentioned tests and viruses has not been satisfactory in many cases, other microorganisms have been considered as indicators for viral pol-

Table 7.1 Chronology of Events Leading to the Use of *Escherichia coli* and Related Bacteria as Indicators of Fecal Pollution

Event	*Reference*
Identification of *E. coli* as an inhabitant of the intestinal tracts of warm-blooded animals	Escherich (1885)
The use of lactose fermentation at elevated temperatures (44.5–55.5°C) to test for fecal (thermotolerant) coliforms	Eijkman (1904)
The development and use of membrane filter (MF) procedures to detect coliforms and *E. coli*	Clark et al. (1951); Toranzos and McFeters (1997)
The finding of *E. coli* in pristine areas not influenced by known fecal contamination	Hazen and Toranzos (1990)
The use of chromogenic and fluorogenic compounds to detect coliforms and *E. coli*	Manafi et al. (1991)

Table 7.2 Bacteria That Have Been Considered for Use as Indicators of Fecal Pollution

Bacteria	*Comments*
Coliforms	A group of microorganisms that can ferment lactose; testing for this group may also detect bacteria that are not of fecal origin.
Fecal (thermotolerant) coliforms	This group of microorganisms is more specific for *Escherichia coli* than the coliform group; however, non–fecal coliforms may sometimes give positive results.
Fecal streptococci, enterococci	Some bacteria formerly classified as *Streptococcus* spp. have been reclassified as *Enterococcus* spp. (i.e., *Enterococcus faecalis*); some studies indicate that these bacteria may be better indicators for viral contamination of marine waters than *E. coli* and other related bacteria.
Bacteroides spp.	They are found in large numbers in the intestinal tracts of humans and other animals; because they are anaerobic bacteria, they do not survive long in aerobic environments. Both *E. coli* and *S. faecalis* were found to survive longer that *Bacteroides* spp. in water (Fiksdal et al., 1985)
Clostridium perfringens	This organism is found in human intestines and sewage and can be used to monitor water sources for fecal contamination; because the spores may survive for long periods, it may indicate historical pollution.

lution (Table 7.2). These microorganisms include fecal streptococci, anaerobic bacteria present in human intestines (*Bacteroides*), and spore-forming bacteria of the genus *Clostridium.* Besides *E. coli* and related bacteria, the enterococci are probably the most commonly used indicator bacteria. *Bacteroides* spp. are present in high numbers in the human intestine, and these bacteria have been detected in sewage and natural waters (Allsop and Stickler, 1984). However, because their numbers decline more rapidly in water that those of *E. coli* or *S. faecalis*, they have not found use as an indicator. Previously, several enteric streptococci were classified in the genus *Streptococcus.* They have now been reclassified as members of the genus *Enterococcus*. The two species most frequently found in humans are *Enterococcus faecalis* and *E. faecium*. The enterococci are distinguished by their ability to grow in 6.5% NaCl and at high temperature (45°C). Enterococci have been found to be more reliable than coliforms in determining health risks in marine waters (Cabelli et al., 1982).

Clostridium perfringens has also been used as an indicator of pollution (Fujioka and Shizumura, 1985). One problem is that this organism produces spores that may survive for long periods in natural environments. Therefore, it may indicate the presence of pollution that occurred long before the sampling.

4.0. METHODS FOR DETECTING INDICATOR BACTERIA

Total and fecal coliforms can be enumerated in standardized tests that use a series of tubes with specific bacteriological media inoculated with serial 10-fold dilutions of the initial sample (Toranzos and McFeters, 1997; Clesceri et al., 1998). The advantage of these tests is that they can be used with both liquid and solid samples. Their main disadvantage is that the most probable number (MPN) of microorganisms calculated using these tests has a relatively high degree of uncertainty. More accurate determination of the actual number of bacteria in liquid samples can be obtained using membrane filtration (MF) procedures. Larger volumes of water can be tested with this test than the MPN or tube tests, and the results obtained reflect more accurately the numbers of bacteria present in a sample (Clark et al., 1951).

Fluorogenic and chromogenic substrates have been incorporated into tests for coliform bacteria and *E. coli*, making the tests easier to perform (Manafi et al., 1991). The enzyme responsible for hydrolysis of lactose, ∃-D-galactosidase (∃-GAL), can also hydrolyze chromogenic substrates such as *o*-nitrophenyl-∃-D-galactopyranoside. The hydrolyzed product can easily be detected by change in color. A positive test is thought to indicate the presence of coliform bacteria. An enzyme that is found in a majority of *E. coli* isolates is ∃-D-glucuronidase (GUD). This enzyme can hydrolyze compounds such as 4-methylumbelliferyl-∃-D-glucuronide (MUG). The hydrolysis of MUG produces a product that fluoresces when irradiated with UV light at a wavelength of 365 nm. These materials are now included in media used for the detection of coliforms and *E. coli* in water (Feng and Hartman, 1982; Brenner et al., 1993). It should be realized, however, that not all *E. coli* strains are able to hydrolyze MUG (Chang et al., 1989).

Indicator bacteria may be injured by a variety of physical and chemical means including exposure to sublethal levels of disinfectants, UV light, high or low temperatures, freezing, copper, and starvation (Speck et al., 1975; Hackney et al., 1979; McFeters et al., 1982; Singh and McFeters, 1986; Kang and Siragusa, 1999). The injured bacteria may not be able to grow on some media used for detecting indicator bacteria and therefore escape detection. However, many injured bacteria can be detected by using procedures designed to give them an opportunity for repair (McFeters et al., 1982).

5.0. CORRELATION BETWEEN INDICATOR BACTERIA AND PATHOGENS IN WATER AND FOOD

Indicator bacteria have been found to be useful in determining the possible presence of pathogens in many cases. Hood et al. (1983) studied the relationship between indicator bacteria (fecal coliforms and *E. coli*) and bacterial pathogens (*Salmonella* spp.) in shellfish. Although *Salmonella* spp. were detected in some samples, none was present when the fecal coliform level in

oysters was less than the level recommended by the National Shellfish Sanitation Program (230 fecal coliforms per 100 g). The authors found that low levels of fecal coliforms was a good indication for the absence of *Salmonella* spp. However, the reverse was not true; high levels of fecal coliforms did not always indicate the presence of *Salmonella* spp. This is understandable because the indicator would likely be present in all cases of fecal pollution, but the presence of the pathogen would be variable and related to the level of infection within a given population. Natvig et al. (2002) compared the survival of *Salmonella enterica* serovar typhimurium and *E. coli* in soil contaminated with manure. The number of *E. coli* in the soil contaminated with bovine manure was always higher than the level of *S. enterica* serovar typhimurium. The authors concluded that *E. coli* was useful as an indicator for *S. enterica* serovar typhimurium under these conditions.

E. coli and *Salmonella* spp. are similar in their physiological characteristics and are likely to originate from the same source. Therefore, a correlation between their numbers and survival in natural environments would be expected. The lack of correlation between pathogens that are normally found in estuarine waters (*Vibrio* spp.) and fecal indicators is not surprising. Thus, Koh et al. (1994) found no correlation between *Vibrio* spp. and several indicator organisms (*E. coli*, enterococci, and total and fecal coliforms) in water from Apalachicola Bay.

Overall, a high correlation between indicator bacteria and viruses in water and food (especially shellfish) has not been found, although the presence or absence of indicator bacteria has been related to the presence of viruses in some cases. Sobsey et al. (1980) studied the levels of enteric viruses in oysters taken from areas closed to shellfish harvesting and those approved for this purpose. Enteric viruses were found in 12% of the oysters samples taken from areas closed to shellfish harvesting but not in samples taken from approved areas. Also, viruses were detected in samples that contained greater than the recommended 230 fecal coliforms per 100 g of oyster meat.

Kingsley et al. (2002) examined imported clams that had been implicated in an outbreak of Norwalk-like gastroenteritis. These authors were able to detect both hepatitis A and Norwalk-like virus genomes in samples of clams. In addition, the clams contained high levels of fecal coliforms (93,000/100 g of clam meat). Because this level was approximately 300 times the recommended level, the finding of virus genomes and the implication of the clams in disease transmission is not surprising.

The level of indicator bacteria in water or food has not been found to be correlated with the number of viruses in several studies. Gerba et al. (1979) examined waters that were approved for recreational use and for shellfish harvesting. The number of indicator bacteria (total and fecal coliforms) and enteroviruses in both the sediment and in the overlaying water was determined over a 3-year period. Enteroviruses were detected in more than 40% of samples from recreational water that met accepted standards for total and fecal coliforms. Enteroviruses were also detected in 35% of the samples taken from areas approved for harvesting shellfish. Goyal et al. (1979) deter-

mined the level of indicator bacteria and human enteroviruses in oysters and in the water overlaying the oyster harvesting areas. These authors isolated viruses from waters that met the bacteriological standards current at the time of the study. They did not find a statistical relationship between the number of viruses and indicator bacteria (total and fecal coliforms) in the oysters.

Ellender et al. (1980) examined oysters and water overlaying the oyster beds for fecal coliforms and enteroviruses. These authors selected two different Mississippi reef areas for the study, one of which was closed and the other open for shellfish harvesting. Viruses were isolated from oysters taken from both open and closed areas. However, 146 viruses were isolated from oysters taken from the closed area and only 12 viruses were isolated from oysters from the approved area. The number of fecal coliforms in water was not correlated with the number of viruses in oysters.

In a similar study, Wait et al. (1983) examined hard shell clams from beds that were open or closed for shellfish harvesting. Although the levels of total and fecal coliforms were higher in water from the closed area, enteric viruses were isolated from oysters taken from both sites. No statistically significant difference was found between the occurrence of viruses in clams from the open and closed sites despite a clear difference in the levels of indicator bacteria in the water from the two sites.

Molecular methods were used by Le Guyader et al. (1993, 1994) to study viruses in shellfish. Genomic probes were used to detect hepatitis A and enteroviruses in cockles and mussels (Le Guyader et al., 1993). No statistically significant difference was found between the presence of viral genomes and fecal coliform counts. Using RT-PCR, Le Guyader et al. (1994) detected enterovirus, rotavirus, and hepatitis A genomes in 22%, 20%, and 14% of cockles, respectively. Again, no relationship was found between viral and bacterial contamination.

Dore et al. (2000) examined oysters from four sites for the presence of *E. coli* and Norwalk-like virus (NLV). All of the samples met the standard of <230 *E. coli* per gram of shellfish meat. NLV was detected in samples from only the most polluted site, as determined by the number of *E. coli* in oysters taken directly from the site and in market-ready oysters that had been taken from the site and treated by depuration. The level of *E. coli* could be used to predict the absence of NLV at the three least polluted sites but not at the most polluted site. Skraber et al. (2004) compared coliforms and coliphages for their ability to predict viral contamination of the Mosells River. They did not detect infectious enteroviruses but did detect the genomes of enterovirus and norovirus, the presence of which was correlated with the levels of bacteriophages but not those of fecal coliforms.

It is clear from these and other studies (Table 7.3) that the level of indicator bacteria may predict the presence of human enteric viruses in some but not all cases. Viruses are more likely to be found at environmental sites and in shellfish meat that are highly contaminated with indicator bacteria, although they may also be found in the presence of low levels of indicator bacteria that meet the accepted standards.

Table 7.3 Correlation Between the Presence of Indicator Bacteria and Viruses in Water and Shellfish

Bacterial Indicator	*Virus*	*Results*	*Reference*
Total coliforms, fecal coliforms	Enteroviruses (as infectious viruses)	Viruses were isolated from estuarine water and oysters even though the water met acceptable standards for indicator bacteria.	Goyal et al. (1979)
Total coliforms, fecal coliforms	Enteroviruses (as infectious viruses)	Viruses were detected in 35% of estuarine water samples that met accepted standards for shellfish harvesting.	Gerba et al. (1979)
Total coliforms, fecal coliforms	Enteric viruses (as infectious viruses)	Enteric viruses were found in 12% of oysters taken from closed areas but were not found in oysters taken from open areas.	Sobsey et al. (1980)
Fecal coliforms	Enteroviruses (as infectious viruses)	The level of fecal coliform bacteria in water did not reflect the level of viruses in water.	Ellender et al. (1980)
Total coliforms, fecal coliforms	Enteric viruses (as infectious viruses)	Enteric viruses were isolated from clams taken from both closed and open areas.	Wait et al. (1983)
Fecal coliforms	Enteroviruses, hepatitis A, and rotaviruses (as detected by RT-PCR)	No relationship between virus and indicator bacteria in shellfish.	e Guyader et al. (1993, 1994)
Fecal coliforms, fecal streptococci	Enterovirus, rotaviruses, and hepatitis A (as detected by RT-PCR)	Viral contamination of river water was correlated with bacteriophages but not with indicator bacteria.	Baggi et al. (2001)
Fecal coliforms	Enteroviruses (as infectious viruses and by RT-PCR); noroviruses (as detected by RT-PCR)	Infectious enteroviruses were not detected in river water; coliform concentrations were not related to the presence of viral genomes.	Skraber et al. (2004)

6.0. DIFFERENTIAL SURVIVAL OF BACTERIA AND VIRUSES

Because of large differences in their size and composition, it is not surprising to note that the length of viral and bacterial survival is different under different environmental conditions. Wastewater solids that were undergoing aerobic treatment were treated with certain physical and chemical methods

that reduced the activity of protozoan predators. This caused a decrease in the adsorption of bacteria to wastewater solids leading to a reduction in their rates of inactivation (Farrah et al., 1985). In contrast, the same treatments did little to change the inactivation rates of several viruses or to change the ability of these viruses to adsorb to wastewater solids. It was concluded that viruses and bacteria were inactivated by different mechanisms during aerobic treatment of wastewater solids.

Baggi et al. (2001) examined the levels of bacteria and viruses during wastewater treatment and after the discharge of the effluent to the river. The wastewater treatment plants reduced the levels of fecal coliforms and fecal streptococci in raw sewage by an average of $2.3 \log_{10}$. In contrast, the levels of three bacteriophages were reduced by only $0.7 \log_{10}$. This is likely one reason that contamination of the river with viruses (enteroviruses, rotaviruses, and hepatitis A) was correlated with bacteriophages but not with fecal indicator bacteria.

The observed lack of correlation between indicator bacteria and enteric viruses in shellfish may at least partly be explained by two factors: (1) selective accumulation and (2) differential survival within shellfish. Burkhardt and Calci (2000) studied the accumulation of indicator bacteria and a bacteriophage (F^+) by oysters over a 1-year period. The most significant finding of this study was the marked change in accumulation of bacteriophages between November and January. Over this period, the bioaccumulation of bacteriophages increased by a factor of 99-fold. In contrast, accumulation of *E. coli*, fecal coliforms, and *Clostridium perfringens* did not change appreciably. This selective accumulation may account for seasonal differences in viral diseases associated with the consumption of oysters and for the lack of correlation between bacterial indicators and viruses in oysters.

Often, shellfish are not sold to consumers immediately after harvest but are exposed to clean estuarine water treated with UV light. After such treatment, the levels of indicator bacteria in oyster meat may be reduced. However, several studies have demonstrated that depuration is better at reducing the levels of *E. coli* and other indicator bacteria but not of viruses. Power and Collins (1989) compared the reductions in *E. coli*, poliovirus, and bacteriophages during depuration by mussels. They found that *E. coli* was eliminated from the mussels at a rate faster rate than that of poliovirus or bacteriophage. This led them to conclude that *E. coli* was an inappropriate indicator for demonstrating virus elimination during depuration.

A significant difference in the elimination of indicator bacteria (*E. coli*, fecal streptococci, and *Clostridium* spores) and bacteriophages by mussels during depuration was also observed by Mesquita et al. (1991). Elimination rates (T_{90}) for the indicator bacteria were in the range of 50 hr while for phages it was 500 hr. In another study, T_{90} values for *E. coli* and bacteriophages during depuration of oysters were 6.5 and greater than 40 hr, respectively (Dore and Lees, 1995). Schwab et al. (1998) found that depuration of oysters and clams reduced the level of *E. coli* by 95% but reduced the titer of Norwalk virus by only 7%.

Formiga-Cruz et al. (2002, 2003) examined indicator bacteria, bacteriophages, and human viruses in shellfish in several European countries. They found that human viruses were related to all bacterial indicators and bacteriophages in heavily contaminated waters and that the current depuration treatments were effective in reducing *E. coli* in shellfish but had little effect on viruses. In another study, oysters that had undergone prolonged depuration (>72 hr), which was sufficient to greatly reduce the levels of coliforms, were implicated in an outbreak of gastrointestinal illness (Heller et al., 1986). Differences in the removal of viruses and bacteria by treatment plants, differences in their accumulation by shellfish, and differences in their elimination during depuration likely contribute to the frequently observed lack of correlation between bacterial indicators and viruses in water and shellfish.

7.0. SOURCE TRACKING

Because *E. coli* and other indicator bacteria are found in the intestines of warm-blooded animals, their presence in a sample may or may not be related to the presence of human pathogens. The lack of correlation between bacterial indicators and human viruses may in part be related to the fact that bacterial indicators in a sample may be of nonhuman origin. Identifying the source of microbial pollution is also important for controlling pollution of an area. Therefore, methods have been developed to determine the source of bacterial indicators (Table 7.4).

The fecal coliform/fecal streptococci ratio was based on the observation that human feces contained relatively more fecal coliforms than animals and

Table 7.4 Methods to Determine the Source of Bacterial Indicators

Procedure	*Comments*	*Reference*
Fecal coliform/ fecal streptococcus ratio	Relies on standard bacteriological tests; the ratio may change with time because of differences in survival rates of bacteria.	Geldreich and Kenner (1969)
Pulsed-field gel electrophoresis (PFGE)	Can detect small genetic differences; highly useful for epidemiologic studies but may be too sensitive for source-tracking studies.	Johnson et al. (1995); Parveen et al. (2001)
Multiple antibiotic resistance (MAR)	Relatively rapid and does not require special equipment; requires a database and results may be valid only for limited geographical areas.	Hagedorn et al. (1999); Kruperman (1983); Wiggins et al. (1999)
Ribotyping	A labor-intensive method that can yield reproducible results; can be used to determine the source of indicator bacteria.	Parveen et al. (1999); Carson et al. (2001)

animals had relatively more fecal streptococci. A fecal coliform/fecal streptococcus ratio of >4.0 was considered characteristic of human pollution and a ration of <0.7 was thought to indicate animal pollution (Geldrich and Kenner, 1969). The test for multiple antibiotic resistance (MAR) relies on the fact that humans and animals are exposed to different types of antibiotics and that their intestinal bacteria show different patterns of resistance to antibiotics (Kruperman, 1983; Hagedorn et al. 1999; Wiggins et al., 1999). The use of this method requires a database to be produced for a specific area, and antibiotic-resistance patterns may change rapidly because of exchange of plasmids between bacteria.

Pulsed-field gel electrophoresis (PFGE) can produce specific genomic patterns of different microorganisms. Although this technique has been used in epidemiological studies to identify the source of bacterial pathogens (Johnson et al., 1995), it may be too sensitive for source tracking studies. Parveen et al. (2001) suggested that PFGE detected small differences in genomes, which may not be associated with specific indicator characteristics such as host range. Ribotyping is a fingerprinting technique that identifies conserved sequences of rRNA. Although this technique is labor intensive, it has been used successfully in source-tracking studies (Carson et al., 2001). Using this technique, Parveen et al. (1999) were able to correctly identify 97% and 67% of *E. coli* isolates from animals and humans, respectively. Although Scott et al. (2003) could not identify the animal source of *E. coli* isolates, they concluded that ribotyping could be used in differentiating human and nonhuman isolates. Some of the methods used for microbial source tracking have been reviewed by Scott et al. (2002).

It is possible that knowing the source of bacterial indicators may make them better indicators for viruses. Current procedures detect indicators that could be from many different sources (e.g., human and non-human). In contrast, most of the viruses are mainly human pathogens. By comparing indicators of human origin with viruses of human origin, it may be possible to obtain better correlations between viruses and indicator bacteria.

8.0. SUMMARY

The association between human enteric viruses and disease is well established. However, determining the presence of all of the many types of viruses that are pathogenic to humans in food and water is not practical at this time. Because enteric bacteria are usual inhabitants of the human intestinal tract, they have been used as indicators of fecal pollution and the possible presence of enteric viruses. Several different types of bacteria have been considered for use as indicators. Currently, most tests for indicator microorganisms rely on the detection of lactose-fermenting bacteria (coliforms, fecal coliforms, *E. coli*). Food and water samples with relatively high levels of these bacteria have frequently been found to contain bacterial and viral pathogens. However, viral pathogens have also been found in food and water samples

with no or acceptable levels of indicator bacteria. It may be necessary to supplement tests for bacterial indicators with tests for other indicators, such as bacteriophages (see Chapter 8). Also, it may be desirable to determine the source of indicators, at least to the extent of determining if they are from human or non-human sources. This may lead to a better correlation between the presence of human indicator bacteria and human enteric viruses.

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