Waterborne Pathogen Prevention and Detection Using Traditional Methods and Microarray Probe Detection

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Abstract

Biotechnology has gained increased interest in scientists and companies for many reasons. Biotechnology uses living organisms to accomplish a certain purpose. In most cases this saves money and it decreases the amount of additional chemicals that would originally be in the process. One world problem that biotechnology has lent its advances is in water quality. The concern for clean healthy water for drinking, household, and livestock purposes runs through the world, but more specifically the developing nations. The issue is not the chemistry of the water itself, but the pathogens received by the waters from usually human feces. Many practices have the implemented and educated throughout such as safe storage, point-of-use disinfection, and sanitation practices. One biotechnological breakthrough there has been with waterborne pathogen detection are DNA microarrays. DNA microarrays are formed from DNA sequences of pathogens put into an array for the purpose of identifying pathogens in water samples. In order to make this process more successful it is combined with the polymerase chain reaction (PCR) in order to amplify the DNA sequences and therefore making the concentration stronger and easier to detect. This is a successful process but it is not approved yet to be a standard method nor does it yet financially meet the desired amount. The effort is being put on this method of waterborne pathogen detection because it is considerably quicker than culture process since microarray detection is simultaneous. Early detection is important when lives may be at risk and with time will be cost effective.

Keywords

Waterborne pathogens, Source Protection, Safe Storage, Point-of-Use Disinfection, Oligonnucleotide-based Microarrays, Polymerase Chain Reaction (PCR)

Introduction

Biotechnology is “any technique that uses living organisms or substances from those organisms, to make or modify a product, to improve pants or animals, or to develop microorganisms for specific purposes” (Office of Technology Assessment, United States Congress). We have used biotechnology for many centuries in procedures such as yeast rising, and brewing but it has recently

been stretched and explored as never before. The first real grasp of this technology newly arose with the Dolly Sheep was cloned using nuclear transfer technology. At this time biotechnology has lend its hand on what we see as priorities: agriculture and medicine. Biotechnology helps us with food quality and new discoveries in insect resistant products and also in finding new antibiotics and vaccines. Some of the major advances that have been made in our decade have been:

- Malaria vaccine
- Stem cell research
- Completion of the human genome map
- 'Golden rice' modified to make vitamin A
- GCSF for increasing the white blood cell count in chemotherapy patients

Another field that has priority in the world is the field of water quality. About half of the world's population is directly impacted by contaminated drinking water. There is about 14,000-25,000 deaths each year due to contaminated drinking water and this population is included in the 18% of the population and does not have access to 'clean' drinking water. (Johannesburg World Summit on Sustainable Development, 2004) The 1.1 billion people that do not have this access are the people that are living in developing nations. The people are contaminating themselves with unsafe sanitation practices and by not knowing how to protect their water source. What is contaminating the water is not its composition, but the waterborne pathogens that enter and thrive in the waters and eventually enter the human system. The following topics will include waterborne pathogens, preventative non-technical measures that can be done in developing countries and new technology that present a great future for all water monitoring.

Waterborne Pathogens

Waterborne pathogens are composed of 3 different types of organisms: bacteria, viruses, and protozoa. These exist naturally and are most likely found on surface waters more than in ground water, unless exposed to surface water. The issue is not with all bacteria or protozoa, but those which harm and cause illnesses. The aim is to destroy all of these pathogens, but that is impossible. The best that can be done is to find preventative measures to reduce the chance of being contaminated. Ways of reducing the chance of contamination include protecting water sources, and practicing good hygiene. (Nicholas John Ashbolt, 2004)

There are several pathogens that are used as indicator organisms for the fact that they are very common. Those pathogens include the following table 1.

Table 1: Table with major waterborne pathogens along with the sources in developing regions

Name of micro-organisms	Major diseases	Major reservoirs and primary sources
Bacteria		
Salmonella typhi	Typhoid fever	Human feces
Salmonella paratyphi	Paratyphoid fever	Human feces
Vibrio cholera	Cholera	Human feces and freshwater zooplankton
Enteropathogenic E. coli	Gastroenteritis	Human feces
Yersinia enterocolitica	Gastroenteritis	Human and animal feces
Legionella pneumophila and related bacteria	Acute respiratory illness (legionellosis)	Thermally enriched water
Protozoa		
Giardia lamblia	Giardiasis (gastroenteritis)	Water and animal feces
Helminths		
Ascaris lumbricoides	ascariosis	Animal and human feces

E. coli

This bacteria lives in the digestive tract of warm-blooded animals including humans. E. coli is the main indicator testers use as a sign of other possible pathogens present as feces. There are many strands of E. coli and they are not all harmful. The strand that we know more commonly for making people sick from water is the serotype strain O157:H7. This is the strain that has causes abdominal pain, bloody diarrhea. The symptoms come in 7-10 days and 2-7% of the infections cause Haemolytic Uraemic Syndrome (HUS) (Moe, 1997; Rice, 1999) This infection causes the destruction of erythrocytes and eventually acute renal failure. To avoid this strand of E. coli, it's important to protect water source, and have a well maintained distribution system (Environmental & Workplace Health 2006).

Legionella bacteria

Legionella is actually naturally living in water and even water surfaces and groundwater (Palmer et al., 1993). I lives and reproduces in all types of environments ranging from 1-63 degrees Celsius and a pH of 5-8.5 (Nguyen et al., 1991). This pathogen is known for severe pneumonia outbreaks that can lead to Legionnaire's disease and Pontiac fever. More early symptoms include confusion, disorientation, nausea, vomiting, and diarrhea (U.S. EPA, 2001). The Mortality rate for Legionnaires' disease is about 15% (Nelson et al., 1985). The greatest sources of infection come from aerosols such as cooling towers, whirlpool baths, shower heads, and mist rooms. General treatment of coagulation, flocculation, and sedimentation will reduce the amount of this pathogen (Environmental & Workplace Health 2006).

Salmonella

Salmonella are etiological agents of gastrointestinal illnesses. This bacteria colonizes on feces and is often the case of contamination in improperly treated water (Environmental & Workplace Health 2006).

Yersinia

This is another organism that is found in feces. The concentration of Yersinia increases in the winter and can actually multiply at low water temperatures. Chlorination of treated water will kill of this organism (Environmental & Workplace Health 2006).

Typical Contamination

All these pathogens contaminate the most vulnerable waters where proper care and disinfection is not done properly. Most of the cases are in the developing nations where technology and education are lacking. Most of their pathogens come from feces from animals and humans that are inappropriately disposed of.

This is known as enteric pathogens (Ashbolt et al., 2001). Enteric pathogens cause 88% of the world's diarrheal disease and 1.7 million deaths (Ashbolt; Science Direct, 2004). All nations, specifically developing ones, will continue to have their population exposed to enteric pathogens until the people are educated about safe and preventative practices. At this point, this has improved, but there are still countries where this is still a daily threat. The same factors apply at almost all poor villages and cities where the quality of the household water is compromised. Those factors include: unprotected water source, inadequate sanitation, animal and fecal matter reaching water source, surface runoff seeping through the ground, water pipes, and wells. Below is an example of a typical rural village and how pathogens reach the water source:

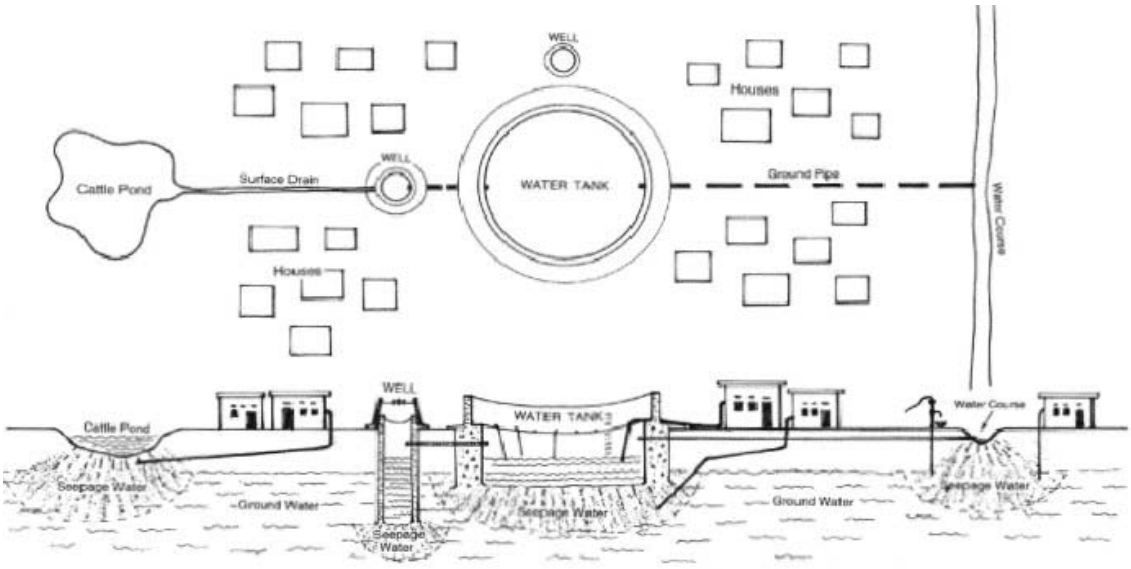

Figure 1: Example of a typical rural village water contamination

In this example, you can see that the cattle pond seems like it is a distance away from the well and other water sources, but the pond does not have a impermeable liner. The water from the cattle pond easily infiltrated down to the ground water source that the village gets their water from. The well may seem like a safe source since it is underground, but it is a false security because surface water has contaminated it. Another common case is the seepage of household water into the groundwater. Not all of this water is harmful, but sometimes families wash and prepare raw food or clean with disinfectants that later drain into the water that they drink and bathe in. One case not shown in the figure that is also a major issue is the case of stagnant water in access of the drinking water supply. Stagnant water gives opportunity for organisms to lay eggs, leave feces and any other bacteria. All of these issues discussed above have practical solutions that can be accomplished even at the level of rural villages.

There are two stages of assuring potable water for the household: disinfecting water as soon as collected (point-of-use disinfection) and collecting water in storage in a way where contamination can be prevented (safe storage) (Mintz, Reiff, Tauxe, 1995)

Point-of-use Disinfection

There have been many procedures of decontaminating water collected, but initial ways have been expensive and or use harmful chemicals that in large doses could dangerous. A more practical and less expensive way to decontaminate water collected has been the use of sodium and calcium hypochlorite. It is safer, easy to use and distribute, and destroys most pathogens. Another method newly used for water decontamination is electrolysis. Electrolysis uses .5% hypochlorite (salt) and water. Manufactures have made this system run on solar power to make it available for those countries that do not have the access to a constant power source. The generators in operation now can generate enough disinfectant for 10,000 people. It is relatively inexpensive ranging from $2.50 to $8 per kilogram of available chlorine. A family of 5 can live daily on 40 liters of water and at most would cost $.25 annually. Overall, treating water at home will reduce pathogens and therefore reduce the risk of getting waterborne illnesses such as cholera and diarrhea (Mintz, Reiff, Tauxe, 1995).

Safe Storage

The other stage of assuring potable water at home is safe storage. Studies have shown that water stored in containers were likely to have more pathogens than at the source (VanDerslice J., Briscoe J., 1993). Researchers tested a source in Peru that had a cholera epidemic and found that their stored water had a thousand-fold increase in mean fecal coliform compared to the municipal tap (Swerdlow DL, 1992). Realizing that this is a major threat to families, there have been newly structured containers that would reduce the amount of pathogens collected. Below is are figures to show comparison of a typical cantero from El Salvador and a container that meets the criteria of the Center of Disease Control and the Prevention/Pan American Health Organization.

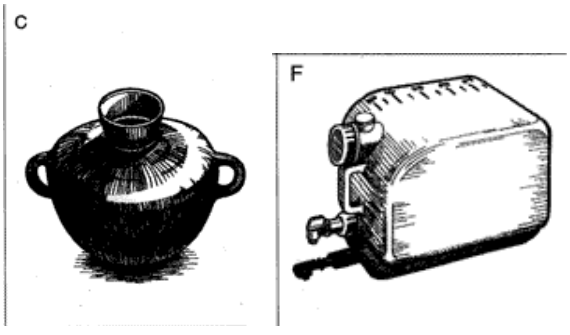

Figure 2: Two examples of water storage containers: canter and a container approved by the Center of Disease Control

In comparison, the cantero on the left is more accessible to bacteria and pathogens for the face that it is an open container. There are no barriers between the water in the cantero and any atmospheric or human interaction. Since there is only one way to take water out, the chances of receiving pathogens is inevitable. The container in the right has two distinctly water ways. The top hole is to receive water and then is tightly closed so once water is in, there is no cross contamination from other water or human touch. The faucet on the bottom makes it possible to families to get water without touching and contaminating the rest of the water. Studies in Sudan showed that using their traditional Zir containers, there were traces of fecal contamination within 2 days. Researchers replaced these with the approved container in figure and even after a month, the water maintain uncontaminated (Hammad, 1982).

Water Sanitation Education

Another way for safe practices to be implemented in families daily lives is to introduce it to families through education. Many times safe practices are not done because the individual doesn't want to, but rather they are not aware of the consequences and benefits of sanitation and personal hygiene. Once families start incorporating proper hand washing and careful food preparation fecal contamination decreased dramatically which shows that most contamination comes from home. All of these practices can be implemented in every home.

New Technologies

There are also newer technologic methods that have recently been introduced. One of the newest technology advancement to water decontamination is DNA mircoarrays. The main reason using DNA microarrays rose interest in people was because of the fear of bioterrorism. Now this technology is being used to aid all nations including rural villages in developing nations. "DNA microarrays are reverse dot-blots for which sequence-specific "probes" are attached to substrate in a lattice pattern" (Schena, 2000). The spots are usually 100-200 micrometers and 200-500 micrometers away from each other and they represent specific probe sequences. Microarrays allows simultaneous detection of specific DNAs from different pathogens. The speed of the detection of pathogens is the major reason this technology is being considered. After cultivation of organisms which takes hours to days, this is high speed and can be detected simultaneously (Call, Borucki, Loge, 2003).

There are two types of microarrays, immobilized oligonnucleotide probes and polymerase chain reaction (PCR) amplicons (Call, Chandler, and Brockman, 2001). It is found that the microarrays are quick, but the inadequate when the concentration of solution is low. PCR amplifies DNA sequences and helps the microarray process. This method uses the combination of several PCRs before

their hybridization on microarrays, for example using 140 amplicons to characterize 18 pathogenic species. PCR materials or oligonnucleotides can be left on any substrate, but usually on modified glass and then attached to probes. In most cases probe adsorption occurs with no specific modifications which reduce the cost of special modifications. Below is a figure describing the PCR process. This reaction takes DNA and it replicates it about 20-30 cycles. This produces a high concentration of DNA sequences which then is hybridized by the Microarray process.

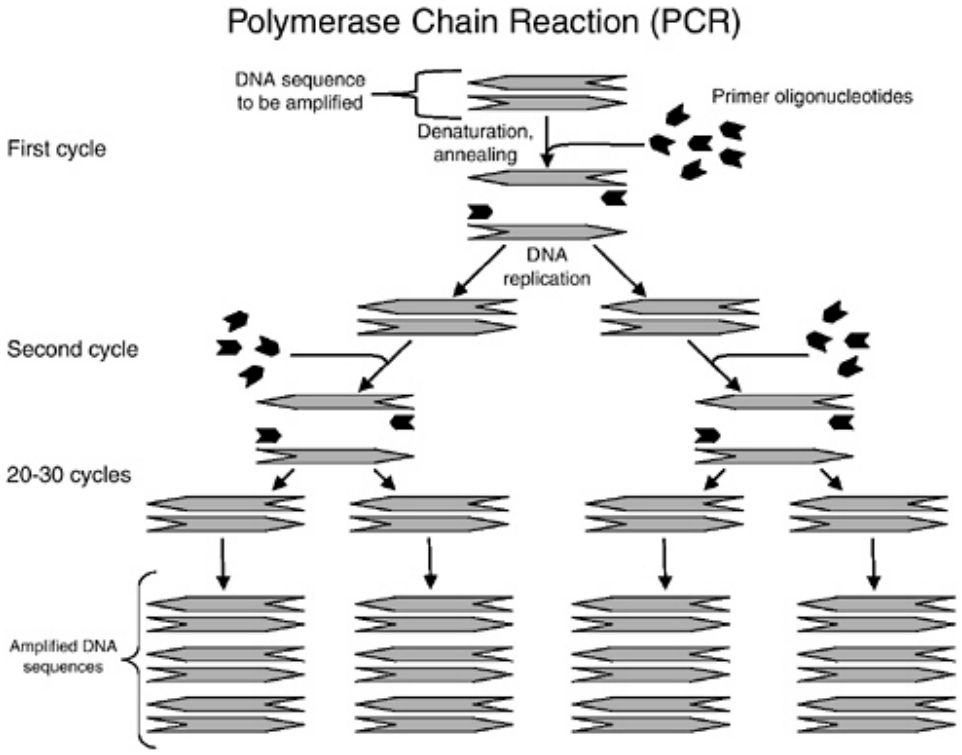

Figure 3: PCR model

At this point, the targets are hybridized into the array. Once the hybridization steps are completed, the arrays are shown as specific light spectra as shown in the figure below. Using single-channel imaging systems, specific bacterial targets may be detected.

To have precise results, factors need to taken into account:

- Assay sensitivity
- sample size
- Efficiency of pathogen isolation
- Efficiency of nucleic acid extraction
- Effect of co-precipitating factors that inhibit PCR

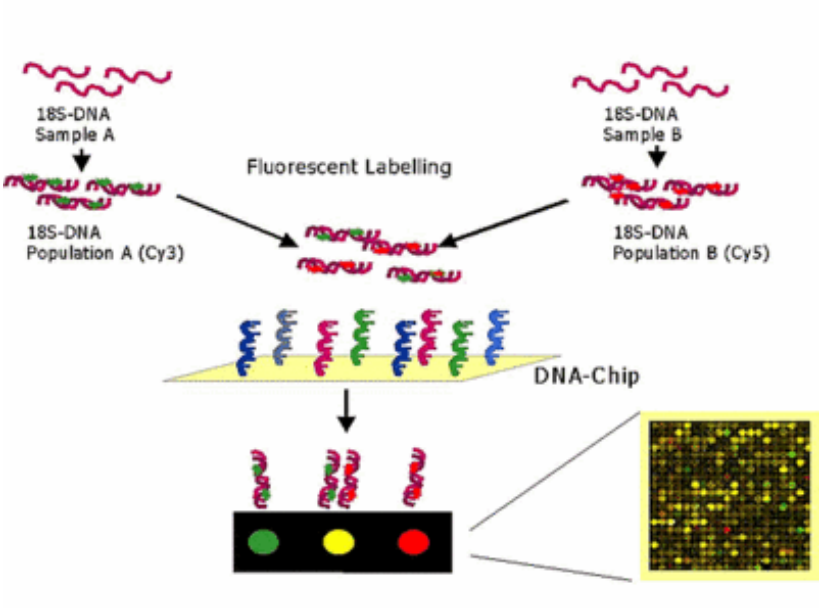

Figure 4: Microarray experiment scheme

When PCR is combined with microarrays as end-point detectors, this amplifies products. The single PCR reaction can simultaneously detect different pathogens because “the probes themselves are located within the polymorphic region that is flanked by the conserved primer sequences.” (Call, Borucki, and Loge; 2003). Another benefit of using the microarrays is because they are “not limited to identification by product length. The reason this is important is because shorter products hybridize better to the arrays and, including PCR, they are produced even more efficiently.

Some restrictions would include identifying the pathogenic-specific sequences before the array is configured. This means that only the pathogens expected or known would be the only pathogens identified on the chip. Any other pathogens would not show up on the chip. Also, the microarrays need to be validated through multiple experiments for confirmation. The overall benefit of this procedure is the fact that the sequence is already identified, the process because

very quick. Below is a figure of the steps taken to detect the pathogens and sequencing process. In this figure to can select to different ways: Microbiological isolation and Nucleic acid extraction and purification (Call, Borucki, and Loge 2003).

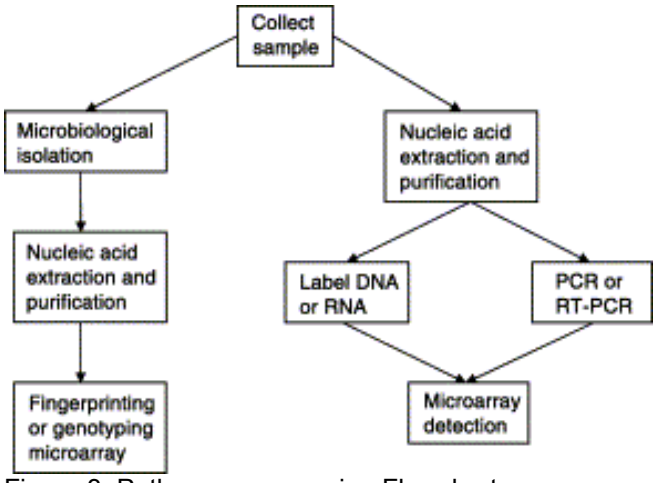

Figure 3: Pathogen sequencing Flowchart

Genotyping with Microarrays

The advantage of microarrays for genotyping is that genomic DNA can directly be put into an array through hybridization. It is used like a fingerprint of different pathogenic DNA as well as discovering new genetic markers. Below is a figure of the steps taken to make a mixed genome microarray.

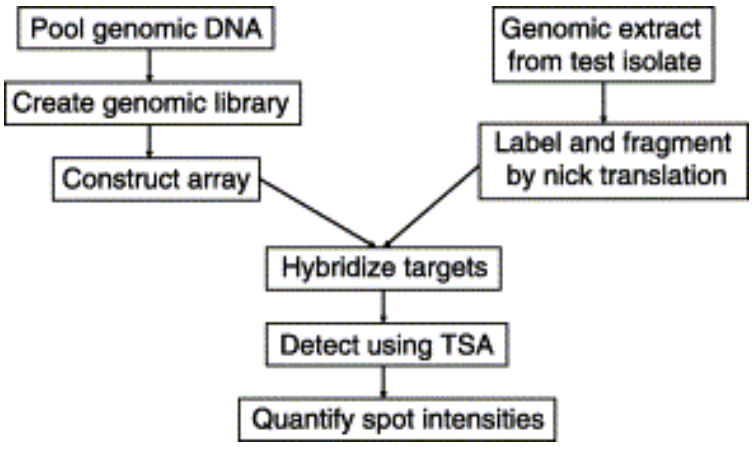

Figure 4: Genotyping Flowchart

The process includes extracting DNA from two serotypes of L. monocytogenes and was hybridized into identical arrays. The figure below show the sequences and the spots that are missing are the sequences that are not in the genome.

In this example, there are 8 spot differences between the two serotypes (Call, Borucki, and Loge 2003).

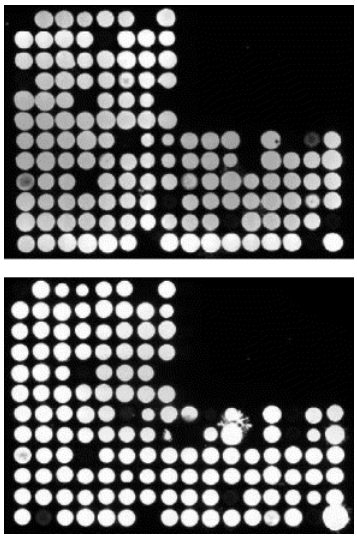

Figure 5: Image of hybridized arrays through the extraction of two serotypes

Limitations

As mentioned above, the main limitation for microarrays is the face that the DNA sequences need to be identified into an array before the microarrays to function. Secondary limitation is the cost at this point is a bit high, but cost decreases considerably as testing increases. If these processes would be implemented as a standard, then the cost should not discourage anyone from using it. Materials used to make deposits to glass include quill pins and solid pins. When it comes to the experiments, it is found that Biotin-streptavidin chemistry used as a labeling scheme is considerably less expensive.

Application in developing countries

Currently, the EPA is looking into the feasibility of introducing DNA microarrays for the detection of water pathogens as a standard instead of the culture process used now which are useless for those pathogens that are non-culturable. At this point microarray technology can only be used in regulatory application because they still need to be monitored until they can be assured that they can detect pathogens consistently and precisely. The government in the U.S. do in fact want to proceed with this technology as the next way to monitor water therefore we will

be seeing more of this technique and using the application in other countries for its usability, and speed. Cost is an issue but with time efficient methods will be developed (*Workshop on the Feasibility of Using DNA/RNA Microarrays and Related Technologies for High Through-Put Detection of Waterborne Pathogens, EPA 2005)*.

Conclusion

Biotechnology is a very important emerging technology for the detection of waterborne pathogens. At this point many developing countries are working on preventative methods to avoid waterborne pathogens such as water source protection, safe storage, and overall education towards better sanitation practices. Once developing nations and control the human aspect of contamination, there is hope of technology that can prevent further contamination from non-human sources. This technology is microarrays in combination with PCR processes to amplify the DNA sequences therefore make a more effective array for detection. It is still fairly new technology in this aspect and many factors are still under investigation, including the precision, the cost, and the fact that the it will only pick up the pathogen DNA sequences that are identified, not all in general. The important piece is that these concerns are already identified and are under investigation. The process is adequate at this time and is being perfected. It can be used and once it is certified and assured, it will ease the pathogen detection process by reducing the time and perhaps the cost. This two factors are especially important for the fact that people should not have to wait to find results of an infected source and shouldn't have to pay extra money for it. Bottom line is that saving peoples' health and lives are the priority and this method gives quick results which in turn results in the prevention of potential sick people.

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