Emerging and re-emerging microbial pathogens in surface water

Stefania Marcheggiani
Dept. Environment and Health- EcoHealth Unit

stefania.marcheggiani@iss.it

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WHO recognizes that access to adequate water supplies is a fundamental human right (28 July 2010, Resolution 64/292)

Water is a the main Reservoir and vehicle of diseases and/or infectious

Water--related diseases are a human tragedy, killing millions of people each year (account for 80% of all deaths in developing countries).

Prüss-Üstün et al., 2008

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Sources, pathways and relative risks of contaminants in surface water and groundwater

The risks related to the use of contaminated water arise not only from direct use of the same but also from indirect routes such as:

- The consumption of molluscs and fish,
- Contact or ingestion of contaminated water during recreational activities,
- From the consumption of fresh vegetables, fruits and vegetables contaminated by irrigation water

Source: Ministry of the environment, Québec, Canada

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Climate changes

Water quality get worse due to the influence of climate changes.

- **Water scarcity** will further exert its negative effects: use of waters of lower quality, worsening of hygienic conditions, higher concentrations of nutrients in surface waters.

- **Floods and heavy rainfalls** can worsen the microbiological quality of waters (run off from agricultural and zootechnical areas, overflow of untreated wastewaters from treatment plants).

- Global warming can increase the incidence of some diseases, like those transmitted in the hottest months, and promote new ones (e.g., transmitted by parasites).
Nowhere are the pressures felt so strongly as at the interface between water and human health.

- Infectious, water-related diseases are a major cause of morbidity and mortality worldwide.

- A significant proportion of this disease is caused by ‘classical’ water-related pathogens, newly-recognized pathogens and new strains of established pathogens are being discovered.
Water quality

Even water that appears "pure" must be tested to ensure that it contains no microorganisms that might cause disease

✓ Microbiological indicators are used to measure suitability for drinking, bathing waters and releasing back into the environment.

Microbiological indicators: useful to define the level of impairment of ecosystems and to assess the health risk.

When they are detected in a water sample, the water is assumed to be unsafe to drink because of the possible presence of pathogenic microorganisms.
<table>
<thead>
<tr>
<th>Directive</th>
<th>Date</th>
<th>Title</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bathing Water Directive (BWD),</strong></td>
<td>4.3.2006</td>
<td>Official Journal of the European Union</td>
<td>L 64/37</td>
</tr>
<tr>
<td><strong>Drinking Water Directive (DWD),</strong></td>
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<tr>
<td><strong>COUNCIL DIRECTIVE 98/83/EC</strong></td>
<td>5.12.98</td>
<td>Official Journal of the European Communities</td>
<td>L 330/32</td>
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*Acts whose publication is obligatory*
How microbes can come into contact with human?

<table>
<thead>
<tr>
<th>Contact</th>
<th>Ingestion</th>
<th>Inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces</td>
<td>Attinomicosi</td>
<td>Poliovirus</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>Infezioni di ferite</td>
<td>Meneingite</td>
</tr>
<tr>
<td>Leptospira</td>
<td>Leptospirosi</td>
<td>Tularemia</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Infezioni di ferite</td>
<td>Legionellosi</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>Otite esterna</td>
<td>febbre di Pontiac</td>
</tr>
<tr>
<td>Vibrio spp</td>
<td>Infezioni di ferite, otite esterna</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Otite, congiuntivite, infezioni urinarie, ascessi, gastro-enterite in neonati</td>
<td>Poliovirus</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Adenovirus</td>
<td>Faringiti, infezioni dell’occhio</td>
<td>Polioenterite</td>
</tr>
<tr>
<td>Naegleria</td>
<td>Meningoencefalite</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Inhalation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavobacterium meningosepticum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Francisella tularensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legionella spp</td>
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</tr>
</tbody>
</table>

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Transmission process

1. Latency
2. Persistence
3. Multiplication

Excreted load of Pathogens

Infectious dose

From Feachemen et al 1983
WHO Water Zoonoses, 2004

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1 Latency

Latency is the time between the excretion of the pathogen and the time that it is infective to a new host.

- Most organisms have no latent period and are immediately infectious after excretion (enteric viruses, bacteria, and protozoa).
- Other organisms require time in the environment to develop into an infectious stage and may pass through one or more intermediate hosts (e.g., helminths, schistosomiasis).

WHO Water Zoonoses, 2004
2. Persistence

The longer an organism can persist, the more likely it is to have the opportunity to come into contact with a susceptible host.

- Survival time in water depends on many physical factors (pH, temperature, sunlight) as well as characteristics of the organism.

- The survival times of water-related pathogens in water range from hours to years.

Some pathogens are capable of entering a dormant state - “viable but non-culturable,” survive longer and maintain their pathogenicity (Salmonella, Campylobacter, and Vibrio cholerae),

Other pathogens have a stage in their life cycle, such as a spore or oocyst, that is environmentally resistant.

- The fate of the organism in wastewater and water treatment processes is also a key element in understanding the risk of waterborne transmission

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WHO Water Zoonoses, 2004
3 Multiplication

The ability of the organism to replicate in the environment is also important. Under favourable conditions, some water-related pathogens can multiply in the aquatic environment.

- all aquatic bacterial can multiply in the environment and may reach high concentrations.
- Some water-related helminth pathogens may be amplified within an intermediate aquatic host

WHO Water Zoonoses, 2004
Infective dose

Infectious dose or degree of water exposure necessary to transmit the infection.

Infectious dose varies widely among environmentally transmitted organisms and is difficult to measure.

The dose that induces infection in 50% of exposed individuals, described as the median infectious dose (ID50), can range from about $10^9$ colony-forming units of Salmonella pullorum (Teunis et al. 1996) to 10–1000 Cryptosporidium oocysts (Teunis et al. 2002).

The ID50 can vary by the strain of microorganism and by the host population, depending on age and immune status

WHO Water Zoonoses, 2004
Waterborne diseases whose pathogens are spread by the fecal-oral route (with water as the intermediate medium) can be caused by bacteria, viruses and parasites (including protozoa, worms and rotifers).

Diarrhoea is one of the most common features of waterborne disease.

Fecal pollution is one of the primary contributors to diarrhoea.
Bradley Classification of disease transmission routes for water-related hazards

**Water-borne Diseases**

Diseases caused by ingestion of water contaminated
- by human or animal excrement, which contain pathogenic microorganisms. By chemicals that have an adverse effect on health. Arsenic, Flouride, Nitrates from fertilizers; Carcinogenic pesticides (DDT); Lead (from pipes); Heavy Metals.

**Water-related Diseases**

Diseases are caused by insect vectors, especially mosquitoes, that breed or feed near contaminated water. They are not typically associated with lack of access to clean drinking water or sanitation services.

**Water-based Disease**

Diseases caused by parasites found in intermediate organisms living in contaminated waters. Includes Schistosomiasis and Dracunculiasis.

**Water-washed Diseases**

or Water-scarce Diseases. Diseases caused by poor personal hygiene and skin and eye contact with contaminated water.


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## Classification of water-related zoonotic diseases

<table>
<thead>
<tr>
<th>Category</th>
<th>Zoonotic examples</th>
<th>Relevant control strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterborne via drinking-water</td>
<td>Salmonellosis, <em>E. coli O157:H7</em>, cryptosporidiosis, giardiasis, campylobacteriosis, microsporidiosis, toxoplasmosis, balantidiasis, yersinia, tularaemia, cysticercosis</td>
<td>Improve microbiological water quality through water treatment; protect drinking-water sources from contamination by animal faeces</td>
</tr>
<tr>
<td>Waterborne via recreational water contact</td>
<td>Leptospirosis, cryptosporidiosis, giardiasis</td>
<td>Protect water source from animal contamination</td>
</tr>
<tr>
<td>Water-washed</td>
<td>Cryptosporidiosis, giardiasis, balantidiasis, hepatitis E virus?</td>
<td>Increase water quantity to improve hygiene; promote hand washing</td>
</tr>
<tr>
<td>Water-based</td>
<td>Schistosomiasis (<em>Schistosoma japonicum</em>)</td>
<td>Protect user, control aquatic hosts, surface water management</td>
</tr>
<tr>
<td>Water-related insect vectors</td>
<td>West Nile virus, Rift Valley fever virus, yellow fever virus, sleeping sickness (African trypanosomiasis)</td>
<td>Protect user, control vector, surface water management</td>
</tr>
<tr>
<td>Inhalation of water/wastewater aerosols</td>
<td>Mycobacteria</td>
<td>Protect individuals who have occupational exposure; limit human exposure to geographic areas impacted by aerosols</td>
</tr>
<tr>
<td>Aquatic food</td>
<td>Paragonimiasis</td>
<td>Avoid ingestion of raw or undercooked crustaceans; prevent faecal contamination of freshwater crab and crayfish habitats, control snails by molluscicides</td>
</tr>
</tbody>
</table>

http://www.who.int/water_sanitation_health/diseases/zoonosessect2.pdf
Emerging and re-emerging infectious diseases

Emergence of infectious disease are the results from dynamic interactions between rapidly evolving infectious agents and changes in the environment and in host behaviour that provide such agents with favourable new ecological niches.

Red = newly emerging diseases
Blue = re-emerging/resurging diseases;
Black = 'deliberately emerging' diseases

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Emerging pathogens are those that have appeared in a human population for the first time (E.coli O157:H7), or have occurred earlier but are increasing in incidence or expanding in areas where they have not previously been reported (i.e. Campylobacter parvum, Legionella spp, Helicobacter pylori, Campylobacter spp, Calicivirus, in USA, Canada and Europe), usually over the last 20 Years (WHO, 1997).

Definition

**Re-emerging pathogens** are those whose incidence is increasing as a result of long-term changes in their underlying epidemiology (i.e. *Giardia duodenalis*) (Woolhouse, 2002).


**Opportunistic pathogens** are microorganisms, commensal, saprophytic or environmental, that are capable of causing infectious only when the host's resistance is lowered, for example vulnerable groups of the population, including infants and children, elderly and immunocompromised individuals. (i.e. *Aeromonas hydrophila, Pseudomonas aeruginosa, Pseudomonas stutzeri, Flavobacterium spp*).

Distribution of emerging pathogens by group

Modified *Emerging issues in water and infectious diseases*  

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Factors key to the emergence of infectious diseases in humans:

1. microbial adaptation and change;
2. human vulnerability;
3. climate and weather;
4. changing ecosystems;
5. economic development and land use;
6. human demographics and behaviour;
7. technology and industry;
8. international travel and commerce;
9. breakdown of public health measures;
10. poverty and social inequality;
11. war and famine;
12. lack of political will; and
13. intent to harm.

https://wwwnc.cdc.gov/eid/article/1/1/95-0102_article
### Examples of Emerging Infectious Diseases

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>ID</th>
<th>Disease</th>
<th>Mortality</th>
<th>Methods of Prevention</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>$10^8$</td>
<td>Cholera</td>
<td>&lt; 1% with treatment</td>
<td>Public sanitation; potential for vaccine</td>
<td>Oral or-intravenous rehydration, antibiotics</td>
</tr>
<tr>
<td><em>Salmonella typhæ</em></td>
<td>$10^6-7$</td>
<td>Typhoid</td>
<td>~600,000 deaths annually</td>
<td>Public sanitation; vaccine</td>
<td>Antibiotics</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>$10^6-7$</td>
<td>Salmonellosis</td>
<td>10–30% of cases in parts of Asia and Africa</td>
<td>Food-related hygiene</td>
<td>Rehydration, antibiotics</td>
</tr>
<tr>
<td>Toxigenic/diarrhoeagenic <em>E. coli</em></td>
<td>$10^{1-9}$</td>
<td>Diarrheal diseases</td>
<td>?</td>
<td>Public sanitation, hygiene</td>
<td>Rehydration, antibiotics</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>$10^1$</td>
<td>Diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome (HUS)</td>
<td>&lt;1% overall; 3–5% with HUS</td>
<td>Food-related hygiene</td>
<td>Rehydration; antibiotics are contra-indicated</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>$10^2$</td>
<td>Shigellosis</td>
<td>~1.1 million deaths/year</td>
<td>Sanitation, hygiene</td>
<td>Rehydration, antibiotics</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>$10^6$</td>
<td>Campylobacteriosis</td>
<td>?</td>
<td>Food hygiene</td>
<td>Rehydration</td>
</tr>
<tr>
<td><em>Leptospiroa</em> spp.</td>
<td>3</td>
<td>Leptospirosis</td>
<td>?</td>
<td>Sanitation, vaccine</td>
<td>Rehydration, antibiotics</td>
</tr>
<tr>
<td><em>Francisella tularensis</em></td>
<td>10</td>
<td>Tularemia</td>
<td>?</td>
<td>Hygiene, sanitation, insect repellents, vaccine</td>
<td>Antibiotics</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>$10^9$</td>
<td>Yersiniosis</td>
<td>Uncommon, except with bactereñia</td>
<td>Food-related hygiene</td>
<td>Rehydration, antibiotics</td>
</tr>
<tr>
<td><em>Aeromonas</em> spp.</td>
<td>$10^8$</td>
<td>Skin and respiratory infections</td>
<td>?</td>
<td>Avoid exposure; boil water if immunocompromised</td>
<td>Antibiotics</td>
</tr>
</tbody>
</table>
Examples of emerging and re-emerging bacteria

- *Salmonella*,
- *E. coli O157: H7*
- *Campylobacter*,
- *Yersinia enterolitica*
- *Mycobacterium avium (ssp. paratuberculosis)*
- *Leptospira*
- *Staphylococcus aureus*
- *Pseudomonas*
- *Aeromonas*
Opportunistic pathogens

Aeromonas, Pseudomonas, Acinetobacter, Flavobacterium

Microbial colonization of water pipe networks-
Biofilm

- Corrosion of pipe
- Changes of organoleptic properties
  (odor and aroma unpleasant)
- Production of colored waters
- Interferences for the detection of coliforms

Low flow and an increase in water temperature.
Pseudomonas aeruginosa

is a member of the family Pseudomonadaceae and is a polarly flagellated, aerobic, Gram-negative rod. When grown in suitable media, it produces the non-fluorescent bluish pigment pyocyanin. Many strains also produce the fluorescent green pigment pyoverdin. Pseudomonas aeruginosa, like other fluorescent pseudomonads, produces catalase, oxidase and ammonia from arginine and can grow on citrate as the sole source of carbon.

Source and occurrence

Pse. aeruginosa is a common environmental organism and can be found in faeces, soil, water and sewage. It can multiply in water environments and also on the surface of suitable organic materials in contact with water. Pse. aeruginosa is a recognized cause of hospital-acquired infections with potentially serious complications. It has been isolated from a range of moist environments such as sinks, water baths, hot water systems, showers and spa pools.

Routes of exposure

The main route of infection is by exposure of susceptible tissue, notably wounds and mucous membranes, to contaminated water or contamination of surgical instruments. Cleaning of contact lenses with contaminated water can cause a form of keratitis. Ingestion of drinking-water is not an important source of infection.
**Pseudomonas aeruginosa**

Human health effects

*Pse. aeruginosa can cause a range of infections but rarely causes serious illness in healthy individuals without some predisposing factor.*

It predominantly colonizes damaged sites such as burn and surgical wounds, the respiratory tract of people with underlying disease and physically damaged eyes. From these sites, it may invade the body, causing destructive lesions or septicaemia and meningitis. Cystic fibrosis and immunocompromised patients are prone to colonization with *P. aeruginosa*, which may lead to serious progressive pulmonary infections.

Most diseases of which *Pse. aeruginosa* are responsible are not due to the ingestion of contaminated water but from contact with it.

Water containing these bacteria can contaminate foods, beverages, and pharmaceuticals, causing deterioration and making it a secondary transmission.

Even structures in contact with water, such as sinks and drains, taps and showers, may be contaminated by *Pse. aeruginosa* and may constitute an infection reserve.

Many strains are resistant to a range of antimicrobial agents, which can increase the significance of the organism in hospital settings

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Emerging diseases in rural areas of the European Region, the example of campylobacteriosis

rural areas are those with a population density below 150 inhab./km²

- Sanitation management often inadequate, higher possibilities of contamination of drinking water supplies;
- small supplies receive less resourcing than large supplies;
- lack of clearly defined responsibilities over management issues;
- lack of community awareness of the potential risks (local people believe their waters safe);
- often groundwater (private wells) is not controlled and used for drinking without any treatment;

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http://www.terragri.eu/zonerurali.asp
Campylobacter spp

Camphylobacter spp are gram negative bacteria, non sporeforming, motile with spiral or S-shaped morphology, microaerophyllic, oxidase positive, urease negative. (Vandamme et al., 2005).

The genus Campylobacter includes 21 species and eight subspecies Debruyne et al. 2010).

At least twelve of these species are associated with human illness; however, the vast majority of infections (80–90%) are associated with Campy. jejuni. Campy. coli is the second most common species associated with campylobacteriosis

Campy. spp. are the most common cause of bacterial gastroenteritis worldwide (Friedman et al. 2004, Silva et al. 2011) C. jejuni subsp. jejuni : the most frequently bacterial cause of diarrhoea in human beings; C. coli, C. lardis and C. fetus : isolated in a small proportion of cases (ECDC, 2009, WHO, 2006).

The infective dose of Campylobacter is less than 500 organisms (CDC, 2008). The incubation period is usually of 2–4 days. The infection is self-limited and resolves in 3–7 days.

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Campylobacteriosis is a zoonotic disease. Wild and domestic animal, especially poultry, wild birds and cattle, may be important reservoirs of Campylobacter spp. C. jejuni has a high infectivity compared with other bacterial pathogens.

Clinical symptoms of C. jejuni infection are characterized by abdominal pain, diarrhoea (with or without blood or faecal leukocytes), vomiting, chills and fever.

Main transmission pathways for Campylobacter infections

Food
- Poultry and poultry products
- Meat and fish
- Vegetables and fruit, lettuce
- Milk unpasteurized milk or its products

Water
Contaminated drinking and bathing waters

Direct contact with animals or persons

Undirect contact
(eg through faecally contaminated soil, especially for children in rural areas)

Cross contamination
(hygiene conditions)
Campylobacter continues to be the most frequently reported gastro-enteric pathogen in the EU and EEA/EFTA countries, with an incidence of almost 47 cases per 100,000. (Annual Epidemiological Report on Communicable diseases, CDC, 2015)

The incidence of campylobacteriosis (ECD, 2015) is much higher in:

● summer months,
● male gender,
● infants and young people.
● travelling people

(Ethelberg et al., 2005- Denmark; Nygard et al., 2004-Sweedden; Kapperud et al., 1992- Norway; Brown et al. 2004- United Kingdom; Brown et al. 2004 - United Kingdom; Norval et al., 2009 -Northeast Scotland)
Gram positivo, cocchi, di diametro 0,5-1,5 µm di diametro, non è mobile, generalmente privo di capsula, asporigeno e anaerobio facoltativo, fermenta il glucosio principalmente in acido lattico e fermenta il mannitolo, catalasi positivo e coagulasi positivo o negativo (importante nell’identificazione di routine di S. aureus, ossidasi negativo. Cresce a tra 15-45 °C ed in alte concentrazioni di NaCl. (15%). (Bergey’s Manual, 2005).

and also known as golden staph, is the most common cause of staph infections. It is a spherical bacterium, frequently living on the skin or in the nose of a person.

*Staph. aureus* can cause a range of illnesses from:

- minor skin infections, such as pimples, impetigo boils, folliculitis, furuncles,
- to life-threatening diseases, such as pneumonia, meningitis, urinary infection, endocarditis, toxic shock syndrom (TSS), osteomyelitis and septicemia.

*S aureus* responded to the introduction of antibiotics by the usual bacterial means to develop drug resistance.
Methicillin- (oxicillin-) resistant S. aureus (MRSA)

MRSA refers to strains of the bacterium that are resistant to β-lactam antibiotics methicillin, oxicillin, penicillin and amoxicillin chloramphenico, macrolides, sulfonamide and tetraciclie.

The meca (2.aKb) gene is genetic determine of penicillin resistance that encode PBP2a protein which has a low affinity for β-lactam antibiotics.

The emergence and global spread of MRSA may be viewed as a process of accelerated evolution. (Oliveria et al, 2002)

The incidence of methicillin-resistant S. aureus (MRSA) has increased greatly in recent years and currently represents a major source of nosocomial infections.


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Antimicrobial resistance surveillance in Europe 2015
Methicillin-Resistant Stap. aureus (MRSA):

- Staphylococci developed resistance to penicillin shortly after this agent was introduced into clinical practice.
- Antistaphylococcal penicillins (methicillin, oxacillin, nafcillin, cloxacillin, dicloxacillin) were developed to counter this problem.
- Subsequently, strains emerged that were resistant to these drugs and to the cephalosporins. (were dubbed MRSA.)
- The incidence of MRSA has been increasing steadily since the 1980's. This pathogen not only causes nosocomial infections in large teaching hospitals, but is also a problem in many non-hospitalized patients as well.

Currently the only available agent for treating infections with MRSA is vancomycin.

The development of vancomycin-resistant MRSA would leave us with no treatment for this very pathogenic microorganism. Vancomycin-resistant *Staphylococcus epidermidis has been reported* in patients. There is tremendous concern that vancomycin-resistant strains of *S. epidermidis or* enterococci will transfer the genes for resistance to MRSA
Open Issues...

✓ There are no standard methods to isolate all pathogens

✓ Available methods are often complex, time-consuming and with low reproducibility.

✓ When pathogenic microorganisms occur in relatively low concentrations in environmental waters their detection is often difficult.

✓ Moreover, the microorganism survival is often limited depending on the environment parameters.

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General remarks

Monitor environmental changes that influence disease emergence and it’s transmission

Improved microbial genetics method for detection, control and prevention

Education on hygiene

Global surveillance
Thank you for your attention