檢討與制定海水水質指標 – 第一階段公眾諮詢

Review and Development of Marine Water Quality Objectives (WQOs) - First Stage Public Engagement

問卷
Questionnaire

問題(1)： 你對「檢討與制定海水水質指標 – 第一階段公眾諮詢文件」(下稱「諮詢文件」)內第 4 節列出的主要的檢討事宜有何意見？

Q(1) : What are your views on the key issues set out in Section 4 of the "First Stage Public Engagement Document for the Review and Development of Marine Water Quality Objectives (WQOs)" (hereafter refers to as the "Engagement Document") ?

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問題(2)： 你對諮詢文件內第 2 節列出的實益用途和敏感受體有何意見？你認爲在檢討中，還應考慮其他實益用途嗎？

Q(2) : What are your views on the beneficial uses and sensitive receivers set out in Section 2 of the Engagement Document ? Are you aware of any other beneficial uses of waters that should be considered in this review ?

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問題(3)： 就保護水域的各種實益用途、敏感受體和敏感生物群而言，你認爲保護的優先次序和程度應如何？

Q(3) : What are your views on the priority and level of protection for various beneficial uses, sensitive receivers and sensitive organisms that should be protected through the WQOs ?

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問題(4)： 除諮詢文件內附錄 A 所列的資料外，下階段的研究還應考慮什麼類型的水質指標或參數？

Q(4) : In respect of Appendix A of the Engagement Document, what other types of WQOs or parameters should be considered in the next stage ?

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問題(5)： 你對諮詢文件內第 5 節提議的水質指標檢討方法有何意見？
Q(5) : What are your views on the review approaches as set out in Section 5 of the Engagement Document ?

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Escherichia coli has been used as the principal pollution indicator worldwide since a century ago. It was generally assumed that the bacterium had survival characteristics in the environment that were similar to many fecal pathogens. However, many recent studies have shown that E. coli can survive in the environment for much longer time than it was assumed. Some survivors can even establish genetically distinct populations (so-called "naturalized") that persist without further pollution input. Disturbances such as heavy rainfall and strong waves can cause naturalized E. coli to emerge from its environmental reservoirs and lead to a surge of E. coli counts not related to any pollution.

Given the high prevalence of naturalized E. coli in tropical and subtropical environments, the effectiveness of using E. coli as a pollution indicator for HK should be re-visited and the use of additional/alternative indicator(s) should be explored.

An ideal indicator bacterium should be (Ishii and Sadowsky 2008):
1) present in the intestine of warm-blooded animals in high abundance
2) non pathogenic
3) present in the water when fecal pathogens are there, and absent in uncontaminated water
4) present in far greater number than fecal pathogens in contaminated water
5) able to survive similarly to fecal pathogens when discharged to the natural environment
6) unable to multiply in the environment, same to fecal pathogens
7) detected and quantified by easy, rapid and inexpensive methods

Although it is almost for certain that ideal indicators do not exist, these criteria should form the guideline for the evaluation of E. coli to remain the (sole) indicator for HK. The same criteria should be used when adopting additional / alternative indicators.

Reference:
Ishii and Sadowsky (2008) Eshcerichia coli in the environment: implications for qater quality and human health. Microbes Environ 23:101-108

問題(6)： 在下階段的研究中，還應考慮那些水質管理概念和水質指標的制定方法？
Q(6) : What broad water quality management principles and WQO approaches should be considered in the next stage ?

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填妥問卷後，請另存或列印此檔案，於 2009 年 12 月 31 日前，以電郵 / 傳真 / 郵遞方式送交環境保護署。

After the questionnaire is completed, please save or print out this document, and send the document to us on or before 31 December 2009 by e-mail, facsimile or mail.

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Microbes and Environments

Minireview

Escherichia coli in the Environment: Implications for Water Quality and Human Health

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Escherichia coli is naturally present in the intestinal tracts of warm-blooded animals. Since *E. coli* is released into the environment through deposition of fecal material, this bacterium is widely used as an indicator of fecal contamination of waterways. Recently, research efforts have been directed towards the identification of potential sources of fecal contamination impacting waterways and beaches. This is often referred to as microbial source tracking. However, recent studies have reported that *E. coli* can become "naturalized" to soil, sand, sediments, and algae in tropical, subtropical, and temperate environments. This phenomenon raises issues concerning the continued use of this bacterium as an indicator of fecal contamination. In this review, we discuss the relationship between *E. coli* and fecal pollution and the use of this bacterium as an indicator of fecal contamination in freshwater systems. We also discuss recent studies showing that *E. coli* can become an active member of natural microbial communities in the environment, and how this bacterium is being used for microbial source tracking. We also discuss the impact of environmentally-"naturalized" *E. coli* populations on water quality.

Key words: *Escherichia coli*, water quality, fecal pollution, health risks, "naturalized" population

Introduction

Contamination of water and food with fecal bacteria is, and remains, a common and persistent problem, impacting public health and local and national economies[95]. Water-related diseases are the major cause of morbidity and mortality worldwide. Among these, diarrheal diseases are estimated to cause 1.8 million deaths each year, mostly in developing countries[113]. Improved water supplies and proper sanitation can reduce the occurrence of gastrointestinal diseases. However, outbreaks of water- and food-borne diseases still often occur, even in developed countries. In the United States, 76 million cases of foodborne illness occur every year, resulting in 325,000 hospitalizations and 5,000 deaths[23]. Pathogenic agents causing these diseases include the enteric bacteria (diarrheagenic *E. coli*, *Shigella*, *Salmonella*, and *Campylobacter*), viruses (norovirus, hepatitis A), and protozoan (*Cryptosporidium* and *Giardia*)[70]. Recently, an outbreak of *E. coli* O157:H7 was reported in the U.S. and Canada during August and September 2006. The source of *E. coli* O157:H7 in this outbreak was spinach, which was most likely contaminated by irrigation water in California[24]. By October 6, 2006, this incident led to 199 infections, 31 cases of hemolytic-uremic syndrome (HUS), and three deaths[24]. In Japan, a large outbreak of *E. coli* O157:H7 was recorded in 1996 in elementary schools in Sakai City, Osaka, causing 7,900 hospitalizations, 101 HUS cases, and three deaths[71]. The source of *E. coli* O157:H7 for this outbreak was identified as school lunch provided on one particular day.

The occurrence of water- and food-borne illnesses has economic and social impacts (medical costs, productivity losses from sick leave, decreasing tourism, etc). The Economic Research Service of the United States Department of Agriculture (USDA-ERS) estimated that in 2001, diseases caused by five major bacterial pathogens in the U.S. resulted in a loss of about $6.9 billion[102]. Consequently, monitoring levels of fecal contamination and the prevention of disease outbreaks is important from both public health and economic perspectives.

Indicators of fecal contamination

Drinking water, ground water, and recreational water are mandated to be monitored for levels of fecal indicator bacteria. These bacteria are used to indicate the potential presence of pathogens in the environment, since detection and enumeration of many types of pathogenic organisms is often difficult due to their low numbers and specific growth requirements[95]. While several bacteria are currently used as indicator organisms for fecal contamination, the ideal indicator bacterium should be: 1) present in intestinal tracts of warm-blooded animals; 2) present when pathogens are there, and absent in uncontaminated samples; 3) present in greater numbers than the pathogen, 4) able to survive similarly to pathogens in the environment; 5) be unable to multiply in the environment; 6) detected and quantified by easy, rapid, and inexpensive methods; and 7) non pathogenic[10].

Historically, total coliforms, fecal coliforms, enterococci, and *E. coli* have all been used as fecal indicator organisms[5,64,103,104]. Coliforms are defined as the lactose-fer-

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menting, gram-negative, *Enterobactericeae*, including *E. coli*, *Enterobacter*, *Klebsiella*, and *Citrobactor*[64]. Thermotrophic coliforms (also called "fecal coliforms"), which can grow at an elevated temperature (44.5°C), were initially recommended as a more "fecal-specific" indicator[103]. However, some members of thermotrophic coliforms, such as *Klebsiella*, can originate from non-fecal sources as well[104].

In order to determine the best indicator of fecal pollution, the United States Environmental Protection Agency (USEPA) correlated bacterial presence to swimming-associated gastroenteric diseases at beaches on the east coast of the U.S.[104]. They reported that enterococci and *E. coli* had the highest correlation to disease incidence at marine and freshwater beaches, respectively. Therefore, enumeration of *E. coli* was recommended as a means to assess fecal loading in freshwater systems and potential health impacts[104]. *E. coli* is also used as an indicator of fecal contamination in the other countries[5]. Based on the U.S. EPA Ambient Water Quality Criteria for Bacteria[104], freshwater beaches should be closed when: (i) *E. coli* counts of a single sample exceed 235 colonies per 100 ml water, or (ii) the geometric mean of *E. coli* counts of at least 5 samples, equally spread over a 30-day period, exceeds 126 colonies per 100 ml water. Some freshwater beaches often exceed these limits, and are closed for many days during summer months[49]. Similar criteria are also used in Japan and other countries for water quality monitoring.

Escherichia coli *in the environment*

E. coli is a rod-shape, gram-negative, gammaproteobacterium in the family *Enterobactericeae*, and is a member of the fecal coliform group of bacteria. The primary habitat of *E. coli* is thought to be the lower intestine of warm-blooded animals, including humans[88]. Greater than one million (10^6) *E. coli* cells are generally present in 1 g of colon material, and are often released into the environment (their secondary habitat) through fecal deposition[88] (Fig. 1). Until relatively recently, however, *E. coli* was believed to survive poorly in the environment, and not to grow in secondary habitats, such as water, sediment, and soil[116]. *E. coli* faces many stresses in the environment, including low and high temperatures[7,48,74,76,93,115], limited moisture[8,13,19,20,30,76,93,115], variation in soil texture[30,39,76], low organic matter content[97,115], high salinity[97], solar radiation[110], and predation[12,14,19,25,93].

Recent studies, however, have shown that *E. coli* can survive for long periods of time in the environment, and potentially replicate, in water, on algae, and in soils in tropical[16,19,20,22,37,38], subtropical[30,93], and temperate environments[9,17,20,48,49,61,99,109]. Relatively high concentrations of nutrients and warm temperatures in tropical and subtropical environments are likely factors enabling *E. coli* to survive and grow outside of the host[22,116]. The addition of nutrients, such as manure, greatly increased the concentration of *E. coli* in Ontario soil[99], suggesting that *E. coli* can grow and maintain their population in temperate environments if favorable conditions exist (Fig. 1).

Byappanahalli *et al.*[20] reported that *E. coli* strains were repeatedly isolated from exclosure-protected temperate forest soils in Indiana, and their genetic structure was different

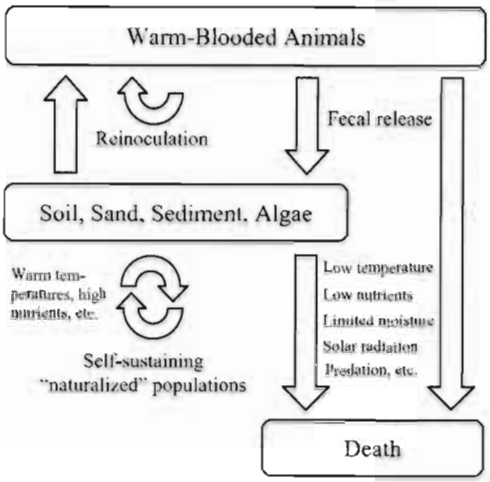

Fig. 1. Schematic diagram of the lifecycle of *E. coli*. Once *E. coli* is released from their primary host (warm-blooded animals) through fecal droppings, the majority of the released bacteria die due to low nutrients and other environmental factors. Some of them, however, become attached to soil, sand, sediment, or algae surfaces, and survive longer. In some conditions, these *E. coli* strains can grow and maintain their populations long enough to become adapted or "naturalized" to the environment. The adapted or naturalized *E. coli* survive and replicated in the environment, and can be reintroduced to animal hosts through contact with water and food.

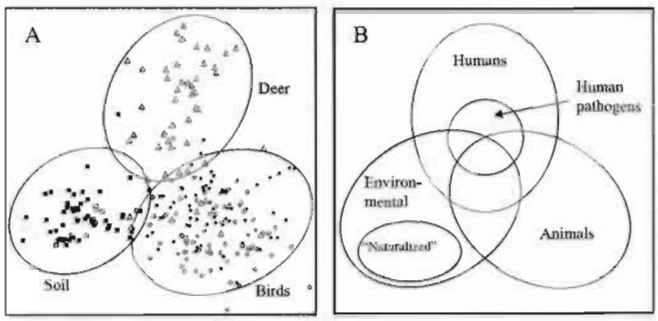

Fig. 2. A) Multivariate analysis of variance (MANOVA) of HFERP DNA fingerprints from *E. coli* strains obtained from soils (■), deer (△), and birds [geese (□), terns (●), and gulls (○)]. The first two discriminants are represented by the distances along the x and y axes (adapted from Byappanahalli *et al.*[20]) B) Conceptual representation of *E. coli* distribution among humans, animal hosts, and environmental reservoirs. Some level of host specificity can be detected in among *E. coli*, but some strains can be found in multiple hosts. Environmentally-adapted "naturalized" *E. coli* strains are unique and different from those found in humans and other animal hosts. Pathogenic *E. coli* strains can cause human diseases, and can be found in other animal hosts and in the environments.

from these bacteria isolated from animals (Fig. 2A). Similarly, Ishii *et al.*[48] reported that genotypically-identical *E. coli* strains were repeatedly isolated from a temperate soil near Duluth, Minnesota. The soil-borne *E. coli* strains had DNA fingerprint patterns distinct from animal-borne isolates, suggesting that they were not recently deposited by animals. The presence of *E. coli* attached to the macroalga *Cladophora* in Lake Michigan[18,21,108] and to periphyton in Lake Superior[61], and in beach sand and sediments[9,49,106] has also been reported. Na *et al.*[74] showed that *E. coli* can enter a viable-but-nonculturable (VBNC) state in natural water held at 4°C. Taken together, these results suggest that *E. coli* can

survive, grow, and become "naturalized" members of soil and algal communities.

The ability of *E. coli* to survive and grow in the environment is likely due to its versatility in energy acquisition. *E. coli* is a heterotrophic bacterium, requiring only simple carbon and nitrogen sources, plus phosphorus, sulfur, and other trace elements for their growth. This bacterium can also degrade various types of aromatic compounds such as phenylacetic acid and benzoic acid, to acquire energy[31]. In addition, *E. coli* can grow both under aerobic and anaerobic conditions, which they may face in a variety of fluctuating environments. Furthermore, *E. coli* can grow over a broad range of temperatures (7.5–49°C), with has a growth optimum of 37°C[47,55]. The long-term survival of *E. coli* under freezing temperature has also been reported[4,13,39]. The ability of *E. coli* to grow and survive under various conditions likely allows them to become an integrated member of microbial communities in a variety of environments.

Pathogenic E. coli

Although most *E. coli* are harmless commensal bacteria, some strains can cause human diseases. Shiga toxin-producing *E. coli* (STEC), including enterohemorrhagic *E. coli* (EHEC), can cause bloody diarrhea as well as potentially fatal human diseases, such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC)[75]. *E. coli* O157:H7 is among the most recognized serotypes of EHEC, and has caused many large outbreaks of food- and water-borne illness. In addition to STEC and EHEC, at least five additional pathogroups of *E. coli* have been identified. Enteropathogenic *E. coli* (EPEC) are one of the major causes of watery diarrhea in infants, especially in developing countries. Enterotoxigenic *E. coli* (ETEC) are the main cause of traveler's diarrhea and enteroaggregative *E. coli* (EAEC) can cause persistent diarrhea, lasting for more than two weeks. Enteroinvasive *E. coli* (EIEC) are genetically, biochemically, and pathogenically closely related to *Shigella*[75,83]. Several, researchers consider *Shigella* as being a subgroup of *E. coli*[84]. While extraintestinal pathogenic *E. coli* (ExPEC), including uropathogenic and avian pathogenic strains, are thought to be harmless while they are in the intestinal tracts, they can cause neonatal meningitis/sepsis and urinary tract infections if acquired by others[107].

Extensive reviews are available on the pathogenesis, diagnosis, and sources of pathogenic *E. coli*[56,67,75,80]. However, the distribution of pathogenic *E. coli* in the environment has not been examined in detail. Several studies have shown that EPEC strains can be more frequently detected in the environment than the STEC[49,63]. Ishii *et al.*[49] and Lauber *et al.*[63] reported the occurrence of potential EPEC strains, but no STEC, at Great Lake beaches. Similarly, Higgins *et al.*[46] reported that the intimin receptor gene *tir*, an EPEC virulence factor, was more frequently detected than *stx* genes (STEC virulence factor) in water samples from urban streams. While cattle and other ruminant animals (sheep, goats, and deer) may serve as major reservoirs of STEC[50,67,80], EPEC strains might be evenly distributed among diverse human and animal hosts[50]. The broad distribution of potential EPEC in a large number of animal hosts may, in part, explain the frequent detection of this pathogen in the environment.

Diversity of E. coli

E. coli is genotypically and phenotypically diverse. Traditional classification of *E. coli* is made based on reaction of antibodies with three types of antigens: the somatic (O), capsular (K), and flagellar (H) antigens[75]. Currently, *E. coli* has been shown to possess 173 O, 103 K, and 56 H antigens, and the number of newly discovered antigens is increasing (The *E. coli* Index [http://ecoli.bham.ac.uk/]). Diverse *E. coli* serotypes, which are defined by the combination of O and H antigens, have been identified. For example, *E. coli* O157:H7 is the most well-known serotype that can cause human disease[80].

E. coli strains also vary in other phenotypic characteristics, such as carbon utilization patterns, antibiotic resistance profiles, flagellar motility, ability to form biofilms, and the ability to cause diseases[3,35,60,79,119]. This is probably due to gene mutations and acquisition of new genes via plasmid- or phage-mediated horizontal gene transfer. Genome sequencing has revealed that horizontal gene transfer plays a significant and important role in gene acquisition in *E. coli*[107]. In addition, mutation can also contribute to the phenotypic diversity of *E. coli*. For example, diversity in carbon utilization ability may be caused by mutations and resulting functional failure of the affected genes. Cooper and Lenski[28] observed that the several lines of *E. coli* that were adapted to glucose medium over thousands of generations lost their ability to utilize several other carbon sources. Similarly, auxotrophic mutants (i.e. mutants that cannot synthesize necessary amino acids for growth) were often obtained from biofilm communities[29]. These studies indicate that some phenotypic variation may be attributed to ecological specialization: thus, *E. coli* adapted to one environment may lose fitness in another.

Diversity of *E. coli* is observed at the genotype level as well. While more than 650 genotypes were observed among 1,535 unique *E. coli* strains based on repetitive element palindromic (rep)-PCR DNA fingerprinting, rarefaction analysis revealed that the diversity observed was not saturated[53]. Similar findings were also reported in other studies[3,66,69,119]. DNA fingerprint patterns are variable even within the same serotype. For example, pulsed-field gel electrophoresis (PFGE) DNA fingerprint patterns of 1,798 *E. coli* strains belonging to the O157 serogroup were only 10% similar[77]. Whole genome PCR scanning analysis revealed that the position and structure of prophages (i.e. viral phage integrated into the bacterial chromosome) were different among 9 representative O157H7 strains[77]. Comparative genomic analysis done by using microarrays also showed that prophage or prophage-related elements contributed greatly (>85%) to the presence of genes in 12 *E. coli* O157:H7 and related strains[114]. These reports indicates that bacteriophage greatly contribute to genotypic diversity. Other factors, such as recombination, can also contribute to genotypic diversity[72,111].

Recent progress in genome sequencing revealed differences in gene content among *E. coli* strains. The complete

genomes of eight *E. coli* strains have been published, including nonpathogenic *E. coli* K12 strains[11,73], EHEC O157:H7 strains[45,81], uropathogenic strains[15,26,107], and an avian pathogenic strain[54]. Genome sequencing projects of 31 other *E. coli* strains are currently in progress (http://www.genomesonline.org/). Genome comparisons among *E. coli* strains, MG1655 (K12), EDL933 (O157:H7), and CFT073 (uropath), revealed that only 40% of the proteins were shared in common[107], further indicating that *E. coli* strains acquired many of their genes by horizontal gene transfer.

While *E. coli* has diverse genotypic and phenotypic characteristics, some characters are shared among strains exposed to similar environments. This is thought to be largely driven by selection pressure. If some of the characteristics among *E. coli* strains can be grouped by origin of isolation (i.e. host animals), then it is possible to use these phenotypes or genotypes as a tool to determine the source of unknown bacteria. This approach is called microbial source tracking (MST), and is discussed below in more detail.

Microbial source tracking

Potential sources of fecal contamination in water, soil, and sediments include human sewage, pets, farm animals, wildlife, and waterfowl. Although recreational beaches are routinely monitored for the levels of fecal indicator bacteria, microbial numbers alone cannot determine the potential sources of these bacteria. The identification of potential sources of *E. coli* and other fecal indicator organisms (such as enterococci and *Bacteroides*) in the environment is of great interest to the public, government regulatory agencies, beach managers, and operators of sewage treatment facilities. MST data can be used to establish proper risk assessment and abatement procedures[96].

Several library-dependent and -independent methods have been developed for MST studies (see reviews by Harwood[41], Sadowsky *et al.*[86], Santo Domingo *et al.*[87], Scott *et al.*[90], Stewart *et al.*[95], Stoeckel and Harwood[96], USEPA[105], Yan and Sadowsky[118]). A library for MST studies contains a dataset of characteristics of the target microorganism from known-source hosts[95]. Both phenotypic (e.g. antibiotic resistance profile, carbon utilization patterns) and genotypic characteristics (e.g. DNA fingerprint patterns) can be used for library-dependent MST methods[60,95,96,118]. Among these, rep-PCR DNA fingerprinting, including horizontal fluorophore-enhanced rep-PCR (HFERP) DNA fingerprinting, has been frequently used as a library-dependent MST method. The technique is reproducible, relatively inexpensive to use, and has relatively high throughput as compared to other molecular methods[118]. Several studies have shown that the HFERP DNA fingerprint patterns of *E. coli* strains could be clustered by animal host groups (e.g. Fig. 2A)[20,33,48,53]. This indicates that some level of host specificity exist in *E. coli* population (Fig. 2B). However, when *E. coli* is used as a target organism for MST studies, a large database is necessary to adequately represent diverse genetic and phenotypic characteristics in *E. coli* populations obtained from multiple hosts[51]. Moreover, since *E. coli* is not evenly distributed among host animal species, the distribution of this bacterium in the environment is patchy[20,49]. The distribution of *E. coli* is also subject to geographical and temporal variability, thus adequate care must be taken in obtaining representative samples for the construction and analysis of libraries. While these issues need to be taken account in the development of any host-source library[95], library-dependent MST methods appear to be useful tools for analysis of fecal contamination in relatively small areas with a limited number of potential input sources. For example, Ishii *et al.*[49] successfully applied HFERP DNA fingerprinting to determine potential sources of *E. coli* contaminating beaches in Lake Superior.

Library-independent MST methods employ host-specific markers, including PCR primers[32,52,57–59,62,78,89,91,92] and gene probes[43,94], to determine sources of fecal pollution. Host-specific markers, targeting 16S rDNA and other genes, have been identified for *E coli*[43,57,58], methanogens[100,101], viruses and coliphage[52,59], member of the Bacteriodales[32,62,78,89], and metagenomic DNA fragments[91,92]. However, before use in field studies these host-specific markers need to be validated by estimating the proportion of false-positives and false-negatives in the target population, and for sensitivity in detecting these bacteria that are present in low numbers in complex matrices, such as soil and sediment. In some cases the primers work well when tested with fecal samples, but have sensitivity issues when used with environmental samples, Although only a relatively few field investigations have been done using library-independent approaches[52,62,78,89,92], this method appears to be promising for future MST studies[86].

While *E. coli* is often used as an organism for both library-dependent and -independent MST studies, and as a metric for fecal contamination, some researchers criticize its use in MST studies postulating that this bacterium may not be distinct enough to be separated into host source-specific groups[42,68]. Gordon and Lee[41] used multilocus enzyme electrophoresis to characterize enteric bacteria and found that only 6% of the genetic diversity in *E. coli* could be attributed to host animals in Australia. Other studies have shown that while the relationship between *E coli* genotypes and animal source groups is not perfectly correlated, there is significant clustering of strains by animal or origin[33,53]. In order to establish a reliable MST method, Malakoff[68] suggested that population genetic studies done using more sensitive and discriminative methods are needed to better understand the relationship between diversity and host specificity in *E. coli*.

Health risk implications and MST studies

One of the main underlying assumptions of all MST studies is that fecal contamination originating from human sources is indicative of greater health risks for humans than is contamination originating from animals and the environment. This hypothesis, however, has not been adequately tested. Most MST methods are, therefore, designed to correctly and accurately separate fecal indicator organisms from human and other animal sources. Although some pathogens, such as *Shigella*, may be specifically harbored by humans[34], others can be distributed among diverse animals and also be resident in various environments[112]. For example, birds, including chickens and turkeys, often harbor *Salmonella* and *Campylobacter*[2,65], and pathogenic *E. coli* can also be found in non-human animals and in several environments (Fig. 2B).

In addition, ruminants, such as cows, sheep, and goats, have been reported to be the major reservoir of STEC[50,67,80]. Based on these, and other findings, it is obvious that the distribution of pathogenic *E. coli* and other human pathogens among diverse animal hosts and in the environment is still not well understood.

Some pathogenic *E. coli*, however, appear frequently in specific lineages[82]. Population genetic studies done by using multilocus enzyme electrophoresis and strains from the *E. coli* reference (ECOR) collection revealed that *E. coli* can be divided into four major phylogenetic groups: A, B1, B2, and D[27,85]. While most STEC strains are found in phylogenetic groups A and B1, ExPEC strains are more frequently identified in phylogenetic group B2 and D[27,40]. Gordon[42] proposed to use virulence factor genes as a MST tool. However, the relationship between phylogenetic groups and host animals is not well understood. Moreover, linking MST studies and potential human health hazards is a challenging but important topic. The construction of microbial risk models is necessary to assess potential human health hazards[6]. For accurate modeling, however, future studies are needed to clarify the distribution of these pathogens in animals and the environment, and the evolutionary and ecological forces leading to their establishment in humans, animals and environmental niches.

Future directions

It is clear from results of numerous studies that alternate fecal indicators need to be developed in order to better predict public health risks. Savichtcheva *et al.*[89] reported that a genetic marker for *Bacteroides* 16S rRNA had a higher predictive value for the occurrence of bacterial enteric pathogens than those based on total and fecal coliforms. Other indicators will likely emerge from ongoing and future epidemiological analyses. Detection and quantification of potentially pathogenic *E. coli* and other enteric pathogens may be another approach to assess human health hazards. Ahmed *et al.*[1] and Ishii *et al.*[50] surveyed *E. coli* strains isolated from water samples by using PCR targeting virulence factor genes. The use of colony hybridization using virulence gene-specific probes is a promising alternate method since it is reliable and can be applied to high-throughput and large-scale studies[86,117]. The use of robots to pick and array *E. coli* colonies allows for the simultaneous analysis of up to 20,736 strains, with minimal time and human input[117].

Another interesting direction for future research is to further investigate the ecology of naturalized *E. coli* strains. Several questions can be asked about these bacteria, chief of which is why these naturalized strains survive and grow better in the environment than other *E. coli*. Other questions also remain, such as: What mechanisms enable these bacteria to better survive and grow in soils relative to non-naturalized strains?, What are the unique genetic characteristics of these strains?, Where can we find naturalized *E. coli* besides soil, sand, and sediments?, and When and how did these strains evolve from a common *E. coli* ancestral lineage? Genome sequencing of the naturalized *E. coli* strains may provide us useful information to answer some of these questions. Comparative genomics of naturalized and other *E. coli* strains (mostly pathogenic strains) is also of interest for ecological perspectives, and the sequencing of some of these environmental strains is currently under way.

In situ evolutionary experiments may also provide new insights into adaptive mechanisms that microorganisms use to survive in soil and water environments. Previous laboratory experiments reported that error-prone DNA polymerase was induced under starvation conditions, and produced mutations at a high rate[36,98]. Since nutrients may limit the growth of *E. coli* in soil, it is possible that error-prone DNA polymerases may be activated and contributes to the genetic variation observed among soil-naturalized *E. coli* strains. This implies that mutation rates in *E. coli* may be different in soil compared to artificial media and the intestinal tract. Other evolutionary mechanisms, such as recombination, plasmid transfer, and the influence of bacteriophage, also need to be studied to understand evolution of *E. coli* in the environment.

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E. coli is highly abundant in fecal matter and is thus utilized by many governments around the world as a fecal pollution indicator. The general assumption is that *E. coli* is not able to survive in the environment and therefore should gradually die off as the impacts of fecal pollution subside. However, many studies have indicated that some *E. coli* strains can survive permanently in the environment as naturalized populations after a pollution event. Disturbances such as heavy rainfall and strong waves can cause naturalized *E. coli* to emerge from its environmental reservoirs and lead to a surge of *E. coli* counts not related to any pollution. This phenomenon seriously impairs the detection of actual pollution and thus the implementation of water resource management strategies.

Persistence of E.coli in secondary habitats. Once released from the animal hosts (primary habitat) into the external environment (secondary habitat), *E. coli* face a large number of biotic and abiotic stresses that are not present in their hosts (reviewed by Rozen and Belkin 2001). It was originally believed that *E. coli*, which has evolved as a gut flora in warm-blooded animals, should not be able to live for long under such stressful conditions. This has been shown in the lab where the relatively simple, controlled and homogenous experimental conditions provided few survival options to the bacteria (Niven et al. 2008, Sinton et al. 2002). However, the situation is completely the opposite in the natural environment. Many studies have reported that *E. coli* persisted in many different kinds of environments, such as river water (Byappanahalli et al. 2003), sediments (Anderson et al. 2005), soils (Ishii et al. 2006), algal surfaces (Olapade et al. 2006) in different climate zones. "Naturalized *E. coli*" is used to describe *E. coli* populations that persist in the environment (Ishii et al. 2006).

Impacts of naturalized E. coli on pollution monitoring. In standard water monitoring practices, it is generally assumed that fecal pollution is the only source of *E. coli* and that the bacterium would gradually die off as the pollution impacts subside. However, these assumptions are seriously violated by the widespread occurrence of naturalized *E. coli*, which impairs the ability of standard water monitoring to identify actual pollution. For example, Solo-Gabriele et al. (2000) reported that riverbank soils were the primary source of *E. coli* in a Florida river because the alternating wet-dry conditions in the soil under tidal influences enabled a large population of *E. coli* to thrive and diffuse into the water. Similar post-storm and wave effects on *E. coli* resuspension have been observed for sediment in Lake Michigan (McLellan et al. 2004) and beach sand in Lake Huron (Kon et al. 2007). In Lake Superior, high abundance of naturalized *E. coli* was found in algal mats, which could release up to 1000 CFU ml^{-1} of *E. coli* into the overlaying water (Ksoll et al. 2007). In New Orleans, serious concerns have been raised about the long-term impacts of the high levels of *E. coli* brought to soils by the fecal-contaminated floodwaters from the hurricanes Katrina and Rita (Sinigalliano et al. 2007).

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