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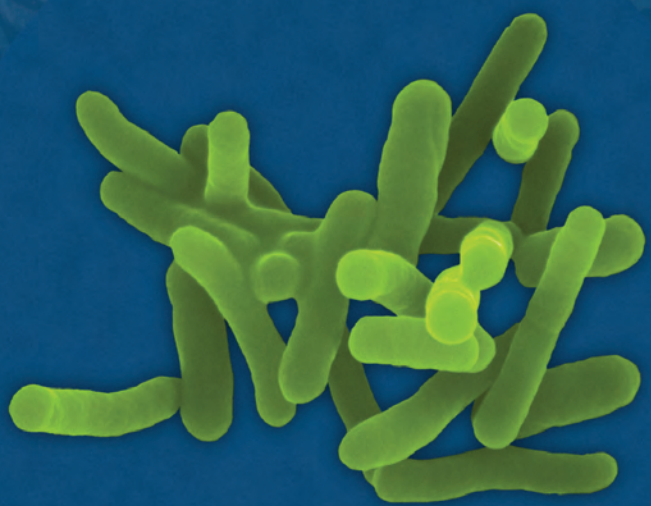
Safety and quality of water used with fresh fruits and vegetables



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MICROBIOLOGICAL RISK ASSESSMENT SERIES

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Safety and quality of water used with fresh fruits and vegetables

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Contents

ANNEXES

TABLES

FIGURES

Acknowledgements

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Declaration of interests

All participants completed a Declaration of Interests form in advance of the meeting. They were not considered by FAO and WHO to present any conflict in light of the objectives of the meeting.

All the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting. All the experts participated in their individual capacities and not as representatives of their countries, governments or organizations.

Abbreviations and acronyms

ALOP	appropriate level of sanitary protection
CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CP	control point
Ct	product of the concentration of a disinfectant (e.g. free chlorine) and the contact time with the water being disinfected
ddPCR	digital droplet PCR
DALY	disability-adjusted life-years
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FC	thermotolerant or faecal coliforms
FFV	fresh fruit and vegetables
FIB	faecal indicator bacteria
FSO	food safety objective
HACCP	Hazard Analysis and Critical Control Points
hAdV	human adenovirus
HAV	hepatitis A virus
HEV	hepatitis E virus
hPyV	human polyomavirus
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
LAMP	loop-mediated isothermal amplification
LRV	log reduction value
mAB	monoclonal antibody
MPN	most probable number
MST	microbial source tracking
NASBA	nucleic acid sequenced-base amplification
NGS	next-generation sequencing
NoV	norovirus
pAdV	porcine adenovirus
PCR	polymerase chain reaction

PO	performance objective
PRPs	prerequisite programs
qPCR	real-time quantitative PCR
RA	risk assessment
RM	risk management
RNA	ribonucleic acid
RV	rotavirus
RT-PCR	reverse-transcription PCR
TAPCs	total aerobic psychrotrophic bacterial counts
TC	total coliforms
SaV	sapovirus
SSP	sanitation safety plans
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WSP	water safety plan
WGS	whole genome sequencing
WWAP	United Nations World Water Assessment Programme

Executive summary

At its 48th session of Codex Committee on Food Hygiene, the Committee noted the importance of water quality and safety in food production and processing. The Committee requested FAO and WHO to provide guidance for those scenarios where the use of "clean water" e.g. water that does not compromise the safety of the food in the context of its use, was indicated in Codex texts and on where it is appropriate to use "clean water". In particular, guidance was sought for the safe use of irrigation water and "clean" seawater and on the safe reuse of processing water.

To facilitate this work, FAO and WHO established a core group of experts and convened two Expert Meetings (21–23 June 2017, Bilthoven, the Netherlands; 14–18 May 2018, Rome, Italy). The report of the first two meetings was published in 2019 (FAO and WHO, 2019). To follow up on the cross-cutting issues raised during the meetings, another Expert meeting was convened on 23–27 September 2019, in Geneva, Switzerland. An overview of the deliberations and outputs of the third meeting is provided below.

The purpose of this meeting was to develop clear and practical guidance on the microbiological criteria and parameters that can be used to determine if water is 'fit-for-purpose' when used in the pre- and post-harvest production of fresh fruit and vegetables (FFV). Practical interventions that could be applied pre- and post-harvest to mitigate food safety risks when water does not meet the requirement of fit-for-purpose were also considered.

During FFV production, water is used for a variety of purposes. At each successive step from the growing stage up to the point of consumption, the microbiological quality/safety of the water used at that step should be of higher quality than that at the previous step or at least of equal quality. An exception is where there is a subsequent validated pathogen reduction treatment (removal or inactivation / kill) before consumption of the final product.

Any water used through the FFV production chain, even that which has been conventionally treated and disinfected, may potentially contain human pathogens, albeit at low concentrations. A risk assessment, appropriate to the national or local production context, should be conducted to assess the potential risks associated with a specific water source or supply in order to devise the appropriate risk mitigation strategies.

The microbial criteria for water quality required for the safe production of FFV should be determined using a risk-based approach, taking into account:

- the availability and suitability of the water for its intended purpose, the method of application, and the production stage at which it will be used, and the potential and the extent of intentional or non-intentional food-water contact;
- the types of FFVs (e.g. soil, vine or tree growth) and any specific characteristics (e.g. leafy vegetables, netted rind melons), the FFV production system (e.g. field, hydroponic) and their intended use (e.g. usually eaten raw or cooked, peeled or unpeeled);
- the water retention and contact time with the edible part of the FFV;
- the potential for decline or proliferation of pathogens or introduction of contamination of FFV after each point of water contact before consumption.

When assessing the potential health risks of water inputs into the FFV production chain a number of qualitative and quantitative microbial water quality targets can be used. These include, but are not limited to, the direct detection of the presence of pathogens and, more often, the indirect determination of pathogen presence by enumeration of groups of microorganisms that can infer pathogen presence. These are referred to in this report as indicator microorganisms and include faecal indicators, index and model microorganisms, and microbial indicators of process control. In the production chain of FFV, the presence of faecal indicator microorganisms is used to indicate unhygienic conditions, the presence of faecal pollution or failures in the performance of sanitary control measures. Emerging evidence indicates inconsistencies can occur in this pathogen/indicator relationship.

Multiple analytical methods are available to assess the degree of microbial contamination of water used in the production of FFV. The choice of microbial methods to assess microbial quality should be based on validated test methods and take into consideration local capacity and resources available.

When applying microbial analyses in assessing the risks of water safety and for trend analysis of microbial quality, the choice of either pathogen presence and/or enumeration of microbial indicators, sampling plans for the microbial targets and the acceptable limits should be proportionate to the risk posed and meet risk management goals. For instance, during baseline water quality assessments, different parameters are suitable for different goals e.g. during validation of the performance of abatement technologies and verification that the control measures are operating as intended.

When considering the use of microbial analyses in risk assessments of water safety use or in risk management programs in FFV production chain, the following should be considered:

- No one water quality microbial indicator is appropriate/useful for all water types and for some water types there may not even be a single useful indicator.
- At present, there is no microbiological indicator/proxy that can reliably predict faecal pathogen occurrence or numbers because bacterial indicators are typically surrogate measures of faecal pollution, rather than measures of the pathogens themselves. It is not possible, with the use of indicators, to accurately predict the presence or concentrations of specific faecal pathogens in the contaminating faecal material.
- It is generally agreed that microbial indicators of faecal contamination, particularly *E. coli* and intestinal enterococci, have been useful and *E. coli* has been widely adopted for monitoring drinking water quality. It is anticipated that *E. coli* and intestinal enterococci will also have wide and useful applications as faecal indicators in the context of water for food production.
- Bacteriophages, especially male-specific coliphages and *Bacteroides*-specific phages, have been found to be effective predictors of human faecal contamination. They can be useful for verification and validation of virus-reduction water treatments. Although their presence does not correlate specifically with the presence of human pathogenic viruses, in groundwater, they may be useful general indicators of the occurrence of viruses.
- There are currently no meaningful indicators (indirect measures) for parasites in water or soil (e.g. protozoa, nematodes and cestodes).
- Correlation between indicator microorganisms and pathogens is stronger in heavily polluted waters, but this correlation is insignificant and biologically uninformative when pollution levels are low.

Quantitative Microbiological Risk Assessments (QMRA) are valuable tools for establishing tailored water quality criteria that are based on human health targets and are suitable for application to water used for food crops such as FFV eaten raw. Existing WHO guidelines provide templates for carrying out the calculations, based on either established health targets or assumed values (WHO, 2016b). However, appropriate data are needed to conduct a QMRA. A QMRA can only be conducted with actual pathogen measurements, or assumptions and cannot be based on microbiological indicator concentrations.

Each geographical region of primary production of FFV can have individual characteristics that preclude generalizations of water quality targets in production and processing of FFV compared with those applied in drinking water supplies.

For example, such characteristics can include varying environmental and socio-cultural conditions among countries, both national and local/traditional practices in primary production, different supply chain dynamics, individual national regulations and levels of oversight, as well as diverse contamination and exposure pathways of contaminants.

For the application of a fit-for-purpose concept to be successful in producing safe FFV, the risk management systems and control measures applied throughout the chain from the farm to the consumer must be complementary, stringent and followed at all times. Water quality criteria for use in FFV supply chains should be established within the framework of national food and water regulations and guidelines and take into consideration local resources, infrastructure and capability.

1

Introduction

1.1 BACKGROUND

At its 48th session, the Codex Committee on Food Hygiene (CCFH), the Committee noted the importance of water quality and safety in food production and processing. CCFH requested the Food and Agriculture Organization (FAO) and World Health Organization (WHO) to provide guidance for those scenarios where the use of "clean water" (i.e. water that does not compromise the safety of the food in the context of its use) was indicated in Codex texts and on where it is appropriate to use "clean water". In particular, guidance was sought for the use of irrigation water and "clean" seawater and on the safe reuse of processing water.

To facilitate this work, FAO and WHO established a group of experts and convened two Expert Meetings (21–23 June 2017, Bilthoven, the Netherlands; 14–18 May 2018, Rome, Italy) (FAO and WHO, 2019).

Reviews were prepared on current guidance and knowledge on water use and safety for 1) fresh fruit and vegetables (FFV) pre- and post-harvest), 2) fishery products (post-harvest) and, 3) water reuse in establishments, and on risk management approaches to ensure the safety of water and food supplies (FAO and WHO, 2019). These reviews provided background information for the experts to consider in the development of a fit-for-purpose concept and a decision support systems approach to safe water use within these sectors.

Cross-cutting challenges were identified by the experts, particularly in the following areas:

- There is a need for guidance on the application of criteria for the microbiological quality of safe water used and reused in food production and processing systems; examples are provided.
  - > There is a lack of guidance for the various types of water used in the food industry along the value chain for verification, operational and surveillance monitoring.
  - > Where they are recommended, there are inconsistencies in the criteria applied by competent authorities among different countries.
  - > Microbial indicators are most commonly enumerated as an alternative to pathogen (bacteria, viruses, parasites) detection in water; however, there is no universal agreement on the most appropriate microbial indicator species or groups for the range of hazards and the scientific rationale for this is controversial.
  - > Current evidences indicate the numbers of E. coli alone are not an appropriate measure of water quality when assessing safe water use and reuse in food safety, as the number of bacteria present is not suitable surrogate for the diversity of bacteria, viruses, and parasites that may be present.
- There is a lack of understanding of the behaviour and the persistence of microbial hazards introduced via water; the interaction of water with the diverse range of food products and within different environments at different steps along the supply chain; the effectiveness of risk reduction measures at these steps to improve water quality; and for concerns of unforeseen contamination in water reuse.
- The availability of qualitative and quantitative data for use in risk assessments for this purpose is very limited and, in some regions, non-existent.
- Education and training tools are required to communicate the value of a risk-based approaches and concepts, such as fit-for-purpose water, for the effective risk management of water use in food production to maintain food safety.

1.2 THE OBJECTIVES OF THE MEETING

The purpose of this meeting was to develop clear and practical guidance on the microbiological criteria and parameters that can be used to determine if water is "fit-for-purpose" for use in the pre- and post-harvest production of FFV. Practical interventions that could be applied pre- and post-harvest to mitigate food safety risk when water does not meet the requirement of fit-for-purpose were also considered.

The main objectives of the meeting were:

- to outline the use of microbial testing to assess the safety of various types of water used for primary production of different types of FFV and,
- to describe the benefits and pitfalls of using these tests for assessing the fitness-for-purpose of the water.

In addressing the objectives, consideration was given of whether the FFVs were to be cooked before consumption or eaten raw, the irrigation methods used (e.g. drip irrigation vs sprinkler and overhead irrigation and the purposes of water use (e.g. pre-/post-harvest, produce washing, cleaning, worker personal hygiene etc.).

This the meeting scope included the following.

- Review of microbial test methods used in risk management, namely:
  - > counts of bacterial indicators (e.g. Escherichia coli, thermotolerant coliforms, spore-formers etc.),
  - > detection of or counts of specific microorganisms that may be waterborne (pathogens and/or non-pathogens and which ones),
  - > bacteriophages or other viruses,
  - > non-culture based microbiological methods (e.g. polymerase chain reaction (PCR), whole-genome sequencing (WGS), microbiome analysis).
- Review of microbial and host source tracking to determine the origin of microbial contamination.
- Review of recommended threshold values for microbial water quality parameters for safety and/or risk-benefit tables that could be applied to water used for different purposes in agricultural food production (in particular FFV) to assess if that water meets or exceeds the quality required to minimize food safety risks in FFV at the point of consumption.
- Ranking of food safety risks of water inputs based on the types of contamination (e.g. from animal waste, wildlife, land run-off, greywater, raw sewage, human activities etc.).
- Consideration of practical interventions to treat water intended for use in FFV production in low- and middle-income countries to achieve an acceptable level of health protection for their consumers (FFV produced for export only are not included), considering a combination of interventions targeting improved water safety as well as safe handling and preparation of FFV (e.g. cooking) and food safety education may be desirable.
- Listing and evaluation of intervention strategies to mitigate food safety risks in FFV where available water pre- or post-harvest exceeds acceptable safety criteria (e.g. harvest withdrawal periods, product re-work, consumer advice/ education on safe food handling/preparation etc.).

2

Codex food safety risk management and WHO water quality management approaches

Guidelines and Codes of Practice are available from the Codex Alimentarius Commission (CAC) for the management of microbial risks in FFV production, including water use (FAO and WHO, 2017), and from the WHO Guidelines for drinking water quality (WHO, 2017a) and wastewater (WHO, 2006a). Aspects of microbial risk management approaches that were relevant to this meeting are summarized in this Chapter.

2.1 CODEX ALIMENTARIUS COMMISSION FOOD SAFETY RISK MANAGEMENT

The CAC provides governments with an overarching risk analysis framework to be used to ensure the protection of public health from foodborne illness built on risk management (RM), risk assessment (RA) and risk communication (FAO and WHO, 2007). Codex provides principles and guidelines for risk managers to use when addressing food safety issues involving known or suspected foodborne hazards (FAO and WHO, 2013). Risk assessment of specific pathogen/product combinations of concern helps ensure an objective and systematic RM process based on relevant scientific information and provides RM options proportionate to the risks posed. Competent authorities may determine a tolerable level of illness in a population (number of illness/year) or a goal linked with a specific foodborne pathogen of concern. A country's health goal is referred to as an appropriate level of sanitary protection or ALOP in global food trade (FAO and WHO, 2008). The ALOP can be operationalized by risk managers by using RA

and relevant dose-response relationships to derive a more practical target, such as the concentration of a microorganism or their toxins in the food at the time of consumption or earlier in the food chain, known as a food safety objective (FSO) or performance objective (PO) respectively.

The Codex General Principles of Food Hygiene provide general principles for ensuring food hygiene and a foundation for further commodity-specific codes of practice and guidelines (FAO and WHO, 2020). These Principles follow a systematic approach that encompasses the food chain from primary production to final consumption. At each stage of the food chain, food hygiene and safety should be ensured using a management system based on pre-requisite programs, PRPs, (e.g. hygiene and good agricultural practices etc.) and application of a Hazard Analysis and Critical Control Point (HACCP)-based system wherever possible (FAO and WHO, 2020).

A Codex Code of Hygienic Practice is available specifically for FFV that addresses practices from primary production to packing, providing guidance on the minimisation of microbial hazards in a manner proportionate to the health risks posed. The Code recognises flexibility is needed to account for the broad range of products and production systems occurring globally (FAO and WHO, 2017).

Water used along the FFV chain can be a potential source of microbial pathogens in products at consumption. Therefore, the risks to public health from water use have to be assessed. Factors to be considered include the microbial quality of the water; the stage in the supply chain and how water is used; whether the water comes into contact with or infiltrates edible FFV parts; the end-use of the crop (e.g. eaten cooked or uncooked); and the efficacy of risk mitigation measures, if applied. RA is a valuable tool that can aid risk managers in identifying and focusing resources on areas associated with potentially high food safety risks. In particular, RA can help identify points for control, i.e., steps or locations in the process where water quality and other process parameters should be controlled to significantly minimise or prevent microbiological hazards in FFV in alignment with a required FSO.

Codex refers in the Code of Hygienic Practice for Fresh Fruits and Vegetables (FAO and WHO, 2017) to the use of water of microbiological and chemical quality suitable for its "intended use" during the pre-harvest and post-harvest production stages. Codex stipulates the use of "drinking" or "potable" quality in the final post-harvest stages where the FFV product will not be subject to further pathogen reduction measures before consumption. Potable water is defined by Codex as "water which meets the quality standards of drinking water such as described in

the WHO Guidelines for Drinking-water Quality". Water of this quality may be sourced from and managed by municipal suppliers or otherwise treated; in these cases, the WHO definition of drinking water is directly applied (WHO, 2017a). In the following sections the term "potable water" is used with this interpretation.

2.2 WHO WATER QUALITY MANAGEMENT

The RM of microbial hazards in drinking water has been developed similarly under the auspices of the WHO and many RM principles for drinking water are based on those used in food safety RM. WHO recommends a comprehensive RA and RM approach encompassing all steps in a water supply system from water catchment to water consumption as the most effective means of consistently ensuring the safety of a drinking-water supply (WHO, 2017a). These approaches are referred to as water safety plans (WSPs) (WHO, 2009). Water Safety Plans have been conceived from other RM approaches, in particular the multiple-barrier approach and HACCP systems commonly used in the food industry. Water Safety Plans are guided by health-based targets and overseen through surveillance of the supply system. Health-based targets for drinking water supplies are based on a judgment of safety and RAs of waterborne hazards. Four types of measurable targets are used: health outcome (e.g. tolerable burden of disease expressed as disability-adjusted life years or DALYs), water quality (e.g. guideline concentrations for chemical hazards), performance (e.g. log reduction of pathogen numbers achieved by an intervention) and specified technology (application of defined treatment processes) targets. A range of guidance documents has been developed to aid policymakers, engineers and water providers to implement WSPs in different settings (WHO, 2011b, 2017a, 2017b, 2017c).

2.3 APPLYING WHO WATER QUALITY MANAGEMENT WITH CODEX FOOD SAFETY RISK MANAGEMENT

In food production, one cannot resort to the exclusive use of drinking-water or potable water along the food chain because of increasing global water scarcity, limited local availability of drinking quality water and costs. Current and future water scarcity, compounded by population growth, climate change and the unsustainable use and exploitation of aquifers will present major challenges to global food production. Moreover, excessive and repeated use of large amounts of water is usually needed in food production. Therefore, groundwater, surface water, and non-conventional sources of water such as reclaimed water, greywater, brackish water, and wastewater are increasingly used to cope with the rising challenges of

water shortages. However, stringent management of these water sources remains imperative to reduce food safety risks (WHO, 2016a, 2006a). It was recommended at a prior Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA)) meetings on this subject that water used in food production chains should be "fit-for-purpose" in order to produce safe food (FAO and WHO, 2019). It was proposed risk managers could benefit from applying this concept in food safety RM systems together with some of the WHO RM approaches for drinking water and other water quality types. For example, for the safe use of wastewater, excreta and greywater in agriculture (WHO, 2006a), risk-based approaches have been adopted applying sanitation safety plans (SSPs) (WHO, 2016a). WSPs should also be applied to produce and deliver safe drinking water from reuse of wastewater (WHO, 2017b).

Water and food safety RM strategies have evolved independently. While there are similar goals and principles involved, there are differences and in many countries RM of water and food supplies is often overseen by separate authorities. The terminology used can differ even when it has a similar meaning. For example, Codex defines food safety as the "assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use" (FAO and WHO, 2020). Food quality is a much broader concept that is related to consumers' needs or expectations, it can be "both objective and subjective", including elements such as nutritional quality, environmental preservation, geographical origin, local traditions, ethical and social quality, animal welfare, etc." (FAO, 2020a). Similarly, safe drinking-water, as defined by the WHO Guidelines, is "water that does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages" (WHO, 2017a). However, drinking- water quality is referred to in relation to guideline target values that are used to protect or improve drinking- water quality and therefore human health.

To assist readers of this report and provide clarity, a table of common terms used to ensure the safety of food and water has been provided in Annex 1.

3

Fit-for-purpose water and fruit and vegetable production

Water is used extensively through the FFV production chain. Water is used at different points for varying purposes and the water quality has to be fit for each specific purpose. The use of water and considerations in determining fit-for-purpose of water when used were addressed.

3.1 WATER QUALITY AND THE INTENDED USE

From the microbiological point of view, potable water is defined as water that meets required microbial quality standards established to ensure it is safe for drinking. i.e. it "does not represent any significant risk to health over a lifetime of consumption" (WHO, 2017a; See Annex 1). Potable water can theoretically be used for any purpose in each stage of the FFV chain without food safety restrictions. The alternative, non-potable water, is recognised as potentially contaminated and has restricted use in food production. Water contamination can originate from various sources, such as soil, industry waste and faeces (farm animals and wildlife, humans), with faeces being the most relevant source of foodborne pathogens. The likelihood of faecal contamination of different types of water differs and generally the risk of microbial contamination of different water sources increases according to the following ranking from low to high risk: 1) protected rainwater, 2) groundwater collected from deep wells, 3) groundwater collected from shallow wells, 4) surface waters and 5) raw or inadequately treated wastewater (WHO, 2012; see Chapter 3). As discussed in Chapter 2 the exclusive use of potable water is not feasible and practical throughout the FFV production chain and in some stages (e.g. growing

and irrigating) and for certain non-food contact purposes (e.g. fire control and steam production), non-potable water can be used if consumer health risks are assessed and adequately managed.

The water quality required for a specific purpose at a particular point in FFV production has to be determined based on an assessment of the health risk for the specific pathogen/product pathway (Chapter 4) and be fit for the specific purpose at the point of use and within the local context. In order to obtain FFV as safe as possible, the microbiological quality of water should increase along the production chain from farm-to-consumer.

3.2 PRIMARY PRODUCTION

In the Codex Code of Practice for FFV, primary production covers the growing and harvesting of FFV and the steps involved can include "soil preparation, planting, irrigation, the application of fertilizers and agricultural chemicals, field-packing and transport to a packing establishment" (FAO and WHO, 2017). Water is used for various purposes during these activities and at each water input point the water should be of known quality, fit-for-purpose and its use managed based on HACCP principles. The purpose and method of water use, the production stage and the types of water available, its storage and distribution hygiene (Chapter 5), can impact on the risk outcome at consumption (WHO, 2006a). FFV type also affects the level of water quantity and quality required. From a microbiological point of view, there are no specifications on the water quality required for FFV usually eaten cooked (and handled safely) where cooking can provide a pathogen kill step before consumption. For FFV (potentially) eaten raw or minimally processed, the microbiological quality of water is highly important. Relevant pre-harvest factors affecting the microbiological safety of FFV are the irrigation method, water/plant contact time and the time intervals between last irrigation and harvest. Irrigation methods that reduce, or even avoid, the contact of the irrigation water with the edible parts of FFV (e.g. localized or subsurface irrigation) can reduce the potential for exposure of FFV to pathogens (CPS, 2014; Solomon *et al.,* 2002). Indirect (subsoil) irrigation methods can allow for the use of lower water quality than a direct method of irrigation (e.g. microspray, overhead or drip irrigation). Also, introducing a validated pathogen die-off period between the last irrigation and harvesting allows for lower irrigation water quality to be used (EC, 2017; CPS, 2014).

Some main uses of water in the FFV chain where there are potential risks of transmission of pathogens to FFV are described based on the publication of Suslow (2010) and the FAO and WHO (2017) unless otherwise stated.

Pesticide, fertilizer and other applications
Water is used as a conduit/carrier for fertilizers, pesticides and other chemicals applied using foliar sprays to aerial portions of plants during cultivation.

Dust abatement
Water can be used to control dust from unpaved farm access roads and harvest buffers to minimise dust dispersal to unharvested or harvested FFV, to equipment and the post-harvest handling areas.

Frost protection
Frost protection is used to protect sensitive plants from frost or freeze injury by insulating the plant and fruit with ice formed by continual application of water or water containing protectants prior to the frost. The solutions can be applied using over- or under-plant sprinklers, micro-sprinklers, surface irrigation and artificial fog.

Irrigation
Different irrigation methods are used in FFV production and they are associated with varying degrees of contact time between the irrigation water and the edible portion of the plant and therefore the level of risk of pathogen contamination via water (Brouwer *et al*., 1985). Examples include:

- flooding (border irrigation) where almost all the land surface is wetted;
- furrows where only part of the ground surface is wetted;
- overhead and sprinkler irrigation where the soil and crops are wetted as for rainfall;
- surface irrigation where the soil surface is only slightly wetted (e.g. drip)
- subsurface irrigation where the subsoil is saturated using tubes buried in the ground
- localized irrigation where water is applied to the root zone of each individual plant at an adjustable rate.

Microorganisms can be internalised in FFV when exposed to contaminated water under certain conditions and the microorganisms may survive and be further translocated within the plant (USA FDA, 2017a). Internalisation can occur through the stomata, entry to buds and flowers, entry to fruit through the stem, stem scar, or calyx, or via damage to natural FFV structures e.g. cuts, splits, wounds, soil spots. Few studies suggested the possibility of internalization via the plant roots. The extent to which internalisation occurs varies depending on the inoculum pressure (Standing *et al*., 2013) and much information is obtained from experimental studies using particularly hydroponic or greenhouse settings. This phenomenon appears to be a more important issue in post-harvest handling where

infiltrations can occur when some FFV types e.g. melons are immersed in water and external pressures such as negative temperature differentials between water and FFV and vacuum systems can drive the process (Macarisin *et al*., 2017).

Hydroponic and aeroponic systems
Hydroponic and aeroponic production systems are where plants are grown in growth media other than natural soil or are suspended, respectively. All the nutrients are dissolved in the irrigation water or misting solution and are supplied on a regular basis to plants/roots.

3.3 POST-HARVEST HANDLING

In the Codex Code of Practice for FFV, post-harvest activities are described as those activities performed incidental to packing and can involve minimal transformation of FFV, such as washing (may include rinsing), sorting, culling, grading, cutting and trimming (FAO and WHO, 2017). In post-harvest handling of FFV, the product may be in contact with water or kept dry.

In general, water that comes in direct contact with edible portions of FFV or FFV contact surfaces during final post-harvest handling should have potable water quality (FAO and WHO, 2017). However, the quality of water used depends on the stage of the operation: for example, during initial washing stages clean water could be used in contrast to final rinses where water should be of potable quality (FAO and WHO, 2017). Examples of the purpose of water use during the various activities in post-harvest handling of FFV are provided.

Receipt at a packhouse
After arrival, field harvested fruit can be rinsed on the transport vehicle with water that often contains disinfectants and that can be recirculated to reduce water usage. Large-scale dump tanks can be used to receive offloaded fruit, reducing fruit damage and transporting it into the packhouse via flumes systems. Attention should be paid that water use in these tanks do contribute to a cross-contamination of product.

Cooling
Cooling FFV post-harvest increases their quality and extends their shelf life. Water-based cooling methods used include - showering FFV with chilled water, moving immersed fruit through a cold-water bath or the use of ice to refrigerate FFV. These methods can help clean product; however, the process has the potential to transfer pathogen contamination from product to water/ice and water/ice

to product, unless water/ice quality is effectively controlled by disinfection and regular monitoring. Cooling water can be recirculated provided water quality is similarly maintained. Microbial quality of ice should be also considered to avoid potential contamination.

Conveyance using flumes
Flumes are troughs with running water that carry product between process steps by suspension, minimising product damage and, at the same time, washing and cooling the product. The water may be recirculated and there can be build-up of the microbial in these systems; therefore, the water quality has to be managed as for cooling above.

Washing
Washing removes soil, organic debris, chemical residues and exudates accumulated on FFV that can be visually unacceptable and/or may contain human pathogens. Regardless of the wash method used, the water should not introduce pathogen contamination and should not facilitate cross-contamination during washing. The microbial quality of water in contact with products to be eaten raw has to be maintained using food grade disinfectants at validated concentrations that prevent cross-contamination and the concentrations have to be monitored. Establishment of minimal residual concentrations in the water is needed to avoid cross-contamination during washing. Other parameters may need to be controlled and monitored also to ensure the efficacy of the specific disinfectant used, e.g. temperature, pH (chlorine-based disinfectants), turbidity etc.

Processing (wax, as an added ingredient)
Food-grade waxes, fungicides, calcium treatment and edible coatings may be applied to some fruits through a water immersion bath or spray nozzles. Waxes are applied to reduce water loss and improve appearance as natural waxes may be removed during washing and cleaning operations.

Refreshing
FFV may be sprayed with water to maintain the moisture content of the product, prevent wilting and extend shelf life. Retaining shelf life can also be achieved with certain crops (i.e. broccoli) using ice packing.

Water use for other purposes
Personal hygiene (handwashing). Water of potable quality should be available for workers to practice good personal hygiene.

Equipment and facility sanitation. Water of potable quality is used in the maintenance and sanitisation of equipment and facility surfaces where FFV will be in direct contact. Water used for non-contact surfaces may be of a lower quality than potable water.

3.4 CONCLUSIONS

- Water is used throughout the FFV production chain at different points and for different purposes. Each point of water use can present specific microbial risks associated with the multiple risk factors that can be present.
- Potable water has the safest quality; however, it is not essential that potable water be used throughout the FFV chain. Water may be available from many different sources and these can vary in microbial quality and present varying types and levels of microbial risks.
- Wherever water is used and is in contact with FFV, the quality of the water has to be chosen based on risk so that it is fit-for-purpose and it should not introduce microbial risks or increase the level of risk during its use.
- During the primary production of FFV, either in soil or alternative cultivation systems, water is used extensively for irrigation, for other horticulture activities and during harvest activities. Risk assessment is required to determine whether water is fit-for-purpose for a specific activity. Some risk-related factors to be considered by risk managers at this stage include the microbial quality of the available water source (wastewaters, surface water, wells, collected rainwater etc.), the method of use (e.g. different irrigation methods result in different levels of pathogen exposure), hygiene in distribution (equipment, pipes) and storage (ponds, tanks), contact with edible portions of the plants and duration of contact.
- In contrast, post-harvest, water in contact with edible portions of FFV or food contact surfaces should be of potable quality and the quality should be monitored and maintained during processing so as not to increase the level of microbial contamination of the final product. Non-potable water may be used for non-food related use; however, this system has to be effectively separated from the potable supply and its use.

4

Overview of relevant risk assessment approaches

In this section, different RA approaches that may be applied during the RM of food safety in FFV production chains are discussed focusing on health risks associated with water use and determining whether water is fit-for-purpose at the point of use. These approaches have been based on Codex Guidelines (FAO and WHO, 2013, 2014) in combination with WHO water safety management guidelines (WHO, 2017a).

Water is used in many and varied steps in FFV production beginning on farm during the growth stage and there can be multiple Control points (CPs) involving water along the FFV chain up to consumption (FAO and WHO, 2017). It is important to remember that CPs can include not only the first occurrence of water use, but also the maintenance of fit-for-purpose water quality throughout the FFV production and supply chain, for instance in water reuse applications or when the same water is used for a certain time at the same production stage.

Microbial water quality can vary widely and rapidly and can be unpredictable. WHO (2017a) emphasised the following cautions:

> When establishing health-based targets for water, care should be taken to account for short term events and fluctuations in water quality along with "steady-state" conditions. This is particularly important when developing performance and specified technology targets. Short-term water quality can significantly deteriorate, for example, following heavy rain and during maintenance. Catastrophic events can result in periods of very degraded source

water quality and greatly decreased efficiency in many processes, or even system failure, greatly increasing the likelihood of a disease outbreak. Events like these provide additional justification for the long-established "multiple-barrier principle" in water safety.

At the request of Codex, FAO and WHO have produced a series of documents, known as the *Microbiological Risk Assessment Series*. These support the conduct of RAs and the application of RA in the RM of various food commodities for which there was evidence of a significant association with foodborne illness and the need to protect public health, and to facilitate trade. These are available from the websites of both FAO (FAO, 2020b) and WHO (WHO, 2020). MRA Series Number 36 (FAO and WHO, 2021) provides guidelines for RA of microbiological hazards in foods, including qualitative, semi-quantitative and quantitative RAs.

WHO (2016b) provides an extensive review of quantitative microbial RA (QMRA) methods relevant to water safety. This guidance illustrates a harmonised four-step framework for the application of QMRA to estimate risks associated with faecal pathogens for drinking water, wastewater and recreational water pathways (WHO, 2016b), and the use of RAs in WSPs for protecting groundwater (WHO, 2006b) and surface water (WHO, 2016a) for health.

We refer the reader to these publications of FAO and WHO for details on RA methodology and summarize the main approaches in this chapter relevant to safe water use in FFV production.

4.1 RISK ASSESSMENT APPROACHES AND FIT-FOR-PURPOSE WATER

RA methods

A broad range of RA approaches and tools are available that provide a continuum from simple and qualitative to fully quantitative. They extend in scope and scale from product-pathogen specific to multi-hazard, and from location-specific to watershed and food network scale. A comparison of the RA approaches based on WHO guidance is provided in Annex 2. The main RA approaches for water safety described by (WHO, 2016b) include:

- Qualitative sanitary inspection: on-site visual evaluation of observable features and conditions at or in the vicinity of the water supply that may present a hazard to water quality.
- Risk matrix: qualitative or semi-quantitative evaluation of the likelihood that a hazardous event will occur and the severity or consequences of the hazard, combined into a categorical risk score or rank.

- QMRA: mechanistic mathematical model of a water system or an empirical approach, combining quantitative scientific knowledge about occurrence and nature of pathogens, their potential fate and transport, routes of exposure to humans and health effects that may result from exposure, as well as the effect of natural and engineered barriers and hygiene measures.

Qualitative risk assessment models sit between risk matrices and QMRAs in terms of the amount of input information and the qualitative/quantitative nature of the output risk estimates. They usually help with identifying systems components and risk-relevant steps, but only assign a qualitative increasing/decreasing/stationary estimate of risk contributed by each step. In addition to mechanistic risk approaches, correlative epidemiological studies, such as cohort and case-control studies, can help in identifying risk factors to consider, or the effectiveness of a large-scale intervention.

Selecting an RA approach

Risk managers should consider a number of factors when selecting a RA approach for determining when water is fit-for-purpose in food production (WHO, 2016b).

- The approach should provide the information that risk managers need as the basis for informed, evidence-based RM decisions or to design RM policies.
- The approach should be feasible to implement in the context of available resources (personnel, skills, analytical and laboratory facilities, access to support institutions).
- Whether the type of data or information can reasonably be expected to be available (e.g. knowledge of the water supply system, types of hazards and hazardous events, exposure routes, water quality data on indicator organisms or pathogens) is sufficient to conduct a reliable RA.

The choice of RA application in water assessments is based on the guiding principle of continuous improvement. It is possible to develop a meaningful and useful preliminary RA in a situation with little resources and not delay progress by waiting for this to improve e.g. more extensive expertise, data collection and analysis. There are simple assessment tools that require only qualitative inputs. As an example, a progressive inquiry process to assess whether the water from a river is fit-for-purpose for FFV washing may start with a visual assessment of the candidate water and a review of potential contamination sources that could impact its quality. Alternative water sources that could feasibly be used should be identified and compared. This process could then lead to follow-up questions that can be answered by additional information, for example:

- Risk factors: there is extensive cattle pasture in the upstream watershed: what is the potential impact on water quality at the point of use?
  - > Water quality measurements: is the river water of better or worse quality than alternate shallow well water, based on sampling results?
  - > Seasonal effects: is river water of different microbiological quality in spring than in summer?
- Potential interventions: can river water be used for FFV washing without any additional precaution, or what type of intervention is needed to improve its quality and reduce risk?

During the JEMRA meeting in Rome on water quality in food production in 2018 (FAO and WHO, 2019), experts considered how a risk-based approach could be practically implemented at even the simplest farm and processing levels. The experts discussed the use of decision support processes and prepared examples. These were intended for use by local regulators, risk managers or agricultural extension agents who should be familiar with local FFV chains and practices and be able to interpret regulations and guidelines. They could then translate, instruct and support local FFV growers and processors in risk- and evidence-based decision making in their individual establishments. The decision support tools were visualised as decision trees and risk mitigation selection tables and can be accessed in the Microbiological Risk Assessment Series No. 33 (FAO and WHO, 2019).

As resources become available, further data could iteratively move the RA along the qualitative-quantitative continuum and towards integration of observational and measured variables. Over time, a more detailed and comprehensive RA could lead to more accurate identification and prioritization of potential risk reduction measures, to the implementation of evidence-based practices or policies, and, hence, to a decrease in hazard exposure. It should be noted that quantitative approaches are not necessarily more effective in leading to risk reduction. Furthermore, qualitative and quantitative variables/observations are not necessarily mutually exclusive and can complement each other (e.g. sanitary inspections and water sample microbial indicator enumeration). Thus, resource prioritization should consider both RA and RM goals, recognizing that RAs are context-specific. In some situations, a focus on implementing risk reduction measures and verifying their proper functioning may advance public health objectives more than extensive data collection or an advanced QMRA. However, monitoring water quality and RA in conjunction with epidemiological surveillance of waterborne illnesses would contribute to the assessment progress towards health-based targets and may direct further action.

4.2 QUALITATIVE RISK ASSESSMENTS: SANITARY INSPECTIONS

Qualitative RAs are based on descriptive or observational information. They aim to evaluate the likelihood and/or severity of events that may compromise the safety of water. They have been widely used in many countries to support the identification and management of high-priority risk factors in small water supply systems; to enhance knowledge of the water supply system (technical, operations, local conditions and practices) and; identify potential sources and pathways of contamination, thereby pointing to required improvements and additional controls. In WHO guidelines, WSPs for public drinking water systems and sanitation systems include sanitary inspections for a range of water sources, e.g. rivers, lakes and groundwater (WHO, 2005). Sanitary inspections are typically based on standardized forms/checklists to identify the most common issues that may lead to the introduction of hazards into a system. This approach has been developed and promoted as a simple and effective tool for small water supplies and, as part of WSPs for small supplies (WHO, 2016b). When a RM plan with PRPs and HACCP-based programs are employed in FFV production, there are steps, e.g. conducting the hazard analysis and determining the CPs, that have similarities with WSP activity (FAO and WHO, 2020, Annex 1). Water use in FFV processing and for equipment and facilities cleaning would be included in prerequisite programs (PRP) and HACCP product flow diagrams.

A sanitary inspection can be repeated to identify changes in hazards and their risk factors and/or risk levels that occur over time and results can be used to evaluate the impact of improvement policies. Results from sanitary inspections are useful at an individual supply level as well as when applied as part of a larger-scale surveillance program to inform regional and national priorities. It is also possible to combine sanitary inspection scores with microbial monitoring results, such as the presence or enumeration of faecal indicator bacteria (FIB) and/or bacteriophages, and in this way gradually include a larger set of relevant variables and more quantitative information. It should be noted that sanitary inspections and other qualitative RA methods are user-friendly tools based on a relatively simple but comprehensive (qualitative or semi-quantitative) understanding of a food system and of water contamination processes. They may be simple to administer, but not necessarily simple to develop as they require a sound understanding of food and water contamination processes.

An example of a qualitative RA can be found in the 'Five keys to growing safer fruits and vegetables' (WHO, 2012) where sources of water for irrigation are ranked from low to high risk of microbial contamination: "1) rainwater, 2) groundwater

collected from deep wells, 3) groundwater collected from shallow wells, 4) surface waters and 5) raw or inadequately treated wastewater" (WHO, 2012). Simply identifying the water source provides an evaluation of potential risks associated with the intended use.

WHO and United Nations Children's Fund, UNICEF (2012, 2017) have indirectly used qualitative RA in assessing the quality of water sources for drinking purposes. Observable features were used to classify water sources as improved or unimproved as follows:

- Improved sources have the potential to deliver safe water by nature of their design and construction e.g. piped supplies (i.e. households with tap water in their dwelling, yard, or plot; or public standposts or tap stands distributing water from one or more taps) and some non-piped supplies (i.e. boreholes, protected wells and springs, rainwater and packaged or delivered water).
- Unimproved supplies include unprotected dug wells and springs, and surface waters.

Thorough knowledge of water systems and their variability across different countries and knowledge on local systems is required when classifying a water source as "improved" and it should not be assumed that it is of the same quality as potable drinking water. The improved/unimproved classification can be complimented using water quality measurements and sanitary inspections as described in the 'Rapid assessment of drinking-water quality, handbook (UNICEF and WHO, 2018; WHO and UNICEF, 2012).

4.3 SEMI-QUANTITATIVE RISK ASSESSMENTS

Semi-quantitative RAs involve more systematically assessing the likelihood and severity of adverse impacts of health hazards in a water source and require more information and expertise compared to a qualitative RA. Semi-quantitative RAs are included in several WHO water-related guidelines, either alone or as part of a more comprehensive approach such as WSPs (WHO, 2009) and SSPs (WHO, 2016c). These WHO Guidelines state the following recommendations:

- They are appropriate for organisations in well-defined regulatory environments, for teams already familiar with PRPs and HACCP programs, or similar frameworks.
- WSPs and SSPs require a planning team to use their knowledge/judgement to assign a likelihood and severity rating to each identified hazardous event that may occur in a water system. Ratings for likelihood and severity are then combined into an overall risk rating or score for each event (e.g. low, medium,

high, or uncertain/unknown, or a numerical score). Multiple hazardous events can then be compared and ranked or prioritised based on their risk rating.

- The team needs to agree on clear definitions of likelihood (e.g. what is meant by unlikely, possible and likely) and severity (e.g. minor or major) and apply them consistently based on the water system and local context, historical data and relevant guidelines, and broader considerations of potential health impacts, regulatory impacts and impacts on community or customer perceptions.
- When assessing severity, the type and concentration of a hazard as well as the magnitude of associated health outcomes are considered. However, the principle of safeguarding public health should never be compromised in any definitions.

4.4 QUANTITATIVE MICROBIAL RISK ASSESSMENT

Quantitative microbial risk assessment (QMRA) is a quantitative mechanistic modelling approach or an empirical approach to estimate exposure and risk of adverse health impacts from an identified microbial hazard and exposure route (WHO, 2016b). While it can be deterministic, QMRAs are most useful when they probabilistically account for variability and uncertainty in variables and parameters, yielding a distribution of risk outcomes. The WHO Guidelines for drinking water quality provide resource material for identification and quantification of health risks related to waterborne pathogens and for establishing health-based targets for water treatment technologies (WHO, 2017a). In addition, QMRA is the RA approach of choice in the WHO Guidelines for the safe use of wastewater, excreta and greywater in agriculture and aquaculture (WHO, 2006a).

It is important to note that when there is insufficient data or as data is accumulated (e.g. levels of pathogens in untreated water) for a QMRA, it may be necessary to rely on assumptions. Iterative updates of a model and estimates provided are warranted as more data become available. WHO provides detailed guidance for the application of water related QMRA for water safety management (WHO, 2016b). Exposure assessments, i.e. estimates of the number of pathogens ingested in an exposure event or in a defined time period, combined with knowledge of the infection process, may also be used as a basis to select RM measures in the absence of appropriate dose-response relationships. However, guidelines have not been developed to guide this process.

Examples of the implementation of QMRA in WSPs can be found in: *Guidelines for drinking-water quality* (WHO, 2017a); *Guidelines on the use of wastewater in agriculture* (WHO, 2006a); *Quantitative microbial risk assessment: application to*

water safety management (WHO, 2016b)*; Evaluating household water treatment options: health-based targets and microbiological performance specifications* (WHO, 2011a); *Potable reuse: Guidance for producing safe drinking-water* (WHO, 2017b). A similar approach is also implemented in *Sanitation Safety Planning* (WHO, 2016c).

4.5 CONCLUSIONS

- There is a wide range of tools for conducting RAs, from simple to complex, qualitative to quantitative; guidance and applications in food and water supply RM programs are provided by Codex, FAO and WHO.
- RAs are most effective when undertaken as part of a comprehensive RA and RM system that for FFV production would encompass the whole FFV chain and any input water supply systems assessed from their source/supplier to point-of-use e.g. through application of PRPs and HACCP-based programs, and WSPs.
- The level of expertise, infrastructure and data available for RAs will vary widely among regions where FFV are produced. An approach should be chosen that provides acceptable results and is at the same time feasible and practical in the local conditions at the time.
- Transition to more complex RA approaches that may be more informative and have fewer assumptions and uncertainty can be undertaken as these limiting factors improve.
- Using a simple RA tool does not mean that the RM measures adopted will be less effective and it is not necessary to wait until perfect data is available. Combining qualitative and quantitative data may provide the most effective assessment and should be considered on a site-specific basis.

5

Examples of water used during fresh fruit and vegetable production

As discussed previously, it is not essential to exclusively use water of potable water quality along the FFV chain (section 2.3). However, the quality of water used for a specific purpose at a specific stage in the chain should be fit for that purpose (Chapter 3). Water used during primary production and post-harvest stages can be available from various sources and be of differing quality, for example wastewater, greywater, surface water, groundwater and rainwater, all with or without treatment, depending upon its use. Potable water may be available from municipal supplies. Water used in post-harvest activities may be re-used or the same water used for a certain time at the same production stage (e.g. for irrigation and in post-harvest processing and for non-product contact activity). Water sources and supplies can be potentially contaminated with microbial, chemical and radiological contaminants (WHO, 2017a). The focus of this report is human pathogenic microorganisms present in water that may contaminate foods.

The likelihood of pathogen contamination of water from different sources varies and decreases from raw or inadequately treated wastewater, down through surface water, shallow groundwater, deep groundwater and roof run-off water to finally rainwater collected safely and potable water (WHO, 2017a). An example of the range of Norovirus concentrations detected in different water sources from a review by Karst (2010) are shown in Table 1.

TABLE 1 Norovirus concentrations in different water sources. Source of data (Karst, 2010).

Source	Range (GC[a]/litre)	Reference
Raw wastewater	$>2 \times 10^2$ to 10^9	Katayama and Jinjé, 2017
Wastewater effluent	$>10^1$ to $> 10^6$	Katayama and Jinjé, 2017
Surface water	2.8×10^{-1} to 3.3×10^4	Katayama and Jinjé, 2017
Groundwater	N.D.[b] to 4.3×10^2	G.S. Fout, unpublished data

a GC – genomic copies
b N.D. – not detected

In this chapter, a brief summary is provided on broad categories of types of water illustrating the potential microbial hazards, treatment systems, the ability of selected treatments to inactivate or destroy pathogens, the log reduction or inactivation values for pathogens using selected treatments and their advantages and disadvantages.

5.1 WASTEWATER

Wastewater is defined by WHO (2016b) as "liquid waste that is discharged from homes, commercial premises, and similar sources to individual disposal systems or to municipal sewer pipes, and which contains mainly human excreta and used water. Wastewater produced mainly by household and commercial activities is called domestic or municipal wastewater or domestic sewage. In this context, domestic sewage does not contain industrial effluents at levels that could pose threats to the functioning of the sewerage system, treatment plant, public health or the environment".

- Industrial wastewater is liquid waste generated by manufacturing and industrial processes.
- Raw wastewater is any untreated wastewater that contains human-derived waste products, including faeces, urine and industrial wastes.
- Treated wastewater or reclaimed water is that which has been treated to reduce the concentrations of organic matter and human pathogens. Some treatment processes also reduce nitrogen and phosphorus-containing chemicals.

Wastewater is mainly composed of the following broad groupings of constituents depending on the source:

- organic matter, inorganic matter (dissolved minerals),
- chemicals including toxic chemicals like pesticides and medicines or heavy metals from industrial processes,

- microorganisms, such bacteria, viruses, protozoa, helminths, including pathogens.

It is estimated 48 % of global wastewater produced is released to the environment untreated (Jones *et al.*, 2021). Wastewater is a year-round source of water and nutrients that has the potential for use for plant growth (WHO, 2006a). It is also a potential source, if untreated, poorly or partially treated, of health hazards including pathogens that can survive in wastewater, on crops and in soil for sufficient time for transmission to humans. Therefore, risk reduction measures or barriers are required to reduce pathogen levels to achieve the health-based targets. Measures include wastewater treatments, alone, or more commonly, in combination with other risk reduction measures.

5.1.1 Risk reduction measures and wastewater use

For production of safe FFV using wastewater during the FFV growing stage a combination of risk reduction measures providing multiple barriers is required (WHO, 2006b, Vol. 2). These can include crop restriction, wastewater application techniques to minimise exposure of edible FFV parts, a withholding period allowing pathogen die-off between application and FFV harvest, safe food preparation and wastewater treatment.

Conventional treatment processes

Conventional wastewater treatment includes chemical, physical and biological processes and operations to remove organic matter, solids and nutrients. The following are processes of different degrees of treatment, in order of enhancing treatment level (WHO, 2006a; FAO, 1992).

Preliminary treatment

Preliminary treatment is applied to eliminate untreatable solids and other large materials often found in untreated raw wastewater (Mara, 2003; Tchobanoglous *et al.*, 2003). Removal of these solids/materials is necessary to improve the operation and maintenance of subsequent treatment units.

Primary treatment

Primary treatment is used to remove settleable organic/inorganic solids by sedimentation and floating material by skimming to reduce biochemical oxygen demand, lower levels of total suspended solids, oils and grease (WHO, 2006a). The outlet from primary sedimentation units is called as primary effluent (FAO, 1992).

Secondary treatment

The main objective of secondary treatment is to remove the residual organics and additional suspended solids remaining from primary treatment (WHO, 2006a). FAO (1992) describes secondary treatment as an "aerobic biological treatment performed in the presence of oxygen by aerobic microorganisms (principally, bacteria) that metabolize the organic matter in the wastewater, thereby producing more microorganisms and inorganic end-products". Many or the microorganisms used in secondary treatment and residual solids are separated from the treated wastewater by sedimentation to release clarified secondary effluent. The biological solids removed during secondary sedimentation and from primary treatment may be used as plant fertilizer (USA EPA, 2003; FAO, 1992). The sludge contains pathogens requiring additional treatment processes or time restrictions between application and FFV harvesting (USA EPA, 2003). Some secondary treatment systems, like stabilization ponds and constructed wetlands, are quite effective in removing pathogens (WHO, 2006a).

Anaerobic digestion is an alternative biological treatment conducted in the absence of oxygen for the breakdown of organic matter and the production of energy with the conversion of organic waste to methane and carbon dioxide gases (Chernicharo, 2007).

Tertiary treatment

Tertiary treatment is a series of additional steps to remove/reduce constituents, including pathogens and nutrients, that cannot be removed by secondary treatment. Tertiary treatment can involve some type of chemical treatment (e.g. ozone, hypochloric acid, hydrogen peroxide), physicochemical treatment (e.g. filtration, coagulation, reverse osmosis, activated carbon adsorption of organics) and other disinfection. Tertiary treatment of wastewater provides additional protection after release of tertiary effluent into lakes or rivers and is especially important when the wastewater is to be reused for irrigation of food crops or for drinking water (Gerba and Pepper, 2019).

Pathogen reduction

The excreted pathogens in untreated wastewater vary globally in association with local disease epidemiology and often may not be measured in some regions (WHO, 2006a, Vol. 1). The reductions in pathogen levels achievable using wastewater

treatments depends on and differs with the pathogen group e.g. bacteria, viruses, protozoan (oo)cysts and helminth eggs. WHO (2006a) estimates 1–6 log reduction of pathogens may be achieved by wastewater treatment overall and provides some ranges of reduction of pathogen groups for the different wastewater treatment processes.

5.2 GREYWATER

Greywater is defined by WHO (2006a) as "wastewater from the kitchen, bath and/or laundry, which generally does not contain significant concentrations of excreta". The main microbial hazards in greywater originate from cross-contamination with faecal material (WHO, 2006a, Vol. 4). Greywater requires handling or plumbing for its collection that keeps it completely separate from wastewater and excreta collection from toilets. Greywater can be collected from one residence or all residences in a community may be connected in one collection system. While greywater contains very little faeces or urine, the levels of these contaminants will increase with the increasing numbers of residences connected. Ottoson and Stenström (2003) reported that a greywater system in Sweden contained 0.04 g of faecal material per person per day. Baker and O'Toole (2019) reported that consuming lettuce irrigated with greywater from a single household posed no risk of norovirus infection. Shi, Wang and Jiang (2018) also reported that the risk of greywater reuse on site is much less than the WHO benchmark of 10^{-6} DALY per person per year.

5.2.1 Risk reduction measures and greywater use

Greywater usually has a faecal load 100–1,000 times less that wastewater and lower pathogen levels than wastewater (WHO, 2006a, Vol. 4). In greywater, a regrowth of *E. coli* sometimes occurs, therefore caution is required in use of this indicator in risk estimation and verification monitoring (WHO, 2006a, Vol 4). A combination of risk reduction measures is required to achieve health goals in FFV production where greywater is used. These can include greywater treatment, crop restriction and application and use of greywater to minimise contamination of edible FFV parts, withholding periods to allow die off between use and FFV harvest, appropriate food preparation and hygiene education (WHO, 2006c).

Treatment options include simple measures e.g. soil infiltration, gravel filters, constructed wetlands and ponds or more complex sand filtration, coagulation/flocculation, biological treatment as used with standard wastewater among others (WHO, 2006a, Vol.4).

Pathogen reduction

WHO (2006a, Vol. 4) estimates pathogen reduction levels achievable in treatment of greywater to be about 3–5 log units based on measured faecal cross-contamination in greywater systems.

5.3 SURFACE WATER

Surface water is defined by WHO (2006a) as "all water naturally open to the atmosphere (e.g. rivers, streams, lakes and reservoirs)". Surface water bodies are fed though precipitation, runoff and groundwater sources. Microbial hazards can be introduced indirectly via overland flow and groundwater and, directly via discharge and runoff from sources such as agriculture, aquaculture, settlements, untreated wastewater and stormwater, commerce and industry effluents and recreational/cultural activity (WHO, 2016a).

The microbial hazards that contaminate these surface water sources can include a range of excreted human and animal pathogens some of which are zoonotic foodborne pathogens that could be transferred to FFV during production. The pathogens of concern in a particular location will vary with local human and veterinary disease epidemiology, cultural practices and the nature of local industries and disease control measures. The contamination of surface water can be complex with multiple risk factors occurring simultaneously and interactively to influence the presence and distribution of the pathogens (WHO, 2016a).

Risk factors affecting pathogen concentrations in water that are common to surface and ground water types include population density, rainfall and water temperature. It is obvious that as the population increases, the number of people shedding pathogenic agents at any given time will increase. These agents will impact surface water quality through sewage effluents, combined sewer overflows and urban runoff. From a study of enteroviruses in wastewater, Brinkman *et al.* (2017) estimated that 2.8% of the population contributing to the wastewater were shedding virus daily. Based upon monthly sampling, wastewater concentrations ranged from 3.8 to 5.9 $\log_{10}$ equivalent copies per litre. In general, wastewater treatment is less effective at viral than bacterial removal (Haramoto *et al.,* 2006; Flannery *et al.*, 2012), but both bacterial and viral pathogens will enter surface water through wastewater effluents. Rainfall can impact surface water through runoff. In countries with combined sewer overflows, rainwater-diluted raw sewage will bypass treatment and flow directly into surface water. Urban runoff may be directed to wastewater treatment plants or directly into surface water. Untreated runoff contributes pathogens to surface water as shown by increased health risk

for recreational surfers (Dwight *et al.*, 2004). Water temperature will impact virus survival. Generally, viruses will survive longer at colder temperatures (Chenar and Deng, 2017; Lee *et al.*, 2015).

In addition to urban runoff and combined sewer overflows, other factors influencing the contamination of surface water include the degree of wastewater treatment and the distance between the treatment outfall and where water is taken for use at a farm. The risk of contamination for surface water will decrease as the level of wastewater treatment increases (tertiary treatment<secondary treatment<primary treatment<<raw sewage). Other sources of contamination include leakage from sewer pipes and latrines and from septage, especially when septic tanks are improperly designed or operated (Borchardt *et al.*, 2011). It can be difficult to find even small creeks free of faecal contamination in some parts of the world due to these sources.

5.3.1 Risk reduction measures and surface water use

The risk reduction measures required when using surface water in FFV production could be identified and managed by applying a WSP that is context-specific for the geographical location and FFV type and production system (WHO, 2016a). Multiple barriers may be required including measures such as careful selection and protection of the surface water source and water storage (WHO, 2016a) and choosing methods for use (e.g. irrigation type) that minimise exposure of edible portion of FFV to effectively reduce risks of microbial contamination (FAO and WHO, 2017). Surface water bodies are a supply of municipal and agricultural water. Treatment processes for potable water supplies are discussed in Section 5.5 Municipal water.

5.4 GROUNDWATER

Groundwater is the water contained beneath the surface in rocks or subsoil. A subcategory of groundwater would be spring water, which is flowing naturally out of the ground. Groundwater provides about 97% of the world's fresh water (WHO, 2006b). It is an important source of potable water in many world regions and can be the single most important source of potable water where surface water is scarce or polluted (WHO, 2006b). Groundwater can provide an economical source of potable water. It can be a more stable and a better quality source than surface waters and may not require treatment to be suitable for drinking. Nonetheless, groundwater can become contaminated with microorganisms.

Unique factors that affect pathogen contamination of groundwater include the local hydrogeology and the depth of the well from which water is drawn e.g. the water table near the surface to deep underground. The simplest way to obtain groundwater is to use buckets to retrieve the water from open holes that are hand dug down to the water table. If care is not taken to prevent surface water from entering the hole opening, this groundwater should be treated as surface water as it is similarly easily contaminated.

Wells may be drilled to tap into shallow groundwater and, if properly constructed, surface water will not directly impact the water quality of these wells. Nevertheless, as pathogens can pass through the soil into the water table, especially if there is an absence of topsoil or if the layers under the topsoil have a high coarse sand/cobble content, shallow water from the water table will have contamination with bacteria and viruses (Fout *et al.,* 2017).

Wells should be drilled into groundwater that is separated from the water table by a sedimentary layer that is not permeable to water movement, if possible. However, because underground hydrogeology can be diverse, even deep wells may have bacterial and viral pathogen contamination.

Similar to surface water, the microbiological quality of the groundwater is affected by multiple and complex interacting natural and animal/human activities and industries (WHO, 2016b; WHO, 2006b). It is therefore important to distinguish between ground water from shallow wells with a relatively high risk of contamination, and water from deep wells.

5.4.1 Risk reduction measures and groundwater use

The risk reduction measures required will be identified during the WSP as for surface water (See section 5.3.1). Protecting the water source and distribution system from contamination e.g. from animal and human activity and from surface water entry are important risk reduction measures (WHO, 2006b). Groundwater is used as municipal water without any treatment in many parts of the world, but a wide variety of treatments may be used to improve its microbiological quality. Very poor-quality groundwater may be treated using conventional treatments used for municipal water (See section 5.5.). In some systems groundwater is disinfected with different water disinfection treatments (e.g. chlorination, ozonation, UV-treatment, etc.). Many households in the United States of America use filters and soft water treatment. However, most household filtration systems will not reduce virus concentrations and water softeners would have no impact on pathogen concentrations (G.S. Fout, personal communication).

5.5 MUNICIPAL WATER

Municipal water can be supplied to communities via taps, containers or tanker distribution systems etc.. The water is often treated in treatment plants, it should be protected during its distribution with monitoring of quality to ensure it meets regulatory compliance for potable water. In some communities, residents may use individual wells. Municipal water can be taken from various sources such as large surface water bodies e.g. rivers, deep wells, lakes or reservoirs (Section 5.3) and groundwater systems (See section 5.4).

5.5.1 Risk reduction measures and municipal water

A WSP for a potable water supply extends from the water source to the point of water use and risk reduction measures may be required throughout this continuum. This can include selection of the highest quality water source and protection of the selected source water and, for FFV production, appropriate use as for surface and groundwater sources discussed in 5.3.1 and 5.4.1.

Some groundwater systems can satisfy all regulatory requirements for potable water without needing any treatment process while others, particularly groundwater under the influence of surface water, need treatment by disinfection or additional treatment steps. Surface water systems are more subject to contamination (See 5.3). National and regional regulations may require surface water to be treated to meet the required targets (WHO, 2017a).

Municipal water can be treated at a central water treatment plant based on the risks assessed for the source water then tested for regulatory compliance before piping to industries and residential homes or treated at the point of use in settings other than piped supplies.

Depending on the quality of the raw source water, multiple treatment processes (e.g. coagulation, flocculation, sedimentation, filtration) and disinfection may be required for the water to meet microbial standards for potable water (WHO, 2017a). The pathogen reduction levels achievable vary between and within microbial groups and for different treatment processes. The treatments may be aggregated and used to provide a multiple barrier approach.

Examples of log reduction values (LRVs) achievable for enteric bacterial pathogens for treatment processes in large community-scale treatment plants are provided by WHO (2017a) and are shown in Table 2.

TABLE 2 Log reduction values for water treatment technologies and disinfectant dosages at water treatment plants supplying large communities. Data sourced from WHO (2017a).

Treatment process	Log reduction values for pathogen groups, disinfectant and UV dosages		
	Bacteria	**Viruses**	**Protozoa**
Pre-treatment	0.2 to > 6 .0	> 2.1 to 8.3	1.0 to 2.3
Coagulation, flocculation and sedimentation	0.2 to 4.0	0.1 to 4.0	0.0 to 2.8
Filtration	0.2 to > 7.0	0.0 to > 6.5	0.3 to > 7.0
Primary disinfection[a,b]:			
chlorine	2 (Ct_{99}[c], 0.04–0.08 min·mg/l; 5°C; pH 6-7)	2 (Ct_{99}, 2–30min·mg/l; 0–10°C; pH 7–9)	2 (Ct_{99}, 25–245min·mg/l; 0–25°C; pH 7–8; mainly *Giardia*)
chlorine dioxide	2 (Ct_{99}, 0.02–0.3min·mg/l; 15–25°C; pH 6.5–7)	2 (Ct_{99}, 2–30min·mg/l; 0–10°C; pH 7–9)	2 (Ct_{99}, 100min·mg/l)
ozone	2 (Ct_{99}, 0.02min·mg/l)	2 (Ct_{99}, 0.006–0.2min·mg/l)	2 (Ct_{99}, 0.5–40min·mg/l)
ultraviolet light	4 (0.65–230mJ/cm^2)	4 (7–186mJ/cm^2)	4 (< 1–60mJ/cm^2)

a-Chemical disinfection: Ct values are given that achieve 2 LRV.
b-UV irradiation: UV dose range is given that achieves 4 LRV.
c-Ct value: the level of reduction (x) resulting from concentration (C) x contact time (t).

Households may treat water from non-piped supplies or when piped supplies fail using similar technologies, adaptations and thermal inactivation methods, some of which may result in higher LRVs (See WHO, 2017a for further detail).

Disinfectants used to inactivate pathogens in water can result in the formation of chemical by-products (WHO, 2017a). However, WHO (2017a) states "the risks to health from these by-products are extremely small in comparison with the risks associated with inadequate disinfection, and it is important that disinfection efficacy not be compromised in attempting to control such by-products".

For further information on risk reduction measures such as managing microbial water quality in piped distribution systems, see WHO, 2017a.

5.6 RAINWATER

Rainwater itself has little or no contamination with human pathogens. However, it can become contaminated by the atmosphere and the microbial quality can deteriorate during rainwater harvesting, storage and use (WHO, 2017a). WSPs are not usually practical for rainwater at the household level. WHO (2017a) recommends sanitary inspections and the use of well-designed harvesting systems with clean catchments, protected storage and hygienic handling practices can reduce health risks.

5.7 CONCLUSIONS

- Any type of water, even municipal water that has been conventionally treated and disinfected, may be contaminated with human pathogens (bacteria, viruses or parasites) albeit with different pathogens and at different levels in different locations.
- The likelihood of pathogen contamination in various types of water differs and in general terms decreases from raw or inadequately treated wastewater, surface water, shallow wells, deep wells and roof run-off water, to rainwater collected safely and potable water.
- Water of different types and qualities could be used in steps in FFV production provided it has been determined to be fit-for-purpose and stringently managed through WSPs and HACCP-based programs. Implementation of a WSP provides an assessment of the level of health risk associated with a water source.
- It is critical to manage the quality of water along the FFV production chain where the water contacts edible portions of the FFV and there are no further pathogen risk reduction steps in the production chain before FFV consumption.
- The risk reduction measures required for the use of a specific water source determined in the risk-based RM plans can be varied. Many are aggregated for a multiple barrier approach and include interventions for the water source, pre- and post-harvest practices related to a specific FFV variety and consumer handling of FFV.
- Risk reduction measures may include the use of water treatment technologies. The efficacy of water treatment technologies varies with the type of water, the pathogens targeted and the proposed water application. Measures may be used aggregately to maximise the LRV achieved in a multiple barrier approach.
- Whatever water safety system is used, the performance of risk reduction measures should be monitored and corrective action taken to correct deviations as required.

6

Establishing tailored threshold values for microbial measures of water quality

Microbial indicator organisms have long been used as a proxy measure of the presence of pathogens in water (Ashbolt *et al.,* 2001). Recognition that correlations between the different groups can be contradictory has led to the recognition of three groups: general (process) microbial indicators, faecal indicators, and index and model organisms. These are defined by Ashbolt *et al.* (2001) as:

- Faecal indicators. Group of organisms that indicate the presence of faecal contamination, such as thermotolerant coliforms or *E. coli*. Hence, they only infer that pathogens may be present.
- General (process) microbial indicators. Group of organisms that demonstrates the efficacy of a process e.g. total heterotrophic bacteria or total coliforms for chlorine disinfection.
- Index and model organisms. A group/or species indicative of pathogen presence and behaviour respectively, such as *E. coli* as an index for *Salmonella* spp. and F-RNA coliphages as models of human enteric viruses.

In this section, microbiological monitoring and the challenge in selecting faecal indicators for measuring microbial water quality and their application when testing water used in irrigation and post-harvest activities during FFV production are discussed.

6.1 FAECAL INDICATOR ORGANISMS AND WATERBORNE PATHOGENS

In most cases, faecal indicators have a dual or even multiple functions and can be used for a range of purposes, including: (i) to indicate the presence and/or the level of faecal contamination in water, acting as a faecal indicator; (ii) to indicate the control and effectiveness of processes such as water treatment (e.g. filtration, disinfection) or produce processing (e.g. washing / disinfection), acting as a process indicator; but as previously mentioned also (iii) as model or index organisms (see below).

To be reliable as indicators of faecal pollution and the potential presence of faecal pathogens in water, WHO (2017a) stipulates a faecal indicator organism should have the following properties:

- be universally present in faeces of humans and animals in large numbers;
- not multiply in natural waters;
- persist in water in a similar manner to faecal pathogens;
- be present in higher numbers than faecal pathogens;
- respond to treatment processes in a similar fashion to faecal pathogens; and
- be readily detected by simple, inexpensive culture methods.

Since no single organism fulfils all these requirements, there is no ideal faecal indicator. Different faecal indicators are suited to different pathogen categories, water sources and use contexts. The absence of an indicator organism does not necessarily mean that all pathogens will also be absent and vice versa. There is no suitable indicator for the presence/absence of protozoa (e.g. *Giardia*, *Cryptosporidium*, *Cyclospora*) and helminth cysts / eggs that are more resistant than bacteria and viruses in water. For this reason, specific tests are required to validate treatments if their presence is suspected. In most cases and depending on the type of water, it is difficult to correlate between counts of indicators and enteric pathogens (Ashbolt *et al.,* 2001).

Microorganisms used as faecal indicators to assess microbiological water quality and those used as process indicators are listed and their advantages and disadvantages summarized in Table 3. The reader is referred to the WHO publications WHO (2017a), Ashbolt *et al*. (2001) and to Figueras and Borego (2010) for further reading.

<u>*Escherichia coli* and thermotolerant or faecal coliforms</u>
Escherichia coli and the faecal coliform group originating from the gastrointestinal tracts of both mammals and birds are commonly used as faecal indicator bacteria

(FIB), even if the thermotolerant (or faecal) coliform group includes bacteria from non-faecal sources. MST can be used to differentiate human and animal host sources (See 7.3). They have a dual function acting as faecal indicators and also as process indicators to validate disinfection treatments.

Total coliforms

The broader total coliform group (TCs) includes bacteria from faecal and non-faecal sources. TCs are common in the environment and for this reason they are not a good indicator of the actual or potential presence of faecal contamination or human pathogens. TCs are mostly used as a process indicator to assess hygiene and the integrity of the water distribution system.

Enterococci

The intestinal enterococci group (e.g. *Enterococcus faecium*, *E. faecalis*, *E. durans* and *E. hirae*) meets many of the requirements of a FIB although present at lower concentrations than *E. coli* in faeces. They may also be used as surrogates for waterborne pathogens (process indicator). MST is required to determine faecal origin (See 7.3).

Clostridium perfringens

Clostridium perfringens is an anaerobic spore-forming bacterium present in faeces. For many years, *C. perfringens* has been widely applied as a general faecal indicator. However, studies of the basic molecular biology of the species contradict the idea that it universally occurs in faecal pollution sources from different hosts (Vierheilig *et al.*, 2013). As the spores are resistant in the environment and have a similar size as some relevant parasites, they have been used as an index for protozoa. *Clostridium perfringens* is used for the validation of disinfection treatments as a process indicator.

Bacteriophages

It is now commonly accepted that FIB are not useful for predicting the presence of pathogenic viruses in water and bacteriophages and some groups of viruses are considered alternative indicators in environmental regulations for that purpose (WHO, 2017a). Bacteriophages are viruses that infect and replicate within bacteria. These viruses share many properties with human viruses (e.g. composition, morphology, structure and mode of replication) which make them useful models or surrogates to assess the behaviour of enteric viruses in water environments and their sensitivity to treatment and disinfection processes (WHO, 2017a). Therefore, bacteriophages have a dual function as faecal indicators and as index or model organisms.

Coliphages, use *E. coli* and closely related species as hosts. Somatic and F-RNA coliphages replicate more frequently in the gastrointestinal tract of warm-blooded animals. Although somatic coliphages can also replicate in water environments, this is very unlikely and consequently, the contribution of its replication in the environment will not influence the number coliphages detected in water environments (Jofre, 2009). Serological sub-types of F-RNA coliphages have been associated with faecal pollution of either human or animal origin. Although coliphages have been shown to better correlate with the presence of viral pathogens compared to the FIB in polluted waters, McMinn *et al.* (2017) found in a review of published data that this correlation cannot be found in other water sources. This is in agreement with a meta-analysis study that used data from 12 groundwater sources and found no direct correlation between coliphages and viruses (Fout *et al.,* 2017). Viruses were usually absent when FIB or coliphages were present, but also were present when FIB and coliphages were absent, hence, the lack of correlation. In spite of the lack of correlation, viruses were more likely to be present when FIB or coliphages were present.

Bacteriophages infecting *Bacteroides* spp., such as *Bacteroides fragilis* phages have potential for use in faecal source tracking (Wu *et al.* (2020). *Bacteroides* spp. are obligate anaerobes that inhabit both human and animal gastrointestinal tracts in large numbers, higher than those of *E. coli*. *Bacteroides* are rapidly inactivated by environmental oxygen levels. In contrast, *Bacteroides* bacteriophages are resistant to unfavourable conditions (Teixeira *et al*. 2020). Cross-assembly phages, also known as crAssphages, are predicted to infect bacteria of the order Bacteroidales and have been suggested as to be used as faecal and process indicators for virus removal in wastewater. Based on Wu *et al.* (2020), crAssphage was strongly correlated with adenovirus and polyomavirus molecular indicators through the wastewater treatment process.

TABLE 3 Common microbial faecal indicators for water quality: benefits, constraints and implementation considerations. (Sources of data Figueras and Borego, 2010; Ashbolt *et al.*, 2001; Saxena *et al.*, 2015).

Microbial Indicators	Advantages	Disadvantages
Escherichia coli	• member of FCs found in the intestines of mammals, including humans. • is usually considered the most suitable indicator of faecal contamination. • indicates recent faecal contamination and that pathogens might be present.	• does not distinguish between human and animal faecal contamination. • may not be a suitable indicator for viruses, protozoans and helminth eggs as less persistent i.e. when absence or low numbers of *E. coli*. • *E. coli* can replicate in environmental waters.
Thermotolerant or faecal coliforms (FC)	• indicate environmental contamination and potential faecal source.	• some thermotolerant spp. (e.g. *Klebsiella*) may not be of faecal origin. • may not be suitable indicator of viruses and protozoans. • re-growth may occur.
Total coliforms (TCs)	• measure of degree of pollution and sanitary quality of water. • positive TCs test can be followed by FC and *E. coli* tests.	• do not necessarily indicate faecal contamination.
Enterococci	• intestinal subgroup relatively specific for faecal pollution. • tend to survive longer in water environments than *E. coli*.	• number present log lower than number of *E. coli* in faeces. • have been shown to replicate in the environment.
Clostridium perfringens	• indicator of prior faecal pollution and sources subject to intermittent contamination • used to evaluate effectiveness of treatment systems for viruses and protozoa.	• higher prevalence and numbers in faeces of some animals than humans. • less often in the faeces of many other warm-blooded animals. • faecal counts normally substantially lower than *E. coli.* • counts in raw water are usually low. • spore survival times likely longer than enteric pathogens.
Bacteriophages (coliphages, *Bacteroides* spp.)	• used as an alternative to faecal indicator bacteria; chosen depending on purpose. • surrogates for human viral pathogens in the environment. • microbial source tracking tools, some specific to human faeces. • models or surrogates to assess the behaviour of human enteric viruses in water environments.	• different excretion patterns phages (continual) versus enteric viral pathogen (during infection only). • detection and counting methods of some phages are more complex and expensive than other phages and for faecal indicator bacteria. • relatively low numbers of some *Bacteroides* spp. in sewage and polluted water environments. • some *Bacterioides* spp. phages exhibit low survival rates in water.

6.2 IRRIGATION WATER

With regards to the microbial contamination of irrigation water, broadly speaking, it is intuitive to consider that higher numbers of an indicator organism will be associated with higher pathogen contamination risks. However, establishing precise figures for indicator / pathogen ratios is difficult. It is also difficult to set a clear-cut threshold above or below which the presence / absence of pathogens is expected.

The strength of the correlation between FIB concentrations, particularly generic *E. coli* counts, with the presence of pathogenic bacteria e.g. *E. coli* O157 STEC or *Salmonella* spp., in irrigation water has varied among studies in different locations. Pachepsky *et al*., (2016) reviewed 81 studies and found a sound relationship between pathogen presence and counts of TCs or generic *E. coli* in surface freshwater used for irrigation in only 28 (35%) of the studies. They proposed microbial standards for irrigation water quality "cannot rely only on concentrations of indicators and/ or pathogens but must" also "include references to crop management". McEntire and Gorny (2017) confirmed enumeration of generic *E. coli* "often has little predictive value regarding the presence or absence of human pathogens for many agricultural surface water sources". A somewhat better correlation between FIB and pathogens might be observed in heavily polluted waters, but this correlation becomes erratic and biologically improbable as dilution occurs (Payment and Locas, 2011). Nonetheless, logistic regression analysis and longitudinal surveys in the United States of America (McEgan *et al*., 2013) and EU (Holvoet *et al*., 2014; Castro-Ibañez *et al*., 2015) have shown high *E. coli* concentrations could reasonably predict the probability of pathogen presence (e.g. STEC and *Salmonella* spp.).

The following are examples of studies of FIB counts and probabilities of pathogen detection.

- Ceuppens *et al*. (2015), Castro-Ibañez *et al*. (2015): *Salmonella*, STEC and *Campylobacter* spp. isolations were more frequently detected in water samples containing high counts of generic *E. coli*, 1.5–2.0 $\log_{10}$ CFU/100 ml.
- Truchado *et al.* (2018): samples from three different irrigation water sources with < 2.35 $\log_{10}$ *E. coli*/100 ml had 90% probability not to be contaminated with enteric pathogens while almost 75% of samples contaminated with *E. coli* at levels > 2.24 $\log_{10}$ CFU/100 ml were contaminated with enteric pathogens.
- McEgan *et al*. (2013): the probability of enumerating *Salmonella* in surface water at different concentrations (3, 5, 10, 15, 20 and 60 MPN/100 ml) increased, proportionally, from the lowest observed level of *E. coli* (1 $\log_{10}$ MPN/100 ml) to the highest (3.2 $\log_{10}$ MPN/100 ml).

Based on these and previous studies, the EU has established a quality criterion of 100 CFU/100 ml (2 $\log_{10}$ *E. coli*/100 ml) for irrigation water intended to be used on crops likely to be eaten uncooked (i.e. ready-to-eat FFV in which irrigation water comes into direct contact with the edible portion) (EC, 2017).

6.3 POST-HARVEST WATER

In post-harvest FFV processes, various microbial indicators can be applied to assure the microbial quality of process water is adequate to avoid cross-contamination of FFV during the process and to determine that the treatment process is performing as required in reducing microbial levels (e.g. water disinfection, Chapter 5). The presence of microbial indicators in the process water is assumed to indicate unhygienic working conditions, faecal pollution or failures in control measures. For this particular use, process indicators and index or model organisms are suitable indicators to validate disinfection treatments. Monitoring for process indicators or index microorganisms can identify deviations from threshold limits and the need for corrective action and can be used to implement new control measures when levels are above a threshold.

When assessing the performance of processes for pathogen reduction, such as water treatment (e.g. filtration, disinfection) or FFV processing (e.g. washing / disinfection), process indicators and/or index organisms should respond to treatment processes in a similar manner to pathogens of concern. However, this will not apply for the removal of every pathogen group by different treatment processes. Thus, a range of indicator organisms, together with measurement of relevant process parameters (e.g. sanitiser levels, pH, temperature etc.), should be considered depending on the different processes and purposes. Since viruses, protozoan cysts and helminth eggs are more resistant than bacteria in water treatment processes, *E. coli* would not be a suitable process indicator for their removal or inactivation. Viruses are also susceptible to inactivation, but little is known about the efficacy of commercial sanitizers against viruses in process water as most of the studies have been performed under lab-scale conditions. On the other hand, protozoan cysts and helminth eggs are large enough to be effectively removed by water and wastewater filtration, or by sedimentation in reservoirs with long retention times (like water reservoirs or waste stabilization ponds), or by wastewater treatment in natural or constructed wetlands. A number of process indicators and index or model organisms that have been used to assess the effectiveness of microbial removal by water treatment processes (Table 4).

TABLE 4 Common microbial process indicators and index or model organisms used for assessing the effectiveness of microbial removal and inactivation by water treatment processes

Microbial indicator	Benefits	Limitations
• *Escherichia coli*, thermotolerant or faecal coliforms, total coliforms	• used as indicators of bacterial inactivation by water treatments. • absence of faecal coliforms or *E. coli* is interpreted as absence of pathogenic faecal bacteria.	• not useful to validate FFV disinfection processes for viruses, protozoan cysts and helminth eggs removal.
• Enterococci	• same as for coliforms and *E. coli*.	• same as for coliforms and *E. coli*. • detection methods for *E. coli* are simpler and cheaper than for enterococci.
• *Clostridium perfringens*	• indicator for effectiveness of disinfection and physical removal processes for viruses and protozoa.	• *C. perfringens* counts can be low, therefore, could be difficult to calculate log reductions.
• Bacteriophages (e.g. for *E. coli, Bacteroides* spp. and enterococcal phages)	• may be adequate viral surrogates, especially in water and wastewater treatment plants.	• different phage types used for specific applications, e.g. as surrogates of human viral pathogens and removal by water and wastewater treatment. • complexity of detection, enumeration and cost varies with phage type and can be greater than tests for FIB.
• Turbidity	• used as a surrogate for protozoan cysts (e.g. *Giardia* and *Cryptosporidium*) removal through physical water and wastewater treatment processes, e.g. filtration.	

Escherichia coli and TCs tests have been used to assess the microbial quality of process water even though their suitability for this role remains controversial (Doyle and Erickson, 2006). As previously discussed in this report, *E. coli* is a more reliable indicator of faecal contamination since it is exclusively of faecal origin. Holvoet *et al.* (2012) found insufficient cleaning and disinfection of washing baths, not regularly re-filling produce wash baths with water of appropriate microbial quality and the using high product/water ratios to result in a rapid increase in *E. coli* in the processing water, with subsequent potential for *E. coli* transfer to the end product (Gombas *et al.*, 2017).

Holvoet *et al.* (2012) assessed the value of total aerobic psychrotrophic bacterial counts (TAPCs) along with TC and *E. coli* counts and pathogens in monitoring water quality when processing fresh produce and found TAPCs were not a reliable indicator of overall quality and best manufacturing practices. Harvested FFV carried high TAPCs and the process water quickly becomes contaminated resulting in little change in TAPCs throughout the production process of a batch.

To measure the effectiveness of disinfection, *E. coli* would be a suitable process indicator of inactivation of bacterial pathogens only. Bacteriophages could be an alternative for evaluating virus inactivation. Measuring other disinfection treatment, parameters such as the disinfectant dose (or Ct values – disinfectant residual concentration vs. contact time) sufficient to inactivate viruses and even protozoan cysts (although difficult, given their high resistance), would also be a meaningful indicator. Examples are provided in Chapter 5, Table 2.

6.4 APPROACHES TO ESTABLISHING TAILORED WATER THRESHOLD VALUES

Post-process water that is in contact with edible portions of FFV should be of potable quality (FAO and WHO, 2017) and threshold values are described in WHO (2017a). This section focuses on pre-harvest water used in FFV production e.g. irrigation water. According to Blumenthal *et al.* (2000), there are three approaches for establishing microbiological guidelines for wastewater use in agriculture, which might also be applied to irrigation water quality in general:

- presence /absence (also referred to as non-detection) of pathogens / or faecal indicators in the water,
- non-detection of excess cases of enteric disease, and
- a model generated risk estimate which is below a defined acceptable risk.

The first approach (1) has been criticised as an unachievable goal of 'zero risks' which unavoidably leads to establishing guidelines that are too strict (Blumenthal *et al.,* 2000; De Keuckelaere *et al.*, 2015). The limitations of microbiological testing and the lack of absolute correlation have been discussed in 6.1. The main criticism of this approach is that it lacks a risk-based perspective.

However, there are irrigation water quality guidelines based on such rationale in use. An example is the EU guideline of 100 CFU *E. coli* /100 ml for irrigation water intended to be used on FFVs likely to be eaten raw that was based on studies of pathogen presence and FIB (See 6.2.1). Another example is the United States of America Environmental Protection Agency (USA EPA) guidelines for agricultural

reuse of reclaimed water for surface or spray irrigation of food crops which are intended for human consumption raw. The guidelines include (i) no detectable faecal coliforms/100 ml, (ii) ≤ 2 nephelometric turbidity units and (iii) ≥ 1 mg/l residual chlorine, to be met after a minimum contact time of 90 minutes and to be attained by secondary treatment followed by filtration and disinfection (USA EPA, 2012). It is assumed that chlorination is used to inactivate bacteria and viruses, whereas filtration is used to remove protozoan cysts.

The second approach (2) takes an epidemiological perspective i.e. there should be no measurable excess risk of infection (or disease) attributable to the consumption of irrigated FFV within an exposed population. Such evidence is difficult to gather; epidemiological studies are usually time- and site-specific, very expensive and may not be able to measure low levels of risk typical of environmental exposure unless extremely large populations are studied. Such approaches may be indirect. For example, the United States of America Food and Drug Administration (USA FDA) guideline for the irrigation of food crops eaten raw is based on the United States Recreational Water Quality Criteria, which was derived from modelling the relationship between swimming-associated illness and water quality (USA EPA, 2012). Values of 126 *E. coli* / 100 ml for the geometric mean (GM) and 410 *E. coli* / 100 ml as the STV (statistical threshold value) approximate the 90th percentile of the water quality distribution and are taken as thresholds that should not be exceeded by more than 10% of the samples used to calculate the GM. These standards are associated with an estimated gastrointestinal illness rate of 36 per 1,000 primary contact recreators. As pointed out Pachepsky *et al.* (2011), the use of recreational water standards is problematic as they were established assuming human health risks posed by full-body contact during swimming, therefore, a rather different exposure from that of consuming irrigated FFV.

In the third approach (3), a QMRA model is used to estimate the risks of infection that can be contrasted to a reference level of acceptable risk (WHO, 2016b, Chapter 4)). QMRA models are pathogen- and scenario-specific and dependent upon the availability of data (at least estimates) on pathogen prevalence in the irrigation water or on the irrigated crops. In the absence of such data, a feasible strategy for microbial water quality evaluation consists of applying a RA approach and assessing the level of public health threat (Pachepsky *et al*., 2018).

A QMRA model for estimating risks of infection arising from the consumption of irrigated FFV, can be comprised of an exposure assessment and a dose-response model (WHO, 2016b; De Keuckelaere *et al*., 2015). Crop contamination can be

estimated based on the volume of irrigation water caught by product (Hamilton *et al.,* 2006; WHO, 2006a) or, whenever possible, from information on pathogen transfer from the irrigation water onto the irrigated crops (Bastos *et al*., 2008). A more detailed exposure model could include an estimation of crop contamination at harvesting taking into account irrigation water and soil transfer to FFV due to splashing of irrigation or rainwater and the daily die-off due to solar radiation (Allende *et al*., 2018).

Exposure and RA should preferably be modelled using a probabilistic rather than a deterministic approach, (Pachepsky *et al*. 2018; Chapter 4). Hamilton *et al.* (2018) published an example of where a database for an RA was established from various sources and then used to generate the probabilistic description of the human health risk associated with the presence of *Cryptosporidium* and *Giardia*.

QMRA modelling can be tailored for specific scenarios, including the FFV type, irrigation scheduling, preharvest environmental conditions and post-harvest processing. It could also be used in a reverse model for setting irrigation water quality standards / criteria by starting with a probability of infection "tolerable risk") then, with knowledge of the exposure input variables, determine the pathogen concentration in irrigation water that achieves the tolerable risk level. This is the basis of the WHO guidelines for the safe use of wastewater in agriculture and could be applied to other types of water (WHO, 2006a). Briefly, the WHO guideline value for the worst-case scenario of irrigation of crops eaten raw with reclaimed water is 10^3 *E. coli* / 100 ml, which is associated with an estimate of annual risk of infection with pathogenic bacteria, viruses and protozoa between 10^{-3} to 10^{-4} per person per year (WHO, 2006a).

A shortcoming for using QMRA is that it needs data on the occurrence of pathogens. As noted by Pachepsky *et al*. (2011) waters used for irrigation are monitored much less intensively than drinking or recreation water and even when it is monitored, in most cases indicator organisms rather than actual pathogens are measured. Alternatively, however controversial it is, a ratio of pathogens / indicator organism concentrations in the water has been assumed. For instance, the WHO guidelines for wastewater irrigation assume the following figures: 0.01–0.1 *Cryptosporidium* oocyst and 0.1–1 rotavirus and *Campylobacte*r per 10^5 *E. coli* in the wastewater (WHO, 2006a).

More detailed examples and evidence can be found in Annex 4.

6.5 CONCLUSIONS

- To indicate the presence of faecal contamination indicator organisms are preferred than presence or concentration level of any specific pathogen. The major indicator organisms are *E. coli* and enterococci; other groups of organisms have been recommended (e.g. *C. perfringens* and Bacteroides and *E. coli* specific phages), but of these other groups, none is widely applicable.
- Faecal indicators can have a dual function acting as process indicators and index or model organisms that can be used to validate the efficacy of water treatments.
- Bacteriophages are better indicators of enteric viruses than FIB, although coliphages cannot be absolutely relied upon as indicators for enteric viruses. Several authors have suggested using a combination of two or more indicators. However, bacteriophages can be proposed as good process indicators to determine the efficacy of water treatments against enteric viruses.
- Protozoa and helminths cysts / eggs are more resistant than bacteria and viruses and there is no suitable indicator of their presence/ absence in irrigation water and specific tests have to be performed if suspected.
- In general, correlation between faecal indicator organisms and pathogens is usually observed in heavily polluted waters, but this correlation becomes erratic and biologically improbable in low contaminated water. The use of logistic regression analysis and longitudinal studies have shown high *E. coli* concentrations could reasonably predict the probability of pathogen presence (e.g. STEC and *Salmonella* spp.) in surface water.
- In post-harvest water, process indicators and index or model organisms can be used to assess the performance of water treatments in pathogen reduction, if they respond to treatment processes in a similar manner to pathogens of concern.
- *Escherichia coli* has been suggested as a suitable process indicator of inactivation of bacterial enteric pathogens. On the other hand, bacteriophages could be an alternative for evaluating virus inactivation.
- There are three main approaches for establishing microbiological guidelines for wastewater use in agriculture, which might also be applied to irrigation water quality including: (i) monitoring faecal indicators or pathogens in the water; (ii) using an epidemiological perspective; and (iii) the use of a risk assessment approach.

7

Testing microbial water quality and microbial source tracking

Microbiological testing of water can be used for a variety of purposes in WSPs and food safety RM programs including PRPs and HACCP programs applied in FFV production (See Chapter 6 for more detail). There is a wide range of different test methods applicable for different purposes and these can include detection or presence/absence tests for pathogens and enumeration of microbial indicator organisms (WHO, 2017a) and tests for markers to track the source of faecal contamination (Ahmed and Harwood 2017).

With the preventive and risk-based approach of WSPs, an increasing number of microorganisms are used to determine the potential presence of waterborne pathogens and for various different purposes and new technologies are emerging for their analyses (Figueras and Borrego. 2010). Research and development of test methods is needed to improve the performance of test systems, reduce complexity and cost, and to provide results in real-time.

Different approaches to measurement of water quality are reviewed in this chapter. A summary of the various test methods and their advantages and disadvantages is presented in Annex 3.

7.1 CULTURE-BASED MICROBIAL METHODS

Culture-based methods are generally considered the standard methods for the detection and identification of bacterial pathogens and enumeration of bacterial indicators. Their advantage lies in assessing cell viability and infectivity, generally

lower cost and ease of use. However, culture-based methods require trained and skilled technicians, preferably complying with a laboratory quality control system (Bain *et al.,* 2012). They tend to be time-consuming, labour-intensive, sensitive to contamination and to inadequate conditions during sample transportation, and exposure to inappropriate temperature ranges. This is particularly challenging where the nearest laboratory is distant from the sites of the water sources. Despite these limitations, culture-based methods remain the gold standard.

Culture methods are classified as quantitative or qualitative. The quantitative methods typically involve counting the number of viable bacteria using the pour plate or spread plate methods or estimating numbers using the most probable number (MPN) method. Membrane filtration is still a preferred method for the microbiological examination of water for target bacterial pathogens or indicator microorganisms. It is relatively easy to apply for small and very large volumes of water and selective media developed for the detection of target bacteria can be used. The development of chromogens and fluorogens that produce a visible signal in the presence of specific enzymes has led to simpler, more rapid, and more specific growth media for target pathogens (Manafi, 2016). Limitations of the culture methods are that some media chromogenic and fluorogenic media are expensive and may not be readily available in all regions. Moreover, other media are not selective enough for specific bacterial pathogens and not all microbial pathogens are culturable, e.g. human norovirus and parasites (Haramoto *et al*., 2018). Another limitation is that some bacteria have been shown to enter the viable but non-culturable state and remain undetected when culture methods are used e.g. *Vibrio cholerae* (Chaiyanan *et al*., 2001).

Culture-based detection of *E. coli* is the simplest for laboratories to perform. Testing methods should be validated and conform to standard methods where possible. Commercial test kits for culture-based enumeration of bacteria in water are available. These range from those that use a proprietary defined substrate technology nutrient indicator (e.g. for E. coli, enterococcus, *Pseudomonas aeruginosa*, etc.) to portable, compact all-in-one microbial test kits which can be used in rural areas without the need for laboratory equipment (Brown *et al*., 2020; Stauber *et al*., 2014). In the food industry, ready-to-use paper thin plates with prepared media for culturing various microorganisms are available. The agar is completely housed in a single unit requiring only the sample to be added, which saves time and is cost-effective.

Qualitative culture methods are used to determine whether bacterial pathogens e.g. *Salmonella* spp., *Listeria* spp. and *Campylobacter* spp. are present or absent in the sample. These detection methods are popular and are simple, less expensive and

quicker than enumeration methods. However, they generate limited information about the level of the pathogen contamination, information that can be helpful in devising solutions for a contamination problem.

Pathogenic microorganisms are generally present in water at low concentrations and the cells can be in a stressed condition. This usually necessitates the concentration of large volumes of a water sample using an enrichment step to enhance detection and a non-selective pre-enrichment step to enhance the recovery of stressed/damaged cells. Target cell concentration steps such as immunomagnetic separation can be used prior to detection using cultural or other methods e.g. flow cytometry for *Cryptosporidia* and *Giardia* (Barbosa *et al.,* 2007; Keserue *et al*., 2011). The typical bacterial pathogen presence/absence test follows the following steps: 1. primary or pre-enrichment, 2. selective enrichment, 3. detection/plating and 4. confirmation.

7.2 NON-CULTURE BASED MICROBIAL METHODS

Non-culture-based methods are used to detect a broader range of microorganisms than bacterial culture methods. These methods include molecular detection procedures such as polymerase chain reaction (PCR), reverse-transcription PCR (RT-PCR), real-time quantitative PCR (qPCR), digital droplet PCR (ddPCR) and RT-qPCR, nucleic acid sequenced-base amplification (NASBA), immunological methods, optical biosensors, next-generation sequencing (NGS), flow cytometry, etc,. A summary of the advantages and disadvantages of the different microbiological test methods are summarized in Annex 3.

Polymerase chain reaction. In PCR based assays, genetic markers target DNA and RNA genes specific for a microorganism, for a group of microorganisms or for genes that encode specific traits (e.g. genes encoding virulence, antimicrobial resistance, host specificity or serotype) that are linked with targeted microorganisms (Ahmed and Harwood, 2017). The sequences targeted and design of primers/probes play a major role as similar non-target sequences may be amplified by poorly designed oligonucleotide primers.

Compared to culture-based methods, molecular methods can have the advantage of being more sensitive and more specific. The time to complete the test can be faster, taking about 2 hours compared to 18-24 hours for a culture method. Molecular tests can be used to distinguish between human and animal sources of contamination (Fuhrmeister *et al.*, 2019; Garcia-Aljaro *et al.*, 2018). Moreover, they can outperform the culture-based methods and immunoassays for their

sensitivity and rapid identification of foodborne pathogens (Naravaneni and Jamil, 2005; Priyanka *et al.*, 2016) which can provide results quickly enough to monitor CPs and allow corrective action. Pathogens such as *Salmonella* and *Campylobacter* may be viable but non-culturable, hence, culture methods may fail to detect them and lead to a false-negative result. Molecular PCR-based methods that detect pathogen-derived nucleic acid (DNA or RNA) would avoid this risk (Vidic *et al.*, 2019). Nonetheless, PCR-based assays may still result in false-negative results for pathogens that present in low concentrations, because only very small volumes can be assayed. In addition, chemical and other substances (humic acid, metal ions, etc.) present in the environmental samples and inhibit the reaction. The major challenges with the PCR methods are generating false positive signals due to binding to non-specific double-stranded DNA sequences (Priyanka *et al.*, 2016), risk of cross-contamination, the lack of standardized methods and controls and the high cost of equipment and supplies. The lack of trained personnel, limited resource and inadequate storage and reliable consistent supply of electricity in developing countries can also pose great challenges to the adoption of molecular tools in some laboratories.

PCR assays generally do not provide an indication of the viability of the target organisms which is important for estimating the infection potential; however, there is ongoing research in this area. For example, microbial cell viability (or more specifically, membrane integrity) has been assessed using qPCR coupled with propidium monoazide) or ethidium monoazide (Reyneke *et al.*, 2017). PCR has been used in combination with virus cell culture to overcome the limitation of both techniques for detection of viruses (e.g. Rotavirus) in environmental samples (Reynolds *et al.*, 2004). The integrated cell culture/PCR test technique enabled more rapid detection and the detection of non-cytopathogenic viruses (e.g. Rotavirus and most wild-type hepatitis A viruses) growing in cell culture. Newer and experimental techniques may not be readily available in many laboratories at this time.

PCR methods that are performed at a single temperature and do not require thermal cycling are available and are simpler to use and more suitable for field testing e.g. gene sequences amplified using an isothermal amplification technique, nucleic acid sequence-based amplification (NASBA) that amplifies RNA (Compton, 1991) and loop-mediated isothermal amplification (LAMP; Notomi *et al.*, 2000).

Digital droplet PCR is a novel sensitive and rapid method for the direct absolute quantification of a target gene in a sample by fragmenting the DNA, partitioning the sample into thousands of nanoliter-sized droplets and PCR amplification of the

target DNA in the droplets. Due to the random, independent segregation of DNA fragments into droplets, Poisson algorithms are used to determine absolute copy numbers in the original sample independently of a standard curve (Pinheiro *et al.*, 2012, Hindson *et al.*, 2011). The method has been claimed to work well for selected pathogens, but initial cost and reagents tend to be more expensive than qPCR.

Next generation sequencing. NGS is a recent DNA-based method that can provide holistic microbial community diversity analysis. It allows untargeted detection identifying several species in a complex matrix through DNA sequencing. The method is extremely powerful and provides opportunities to examine and track changes in food microbiomes when food is subject to environmental perturbations (Jagadeessan *et al.*, 2019; Jongman *et al.*, 2020). The method has enabled the identification of novel host-associated viruses in faecal and wastewater samples based on their relative abundance (Ahmed and Harwood., 2017). As this technology advances and becomes more widely accepted, the cost is decreasing but it is, nevertheless, currently expensive. NGS requires skilled bioinformatics analysis to interpret the data. Ion torrent uses a semiconductor sequencing that is based on the detection of hydrogen ions released during DNA polymerization. The ability of semiconductor sequencing in recovering plant associated fungal biomes showed limitations of the technology underestimating diversity due to the presence of unknown taxonomic affiliations (Jongman *et al.*, 2020). Other NGS technologies supersede ion torrent in this regard. Recently, real-time NGS had become available although sensitivity is still an issue and more field testing is needed before it can be used in a practical way. The technology has allowed for sequencing across a broad spectrum of applications in genomics, transcriptomics and epigenomics (Jongman *et al.*, 2020). Nanopore sequencing was also recently developed and provides a real-time analysis, produces long reads and is capable of single molecule sequencing devoid of PCR amplification.

NGS can provide Whole Genome Sequencing (WGS) information on bacterial isolates and pathogen identification (Moran-Gilad, 2017). This is of particular advantage in epidemiological investigations of food/water outbreaks by linking clinical and non-clinical isolates and can be used to link to other outbreaks from other times and locations. New WGS platforms have been established such as the GenomeTrakr Network providing a global rapid assessment tool to link clinical and food or environmental related isolates (Timme *et al.*, 2018; FDA, 2020). However, it cannot differentiate between infectious and non-infectious cells/particles.

Microarrays. Microarrays are a multiplex lab-on-a-chip, typically as a two-dimensional array of spotted genes with a specific DNA sequence on a solid substrate

that can be hybridized to a target that is detected and quantified by fluorescence. Microarrays have the advantage of being high throughput (thousands of genes can be analysed) and can be custom made to target pathogens or indicators of interest. For example, Li *et al.* (2015) developed a microarray targeting human viruses, viral indicators and antimicrobial resistance genes. Their results showed that host-specificity ranged from 83 to 90%, but sensitivity for this method was rather low (21 to 33%) and could be improved.

Biosensors. Many biosensors have been developed and used for environmental monitoring. Compared with culture- and molecular-based methods, biosensors have unique advantages of rapid detection and being relatively easy to operate. Generally, biosensors consist of three elements: bioreceptor, transducer and detector. By far, the most reported biosensors used in environmental monitoring use antibodies as biological elements. The optical signal detected can be easily correlated to the concentration of the analyte of interest and multiple analytes can be detected. Many biosensors have been successfully commercialized. They typically take about 3–12 h to obtain results with a detection limit as high as ~100 CFU/ml. There are several commercially available kits that employ fluorescence optical sensors for the detection of faecal indicators in water.

Flow cytometry. Flow cytometry is another optical method that identifies and enumerates cells based on the fluorescence signature of particles. A pre-concentration step may be necessary to meet required sample test volume. The technique involves exciting a stream of cells flowing single file with a laser and detecting the fluorescence. The method is very rapid and thousands of cells can be analysed in seconds. Specific detection of bacteria and some viruses requires the use of DNA or antibody-based probes or aptamers and viability stains can be used to differentiate membrane intact and membrane compromised cells (Berney *et al.,* 2007). Online flow cytometry systems have recently been implemented at water treatment plants to monitor total bacterial counts. However, in more complex matrices, such as different water sources for agricultural water use, this method cannot be successfully applied as it may be challenging to obtain reliable counts for environmental samples containing large amounts of non-cell particles (Safford and Bischel, 2018). Additionally, validation of specific water disinfection treatments such as UV-C treatments cannot be performed using flow cytometry.

Immunological methods. Immunological based methods rely on antibodies to bind to specific targets or antigens of interest. Monoclonal antibodies (mAbs) have been broadly used in many applications, including enzyme-linked immunosorbent assays (ELISA), Western-Blot, flow cytometry, etc. (Ndoja and Lima, 2017). The mAbs provide high specificity (detect only one epitope on the antigen) and high

affinity while the homogeneity of mAbs is very high relative to polyclonal antibodies. However, there are some limitations in mAbs, e.g. they are time-consuming and costly to make and sometimes there are unexpected cross-reactions with unrelated antigens and in unpredictable instances, only low-affinity mAbs can be generated against certain antigens. Also, there are animal ethics concerns in the production of mAbs.

Adoption of non-culture-based methods. Often scientific technologies are advancing much faster than they can be implemented in practice. One of the limitations of non-culture-based assays is the lengthy process for approval or in obtaining their adoption in national surveillance programs; however, this is changing. For example, in the United States of America, the recreational water quality guidelines have been revised to include guidelines based on epidemiological and QMRA studies for sewage specific markers, as well as human enteric pathogens in order to determine the health risks for bathers in all recreational waters. The document recently included information regarding the use of rapid indicator methods such as molecular qPCR, which can allow beach managers to make faster decisions to protect bathers, in contrast to traditional culture-based methods which provide estimates of water quality a day or two after the actual exposure (USA EPA, 2015). However, most other countries have not adopted molecular methods in their guidelines.

7.3 MICROBIAL SOURCE TRACKING

Microbial source tracking (MST) markers have emerged in response to the need to better identify the source(s) of faecal pollution in water sources when developing RM strategies to safeguard human health and in the investigation of contamination incidents.

Sensitivity and specificity of markers for the host of origin are the key criteria in evaluating the suitability of markers for source tracking. Some of these microbial indicators/pathogens are listed in Table 5. Ahmed and Harwood (2017) reviewed a variety of human and animal enteric viral markers for tracking the sources of faecal pollution, including human adenovirus (hAdV), human polyomavirus (hPyV) and pepper mild mottle virus. The authors concluded that hAdV and hPyV were good MST markers for human faecal pollution, but that many of the animal MST viral markers were not very specific and further research was needed to develop, evaluate and validate new qPCR assays. Another challenge that was highlighted is the difficulty in effective quantitative recovery of viruses from environmental water samples. Recently, a novel bacteriophage, crAssphage, was discovered through

metagenomics mining and reported to be abundant in and closely associated with the human faecal waste (Stachler *et al*., 2017). The qPCR genetic markers for crAssphage were shown to be highly abundant in raw sewage and sewage-impacted water samples both in the United States of America (Stachler *et. al*. 2017) and Thailand (Kongprajug *et. al*., 2019) and appear to be promising MST markers of human faecal contamination.

TABLE 5 Selected qPCR-based MST markers for differentiating human and animal sources of faecal contamination.

Microbial Indicator/ Pathogen	MST markers		Reference
	Human source	Animal source	
Bacteroidales		BacCoW	Fuhrmeister *et al*. (2019)
	HF183, BFD, BVulg	BoBac (bovine marker)	Ravaliya *et al*. (2014)
	GenBac3 (B.theta-ionomicron)	BacR (ruminants)	Harris *et al*. (2017)
Coliphages	F+RNA GII, GIII MS-2, PRD1, ΦX-174, Qβ and fr	F+RNA GI, GIV	Gerba *et al*. (2014)
Enterococci	*E.faecium, E. faecalis*	*E.casseliflavus, E.mundtii*	Bahirathan *et al*. (1998), Ferguson *et al*. (2005)
Norovirus	GI, GII, GIV	GIII, BNoV (bovine)	Aw *et al*. (2009), Ahmed and Harwood (2017)
Adenovirus	Ad40, Ad41	BAdV (bovine), PAdv (porcine)	Ahmed *et al*. (2010), Ahmed and Harwood (2017)
Shigella	Group A, B, C, D		
crAssphage	CPQ_056, CPQ_064		Stachler *et al*. (2018), Kongprachug *et al*. (2019)
Polyomavirus	HPyVs (JCVs and BKVs)	OPyVs (opine), BPyVs (bovine)	Ahmed and Harwood (2017)

The properties and pro/cons of the different microbial indicators have been reviewed extensively by several authors (e.g. Korajkic *et al*. (2018), Ahmed *et al*. (2017), Saxena (2015) and Ashbolt (2001)).

7.4 CONCLUSIONS

- There are many approaches to testing for pathogen presence and enumeration of microbial indicator organisms in water and similarly many individual test methods have evolved based on common basic principles. The choice of test method should take into consideration the purpose of testing, as well as a number of practical factors e.g. facilities and technical expertise available, ease of application, affordability and availability of test materials and equipment.
- Culture-based methods are generally considered the standard methods for the detection and identification of bacterial pathogens and for the enumeration of bacterial indicators. They are limited to the detection of bacterial hazards and require laboratory facilities, technical expertise and a quality control program to ensure reliability. Field test systems are available for simple and rapid indicator tests.
- Non-culture-based methods are available for a wider range of microorganisms and are increasingly used for both culturable and non-culturable microorganisms and for both detection and enumeration and for the presence of specific traits e.g. virulence and antimicrobial resistance genes.
- Non-culture-based methods vary widely (e.g. from PCR to WGS) in their requirements for special technical expertise, the need for specialised equipment and in the cost. Examples are various PCR based methods, genome sequencing, immunoassays, micro-assays, flow cytometry and biosensors.
- Microbial source tracking (MST) markers can assist in identifying of the source(s) of faecal pollution in water. Host sensitivity and specificity are the key criteria in evaluating the suitability of markers for source tracking.
- New and novel approaches to test methods continue to evolve with the aim to improve the performance of the tests, to make them more practical and affordable and to provide results in real time. Regulatory approval is required for the use of test methods not approved in regulatory standards.

8

Microbial monitoring of water quality

Monitoring is an essential activity in both food safety RM programs including PRPs and HACCP systems in food production (FAO and WHO, 2020) and WSPs in drinking water supplies (WHO, 2017a) and water use in agriculture (WHO, 2006a). Monitoring includes a planned sequence of observations or measurements of control parameters to assess whether a risk reduction measure is under control (Annex 1).

The WHO provides guidelines for monitoring of drinking water to ensure that safety compliance is maintained throughout the water supply system (WHO, 2017a). In the WHO guidelines for the use of various water types in agriculture (WHO, 2006a) monitoring is described as having three different purposes used at different times: validation, operational monitoring and verification (WHO, 2017c). As the CAC guidelines for FFV do not provide specific guidance on monitoring, the WHO guidelines could provide an approach to the management of the quality of water inputs into FFV production.

8.1 VALIDATION

Validation of a control process is defined by Codex as "evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome" (FAO and WHO, 2020). WHO (2017a) describes validation in the drinking water quality guidelines as:

> Validation is an investigative activity to identify the effectiveness of a control measure. It is typically an intensive activity when a system is initially constructed or rehabilitated. It provides information on reliably achievable water quality in preference to assumed values and also to define the operational criteria required to ensure that the control measure contributes to effective control of hazards.

Validation is a means for obtaining evidence on how control measures are performing; therefore, it has to be based on accurate and reliable technical information. Validation approaches are adapted to a particular control measure under normal and exceptional circumstances and include site inspections, using existing data and literature or targeted monitoring programmes (WHO, 2017a).

The reason treatment processes are validated is to show that the processes can operate as expected and achieve required levels of microbial hazard reduction. Validation can be undertaken at different stages of the FFV production chain, based on the fit-for-purpose water use approach.

Periodic checking of health outcomes in the worker populations or professionals involved in treatment processes and populations consuming the product is also useful e.g. measurement of health outcomes for each of the treatments.

Points to consider:

- Validation is not carried out daily in the operation of the water supply system.
- Existing data should be assessed thoroughly to understand the water supply systems that are in operation.
- Specific conditions/requirements and hazard agents/indicators in the water supply system should be well understood.
- Validation should not be confused with operational monitoring.
- Validation can lead to systematic improvements of the water supply.

8.2 OPERATIONAL MONITORING

Operational monitoring in drinking water quality management is defined by WHO (2017a) as:

> a planned and routine set of activities used to determine that control measures continue to work effectively.

Where water is an input to FFV production, routine monitoring of the input water supply and the water quality is required to ensure the water does not compromise

the overall safety of the FFV at the point of consumption i.e. it is fit-for-purpose. RAs provide direction on the risk reduction measures and the level of risk reduction required and will be specific to a FFV production chain (Chapter 4). A manager will have to assess how the entire input water supply system operates and determine the appropriate control measures.

Some points to consider include the following (WHO, 2017a):

- Determining the control measures requires a sufficient understanding of the water supply and the water use in FFV production, the control measures and the likelihood and consequences of loss of control of microbial hazards at specific points in the chain. They can include observational activities (site inspection) or measurements of parameters (e.g. sanitiser and disinfectant levels, pH, turbidity etc.).
- Operational monitoring parameters should indicate the effectiveness of control measures at the CP and assess performance with sufficient frequency to identify failures of the measures in a timely manner to allow a rapid response. Examples of operational monitoring parameters are provided by WHO (WHO, 2017a, 2009).
- Testing for enteric pathogens or microbial indicators can be of limited use for operational monitoring when the time taken for the analysis does not allow corrective action to be taken before use of the water supply. The indicator organism or pathogen has to be selected carefully, as only a limited number of tests can be performed during routine programs.
- Control measures require operational monitoring and a plan for remedial action if improvement is required. The plan should detail how to improve performance, the acceptability for human consumption of FFV that were treated with water that was not fit-for-purpose, and additional risk reduction measures (e.g. thermal treatment/cooking) if required for safety of FFV at consumption.

8.3 VERIFICATION

In food safety RM, verification is an activity in the PRPs and a HACCP system-based food safety plan where methods, procedures, tests and other evaluations, are applied in addition to monitoring to determine compliance (FAO and WHO, 2020). Verification in drinking water quality management similarly provides a final check on the performance of the water supply system and that the water quality is meeting the required health targets (WHO, 2017a).

WHO (2017a) states:

> In addition to operational monitoring of the performance of the individual components of a drinking-water system, it is necessary to undertake final verification for reassurance that the system as a whole is operating safely. Verification may be undertaken by the supplier, by an independent authority or by a combination of these, depending on the administrative regime in a given country. It typically includes testing for faecal indicator organisms and hazardous chemicals, as well as auditing that WSPs are being implemented as intended and are working effectively.

Water with a range of qualities may be fit-for-purpose at individual steps and CPs in FFV production chains. Municipal suppliers of potable water would be expected to comply with the WHO specifications (WHO, 2017a). For all other water in contact with edible portions of the FFV, verification is required that the water quality is within limits defined as fit-for-purpose at a CPs and that the WSPs are working effectively.

Points to consider when verifying microbial water quality:

- Selection of indicator organism (see also Chapter 6). *E. coli* may not always be the best indicator as new studies show that, despite the low concentration of *E. coli*, source waters may contain pathogenic enteric viruses and bacteriophages may be more reliable indicators.
- Selection of tests for the types of indicator organisms (Chapter 7). These could be based on culture methods to non-culture gene-based (e.g. PCR) techniques.
- Designing a sampling regimen and its periodicity. These should be feasible and fit the budget of the water suppliers and FFV production managers.
- The tests have to be validated and appropriate for the local settings.

The importance of verification that water used if FFV production is illustrated in an investigation of an *E. coli* O157:H7 outbreak in 2018 linked with consumption of romaine lettuce in multiple American states and Canadian provinces (USA FDA, 2019).

The results of the FDA investigation included:

- **On farm**: *E. coli* O157:H7 was detected in one sediment sample from an on-farm water reservoir. Using WGS, the isolate was indistinguishable from the outbreak strain, indicating that the outbreak strain was present in the water of this on-farm reservoir.

- **Lack of water treatment verification**: The farm treated the agricultural water with a sanitizer before use and had a procedure for periodical analysis of reservoir agricultural water including generic *E. coli* as a microbial indicator for treatment efficacy. It was found that the verification of the water sanitizer concentration at levels to ensure the safety of the water used in direct contact with the romaine lettuce at harvest, during postharvest handling, and to wash/rinse harvest equipment food contact surfaces, was not implemented and recorded.
  - > This was a critical mistake as the water tank sanitizer treatment systems had undissolved sanitizer cakes and the optimal sanitizer treatment of the agricultural water was likely affected.
  - > Hence, untreated water from the contaminated reservoir was used in the harvest/postharvest handling. It was also used for spraying roads for dust abatement and these roads were trafficked by harvest equipment prior to commencing the harvesting operations. Again, another factor that can contribute to transmitting the pathogen to the lettuce.

The investigators found untreated water used for irrigation and post-harvest activities was the probable source of the lettuce contamination. They emphasized that water treatment verification procedures and record-keeping are important management components in preventing hazards and in ensuring that the water used in direct contact with produce and equipment food contact surfaces is not contaminated with pathogens. These include periodically checking and assessing that the concentration of the sanitizer is appropriate and the treatment procedures are correctly implemented.

9

Fit-for-purpose water, knowledge gaps and limitations

The purpose of JEMRA meeting on water use in fresh fruits and vegetables convened on 23-27 September 2019, in Geneva, Switzerland was to develop clear and practical guidance on the criteria and parameters that can be used to determine if water is 'fit-for-purpose' for use in the pre- and post-harvest production of FFV. Practical interventions that could be applied pre- and post-harvest to mitigate food safety risk when water does not meet the requirement of fit-for-purpose were also considered. When addressing the meeting objectives, many gaps in relevant scientific knowledge, a lack of data to underpin a risk-based approach and limitations of existing tools for determining water quality were found. Additional data on following specific topics will allow for the development of more robust RA and more precise recommendations for microbiological criteria of water used in FFV production:

- Data of water contamination used for FFV production and processing, especially in Low and Middle Income Countries.
- Epidemiological data on human diseases associated with FFV consumption in various countries.
- Dose-response relationships and the impact of immune status (population-specific) on dose-response from consuming contaminated vegetables and becoming ill.
- Data on pathogen transmission routes in the FFV chain; for example, additional data to support the on the association between microbiological quality of and the microbiological quality of irrigation water, including the role of water in contaminating FFV with antimicrobial resistant bacteria.

- Better indicators for water contamination with waterborne viruses, parasites and helminths.
- Impacts of the contact between water and FFV and the contact duration on the subsequent safety of the FFV
- More precise source tracking tools and associated global databases.
- Assessment of irrigation water interventions and controls on farms, especially those applicable to low resource settings.
- Data on the survival of various pathogens under real-world water quality conditions to support lab-based observations.
- Increase community empowerment and partnerships that support irrigation water management.
- Improved education and training for different stakeholders on irrigation and water quality management.

10

Conclusions

Key conclusions drawn by the Expert Group for the meeting objectives are summarized below.

- The risk of pathogen contamination of FFV via water can be reduced by adopting a PRPs or a HACCP-based risk management system (FAO and WHO, 2020) including the use of fit-for-purpose water in both primary production and food processing. WSPs (WHO, 2017a) are an effective risk management approach to ensure the safety of the water that could be used in the production of FFV, particularly for small producers, through identifying the water contamination pathways and establishing appropriate control measures.
- Key factors to consider in the assessment of the microbiological quality of water in the safe production and processing of FFV include: the sources of available water, potential source contamination and risk factors, how water is applied and used and at what step in the chain, the type of FFV and any further microbial inactivation steps applied after water use and before consumption of the final product. These factors are dynamic and may vary over time and space due to cultural, climatic and other factors.
- The establishment of microbiological food safety metrics for water used for the safe production of FFV should be risk-based, taking into account:
    - water availability and whether it is fit-for-purpose at the production/processing step where used, including the potential and extent of intentional or non-intentional food-water contact;

  - > the type of FFV and any specific characteristics (e.g. leafy vegetables, netted rind melons), the production system (e.g. root or row crop, vine/tree, hydroponic), whether they are usually eaten raw or can be cooked, peeled or unpeeled;
  - > retention levels and timing of contact of water with the edible portions of the product prior to consumption;
  - > potential for decline or proliferation of pathogens and cross- or re-contamination after each water contact;
  - > inclusion of the whole food chain from farm to consumption when managing food safety risk;
- Fit-for-purpose water is a relative concept. At each successive step from the growing stage up to the point of consumption, the microbiological quality/safety of the water used at that step should be of higher quality than that used at the previous step or at least of equal quality. An exception is where there is a subsequent validated pathogen reduction treatment (removal or inactivation / kill) before consumption of the final product. Without such treatment, potable water is required in the final steps where water contacts edible parts of FFV.
- Any water, even that which has been conventionally treated and disinfected, may potentially contain human pathogens albeit at low concentrations. A risk assessment appropriate for the national or local FFV production context should be conducted to assess the potential risks associated with the use of a specific water source or supply and to determine appropriate risk mitigation strategies.
- Risk assessments can make use of a number of qualitative and quantitative water quality variables when assessing water for health risks. QMRAs are pathogen- and scenario-specific and depend upon the availability of data (at least estimates) on pathogen prevalence or alternatively, on the use of a ratio of pathogens / indicator organism concentrations. Data available for input into QMRAs can be limited and often generated in specific settings, resulting in large uncertainties and important limitations in the QMRA. One alternative is the use of the indirect measurement of the concentration of microorganisms indicative of the presence of faecal contamination, often referred to as "indicators". In this case, the QMRA is replaced by a quantitative microbial exposure assessment
- Scientific evidence that should be considered when choosing whether to include microbial indicators as RA inputs or when choosing specific indicators and appropriate thresholds levels, include:
  - > No one water quality indicator is appropriate/useful for all water types and should be selected based on the purpose and information needed.
  - > At present, there is no reliable microbiological indicator/proxy that can reliably predict pathogen occurrence or numbers because bacterial

indicators are typically surrogate measures of faecal pollution rather than measures of pathogens themselves. It is not possible, with the use of indicators, to predict the presence or concentrations of specific pathogens in the contaminating water.

> It is generally agreed that indicators of faecal contamination have been useful for monitoring water quality, in particular *E. coli* and intestinal enterococci have both been widely adopted.

> Bacteriophages, especially male-specific coliphages and *Bacteroides* phages and more recently cross-assembly or crAssphages, were found to be effective predictors of human faecal contamination. They can also be useful for verification and validation of virus-reduction treatments. However, although coliphages have been shown to better correlate with the presence of viral pathogens compared to the FIB in polluted waters they cannot be absolutely relied upon as a unique indicator for enteric viruses.

> There are currently no meaningful indicators for parasites in source water (e.g. protozoa and cestodes, helminths); however, sulphite-reducing spores of clostridia and of aerobic spore -forming bacteria, may be used for determining the effectiveness of parasite reduction treatments.

> Correlation between indicator organisms and pathogens is stronger in heavily polluted waters, but this correlation is insignificant and biologically uninformative when pollution levels are low.

> In many cases faecal indicators have a dual function acting both as faecal indicators and as process indicators that are used to validate the water disinfection treatments.

- In the processing of FFV, the presence of faecal indicator organisms is assumed to indicate unhygienic working conditions, faecal pollution of water, or failures in control measures.
- Microbiological testing of water has a role in initial water quality and environmental assessment and in verification, validation and monitoring during production and processing. It can be used together with other non-microbiological process parameters.
- Multiple analytical methods are available to assess the degree of microbiological contamination of water involved in the production of FFV. The choice of microbiological assessment methods for water quality should be based on validated test methods, the capacity and resources available. It is recognized that in some situations water testing may not yet be feasible and hence source water quality is highly uncertain. In such scenarios, conservative assumptions should be made and a simple RA applied, until more data become available.
- Sampling plans for microbiological targets used to determine water quality, including pathogen detection or concentration of microbiological indicators,

should be based on RA and RM goals. For example, baseline water quality assessment, validation of abatement technology and verification may require different parameters suited to their different goals.

- QMRA is a valuable tool for establishing tailored water quality criteria based on health targets and/or process criteria for FFV. Existing guidelines provide templates for how to carry out the calculations, based on either established health targets or assumptions. However, appropriate data are needed to conduct a QMRA. A QMRA cannot be based on microbial indicator concentrations only, it requires pathogen measurements or assumptions on their occurrence and levels. Exposure assessment can also be used as a basis to develop water quality standards, at least as an initial step, where no applicable quality targets have been established and no reliable dose-response relationship is available. Exposure assessments based on the demonstrated association of concentration of indicator microorganisms and the presence/absence of a specific pathogen is a suitable approach.
- Each country has individual characteristics that preclude generalisation of water quality targets in food production and processing compared with drinking water supplies e.g. varying environmental and sociocultural conditions among countries, both national and local/traditional practices in food production, different supply chain dynamics, individual national regulations and levels of oversight, and the diverse exposure levels and pathways of contaminants in the water to food vary among the countries and regions.
- For application of a fit-for-purpose concept to be successful in producing safe FFV, the risk management systems and control measures applied along the value chain pathway must be complementary, stringent and followed at all times. Water quality criteria for use in FFV supply chains should be established within the framework of national food and water regulations and guidelines and take into consideration local resources, infrastructure and capability, etc..

11

Recommendations

- Metrics for the safety of FFV and for water used in FFV production are context-specific. General concepts for the safety metrics should be considered at a country level and acceptable thresholds values would be further tailored based on national epidemiological evidence and health risks, scientific evidence and studies of the microbial hazards in individual FFV production chains.
- Water quality indicators, such as *E. coli* and intestinal enterococci, are suitable indicators for the presence of recent faecal contamination of water, but are not reliable indicators of (i.e., do not predict) the presence of non-faecal foodborne pathogens linked with FFV (e.g. some viruses and parasites).
- Process indicators, such as *E. coli*, are reliable indicators for monitoring the pathogen reduction measures, including non-faecal foodborne pathogens.
- There is a need for inexpensive, specific, sensitive and rapid tests that would indicate the presence and concentration of microbial hazards in water used in food production.
- Setting safety metrics in a comprehensive risk management program can be challenging when there is a scarcity of information available for this purpose e.g. in developing countries.
- Related recommendations from the previous JEMRA report (FAO and WHO, 2019) may be useful to overcome these challenges in countries that do not have national guidelines and relevant data. Piloting of the decision trees in local contexts across regions is recommended.

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Annexes

Annex 1

Comparison of terms used in management of the microbiological safety of food and water

Risk management

Codex food safety risk management	WHO guidelines for drinking water quality
Appropriate level of sanitary protection (ALOP): the level of protection deemed appropriate by the country establishing a sanitary measure to protect human life or health within its territory. (This concept may otherwise be referred to as the "acceptable level of risk") (FAO and WHO, 2008).	**Health outcome target**: defined tolerable burden of disease. High-level policy target set at national level, used to inform derivation of performance, water quality and specified technology targets (WHO, 2017). WHO guideline defines a tolerable burden of disease of 10^{-6} DALY per person per year (WHO, 2017).
Food quality: a much broader concept that is related to consumers' needs or expectations, it can both objective and subjective, including elements such as food safety, nutritional quality, environmental preservation, geographical origin, local traditions, ethical and social quality, animal welfare, etc. (FAO, 2021).	**Drinking water quality**: is referred to in relation to guideline target values that are used to protect or improve drinking water quality and therefore human health (WHO, 2017).
Food safety: the assurance that food will not cause adverse health effects to the consumer when it is prepared and/or eaten according to its intended use (FAO and WHO, 2020).	**Safe drinking-water:** as defined by the WHO guidelines, does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages (WHO, 2017).
Food safety objective (FSO): the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP) (FAO and WHO, 2018).	**Health-based target:** measurable health, water quality or performance objectives that are established based on a judgement of safety and on risk assessments of waterborne hazards (WHO, 2017). WHO (2017) describes four distinct types of health-based targets, applicable to all types of hazards and water supplies: 1) health outcome targets; 2) water quality targets; 3) performance targets; 4) specified technology targets.
Microbiological criterion: a MC is a risk management metric which indicates the acceptability of a food, or the performance of either a process or a food safety control system following the outcome of sampling and testing for microorganisms, their toxins/metabolites or markers associated with pathogenicity or other traits at a specified point of the food chain (e.g., the microbiological limit associated with a 2-class sampling plan) (FAO and WHO, 2013b).	

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Codex food safety risk management	WHO guidelines for drinking water quality
Monitoring: term used in Microbial risk management (MRM) and HACCP systems In MRM: an essential MRM process including the on-going gathering, analysing and interpreting of data related to the performance of food safety control systems, which, in this context is referred to as monitoring. Monitoring is essential to establish a baseline for comparing the effectiveness of new MRM activities. It also may provide information which the manager may use to determine what steps may be taken to achieve further improvements in the extent or efficiency of risk mitigation and public health. Risk management programs should strive for continual improvement in public health. (FAO and WHO, 2013a). Monitoring activities can include the collection and analysis of data derived from: • surveillance of clinical diseases in humans, as well as diseases in plants and animals that can affect humans; • epidemiological investigations of outbreaks and other special studies; • surveillance based on laboratory tests of pathogens isolated from humans, plants, animals, foods and food processing environments for pertinent foodborne hazards; • data on environmental hygiene practices and procedures; • behavioural risk factor surveillance of food worker and consumer habits and practices. In HACCP systems: the act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control (FAO and WHO, 2020).	**WHO microbial monitoring** (WHO, 2017)**:** Microbial monitoring can be undertaken for a range of purposes, including: • validation • operational monitoring • verification • surveillance • source water monitoring for identifying performance targets • collecting data for QMRA the supporting document. **Operational monitoring:** operational monitoring is the conduct of planned observations or measurements to assess whether the control measures in a drinking-water system are operating properly (WHO, 2017).
Performance objective (PO): the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable (FAO and WHO, 2018).	**Water quality targets:** guideline values: Chemical hazards; based on individual chemical risk assessments. Microbial water quality targets are not normally applied ; *Escherichia coli* is used as an indicator of faecal contamination and to verify water quality (WHO, 2017).
Performance criterion (PC): the effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO (FAO and WHO, 2018)	**Performance target:** specified removal of hazards. Microbial hazards (expressed as log reductions). Specific targets set by water supplier based on quantitative microbial risk assessment and health outcome targets or generic targets set at national level. Chemical hazards (expressed as percentage removal). Specific targets set by water supplier based on chemical guideline values or generic targets set at national level WHO, 2017, 2016).

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Codex food safety risk management	WHO guidelines for drinking water quality
Risk analysis: an overarching framework for managing food related risks to human health. A process consisting of three components: risk assessment, risk management and risk communication (FAO and WHO, 2007, 2018). **Risk assessment:** a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment and (iv) risk characterization (FAO and WHO, 2018). It should be based on scientific data and take a whole of food chain approach (FAO and WHO, 2007, 2018). **Risk management:** The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options (FAO and WHO, 2018). **Risk communication:** The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions (FAO and WHO, 2018). **Hazard analysis and critical control point (HACCP) plan:** Documentation or set of documents, prepared in accordance with the principles of HACCP to ensure control of significant hazards in the food business (FAO and WHO, 2020). **Hazard analysis and critical control point (HACCP) system:** The development of a HACCP plan and the implementation of the procedures in accordance with that plan (FAO and WHO, 2020).	**Water safety Plan (WSP):** a comprehensive risk assessment and risk management approach that encompasses all steps in water supply from catchment to consumer. It draws on many of the principles and concepts from other risk management approaches, in particular the multiple-barrier approach and HACCP (as used in the food industry) (WHO, 2017).
Validation: obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome (FAO and WHO, 2020).	**Validation:** is concerned with obtaining evidence on the performance of control measures (WHO, 2017).
Verification: the application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended (FAO and WHO, 2020).	**Verification:** provides a final check on the overall performance of the drinking-water supply chain and the safety of drinking-water being supplied to consumers (WHO, 2017).

Risk assessment steps

Note: aligning these steps in the columns and in numbering does not imply they are equivalent

Codex microbiological Risk assessment guidelines (FAO and WHO, 2014)	WHO harmonised QMRA framework (WHO, 2016)
1. **Hazard identification:** the identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.	1. **Problem formulation:** The overall context (reference pathogens, exposure pathways, hazardous events and health outcomes of interest) of the risk assessment is defined and constrained in order to successfully target the specific risk management question that must be addressed.
2. **Hazard characterisation:** the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents, which may be present in food. For the purpose of Microbiological Risk Assessment the concerns relate to microorganisms and/or their toxins.	2. **Health effects assessment:** Dose–response relationships (linking exposure dose to probability of infection or illness) and probability of morbidity and mortality (depending on the health end-point of the assessment) are identified for each reference pathogen.
3. **Exposure assessment:** the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant	3. **Exposure assessment**: The magnitude and frequency of exposure to each reference pathogen via the identified exposure pathway(s) and hazardous events are quantified.
4. **Risk characterisation:** the process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on 1-3.	4. **Risk characterisation:** The information on 2 and 3 are combined to generate a quantitative measure of risk.

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Annex 2

Comparison of risk assessment approaches for water quality management

Information in the tables is based WHO Quantitative microbial risk assessment Application for water safety management, 2016.
(available at https://www.who.int/publications/i/item/9789241565370).

Risk assessment approach	Features	Examples of applications	Strengths	Weaknesses	Level of complexity	Expertise required	Resources required	Costs
Qualitative risk assessment (Sanitation inspection)	Descriptive RA; assists with identification of most important hazards, contamination pathways and control options in a location. Inspection can include a checklist of risk factors used to calculate a risk score and classification. Value can be increased by combining risk score/ classification with water quality monitoring results e.g. *E. coli* count.	Suited to small water supplies. Used to inform more complex RAs and regional and national priorities. Can be used in surveillance programs.	Simple, requiring minimal resources. Valuable for small water supply systems.	Based on limited visual information and does not account for variability in conditions and practices. No prioritisation of risk factors.	Lowest. Simple. Can be based on site visit, qualitative information and observational survey.	Lowest. Sanitary inspectors and/ or community members with relevant experience/ knowledge.	Few, Site visit. Can be based on standardised WHO forms/ checklists modified for local context.	Lowest
Semi-quantitative risk assessment (Risk matrix)	Quantitative or semi-quantitative risk evaluation combining likelihood of a hazard occurring and severity of the consequences.	Used to evaluate range of risks to water quality.	More comprehensive; based on more extensive information on hazards and hazardous events. Provides a risk score or rating.	Relies on expert judgement and can be subjective.	Medium	Medium. Requires expertise in broad water management skills area and sound judgement. Can be supported by reference databases and tools.	Databases/ historical records of frequency of hazardous events and severity of hazards.	Medium
Quantitative risk assessment	Systematic process based on scientific data and statistical inference with a numerical output.	Used for evaluation of risk management priorities or control strategies.	Precise, evidence-based, objective and transparent approach. Assumptions, uncertainties and variability can be considered.	Limited data available on pathogens, their behaviour in water, in food production systems and in risk mitigation processes.	Highest	Highest; requires team with multiple skills e.g. risk management, technical knowledge and QMRA expertise.	As above plus quantitative data or assumptions on pathogen occurrence, exposure and health outcomes. Computerised analytical tools may be required.	Highest

Annex 3

Advantages and disadvantages of the different testing methods for microorganisms

Method	Advantages	Disadvantages	References
Culture-based methods			
Total viable counts	• Easy to apply • International standard methods available	• Expensive • Timely • Non-specific	
Enrichment	• Amplifies target microorganism • Recovery of injured cells	• Costly • Timely • Loss of quantitative measurement, except for most probable number (MPN) methods.	
Non-culture-based methods			
Polymerase chain reaction (PCR)	• High specificity and sensitivity • Reliability • Can be automated	• Can detect viable and non-viable cells • Sensitive to PCR inhibitors • Requires effective primer design • False-positive results (very sensitive to contamination) • Requires post-PCR procedures, e.g. gel electrophoresis	Mandal *et al.*, 2011, Park *et al.*, 2014, Zhang, 2013
Multiplex PCR (mPCR)	• High specificity and sensitivity • Reliable • Detect multiple (≥ 5) targets/ reaction • Can be automated • Saves time	• Same as PCR (above) • Optimization and troubleshooting more difficult than PCR	Chen *et al.*, 2012 Mandal *et al.*, 2011, Park *et al.*, 2014, Zhang, 2013,
Real-time/ quantitative PCR (qPCR)	• High specificity and sensitivity • Reliable • Automated • Real-time monitoring • High throughput analysis	• As above • Availability of reagents • High operational cost • Equipment & replacement costs • Equipment maintenance • Need for standardization	Mandal *et al.*, 2011, Park *et al.*, 2014, Zhang, 2013

(cont.)

Method	Advantages	Disadvantages	References
Digital droplet PCR ddPCR	• High specificity and sensitivity • Reliable • No PCR product visualization • High throughput analysis • Simplified quantification approach	• Sensitive to PCR inhibitors • Highly skilled operational personnel • Potential for cross-contamination bigger • Costly equipment & replacement costs • Equipment maintenance	Hindson *et al.*, 2011
Nucleic acid sequence-based amplification (NASBA)	• Distinguishable non-viable cells • High throughput analysis • Sensitive and specific • Low cost • No thermal cycling required	• Will not detect non-viable organisms (evidence of past contamination or effective disinfection)	Zhao *et al.*, 2014, Simpkins *et al.*, 2000
Loop-mediated isothermal amplification (LAMP)	• High specificity and sensitivity • Low cost • Easy operation • No thermal cycling required	• Complicated primer design • Additional confirmation steps recommended (Amplified targets should sequenced	Zhao *et al.*, 2014
Oligonucleotide DNA microarray	• High throughput analysis • Cost-effective • Detection of multiple pathogens • Detection of specific serotype	• Difficult to distinguish between viable and non-viable cells • Skilled personnel required • Labeling of target genes required • Oligonucleotide probes required • Less sensitive than qPCR • High initial equipment costs & maintenance	Mandal *et al.*, 2011, Park *et al.*, 2014
Flow Cytometry	• Automated and high throughput • Can be real-time • Quantitative	• High cost • Requires label for non-auto-fluorescing cells • High initial equipment costs & maintenance	Van Nevel *et al.* 2017, Wang *et a*l. 2010
Biosensor-based methods			
Optical biosensors	• Easy to operate • Sensitive • Absence of reagents • Detection in real-time / near real-time • No sample pre-enrichment • Automated and rapid throughput	• High cost • Shelf life of reagents	Taylor *et al.*, 2006, Zhang, 2013
Mass-based biosensors	• Easy to operate • Cost-effective • Real-time detection • Absence of reagents • Does not require sample pre-enrichment	• Lower specificity / sensitivity • Multiple washing and drying steps • Long incubation period • Crystal surface regeneration potentially problematic	Mandal *et al.*, 2011 Zhang, 2013
Electrochemical biosensors	• Easy to operate • Absence of reagents • Capacity to handle large number of samples • Automated • No sample pre-enrichment	• Low specificity • Laborious • Large number of microorganisms required in sample • Food matrices may interfere with analysis	Zhang, 2013

(cont.)

Method	Advantages	Disadvantages	References
Sequencing			
Pyro sequencing	• High throughput metagenomics	• Indicates microbial presence not activity or viability. • However, presence of target DNA implies activity at some point.	Higgins *et al*., 2018
Whole genome sequencing	• High specific	• Requires data analyses, bioinformatic expertise / knowledge • Costly	Moran-Gilad, 2017
Illumina sequencing	• High specificity and sensitivity • Sequencing across a broad array of applications in genomics, transcriptomics and epigenomics.	• Require high level of technical input and bioinformatics expertise • Costly • Sample loading challenges resulting in overlapping clusters and poor sequence quality and sequence complexity requisites have been reported to impede absolute success of NGS platforms	Slatko, Gardner and Ausubel, 2018
Immunological based methods			
Enzyme-linked immunosorbent assay (ELISA)	• Specificity for certain serovars • Bacterial toxin detection • Can be automated • Time-efficient • Suitable for high throughput	• Low sensitivity • Cross-reactivity may occur in closely related antigens leading to false positive results • Pre-enrichment required • Skilled personnel required • Antibody or antigen labeling required	Zhang, 2013, Zhao *et al.*, 2014
Lateral flow immunoassay	• Easy to use • High specificity and sensitivity • Bacterial toxin detection • Low cost	• Antibody or antigen labelling required	Zhao *et al.*, 2014
Other methods and technologies			
Denaturing Gel Gradient Gel Electrophoresis	• Can be used to study diversity • Effective to study pathogen activity	• Require technical input in running system • sensitivity of next generation profiling techniques supersedes that of DGGE	Ercolini, 2004
MALDI-TOF MS	• Automated, high throughput • Fast • Inexpensive once established	• Initial cost expensive	Jadhav *et al*., 2018

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Annex 4

Example from literature

The following examples have been constructed from peer-reviewed publications and serve to contextualize specific aspects of MRA in an effort to guide the reader how the work published here can be applied in practice, and to illustrate the role of water management in the microbiological contamination of fruits and vegetables. The reader is reminded that some of the examples make reference to specific national and value chain contexts. The data presented here may not be suitable to generalize the findings beyond the context of the example given.

A4.1 SELECTED FRESH LEAFY VEGETABLES AND HERBS EATEN RAW

A4.1.1 Ghana (lettuce)

A4.1.1.1 Background

Amoah *et al* (2007), in providing background for their case study, described Ghana is "a typical low-income sub-Saharan African country facing significant sanitation challenges. In Ghana, fresh salads are not part of the normal diet, but have become a common supplement to urban fast food served in streets, canteens and restaurants. In Accra, about 200 000 people consume such supplements every day" putting many segments of the population at risk, including all income classes, adults and children alike. Studies are described that were carried out to address this public health issue.

A4.1.1.2 Evidence and data collection

Situation analysis of water use in lettuce production from farm to consumer

A QMRA study was undertaken (Amoah *et al*, 2007). Supporting data was gathered from a study in Accra and Kumasi to determine the extent of water pollution in urban and peri-urban areas where 95% of the lettuce consumed in the cities is produced, the agricultural practices used, and the risk groups. Over 12 months, 2003-2004, lettuce samples from the same production sites were tested along the "farm to fork" pathway for total faecal or thermotolerant coliforms (FC) and helminth egg counts. The irrigation water was drawn in Accra from drains and streams and in Kumasi from streams or shallow wells close to streams of shallow valleys. One or two sites used piped water over several years. The study revealed:

- All irrigation water sources sampled, except piped water, had FC counts exceeding the WHO recommended geometric mean count of $1x10^3$ CFU/100ml (Mitchell, 1992).
- There were no significant differences in FC counts on lettuce at different points from farm to retail (i.e. farm, wholesale and retail markets) and, irrespective of the irrigation water source, the mean FC counts exceeded recommended standards ($1x10^3$ CFU/100g wet weight). Some lettuces had higher FC counts during the rainy season though the water used varied between cities.
- The helminth egg counts on lettuce ranged from 1-6 eggs/100 g wet weight. Most samples irrigated with polluted water had higher helminth counts and counts on lettuce from the same original stock and irrigation water source did not significantly differ from farm to retail.

Amoah *et al* (2007) identified the farm as the main point of lettuce contamination with some correlation between the quality of irrigation water used and the lettuce contamination levels. Piped water (clean) irrigated lettuce had relatively lower pathogen levels that was possibly due to contamination of produce from already contaminated soil. Despite poor sanitary conditions in markets, post-harvest handling and marketing did not further increase the farmgate contamination levels.

QMRA models for irrigation water and lettuce production

Using the above data QMRA models were used to determine the risks of rotavirus (RV) and *Ascaris* infections for farmers and for consumers of irrigated lettuce with different water qualities after allowing for contamination in post-harvest handling (Seidu *et al*., 2008). To quantify the RV in the RA models, the authors used published reports of FC data in literature sources and converted them to RV counts using a ratio of 1 RV to 10^5 FC as applied by Shuval *et al.* (1997). The same approach was followed by Mara *et al.* (2007) but with an assumption of a ratio of 1 RV to 10^5 *E. coli*. A tolerable risk of infection of 7.7×10^{-4} and 1×10^{-2} per person per year were used for RV and *Ascaris* respectively (Keatinge *et al*., 2012).

The authors reported the median annual risk of *Ascaris* infection was $10^{-2} \pm 1 \log_{10}$ for farmers accidentally ingesting drain or stream irrigation water, 10^0 for farmers ingesting farm soil accidentally, and 100 for farmers ingesting any of the irrigation waters and contaminated soil. For farmers using piped water there was a very low risk (10^{-5}) of *Ascaris* infection. The annual risks of *Ascaris* and RV infections for consumers were 10^0 and 10^{-3} for drain and stream irrigated lettuce respectively, and there were slight increases for RV infections during post-harvest handling. For pipe-irrigated lettuce the risk of a RV infection was 10-4 and that did not change

during post-harvest handling. On-farm soil contamination was found to be the most significant health hazard.

Conclusions from QMRA

The study authors concluded that to mitigate health risks for farmers using different quality irrigation water and for consumers of irrigated lettuce, local guidelines for interventions were required. These interventions could be implemented in the short-medium-long terms and account for the different quantifiable tolerable risk levels associated with the reuse of irrigation water of different qualities for farmers and consumers. Establishing guidelines based on quantitatively verifiable health risks could also establish a productive relationship among the different stakeholders concerned with public health issues.

Risk factors for produce contamination and wastewater use

In 2012, Antwi-Agyei *et al.* (2015) conducted a study using a HACCP type approach to identify the key risk factors for produce contamination at different entry points of the food chain in the dry and wet seasons in Accra, Ghana. Lettuce, soil and irrigation water samples were collected from wastewater irrigated fields, while lettuce and cabbage were collected from local markets and ready-to-eat salad samples from restaurants. Samples collected were analysed for *E. coli* counts, human adenovirus (hAdv) and norovirus (NoV) genomes I and II. Critical exposures associated with microbial quality of produce were assessed by observations and interviews.

The following were the main findings for produce quality and infection risk associated with salad consumption:

- *E. coli*: > 80% samples were positive, median counts were 0.64 - 3.84 $\log_{10}$ CFU *E. coli*/g. Salads prepared at street vendors had the highest counts (4.23 $\log_{10}$ CFU *E. coli*/g) and consumption levels exceeded acceptable health limits.
- Median NoV infection risk for the consumption of 10–51 g of lettuce salad for 2–4 days per week varied across the different exposure models and ranged between 2.6 x 10^{-3} and 0.32 pppy and was highest with the street food salad model.
- Estimated infection risks from the irrigation water model, restaurant and street food salad models all exceeded WHO guidelines (Mitchell, 1992). Only the risks from the consumption of produce of average contamination levels at farms and markets were marginally within the acceptable NoV infection risks.
- Key risk factors for produce contamination were irrigation water and soil on farm, produce storage duration and temperature at markets and contact with dirty rinse water before sale.

The authors recommended produce safety risk factors should be addressed at all domains along the food chain while it would be more effective to focus on markets and kitchens as a priority due to cost, ease of implementation and health significance.

Helminths in wastewater for irrigation and soil

Amoah *et al* (2016) further assessed the occurrence of soil transmitted helminths both in wastewater used for irrigation and in the soil in Ghana by measuring the concentration of soil transmitted helminths in wastewater sourced irrigation water, the actual load of helminth ova in the stool of farmers and their family members and in a control group of non-farmers during a wet and a dry season. Ova of *Ascaris* spp. and hookworm were identified in both the irrigation water (1.38-2.05 hookworm eggs / L and 2.62-2.82 *Ascaris* eggs / L) and in soil (1.67-2.01 hookworm eggs / L and 2.90-3.70 *Ascaris* eggs / L). In general ova concentrations were higher in the wet season than the dry season for both irrigation water and soil samples.

The study results indicated a positive correlation between soil transmitted helminth concentrations in irrigation water/soil and soil transmitted helminth ova in the stools of the exposed farmer population. Farmers and family members exposed to irrigation water were three times more likely, as compared to the control group of non-farmers, to be infected with *Ascaris* (OR = 3.9, 95% CI, 1.15–13.86) and hookworm (OR = 3.07, 95% CI, 0.87–10.82). Also, higher odds of infection were identified during the wet season.

A4.1.1.3 Summary

This example demonstrates the development of a risk- and evidence-based food safety risk management (RA) strategy for irrigated lettuce and for occupational protection of farmers by implementation of an ongoing series of studies providing relevant data to support a QMRA and by identification of risk factors that were used to recommend context specific risk reduction measures.

A4.1.2 Egypt (lettuce)

A4.1.2.1 Background

Use of irrigation water contaminated with human faeces can result in the transfer of enteric viruses, (e.g. human adenovirus (hAdV), hepatitis A virus (HAV), Rotavirus (RV) group A and norviruses (NoVs)) to the soil and/or vegetables cultivated (Garcia *et al.* 2015). The crops may be contaminated also through poor hygiene practices of infected workers (Seymour and Appleton, 2001; Bosch *et al.*, 2011). Monitoring for the presence of faecal indicator organisms in agricultural

water is recommended as a measure of the potential risk of human faecal pathogen presence (Chapter 6). WHO (2017) recommends targeted surveys of specific pathogens be carried out for specific purposes such as outbreak investigations, research and watershed evaluations to target point sources of contamination, and to provide additional relevant information on faecal indicators bacteria (FIB) for use in RAs and in developing risk management (RM) strategies.

A4.1.2.2 Evidence and data collection

Survey of enteric viruses in FFV and irrigation water

Shaheen *et al.* (2019), tested for the presence of enteric viruses, hAdV, HAV, RV A and norovirus GI, in 128 fresh produce (green onion, leek, lettuce, watercress) samples and irrigation water samples used for these fresh produce varieties from two cities (Mansoura and Cairo) in Egypt, during September-December 2017, using qPCR. The survey results included:

Water samples (n=32).

- 27/32 (84.3%) were positive for at least one of the viruses.
- 30/32 (94%) were positive for hAdV with a mean viral load = 1.5×10^7 genome copies/L (GC/L). This was the most commonly detected virus in water samples, followed by RV group A (16/32, 50%, with a mean viral load = 2.7×10^5 GC/L), HAV (11/32, 34%, with a mean viral load = 1.2×10^4 GC /L) and NoV GI (10/32, 31%, with a mean viral load = 3.5×10^3 GC/L).

Fresh produce (n=128).

- 99/128 (77.3%) were positive for at least one of the viruses.
- 71/128 (56%) were positive for hAdV with a mean viral load = 9.8×10^5 GC/g). This was the most commonly detected virus in the fresh produce, followed by NoV GI (43/128, 34%, with a mean viral load = 4.5 × 103 GC/g), HAV virus (33/128, 26%, with a mean viral load = 6.4×10^3 GC/g) and RV group A (25/128, 20%, with a mean viral load = 1.5×10^4 GC/g).

A4.1.2.3 Conclusions

Shaheen *et al. (2019), concluded* the high prevalence of the viruses in both fresh produce and surface water samples may indicate a widespread circulation of these viruses among the Egyptian population. Furthermore, the occurrence of these viruses on fresh produce, which grows at ground level, may be a consequence of an irrigation process that uses contaminated surface water. Further investigation of these viruses in the population and food supply is indicated.

A4.1.3 Jordan (lettuce and tomatoes)

A4.1.3.1 Background

In Jordan, water has historically been scarce and this situation has been worsening with population growth and climate change (Halalsheh and Kassab, 2018). In agriculture, the reuse of treated wastewater has become an accepted practice to supplement the limited water resources available for use in food production. Microbiological contamination has occurred during the cultivation of FFV due to the use of effluent from wastewater treatment plants and due to agricultural drainage and runoff. This issue necessitated the need for implementation of a RM plan and required close coordination between different authorities and also stakeholders.

Supply of irrigation water is limited according to water end use and the crop type. The Jordan Ministry of Agriculture requires some constraints on the use of water and the Water Authority of Jordan limits use of water resources e.g. by issuing contracts by which treated effluent can be used only for irrigating fodder crops and fruit trees. Vegetables that are eaten raw are not allowed by law to be irrigated with treated wastewater effluent. More than 70% of treated effluent is being disinfected after secondary treatment.

The Jordanian Standard of 2006 (893/2006) covering wastewater use requires monitoring of the quality of treated effluent and it must conform to the standards corresponding to the water end use (Halalsheh and Kassab, 2018). The standard specifies water quality "tests must be performed by both the monitoring and operating entities according to the sampling frequency specified in the standards" and when deviations occur corrective action is required.

The microbiological tests currently used for assessing treated wastewater quality are *E. coli* counts (e.g. limits are set in relation to crops) and the presence/count of nematode eggs. The latter became an issue with the recent human displacement caused by political unrest in neighbouring countries and as displaced persons used raw wastewater for irrigating crops eaten raw. Consequently, a few samples collected from effluents of wastewater treatment plants at the beginning of the migration showed helminth eggs were in exceedance of allowable limits (Laboratories of the Water Authority of Jordan). Accordingly, the treatment plants were instructed to put maturation ponds in service for removal of helminth eggs.

Recently, an update of the Jordanian Standard 839/2006 is being drafted by the Jordan Standards and Metrology Organization (Halalsheh and Kassab, 2018) taking into account the WHO Guidelines (WHO, 2006). Irrigation water use is regulated regardless of the source and the level of crop restriction is based on irrigation water quality and the irrigation system. Irrigating vegetables eaten cooked with treated effluent is prohibited, no matter what treatment level and effluent quality.

A4.1.3.2 Evidence and data collection

Investigation has shown that disinfected effluent is being re-contaminated down-stream, mostly due to agricultural drainage and runoff from the catchment areas. Managing down-stream water necessitated the adoption of the WHO guidelines (2006) at the national level by establishing the new Jordanian Standard (1766/2014). It was shown after testing different types of crops including cabbage, zucchini, green pepper, tomato and lettuce that control measures can be established down-stream of wastewater treatment plants and can result in a reduction of pathogens in produce (Halalsheh *et al.,* 2008). Application of control measures such as drip irrigation systems, covering soil with plastic sheets and ceasing irrigation 2 to 3 days before harvesting crops was shown to be effective as a multi-barrier approach to reducing microbiological contamination of produce. For a crop such as lettuce, subsurface irrigation coupled with ceasing irrigation 2 days before harvesting was shown to be effective. It was also shown that contamination might occur during harvesting (Halalsheh *et al.*, 2018). Such measures were included in the Jordanian Standard (1766/2014); however, implementing the new standards was frozen due to the absence of a clear implementation plan i.e. a Sanitation Safety Plan.

A4.1.3.3 Summary

A series of round table discussions with decision makers from different relevant authorities and control groups were held in order to boost implementation of the new Jordanian Standard by defining responsibilities of each authority based on its mandate and the existing capacities (Halalsheh and Kassab, 2018). However, implementation is still pending due to a lack of required capacities and infrastructure, and due to the complexity related to the high number of small farms. The next steps will include:

- piloting a "safety management plan" at a catchment area and strengthening existing capacities,
- including the full production chain from farm to consumption and
- replacing Jordanian Standard 893/2006 with Jordanian Standard 1766/2014.

A4.1.4 Morocco (coriander)

A4.1.4.1 Background

In Morocco, the incidence of helminthiasis was reported to be higher in consumers of sewage-irrigated crops than in control groups and helminth eggs were detected in raw vegetables at markets (Hajjami *et al.*, 2013). In response, a study was conducted to assess the risks of wastewater reuse on the contamination of fresh vegetables with parasites (Hajjami *et al.*, 2013).

A4.1.4.2 Evidence and data collection

Raw and treated wastewater from two wastewater treatment plants (pond systems) were collected as well as crops (mint, coriander, alfalfa and cereals) and soil from nearby farmland where the crops were irrigated with treated wastewater from the plants. Samples were tested for the presence of helminth eggs. In addition, field experiments were conducted using freshwater, raw and treated wastewater from one wastewater treatment plant to irrigate coriander, parsley and radishes. The results were:

- Irrigation water: helminth eggs were found at mean concentrations of 8.98 eggs/L and 0.13 eggs/L in raw (n=60) and treated (n=60) wastewater samples, respectively. Fresh water samples (n = 16) were always negative for helminth eggs.
- Irrigated crops: fifty percent (35/69) of the wastewater irrigated crops from farmland were contaminated by helminth eggs, with an average concentration of 8.4 eggs/100 g.
- In the experimental study, helminth eggs (including *Taenia* sp., *Ascaris* sp., *Toxocara* sp. and *Strongyle* eggs) were found at mean concentrations of 35.62 eggs/100 g, 9.14 eggs/100 g on crops irrigated with raw wastewater and treated wastewater, respectively. No eggs were found on crops irrigated with freshwater.
  - The load of helminth eggs varied with the plant type and closeness with soil and contact of edible portions with irrigation water e.g. radishes, a root crop, had higher counts than those for the parsley and coriander growing above ground.
- Soil: the mean concentration of helminth eggs in soil obtained from fields irrigated with raw and treated wastewater was 2 eggs/10 g and 1.67 eggs/10 g, respectively, for pathogen helminth eggs and 2 eggs/10 g and 1 egg/10 g for *Strongyle* eggs, respectively.

A4.1.4.3 Conclusion

The authors concluded the agricultural reuse of untreated wastewater on raw vegetables presented a risk for human health both via irrigation and soil contamination of crops in the field. It was recommended the use of untreated wastewater should be prohibited.

Treated wastewater was also contaminated at levels that did not meet the WHO recommended standard of 1 helminth egg/L (Mitchell, 1992). It was recommended its use should be restricted and not used for irrigation of green leafy vegetables eaten raw and consumers should apply disinfection measures to reduce contamination levels on vegetables.

A4.1.5 Lebanon (lettuce, radish, parsley)

A4.1.5.1 Background

Lebanon is a country that is known to be rich in its natural water resources; however, water availability is diminishing given the effects of climate change and over-exploitation of underground water. Water is one of the main sources of microbial contamination for FFV. In Lebanon, most of the natural water resources are polluted by untreated wastewater and industrial effluent due to a lack of adequate water resources management policy, regulatory enforcement, and wastewater management (Faour-Klingbeil and Todd, 2019; Khatib *et al*., 2018; Houri and Jeblawi, 2007). For example, the Litani River, the major water source for farms in the most extensive Lebanese agricultural area, the Bekaa Valley, is known to be polluted with high levels of chemical and bacterial contamination. Farmers are also resorting to using untreated wastewater for irrigation to enhance agricultural production. Surveillance data on foodborne diseases that are linked with fresh produce are limited in Lebanon. Hence, this study described below of Faour-Klingbeil *et al* (2016) aimed to: (1) identify the risk factors and transmission routes of microbial hazards from the farm to the central FFV market in Beirut, (2) study the microbiological quality of vegetables and water used on-farm and post-harvest stages, and (3) estimate the relationship between the microbial quality of irrigation and post-harvest wash water and the microbiological quality of FFV traced to the markets.

A4.1.5.2 Evidence and data collection

Vegetables During the hot summer seasons between July–August 2013 and July 2014, leafy vegetables and radishes (n=90) were collected from ten major farms in the Bekaa Valley, two crop washing facilities and the wholesale market in Beirut, a major supply point of fresh raw vegetables for supermarkets, distributors,

groceries, and restaurants (Faour-Klingbeil *et al.*, 2016). Each type of vegetable was sampled from different points of the same growing field and tested for the presence of pathogens and hygiene indicator organisms, i.e., *S. aureus*, *Salmonella* spp., *L. monocytogenes*, total aerobic plate counts (TAPC), *E. coli* counts and TC.

Water For irrigation and wash water microbiological assessments, *E. coli* and TC were enumerated. Samples (n=5) were collected from different points of the crop washing ponds (1L in 250-ml samples) or from wells (1L bulk samples) and from streams (n=6, 100ml samples).

Water source Non-potable river water was used for irrigation and post-harvest washing on farms. In the summer when water sources were diminished, farmers used private wells for irrigation and filling the washing ponds. In some farms, raw sewage water was used both as irrigation and as a nutrient fertilizer for economic reasons.

Microbiological analysis of vegetables

- 62% samples had TAPCs above 6 log CFU/g, geometric mean 3.50 to 8.39 $\log_{10}$ CFU/g
- 69% had TC counts ≥ 5 log CFU/g), range 1.69 to 8.16 $\log_{10}$ CFU/g
- *E. coli* was detected in 45.5% samples, range < 0.7 to 7 $\log_{10}$ CFU/g
- *L. monocytogenes* was isolated from 20% of the samples in the fields and after washing, though decreased to 8% by the time the product reached the market.
- *Salmonella* spp. were detected in 6.7% samples from the post-harvest washing facilities overall.
- TC and *E. coli* counts on raw vegetables increased significantly from the farms to post-harvest washing and packing. A slight decrease in TC and *E. coli* counts was observed on samples from the market, these were still higher than counts at harvest.

Microbiological quality of irrigation and vegetable wash waters

- *E. coli* mean counts for well water and wash water samples ranged from <0.7 to 135 CFU/100ml and 15-140 CFU/100ml, respectively.
- TC counts were too numerous to count/100 ml.
- Water from wells and river streams used for post-harvest washing and crop irrigation in 5 farms each contained high levels of TC and *E. coli* >100 cells/100ml.
- On one farm, the wash water used in washing ponds and initially sourced from well water with no detectable TC and *E. coli* became contaminated to

levels similar to that of nearby river water. This indicates that inadequate controls of water quality, lack of treatment and water replenishment cycles allowed unacceptable environmental contamination on these farms.

Association between *E. coli* counts in agricultural and wash water, and on exposed vegetables

A significant association between *E. coli* counts on raw vegetables and the microbial quality of agricultural and wash water was reported. Regression analysis showed that *E. coli* counts on fresh produce increased by 0.799 for each CFU/100ml of water to which they were exposed on a farm. These data indicated that, at the same sampling locations, the microbiological quality of agricultural and wash water was a useful predictor of the *E. coli* contamination levels on fresh produce exposed to theses water sources.

A4.1.5.3 Conclusion

Faour-Klingbeil *et al* (2016) concluded the study findings demonstrated that the *E. coli* levels in wash water were a useful predictor of microbial contamination on washed vegetables sampled from the same areas. *E. coli* and TC counts increased as the vegetables moved along the supply chain from farm to market; the contamination with pathogenic microorganisms was evident at all stages of the food chain but to a larger extent at the post-harvest washing step.

They further concluded the findings emphasized the importance of conducting national risk assessment and mitigation strategies depending on the supply chain characteristics. There is a need for comprehensive solutions while addressing the economic hurdles and water scarcity by promoting appropriate wastewater treatment and management plans, vigilant sanitation measures and risk-based preventive controls to minimize the microbiological hazards that are known to be associated with the water used at each stage of the supply chain.

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A4.2 BERRIES

A4.2.1 Background

A variety of berries, both local and imported, have been associated with outbreaks of viral gastroenteritis in multiple countries worldwide (Palumbo *et al.*, 2013). In response, many countries have sought to establish RM strategies for the protection of consumers from viral illnesses transmitted by berries. Various activities have been undertaken in developing RM strategies and have included epidemiological investigations, observational and experimental studies and microbiological surveys to identify the source and transmission routes for the viruses and the control points. Risk mitigation strategies have involved management of water quality and use, among other control measures. Examples of the approach to the problem in different countries are provided.

A4.2.2 Evidence and data collection

A4.2.2.1 Europe

A multinational study was conducted to investigate contamination routes of berries with human enteric viruses in Europe (Kokkinos *et al., 2017; Maunula et al.*, 2013). Samples (n=785) were collected throughout the entire berry food chain in farms in Finland, Poland, Serbia, Czech Republic and included irrigation water, swabs from food handlers' hands, swabs from toilets on farms, animal faeces, conveyor belts in processing plants and strawberries/raspberries at point-of-sale from farms (Maunula *et al.*, 2013). RT-qPCR procedures for a panel of human and animal viruses were used to detect viral pathogens and for source tracking (see Chapter 7) as follows:

- human pathogenic viruses (norovirus, NoV GI, NoV GII and HAV),
- index viruses for human and animal source tracking hAdV, porcine adenovirus, (pAdV) and bovine polyomavirus, bPyV)) and,
- zoonotic viruses (hepatitis E virus, HEV).

The following results were reported.

Berry production

- NoV GII was detected in 3.6% (2/56) irrigation water samples (used for both spray- and drip-irrigation of berries); no HAV was detected in any sample.
- Source tracking. hAdV was detected in irrigation water (9.5%), on food handlers' hands (5.8%) andon toilets (9.1%) indicating human faecal sources.

Berry processing

- HEV was detected on frozen raspberries once (2.6%).
- Source tracking. hAdV was detected on one food handler's hands (2.0%) and in fresh raspberries (0.7%), frozen raspberries (3.2%) and fresh strawberries (2.0%) indicating human faecal sources.
- pAdV and bPyV were detected in one water sample and pAdV was detected in point-of-sale fresh berries (5.7%) and frozen berries (1.3%) indicating animal faecal sources.
- Food handler's hands were identified as another important source of contamination.

Irrigation water (n=108) was further studied in 5 berry fruit farms in Finland, the Czech Republic, Serbia and Poland (Kokkinos *et al.*, 2017). The following results were reported.

- NoV GII was detected in 3.6 %, (2/56). No HAV or HEV or NoV GI was detected.
- Evidence of human, 8.3% (9/108), porcine, 4.5% (4/89) and bovine, 1.1% (1/89 faecal contamination was detected.
- Farms included in the study used both ground and surface water as sources for irrigation, with groundwater being a frequent source of contamination (2/56).

Conclusions

The studies provided insights into the contamination of berries with viruses along the food chain, the potential point for contamination and the vehicles of transmission. Both irrigation water and food handler's hands were possible vehicles of transmission of pathogenic viruses during berry production and processing in these countries and source tracking provided useful additional evidence on whether potential faecal contamination was of human or animal origin and demonstrated the utility of this tool.

With regard to RM, the information resulted in recommendations that the Codex guidelines as well as regulations on the use of irrigation water of appropriate quality should be followed.

A4.2.2.2 Republic of Korea

Two studies from Republic of Korea are described where the prevalence of enteric viruses in FFVs and various potential field sources were monitored and correlation with viral and bacterial indicators of faecal contamination was assessed.

Study 1. Human enteric viruses were monitored in a range of FFVs, including strawberries and agricultural environmental samples (irrigation water, soil and worker's gloves) to provide data on their seasonal and geographical prevalence for development of RM strategies for their control (Shin *et al.*, 2019). The viruses tested included a suite of human enteric viral pathogens (NoV GI and GII, hAdV, astrovirus, RV, HAV) and a male-specific coliphage and hAdV were used as indicators of human faecal contamination (See Chapter 7).

Little contamination was found overall. Notably, a sample of strawberries and from workers gloves at the same farm were positive for HAV, though the faecal indicator viruses were not detected in either. Two of 14 irrigation water samples were positive for NoV (both GI and GII) and three of 56 for coliphage; however, they were on different farms. Irrigation water samples were from both surface and groundwater sources, but the report does not differentiate between the sources.

The authors recommended the use of male-specific coliphage for monitoring, although their results showed inconsistencies (i.e. sometimes coliphage was detected but not viral pathogens and sometimes the reverse). The number of positive samples in this real-life field setting was low overall limiting analyses. Measuring coliphage was nevertheless, considered easier than measuring pathogens directly and was a culture-based method.

Study 2. Raw vegetables, although no berries, and irrigation groundwater samples, were monitored monthly on 3 farms to determine the prevalence of viral contamination and the role of irrigation water as a contamination source (Cheong *et al.*, 2009). The presence of enteric viruses (NoV, enterovirus, AdV, RV) was detected by RT-PCR together with FIBs (TCs, FCs and enterococci). Detected virus strains were sequenced to further track potential contamination sources.

Of 29 groundwater samples tested, 17% were positive for enteric viruses, while 10% of the vegetable samples were positive. The groundwater samples were positive for enteroviruses and infectious AdV while the vegetable samples were positive for NoV GII and infectious AdV.

Overall, the results showed that levels of TCs, FCs and enterococci were below recommended levels in groundwater and were not correlated to the molecular detection of viruses. The rate of virus detection in groundwater (5/29; 17%) was higher than that for enterococci (4/29; 14%), that are commonly used to assess the microbial quality of groundwater. The sequence analyses demonstrated a close relationship of the isolated strains with reference or clinical strains of the viruses.

A4.2.3 Example on berries in other countries

In addition to the above examples, we reviewed reports from Australia (Torok *et al.*, 2019), Czech Republic (Dziedzinska *et al.*, 2018) and Italy (Purpari *et al.*, 2019). QMRA studies of norovirus contamination on strawberries have also been undertaken by Jacxsens *et al.* (2017) and Bouwknegt *et al.* (2015).

A4.2.4 Summary

The key messages from the example were as follows:

- Microbiological monitoring and source tracking can provide insight into pathogen source and transmission routes in the FFV chain and data for use in RAs and RM decision making e.g. on farm water and environmental contamination, and human contamination.
- For example, while irrigation water may be a potential source of contamination, some of the studies have highlighted that there could be higher contamination of virus through food handlers and possibly through post-harvest pesticide application.
- Viral indicators of faecal contamination, such as the molecular based markers, were effective at discriminating animal and human sources and showed a good correlation with viral pathogens measured by the same method.
- The use of coliphage (male-specific) was a little more inconsistent, with presence of pathogen occasionally not concomitant with the indicator.
- Bacterial indicators were even more problematic and did not show consistent correlations with the presence of viral pathogens.

A4.2.5 References

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A4.3 CARROTS

A4.3.1 Background

This example summarizes the approach to assessment of health risks and development of RM strategies for carrots, a root crop eaten raw.

A4.3.2 Evidence and data collection

A4.3.2.1 Production

A summary of the main processing steps in fresh carrot production is shown in Figure 1. In brief, commercial production occurs in open fields where main inputs can include irrigation water, usually delivered via sprinklers, pesticide application with overhead sprayers and organic fertilizers (e.g. manure). Carrots may be manually or mechanically harvested and usually no water is used during harvest except possibly for equipment cleaning. Howver, preliminary product cleaning may occur in the field immediately after harvest, possibly involving water, but this does is not a common practice in medium-large scale operations. After harvesting, depending on the season and region, field heat may be removed, e.g. by hydrocooling or in a rotating drum that places the carrots in contact with water at 4 ^{o}C. Carrots then undergo a main washing step, e.g. in large vats of water with the aid of scrubbers to remove soil and debris. In some production chains, there are separate cleaning/soil removal and washing steps, or multiple washing and polishing steps in sequence, all of which can involve water spraying or submersion. After the final washing or polishing, carrots are sorted and packaged. In addition, water is commonly used to clean harvest and post-harvest equipment and containers and to clean the facility (e.g. floors, contact and non-contact surfaces), with potential for cross-contamination.. Processes may vary by country and scale of operations, hence not all steps may be present in every production chain. In general, the process is similar to fresh cut processing, minus the cutting step, unless carrots are further processed e.g. peeled, shredded, or grated, or shaped into baby carrots.

A4.3.2.2 Epidemiological evidence

Based on conservative estimates reported by the United States of America Centers for Disease Control, between 1998 and 2017, 40 foodborne outbreaks, 849 illnesses, 22 hospitalizations though no deaths, were linked to carrots, including carrot products such as juice and shredded carrots (CDC, 2020). Foods implicated in outbreaks included multi-ingredient foods including carrots (e.g. salads) where the specific pathogen vehicle was unclear and these foods were likely contaminated during preparation and at food service (Erickson, 2010). In outbreaks with

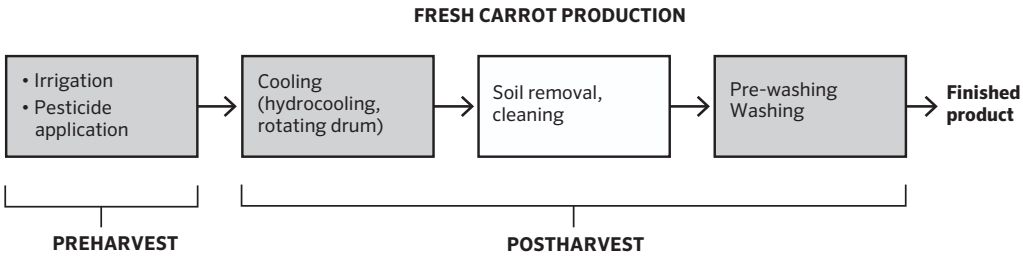

FIGURE 1 Main handling and processing steps in commercial carrot production, from preharvest to retail. Steps that involve water are highlighted in grey.

known aetiology in the United States of America, the most commonly implicated pathogen was NoV (20%), but outbreaks have also been associated with *Bacillus cereus*, *Salmonella*, sapoviruses (SaV), *Clostridium botulinum*, *Shigella* and *Staphylococcus aureus* (CDC, 2020). A *Yersinia pseudotuberculosis* and erythema nodosum outbreak (53 illnesses) in school cafeteria in Finland in 2004 was directly traced to "poor-quality" carrots and to a production facility where prolonged cold storage before distribution that may have facilitated *Yersinia* growth (Rimhanen-Finne *et al*., 2009). A *Cryptosporidium hominis* outbreak occurred in Denmark in 2005 (99 illnesses), possibly due to an ill consumer cross-contaminating a salad bar, including a water bowl used to freshen the produce (Ethelberg *et al*., 2009). In 2007, baby carrots included in packaged salad from a company sickened four people with *Shigella* in Canada (Health Canada, 2007). In 2018 two United States outbreaks of *Cyclospora cayetanensis* were traced back to fresh vegetable trays and salads, both containing carrots, and no specific vegetable was identified as the vector of contamination (Casillas *et al*., 2018; Hadjiloukas and Tsaltas, 2020). Another *Cyclospora* outbreak in the United States of America linked to mixed salad containing carrots occurred in 2020 (Hadjiloukas and Tsaltas, 2020).

A4.3.2.3 Microbial dynamics during carrot production

The microbiological quality of carrots can vary through the production process due to the introduction, cross-contamination, or abatement of microbial contaminants (as well as potential growth and die-off processes not explicitly considered here). A qualitative assessment of potential microbial dynamics through the production chain is shown in Figure 2.

The microbial quality of produce at harvest is mainly determined by the microbiological quality of field inputs such as irrigation and pesticide spray water, organic fertilizers (if used) and soil contamination, as well as exposure

to other possible contamination sources (e.g. wildlife, floodwaters, dust, workers). Harvest tools and containers cleaned using contaminated water can be a vehicle for contamination or cross-contamination of product during contact.

All intermediate processing steps have the potential to reduce pathogen levels as well as redistribute pathogens via cross-contamination. Steps involving water contact have the potential to introduce contamination. Throughout post-harvest processing, indirect cross-contamination in the facility can occur and may be associated with water, e.g. water for cleaning floors and equipment (contact surfaces and splashes from non-contact surfaces). Washing or polishing are the last steps before packing and hence can significantly influence the microbial quality of the final product.

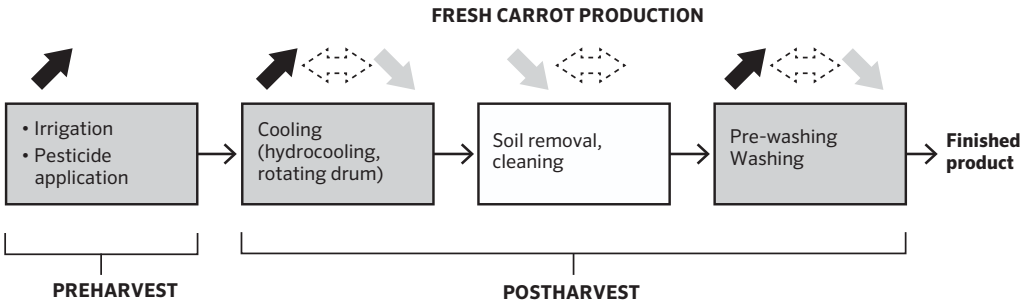

FIGURE 2 Qualitative assessment of main microbial dynamics along the carrot production chain. (Black arrows: processes where contamination may be introduced. Grey arrows: processed that may reduce microbial loads. Dotted arrows: potential for cross-contamination. Growth and die-off not due to antimicrobial treatments are not included. Additional contamination input from the production environment, not explicitly depicted in the figure, is also possible.)

Several characteristics of carrots (whole, peeled, or shredded) can influence interactions with waterborne microorganisms. As carrots are root crops, the upper root and leaves can be exposed to irrigation water that, if contaminated, can result in microbial contamination of the carrots (Armon *et al.*, 1994; Okafo *et al.*, 2003). Bacterial pathogens introduced in carrot fields via soil, amendments, or irrigation water can persist on carrots for over 100 days (Ingham *et al.*, 2004; Islam *et al.*, 2004, 2005). Soil and possibly water can adhere to the root during harvest and be transported into production facilities. The shape, rugosity and surface topography

of the carrot peel, as well as the presence of "hair", can provide niches for soil and/or microbes to attach to and be protected from the action of washing and sanitizers (Burnett and Beuchat, 2001). Peeling can reduce or eliminate risks due to attached microorganisms; however, cross-contamination via equipment should be avoided.

Cross-contamination in (pre-)wash water could occur. No data were found for the likelihood and extent of cross-contamination during carrot washing. The presence of soil residues and likely organic matter on carrots at the pre-washing (or hydrocooling) stage can reduce the effectiveness of chlorine sanitizers if added. No data was found on pathogen transfer from surfaces (wet or dry) to and from carrots. Human pathogens have been observed on washed, unpeeled carrots (Määttä *et al.*, 2013; Erickson, 2010), suggesting that commercial washing may not eliminate them. It is possible that pathogens may have a different degree of attachment to peeled or shredded carrots, compared to unpeeled carrots. Higher attachment ratios have been observed in cut versus uncut cabbage for *Listeria* spp. (Ells and Hansen, 2006). In addition, cutting or shredding carrots can release juices that may support bacterial growth (Abadias *et al.*, 2012; Gleeson and O'Beirne, 2005).

A4.3.2.4 Fit-for-purpose requirements for water inputs

Pre-harvest. How fit-for-purpose is defined for pre-harvest waters is relative to the other downstream production steps, as well as other pre-harvest inputs (See Annex 4, 3.1). A simple risk matrix was constructed at the previous JEMRA meeting to support MRAs for irrigation water used for fresh produce, and for use in data-poor settings (see Figure 2 in the MRA 33 meeting report, FAO and WHO, 2019). A more complex decision tree including water and other pre-harvest processes, pointing at existing guidelines, was developed to guide subsequent selection of risk reduction measures (see Figure 3 in the MRA 33 meeting report, FAO and WHO, 2019).

Post-harvest. Since (a) cooling and washing waters come into direct contact with the raw product, (b) there is no further risk-reduction step before consumption of raw carrots, and (c) the product is commonly eaten raw, the risks potentially introduced in post-harvest steps are largely determined by the microbiological quality of water inputs and these will be additional to any risk posed by hazards already present on the product at the start of a processing step. Cross-contamination via immersion in water and from contact surface and environmental sources in the processing area are described in Annex 4, 3.3. While difficult to characterize, cross-contamination processes cannot be ignored and should be considered in a risk-based approach (Maffei *et al.*, 2017).

The Codex Guideline for FFV defines clean water as "water that does not compromise food safety in the circumstances of its use" (FAO and WHO, 2017). Therefore to operationalize this definition, the tolerable number of pathogens introduced by water at a processing stage depends on the level of pathogens present on the product at that stage, the potential pathogen fate and transfer, and the pathogen level reduction achievable with subsequent steps, in particular the last washing step. For instance, multiple processes can occur during cooling and washing as pathogens may be introduced by water, if contaminated, pathogen levels on carrots may decrease due to the washing process, pathogen transfer can occur from one produce unit to another via water-mediated cross-contamination; and pathogen levels can be reduced in the water via a microbicidal treatment. Naturally, the tolerable amount of hazard introduced by water also depends on the acceptable level of risk in the final product.

According to CAC Guidelines for hygienic production of FFV (FAO and WHO, 2017), recommended water quality standards or risk reduction measures exist for several of the water input steps, but gaps exist in how to move from the general Guideline to its implementation:

- **Hydrocooling, soil removal, or pre-washing:** according to the CAC guidelines the soil removal, hydrocooling and/or pre-wash steps are the only ones where non-potable water may be used, provided water meets fit-for-purpose criteria (FAO and WHO, 2017). The fit-for-purpose quality of pre-wash water (for simplicity, steps using water- before the final wash will here be considered as one) should be at a level *equal to or better* than that of the product. The operational definition of *equal to or better than* would need to be determined via RA/QMRA.
- **Washing:** The downstream final carrot washing or polishing with potable water, possibly with a disinfectant (see next point), provides the final risk reduction step since there is no downstream critical control point before consumption if eaten raw. The extent of pathogen reduction necessary has to be determined based on risk e.g. via RA/QMRA. If the water source available for washing does not meet potable water standards, risk reduction measures should be implemented to bring the water quality to potable standards (WHO, 2006). See also the previous meeting report MRA, 33, the risk reduction measure RR6 in Fig.3 (FAO and WHO, 2019).
- **Cross-contamination:** If there is potential for cross-contamination, especially without any downstream risk reduction measure, measures should be put in place to minimize cross-contamination (FAO and WHO, 2017). See also Refs B and RR6 in Figure 3 of MRA 33 (FAO and WHO, 2019). For example, for water-mediated cross-contamination during washing, a disinfectant

concentration should be maintained in the water adequate to inactivate pathogens (see washing step). For facility cross-contamination (e.g. water splashes, utensils and surfaces), sanitary operating procedures pre-requisite to HACCP plans should be used (FAO and WHO, 2017).

Existing WHO guidelines provide templates for how to carry out risk assessment calculations, based on either established health targets or assumed values (Chapter 4; WHO, 2016).

A4.3.2.5 Fit-for-purpose determination for cooling/pre-washing water

QMRA approaches may be used to determine fit-for-purpose criteria for water used at steps such as hydrocooling or pre-washing, (Chapter 4) while also allowing for the principle of continuous improvement. This section outlines a proposed quantitative framework to establish fit-for-purpose criteria.

First, a model processing flow chart should be defined, for the purpose of risk-based calculations. In this example, a simple flow chart including only the pre-washing and final washing steps are required to illustrate the proposed approach. The first calculation aims to determine acceptable fit-for-purpose microbial water quality criteria for the pre-washing step, for one or multiple pathogens. This example focuses on one model bacterial pathogen (e.g. *Yersinia*) and could be adapted for other pathogens. The calculation can be initially carried out deterministically and then refined accounting for the variability and uncertainty in parameters. Key processes and variables to be included need to be defined (Table 6).

TABLE 6 Key variables or processes involved in the determination of fit-for-purpose water quality criteria for carrot pre-washing water

Variable/Process	Unit/Parameter explanation
Concentration on incoming produce	CFU/g (or other appropriate unit)
Transfer from pre-wash water to produce	Transfer ratio (or Log transfer ratio)
Pre-wash reduction	Log reduction of CFU/g on product
Water-mediated cross-contamination at pre-washing	Metric may vary, e.g. change in concentration distribution, or transfer ratio
Water sanitizer impact in pre-wash	Log reduction in CFU/100ml in water (dynamic process)
Log reduction at final wash step	Log reduction of CFU/g on product

The potential impact of each microbiologically relevant sequential process on pathogen levels on produce can be calculated and the impact of individual processes summed, subtracted, or otherwise combined as appropriate to derive an estimate of their cumulative effect. As a simplified example, product may be entering the pre-wash step with P Log CFU/g and the pre-washing process may yield X Log reduction due to the effect of washing (with or without a sanitizer), while pathogen transfer from wash water to produce may occur at a transfer coefficient T (in Log scale). The added pathogen load (concentration on produce) associated with transfer from water is then T*W, where W is the pathogen concentration in the water. The product concentration at the end of this step, C, would then be: C = P + (T*W) – X (all in Log CFU/g scale). While this example is a simple illustration, in reality these processes interact with each other dynamically and are usually not at steady-state. Ideally experimental data could be made available that quantify the combined effect of washing and water-mediated transfer together, under different conditions. Mechanistic physical/engineering models, combined with experimental data, can be used to dynamically simulate multiple concurrent processes over time and under different inputs and system settings, e.g. transfer of pathogens from product to water, from water to product and die-off or removal in water due to a treatment or water in/outflow. Outcomes of dynamic models may then be simplified for inclusion in risk-based calculations, e.g. as a probabilistic distribution of water concentrations capturing fluctuations over time. The validity and impact of any simplifying assumption would need to be assessed.

The estimated pathogen level on product at the end of the pre-wash can then be compared with the log reduction that can be reasonably expected in the final washing step. For example, if washing with potable water yields e.g. 0.5 log CFU/g reduction, then pathogen levels on produce entering the washing step could be at most 0.5 Log CFU/g, if a performance objective (PO) of zero CFU/g as mean concentration in the final product is agreed upon. The same reasoning holds if calculating risk to consumers from ingestion of carrots after the final wash. Any difference between estimated pathogen level on product at consumption and the required food safety objective (FSO) would need to be reconciled by improving the quality of pre-wash water or by changing other process parameters. Probabilistic considerations should be included in the calculations to account for variability and uncertainty in parameters; safety buffers could also be considered. If pre-wash water quality is the only parameter to be changed, the algorithm above can be run within an optimisation algorithm to calculate the minimum pre-wash water quality that reliably meets the FSO and maximum acceptable level of risk (or ALOP), i.e. water quality that is FFP for the considered processing step.

As processes and FSOs may differ among pathogens, the same risk-based approach outlined above can be repeated for multiple key pathogens and risk reduction interventions such a pre-wash water treatment can be selected to meet health targets for multiple hazards. Also, since carrot processing chains differ across regions and final product, the model should be adapted to the specific production context, also accounting for a country's health targets or ALOPs.

A4.3.2.6 Scientific evidence in support of fit-for-purpose water criteria determination

While some data are available to assess fit-for-purpose water criteria in carrot supply chains, significant gaps exist, some of which are common to other FFV. Further scientific evidence, ideally quantitative, that is needed to support FFP determination for cooling or "intermediate washing" water includes:

- **Irrigation water**: (1) updates on water-to-produce transfer for ready-to-eat root crops (water to soil to root), also as function of soil type; (2) persistence in soil at different depths, (3) review and updates on occurrence and levels of different pathogens in different water sources, esp. data gaps e.g. helminths and protozoa, (4) evidence of infection/illness where there is no processing step at all after harvest;
- **Transfer** of pathogens from water to produce during different processes (i.e. log increase on product as a function of water quality and other processing parameters);
- **Cross-contamination** in the water vat (e.g. change in the concentration distribution shape) vs. in rotating drums and during other processing steps;
- Impact of **product shape and size** on washing effectiveness and on potential for transfer and microbial attachment (including presence of small lateral roots, with greens removed or not, etc.)
- Impact of **multiple steps**: how do water FFP criteria change at multiple water-using steps up to the second last step, i.e. before the final wash? How do multiple steps add to each other?
- **Clustering**: **soil** clumps that are not removed may lead to higher doses. Are there more soil clumps on carrots than on other fresh produce?
- Role of **product quality** e.g. do cuts etc. on FFV and presence of spoilage organisms impact FFV vulnerability to pathogen transfer, attachment and internalization.
- Impact of **storage, consumer handling** and other market and post-market steps in survival or growth of bacterial pathogens.

A4.3.3 Summary

Fresh carrots can be vulnerable to microbial contamination at multiple steps in their production. While outbreaks linked to bacterial pathogens and parasites have been documented, there is also potential for viral and helminth contamination. Water, usually in direct contact with the raw product, is used at multiple pre-harvest and post-harvest stages. A quantitative risk-based conceptual approach is presented to illustrate the impact of multiple microbial processes potentially occurring in washing steps. In general terms, guidelines have been developed for most water-related processing stages, usually common to other FFV, but gaps exist in data necessary to conduct QMRAs and on guideline implementation strategies.

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A4.4 MELONS

A4.4.1 Background

Melons referred to in this study include cantaloupe (rockmelon), honeydew, watermelon and variety melons. The two examples presented demonstrate approaches to the risk management of the safety of water used in melon production. The focus is specifically on bringing together the accumulation of evidence, the development of a fit-for-purpose approach, the use of microbiological testing, and test criteria as presented in the main report. The first study summarizes the work of JEMRA as an advisor to CCFH (risk manager) when developing Codex international guidelines for melons. The second follows the development of the United States of America's guidelines and regulations for their melon industry.

A4.4.2 Codex Committee on Food Hygiene

In a risk profile of FFV prepared for CCFH based on the level of public health concern and negative consequences on trade, melons were ranked second after leafy vegetables and herbs. Subsequently, a JEMRA consultation was conducted in 2011 and provided a report with scientific advice to CCFH on food safety hazards and melons (FAO, 2011).

Key points of relevance with regards to water safety quality and water use during melon production were noted by JEMRA.

A4.4.2.1 Epidemiological evidence

From 1950 to May 2011; 85 outbreaks attributed to melons were identified, mainly in North America. The most common aetiological agents were *S. enterica* (47.1%). Norovirus (22.4%), *E. coli* O157:H7 (5.9%), *C. jejuni* (3.5%), *S. sonnei* (2.4%), *L. monocytogenes*, *Cyclospora* sp. and a suspected combination of *Staphylococcus aureus* and *B. cereus*. Main contributing factors identified were cross-contamination, poor melon washing, infected food handlers, poor hygiene and poor control of melon holding temperatures.

A4.4.2.2 Characteristics of melons, microbial interactions and managing risks

Topography of melon rind influences the attachment and protection of microorganisms on the melon surface. Cantaloupes, also known as rockmelons, in particular, have a waxy and highly hydrophobic surface matrix on their rinds that enhances attachment and provides niches where microorganisms can be protected during washing and sanitisation.

Foodborne bacterial pathogens can survive and grow on melon rinds and flesh. Microorganisms have been shown experimentally to infiltrate both the root

system of melon vines and fruit. Infiltration via the growing plant was considered transient and of lesser importance than infiltration into whole fruit post-harvest. Transfer of water and any microorganisms, if present, can occur across intact rinds of cantaloupe when there is a negative temperature differential during their immersion in water and can also occur via wounds caused by physical damage or pests, via splits, the ground spot and the stem scars.

A4.4.2.3 Water use and quantitative data on microbiological contamination levels

The JEMRA experts acknowledged limitations when establishing scientific evidence on the role of water as a source of pathogens for melons and the relationship of pathogen presence and water quality targets, because methodological insensitivities due to low prevalence and concentration of pathogens may limit meaningful investigations due to limited analysable data (Chapters 6,8). Under natural conditions and good agricultural practices, the prevalence and levels of enteric pathogens and also FIBs in water and on melons can be very low, intermittent and non-homogeneously distributed, all of which can limit investigations and lead to reliance on extrapolation of experimental inoculation studies. For example, Castillo *et al.* (2004) statistically analysed the relationship between *E. coli* counts and *Salmonella* spp. detection in cantaloupes, water and environmental samples at six cantaloupe farms; however, they were unable to conclude *E. coli* was a reliable FIB as the low counts and few samples with detectable *E. coli* did not allow reliable analysis (Table 7).

TABLE 7 Frequency of isolation of *Salmonella* and *E. coli* from samples of irrigation water collected at cantaloupe farms in the United States of America. Adapted from Castillo *et al.* (2004).

Source of water sample and use	No. of positive samples/total analysed (%)[a]	
	Salmonella	***E. coli***
Irrigation water (e.g. river, aquifer, or underground water)	9/70 (12.8)	19/70 (27.1)
Before filter	0/5 (0)	4/5 (80.0)
After filter	0/5 (0)	1/5 (20.0)
Reservoir water used for irrigation or for washing produce in the packing shed prior to packing	1/15 (6.7)	2/15 (13.3)
Irrigation water delivered in the field either by a drip emitter or by irrigation channel	2/25 (8.0)	4/25 (16.0)
Water sampled from the field pipe used for irrigation	1/20 (5)	2/20 (10.0)
Total	13/140 (9.2)	32/140 (22.8)

a Total percent values within columns followed by same letter are not significantly different ($P > 0.05$).

Irrigation

Duffy *et al.* (2005) determined risk factors for contamination of cantaloupes and irrigation water using data on the presence of *Salmonella* and *E. coli* and *E. coli* counts (Table 8). They did not find corresponding values or a relationship between these parameters, although they quoted Geldreich and Bordner (1971) who found that when the faecal coliform density was above 1 000 CFU/ 100 ml in various stream waters, *Salmonella* occurrence reached almost 100% frequency.

TABLE 8 Prevalence of *Salmonella* and prevalence and count of *E. coli* in irrigation water and cantaloupes in the field in Texas, the United States of America. Data from Duffy *et al.* (2005)

Source of sample	No. of positive samples/total analysed (%)		*E. coli* mean count (log CFU/100ml)
	Salmonella	*E. coli*	
Irrigation water	16/170 (9.4)	67/179 (39.4)	0.4 ± 0.5
Cantaloupes in field	0/100 (0.0)	13/100 (13.0)	2.2 ± 0.8

Irrigation water can be a vehicle for exposure of vines, fruit or root systems to pathogens and the application method was a risk factor for contamination of soil and fruit. Experimentally, *S. enterica* inoculated in large numbers into soil via furrow and drip irrigation systems during cultivation resulted in contamination of the soil during the growing season though it was not detected in the vines or fruit at harvest (Suslow *et al.,* 2010). Rind surface of furrow irrigated fruit was contaminated during heavy rain.

Irrigation water for melons can be contaminated with FIB and *S. enterica* at both the source, in surface waters, poorly maintained wells and irrigation canals and in holding ponds (Duffy *et al.*, 2005; Castillo *et al.*, 2004; Gagliardi, *et al.*, 2003). For example, on cantaloupe farms:

Duffy *et al. (2005).*

- *E. coli* detected in irrigation water sources (39.4% of 179), mean count of 0.4 $\log_{10}$ CFU/ml in well water (10/10), furrow (15/20), reservoir (15/30) and dirt canal (15/30) sources. The well and reservoir waters had the highest mean *E. coli* counts of 0.7 ± 0.3 and 1.0 ± 0.7 $\log_{10}$ CFU/ml.
- The structure with cement irrigation canals were significantly less contaminated than dirt canals.
- S. enterica was detected in 16/170 (9.4%) of irrigation water samples, the frequency of source being reservoir, dirt canals, furrow, cement canals and there was no positive well or riverine irrigation waters.

Castillo *et al*. (2004)

- Detected *S. enterica* (12%) and *E. coli* (23%) in irrigation water sources on six farms.
- Positive samples were mainly from one farm using water from an irrigation canal compared with the others using well or pond water.
- When *E. coli* was evaluated as a potential FIB in irrigation water, the low counts obtained and the few samples showing detectable *E. coli* made any data analysis unreliable.

The JEMRA Experts noted that the relationships between *S. enterica* serovars detected in irrigation water and those on melons at the same farm and washing water and melons in processing can be different when investigated using genetic fingerprinting (Duffy *et al.*, 2005; Castillo *et al*., 2004). This may also reflect insensitivities in methodology available as mentioned above.

Cooling

When hydrocooler water is poorly controlled it can have significant levels of faecal indicators and contaminate cantaloupe rinds with up to 3.4 $\log_{10}$ CFU/g (Gagliardi *et al*., 2003).

Washing and sanitising

In several studies it was found pathogens (*S. enterica*) were introduced and the bacterial load of aerobic bacteria (Akins *et al.*, 2008), *E. coli* (Duffy *et al.,* 2005; Castillo *et al*., 2004), FCs and faecal enterococci (Gagliardi *et al*., 2003) on cantaloupes increased between pre- and post- harvest. It is uncertain whether processing released bacteria from the netted rinds and the extent of introduction of contamination or both were involved (Duffy *et al*. 2005).

A4.4.2.4 Conclusions

- Netted rind melons can facilitate microbial contamination; elimination of contamination is extraordinarily difficult and infiltration of human pathogens into whole melons can occur with exposure to contaminated water.
- Much of the contamination during cantaloupe processing has been traced to primary wash tanks and hydrocoolers.
- Once disinfectants have reduced microbial populations, there is an increased risk of contamination of melon rinds highlighting the importance of maintaining the cleanliness and hygiene of all contact surfaces post-sanitisation and any water used should not be a source of further contamination.
- Generally, sanitisers control microbial populations in the wash water and reduce the potential for croos-contamination, rather than disinfecting the melons (Castillo et al., 2009; FAO and WHO, 2008).

- Control Points identified were at cooling and washing steps where contamination can be spread if not adequately controlled and where there is the potential for infiltration of pathogens into melons e.g. uncontrolled temperature differential between fruit and wash water.
- Monitoring tools at these points should include disinfectant concentration, temperature, turbidity, pH (chlorine based) etc. Microbiological analysis can be used for process verification.

A4.4.3 United States of America

A4.4.3.1 Epidemiological evidence

Walsh *et al.*, (2014) reviewed outbreaks linked to melons in the United States of America between 1973 and 2011. They found *Salmonella* (56%) followed by NoV (15%) were the most common aetiological agents reported in 34 outbreaks caused by a single melon type of which cantaloupes were responsible for more than half Pathogen contamination in most outbreaks occurred during production when melons were in contact with soil. The most common *Salmonella* serotypes involved, Poona and Javiana, had been associated with reptilian reservoirs. They also found post-harvest, factors contributing to melon contamination included poor sanitary practices in packing sheds, inadequate monitoring of chlorinated wash water, improper cooling and cold storage practices and contaminated equipment. In 2011 a significant multi-state outbreak of listeriosis including 147 cases and 33 deaths was linked with whole cantaloupes. The involvement of whole fruits compared with pre-cut products was un-precedented. Investigations identified inadequate control of *L. monocytogenes* in packhouses (CDC, 2012).

A4.4.3.2 Melon industry guidelines

In response, a series of guidelines and regulatory measures to assist producers in the development and implementation of food safety RM plans have been developed based on scientific and public health evidence, quantitative data where available, industry and regulatory experience and cooperation). *Commodity specific food safety guidelines for cantaloupes and netted melons* were developed as this group was identified as presenting a higher consumer health risk among melon varieties (USA FDA, 2013). Key points addressed in these Guidelines were similar to those identified by JEMRA (See 4.1).

Where water was used pre-, during and post-harvest it was recommended water was of a quality fit-for-purpose or that it should not increase the risk of melon contamination. It was recommended overall that sanitary design and sanitation programs were critical to ensure melons exiting unit operations do not experience

a net increase in microbial populations. This is key in determining criteria for fit-for-purpose water at sequential steps. The use of potable water was specified for use when in contact with cantaloupes after harvest.

A4.4.3.3 Microbiological testing of water

In the Guidelines (USA FDA, 2013) microbiological testing was considered a useful tool to evaluate water quality to_verify the effectiveness of sanitation practices provided sampling plans and methodology were properly designed and performed for the intended use of the information. An action plan was required in the event a test failed safety requirements. Main points recommended or noted in the Guidelines at pre- and post-harvest stages are summarized:

Pre-harvest

- Agricultural water should be tested at least annually (note this would be adapted later to conform with the Regulation i.e. Produce Final Rule). The testing frequency depended on the source water, intended water use (degree of contact with the cantaloupe and time until harvest) and the risks of environmental contamination, including intermittent or temporary contamination (e.g., heavy rain, flooding). Frequent water tests may be useful to establish the baseline for assessment of water quality.
- Preventive measures included the use of irrigation water of known and acceptable quality together with the use of irrigation and cultivation methods that did not increase the risk of cantaloupe contamination by direct or indirect exposure to water.
- If the water source was found to have levels of indicator organisms or pathogens detected indicating the potential for pathogen contamination, corrective actions were to be taken and documented to ensure that the water was not a source of contamination for melons.

Harvest/post-harvest

- Water in direct or indirect contact with cantaloupes should be of potable water quality.
- Recycled water must be disinfected.
- Any pathogens which may be present on the rind may be reduced but are unlikely to be eliminated by washing.
- Water pH (where appropriate for oxidizer), sanitiser concentration, soil load, turbidity levels, water hardness, product through-put capacity and resident or contact time e.g.in dump tank water, should be controlled and monitored to ensure the efficacy of any antimicrobial water treatment.

A4.4.3.4 Regulatory measures

The Food Safety Modernization Act (FSMA) Final Rule on Produce Safety went into effect in 2016: *FSMA Final Rule on Produce Safety Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (USA FDA, late update 2020). This rule* specifies actions that must be taken at points in produce production to prevent contamination with foodborne hazards and would apply in the melons industry guidelines.

The Final Rule specifies regulatory requirements for the quality of water used in produce production and applies to melons. All agricultural water (e.g. water used in melon pre-and post-harvest steps) likely to contact produce or food contact surfaces must be of adequate sanitary quality for its intended use.

Two sets of criteria were established for microbial water quality, both of which are based on the presence of generic *E. coli*, which is used to indicate the presence of faecal contamination.

The Final Rule stated:

- No detectable generic *E. coli* are allowed for certain uses of agricultural water in which it is reasonably likely that potentially dangerous microbes, if present, would be transferred to produce through direct or indirect contact. Examples include water used for washing hands during and after harvest, water used on food-contact surfaces, water used to directly contact produce (including to make ice) during or after harvest, and water used for sprout irrigation. The rule establishes that such water use must be immediately discontinued and corrective actions taken before re-use for any of these purposes if generic *E. coli* is detected. The rule prohibits use of untreated surface water for any of these purposes.
- The second set of numerical criteria is for agricultural water that is directly applied to growing produce (other than sprouts). The criteria are based on two values, the geometric mean (GM) and the statistical threshold (STV).

A summary of the scientific rationale applied in the development of these criteria has been provided and is summarized below. It can be accessed at https://www.fda.gov/files/food/published/FSMA-Final-Rule-for-Produce-Safety--How-Did-FDA-Establish-Requirements-for-Water-Quality-and-Testing-of-Irrigation-Water--PDF.pdf.

The goal of the regulatory water quality criteria is to understand and describe water sources and water distribution systems (USA FDA, 2020). After a review

of scientific literature, *E. coli* found in the intestinal tract of humans and animals was concluded to be a consistent indicator of the presence of faeces. In assessing the safety of agricultural water identifying faecal contamination was considered important; increases in faecal contamination coincided with increasing likelihood of the presence of disease-causing microorganisms.

As a starting point in defining the numerical criteria, the Environmental Protection Agency's (EPA) recreational water criteria based on recent human epidemiological studies were considered (See discussion in main report section 6.4). The scientific evidence showed people became ill by swallowing recreational water that was contaminated with faeces. Other technical information was also considered e.g. WHO water safety information resources (WHO, 2017), data on post-irrigation microbial die-off and microbial removal and recommendations on circumstances unique to produce growing.

For untreated water two criteria outlined in the Final Rule must be met as follows:

- a Geometric Mean (GM) of samples of 126 CFU or less of generic *E. coli* per 100 ml of water,
- a statistical threshold value (STV) of 410 CFU or less of generic *E. coli* in 100 ml of water.

These reasons provided for including two criteria addresses two perspectives on the distribution of levels of generic *E. coli* in a water source.

- The GM measures the central tendency or the average amount of generic *E. coli* in a water source.
- The STV represents the amount of variation in the *E. coli* levels, e.g. as can occur with heavy rainfall and measures the expected deviations from the average for a water source.

Using both criteria, the regulators proposed a more complete description of water quality is obtained accounting for the variability of *E. coli* levels that occurs in water sources in nature. This means a farm will not have to discontinue use of its water source due to small fluctuations in water quality that occur naturally.

Some flexibility for compliance with the rule to account for potential differences among regions, commodities and farming practices is provided (USA FDA, 2020). If the required criteria are not met then corrective measures could be applied such as:

- Applying specific withholding times "to allow time for potentially dangerous microorganisms to die off e.g. between last irrigation and harvest (up to a

maximum four days) and/or between harvest and end of storage". Alternatively, farms could use a calculated log reduction during activities such as commercial washing of the exposed produce.
- "Re-inspecting the entire affected agricultural water system under the farm's control and, among other steps, making changes to ensure that its water meets the criteria".
- "Treating the water".

Farms can also use alternative water quality criteria if they can be scientifically proven "to provide the same level of public health protection as is provided by the Final Rule and do not increase the likelihood that the produce will be unsafe or otherwise adulterated" (USA FDA, 2020).

Testing regimens under the Final Rule (USA FDA for untreated surface and ground water differ in the sampling regime because of the increased vulnerability of surface water to contamination and the potential for greater variability in generic *E. coli* levels. These include:
- Surface water testing includes a minimum of 20 initial samples collected over two to four years followed by a minimum of five samples a year. The microbial water quality profile will thus be updated annually on a rolling basis using a minimum of 20 samples. The calculation of the GM and STV will be based on the five new samples and 15 of the most recent earlier samples.
- Ground water testing will require a minimum of four initial samples over one year, followed by a minimum of one new sample each year. The profile will be updated annually using at a minimum the most recent four samples.

A4.4.4 Summary

The study based in the United States of America provides an example of the development of safety criteria for water in a produce commodity, melons and more specifically cantaloupe that had been attributed to increasing illness rates, in the United States of America. The recommendations of use of water of "quality sufficient for the intended purpose" or "fit-for-purpose" has become more specific over time with continuous improvement in regulation that at present defines assessment of water quality and related metrics using sampling plans and criteria based on *E. coli* as an indicator of faecal contamination with stringency depending on the water source and its intended use. These have been developed using public health data, risk assessment, knowledge of the behaviour (growth and survival) of the pathogens in water and the environment and the special characteristics of produce and developed by collaboration between all stakeholders.

A4.4.5 References

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Microbiological Risk Assessment Series

During fresh fruit and vegetables (FFV) production, water is used for a variety of purposes. Even the water was conventionally treated and disinfected, it may still potentially contain human pathogens, albeit at low concentrations. A risk assessment, appropriate to the national or local production context, should be conducted to assess the potential risks associated with a specific water source or supply in order to devise the appropriate risk mitigation strategies.

Since the 48th session of Codex Committee on Food Hygiene (CCFH) noted the importance of water safety and quality in food production and processing, FAO and WHO has undertaken the work on this subject. This report describes the output of the third in a series of meetings, which examined appropriate and fit-for-purpose microbiological criteria for water used with fresh fruit and vegetables. The advice herein will support decision making when applying the concept of fit-for-purpose water for use in the pre- and post-harvest production of fresh fruit and vegetables.

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