Diploma Thesis

Water Quality in Developing Countries with Special Attention to the Water Supplies of a Peripheral Hospital in Kakkaveri, Tamil Nadu, Southern India

Submitted by

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To attain the academic degree

Doctor medicinae universae
(Dr. med. univ.)

At the

Medical University of Graz

Conducted at the

Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine

Under supervision of
Univ. Prof. Dr. Andrea Grisold
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Graz, 22nd January 2019

Statutory Declaration

I hereby declare that I have authored this thesis independently and without any support from third parties, that I have not used other than the explicitly marked sources/resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, 22nd January 2019 *Isabella Dicker eh.*

Acknowledgements

To start with, I want to thank my supervisor Prof. Dr. Andrea Grisold for giving me the opportunity to go to India and also for steadily providing me with constructive advice, before and during the writing process. My gratitude goes to the team of Global Health and Development, the six persons with whom I have been travelling to India. Dr. Andreas and Angela Schöpfer, Angelika Schirnhofer, Anneliese Pfeifer, Karla Thomas and Sylvia Sammer took me in their team with great warmth, amity and support. Very special thanks to Dr. Sr. Francina from Salem, India, who showed me around at the hospital's premises, did never get tired of my questions and was always answering them patiently. Furthermore, I thank Sabine Platzer and Jürgen Hautz from the water laboratory for the analysis of the samples, for introducing me to the different methods and for answering all my questions. Also thanks to Prof. Dr. Franz Mascher, who supported me with helpful comments about water systems and borrowed me his books.

On a more personal level: very special thanks to Elisabeth and Karla for leaving comfort zones together and for unforgettable memories. Big thanks also to Andreas for being always patient and supportive.

Special thanks go to my sister Marlene who is always listening to my problems and helps me resolving difficulties with all her ingenuity, at day and night time. Coming to an end, I am particularly grateful to my parents for their infinite support and for always being there. You always helped me find my way, without pointing at it.

Zusammenfassung

Hintergrund: Weil es für unsere Gesundheit unabkömmlich ist, muss Trinkwasser definierte Qualitätsstandards erfüllen. Die vorliegende Diplomarbeit präsentiert die Trinkwassersituation in Entwicklungsländern beispielhaft anhand der Untersuchung der Wasserqualität eines Krankenhauses in Kakkaveri, Südindien. Weiters werden ein Überblick über die Richtlinien der Weltgesundheitsorganisation (WHO) und vergleichend die Trinkwasserstandards von Österreich und Indien dargestellt.Diese Arbeit wurde im Rahmen eines Projektes des Department for Global Healthand Development (GHD) der Medizinischen Universität Graz, Österreich und des Doctor Typhagne Memorial Charitable (DTMC) Trust in Salem, Südindien verfasst.

Für hohe Wasserqualität kann nur garantieren, wer diese auch regelmäßig überprüft. Analysen in Wasserlaboratorien sind aber einerseits manchmal kostenintensiv und andererseits in ländlichen Regionen von Entwicklungsländern nicht immer zugänglich. Aus diesem Grund müssen in manchen Fällen andere Methoden gefunden werden, um die Wasserqualität zu überwachen.

Methoden: In dieser Diplomarbeit wird ein Point-of-care-Testsystem als ortsnahe Diagnostikmethode (3M™Petrifilm™*E. coli*/Coliform Count Plate) mit den Ergebnissen eines Wasserlabors verglichen. Zu diesem Zweck wurden im November 2017 im Krankenhaus von Kakkaveri, Südindien, an verschiedenen Stellen insgesamt 15 Wasserproben entnommen. Jede Probe wurde dem Point-of-care-Test, einer physikalisch-chemischen und einer laborgestützten, bakteriologischen Untersuchung unterzogen und die Ergebnisse verglichen. Liefert ein leicht zuhandhabendes Point-of-care-Testsystem vergleichbare Ergebnisse wie ein Wasserlabor, jedoch zu geringeren Kosten, kann dieses eine günstigere Alternative zur Überwachung der Wasserqualität darstellen.

Ergebnisse und Schlussfolgerungen: Die eingesetzten Point-of-care-Tests wurden sowohl bei 37°, als auch bei 29° Celsius (Raumtemperatur) bebrütet. Zieht man in Betracht, dass bei den Point-of-care-Tests (Petrifilm™) im Vergleich zu den Laboruntersuchungen nur 1 ml Probenvolumen (statt 100 ml) verwendet werden, so fanden sich in 66.6 % (4/6; 37°C) und 50.0% (3/6; 29°C) Fällen Bakterien im Wasser. Die Untersuchungen im bakteriologischen Labor zeigten in 100 % (15/15) der Proben eine

Überschreitung der lokalen Richtwerte. Die chemische Untersuchung der Wasserproben zeigte keine gesundheitsgefährdenden Überschreitungen.

Das verwendete Point-of-care-Testsystem kann, auch bei eingeschränktem Zugang zu Laboren, in dieser Studie nur sehr eingeschränkt als Alternative zu Untersuchungen durch ein mikrobiologisches Laborempfohlen werden.

Abstract

Background: Water is essential for health. Drinking water for human consumption, therefore, needs to meet certain quality standards. This diploma thesis presents the drinking water situation in developing countries exemplarily with a survey of the water situation of Kakkaveri hospital in rural southern India. Furthermore, it gives an overview over the guidelines of the World Health Organisation (WHO) and takes in the drinking water standards of Austria and India. This thesis was written within the framework of a cooperation of the Department for Global Health and Development (GHD) at the Medical University of Graz and the Doctor Typhagne Memorial Charitable (DTMC) Trust.

High quality drinking water can only be granted if there are regular verifications of the quality. Analyses at water laboratories on the one hand are partly expensive and on the other hand not unlimited accessible in rural regions of developing countries. Therefore, other methods to survey the water quality need to be determined.

Methods: In this thesis, a point-of-care-testing-system as a decentralized diagnostic method (3M™ Petrifilm™ *E. coli* and Coliform Count Plate) and the results from a water laboratory were compared. For this purpose, 15 water samples (from different locations at Kakkaveri hospital) were collected in November 2017. Every sample underwent the point-of-care-testing, a physical-chemical and a bacteriological analysis. A comparison of the results was conducted. With an easy to use point-of-care-testing-system that provides measurements with a comparable reliability as a laboratory, but at lower costs, an alternative surveillance system can be implemented.

Results and conclusion: The applied point-of-care-testing-system was incubated at 37° and 29° Celsius (room temperature). Taking into account, that the point-of-care-testing (Petrifilm™) needs inoculation with 1 ml sampling volume (rather than 100 ml for laboratory analysis) the results matched in 66.6 % (4/6; 37°C) and 50.0% (3/6; 29°C) of the cases only. The bacteriological analysis in general showed bacterial contamination in 100 % of the samples (15/15). The chemical analysis did not show any health concerns. Even if the access to microbiological laboratories is limited, the applied point-of-care-testing-system in this study could not replace the routine screening.

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List of Abbreviations

AOAC	Association of Official Agricultural Chemists / Association of Official Analytical Chemists
AST	Antimicrobial Susceptibility Testing
CFU	Colony Forming Units
DAEC	Diffuse Adherent *Escherichia coli*
DALYs	Disability-/ Disease-Adjusted Life Years
DIN	German: *“Deutsches Institut für Normung”*
DTMC Trust	Doctor Typhagne Memorial Charitable Trust
EAEC	Enteroaggressive *Escherichia coli*
ECC plate	*Escherichia coli* and Coliform Count Plate
ECG	Electrocardiography
E. coli	*Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GHD	Global Health and Development
GI-tract	Gastrointestinal-tract
HIV	Human Immunodeficiency Virus
ISO	International Organisation for Standardisation
JMP	Joint Monitoring Program
MALDI-TOF MS	Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight Mass Spectrometry
MDG	Millennium Development Goals
NTU	Nephelometric Turbidity Units
NTS	Non-Typhoidal-Salmonella
PCR	Polymerase Chain Reaction
ppm	Parts Per Million
pH	Potential of Hydrogen
R. O.	Reverse Osmosis

SDGs	Sustainable Development Goals
S.F.S	Saint Francis de Sales
sp.	*species*
spp.	*species pluralis*
STI	Sexually Transmitted Infections
TDS	Total Dissolved Solids
TM	Unregistered Trade Mark
TOC	Total Organic Carbon
UN	United Nations
UNICEF	United Nations International Children's Emergency Fund
USEPA	United States Environmental Protection Agency
WaSH	Water, Sanitation and Hygiene
WHO	World Health Organisation

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1 Introduction

The existence and access to safe drinking water is essential for the daily life and therefore also for healthcare centres and hospitals. Water quality is a crucial factor for health, especially for patients when medical care is needed. In regions with low infrastructure and changing political situation, providing high quality drinking water may be challenging.

This thesis includes a theoretical part concerning the problem of not accessible safe water sources and the resulting health issues (worldwide 663 million humans are currently using unimproved water sources (1)) as well as the presentation of the investigation of water samples, collected in South India in November 2017. This thesis was performed within the framework of a project of the Department of Global Health and Development of the Medical University of Graz (GHD) and the Doctor Typhagne Memorial Charitable (DTMC) Trust in Salem. The general part of this thesis concentrates on different drinking water ordinances and the recommendations of the World Health Organisation (WHO) on drinking water hygiene.

For the practical part of this study, the water system and quality of drinking water in the S.F.S. (Saint Francis de Sales) Health Centre in Kakkaveri in rural South India was systematically inspected and documented. The aim is the future implementation of a surveillance system to ensure safe drinking water. Therefore, a comparison of the results of a point-of-care-testing-system (3M™ Petrifilm™ *E. coli* and Coliform Count Plate to detect *Escherichia coli* and other coliform bacteria, in the following referred to as Petrifilms™) and the results from a professional water laboratory was conducted. With an easy to use point-of-care-testing that provides measurements comparably exact to the laboratory results, but at lower costs, regular check-ups can be implemented. Restrictions of the study include a limited amount of samples due to the limited space in the sample incubator and the limited allowed weight on flights.

The theoretical part of this thesis points out the importance of high quality drinking water to health. Worldwide actions taken in favour of safe drinking water are described, as well as the country-specific drinking water standards for Austria and India, as well as a comparison of those two standards. Furthermore, an overview over the microorganisms causing health problems when appearing in drinking water is given. A summary about the study area and the cooperation between the Austrian Team of the Department for Global Health and Development, and the Indian organisation Doctor Typhagne Memorial Charitable Trust completes the theoretical part.

1.1 Drinking Water Quality and Hygiene

In 2015 the 17 Sustainable Development Goals (SDGs) with 169 targets were set by the United Nations (UN) with the aim to fulfil these goals until 2030. Especially goal number six of the 17 SDGs is to 'ensure availability and sustainable management of water and sanitation for all'.(1: p.14) The targets defined in goal six will be described in the following.

1.1.1 The Sustainable Development Goal Concerning Water

Since 1990 the Joint Monitoring Program (JMP) for Water Supply and Sanitation of the WHO and the United Nations Children's Fund (UNICEF) exists and monitors the worldwide progress on drinking water by following the SDGs. (3) The SDG number 6 is named 'Clean Water and Sanitation'. It focuses not only on the access to safe water and sanitation, but also stands for sustainable and high-quality water sources. (1) Therefore, the program sets itself six targets to be put into practice by 2030.(4)

The first target is the achievement of 'universal and equitable access to safe and affordable drinking water for all'. (2: p.2) '*Universal*" refers to the settings and includes households, and public spaces like schools, health facilities and workplaces. The '*access*' to the water source which is used at home should be nearby. The word '*safe*' in the definition means that the water has to be free from pathogens and other contaminations such as toxic substances at all time. '*Drinking water*' is water suitable for human consumption and personal hygiene. Furthermore, the water has to be '*affordable*', which means money must not stand between human needs and use of adequate water. It refers to water as a human right. '*Equitable*' and '*for all*' refer on the one hand to the reduction of inequalities and on the other hand to water, that should be possible to use for populations of all genders and ages, as well as people with disabilities. (4)

To realize this plan, standards and definitions were set. Table 1 presents the drinking water ladder, according to the JMP Wash in the 2030 Agenda. Given are also ladders for sanitation and hand washing. The drinking water ladder shows which levels of water supply are possible. On the top, safely managed drinking water is located. This source has to be improved, on the premises, available when needed and free from contamination. (3)

Safely managed	▪ improved source ▪ free from faecal and priority contamination ▪ located on premises ▪ available when needed
Basic	▪ improved source ▪ collection time <30 min for a roundtrip including queuing
Limited	▪ improved source ▪ collection time >30 min for a roundtrip including queuing
Unimproved	▪ unprotected dug wells ▪ unprotected springs
Surface water	▪ E g.: rivers, ponds, lakes, dams, canals, irrigation channels, etc.

Table 1: Drinking water ladder – JMP Wash in the 2030 Agenda (3)**: p.4**

The program defines: '*'Improved'* sources are those that are potentially capable of delivering safe water by nature of their design and construction, including 'piped water, boreholes or tube wells, protected dug wells, protected springs, and rainwater'.(4: p.13)

1.1.1.1 Water Supplies in Healthcare Facilities

The WHO Guidelines for drinking water list water for the use in health care facilities as a specific circumstance, as it should be able to be used not only for drinking, but also for personal hygiene.(5)

According to the JMP for water supplies and sanitation, only one-third of the healthcare facilities in low and middle income countries are able to acquire water from an improved source within 500m. There is a special service ladder (Table 2) for water supplies which is not just for drinking water, but for use of water in general. In healthcare facilities, vulnerable persons are likely to be present. Vulnerable populations include infants and pregnant women, as well as immunosuppressed individuals. (4)

Examples for bacteria and microorganisms, which are of no danger for healthy populations or patients with a functioning immune system, but might be a health risk for immunosuppressed patients are *Pseudomonas aeruginosa*, the group of *mycobacteria*, *Acinetobacter* or *Aspergillus* species.(5)

Advanced service in healthcare facilities is meant to be defined at national level, whereas basic service is water from improved sources on the premises. This is followed by limited service, where the improved source is not located on the premises. Finally, no service includes an unimproved source or no water source at all. (4)

For special uses, such as dialysis wards, washing endoscopes or wound management or water used to cool dental drills, the WHO recommends further processing, for example, sterilization of the water used for the listed purposes or others.(5)

Advanced service	Defined at national level e.g. for India it is defined in the *Drinking Water – Specification* published by the Bureau of Indian Standards
Basic service	Water from improved sources available on the premises
Limited service	Improved source, not on the premises or water is not available
No service	No water source or an unimproved source

Table 2: Water Supply in Health Care Facilities – JMP 2017 Safely Managed Drinking Water (4): p.15

1.1.1.2 Progress on Water Quality Worldwide and Further Actions Taken

After the first 15 years, the progress in the achievement of the SDGs was monitored and a report was published in 2016. Concerning goal 6, improvements have been perceived. In 2000, 82% of the global population had access to improved water sources. This percentage has increased till 2015, when 91% used improved water sources. Furthermore, there are still 663 million humans using unimproved water sources and surface waters. For south Asia, the part of the population using improved water sources rose from 81% up to 93%. Nonetheless, estimations made in 2012 and described in the 2016 report claim that at least 1.8 million people use faecal contaminated drinking water, due to unsafe water from improved sources.(1)

Worldwide, 2 billion people suffer from water stress in 2012 and the numbers are increasing. Water stress is defined as the ratio between the total freshwater withdrawn in a country to the total renewable freshwater resources. If this ratio exceeds 25%, one is speaking of water stress. (1)

Additionally, the period of time from 2018 to 2028 is declared "Water Action Decade" by the General Assembly of the UN. The Water Action Decade follows the "Water for Life"-Decade (2005-2015). The Action Decades objectives include a greater focus on sustainability of water resources, the implementation of related programmes and projects and the achievement of international water-related goals and targets, including the targets from the 2030 Agenda. (6)

1.2 Standards for Drinking Water in India and Austria

1.2.1 Standards for Drinking Water in India

In 2012, the Bureau of Indian Standards published the guideline *Drinking Water – Specification (Second Revision)*. This document gives an overview of the necessary requirements for water save to use. It is based on WHO guidelines, United States Environmental Protection Agency standards (USEPA) and European Union (EU) directives and contains requirements, as well as methods of sample taking and testing. These Indian standards define drinking water as: "[…] water intended for human consumption for drinking and cooking purposes from any source. It includes water (treated or untreated) supplied by any means for human consumption.".(8: p.1)

The Indian standards are subdivided into the categories bacteriological, virological and biological requirements. Also, acceptable limits for physical and chemical parameters are listed. (7)

1.2.1.1 Bacteriological Standards

Bacteriological requirements of drinking water and treated water entering or in the distribution system in India are that neither *Escherichia coli* nor thermo tolerant coliform bacteria are allowed to be detectable in a 100ml sample. Additionally, water entering or in the distribution system must not show any coliform bacteria (total coliform bacteria not detectable in any 100ml sample). There is also the recommendation that actions shall be taken if *E. coli* or total coliform bacteria are detected. (7)

1.2.1.2 Virological and Other Biological Standards

The virological requirements state that the water in the distribution system should be free of viruses. If the virus *Bacteriophage MS2* is detected in the water sample, a polymerase chain reaction (PCR) of 100 litres of water is indicated. (7)

Furthermore, the biological requirements and restrictions refer to biological organisms as protozoa, parasites, zooplankton, rotifers, and algae. Those organisms should not be detectable in the water samples. Species of interest are *Plumatella, Dreissena, Asellus,* amoebic cysts*, Giardia, Cryptosporidium,* etc. (7)

There is no guideline for parasites and other health concerning microorganisms, but the drinking water ordinance states, to check the water for such organisms, if *Clostridium perfringens* is above the reference value. In this case, the authorities must be made aware of the exceedance.(8)

1.2.2 Standards for Drinking Water in Austria

In accordance with the Austrian drinking water ordinance (federal law gazette II No. 362/2017), drinking water has to be use- and consumable without any hazardous potential for health. Therefore, a defined concentration or number of microorganisms, parasites, and chemical agents needs to be limited or must not exist. Furthermore, the Austrian drinking water ordinance defines indicators, which are parameters used to control the water. It also specifies the sample taking points pursuant to the size of the tested water system. In general, Austrian drinking water is analysed once a year. According to §6 of the Austrian drinking water ordinance, the operators of the water supply system have to inform the consumers about the following parameters: nitrate, pesticides, total hardness, carbon hardness, potassium, calcium, magnesium, sodium, chloride, sulphate. (8)

1.2.2.1 Bacteriological Standards

The table hereafter (Table 3) shows the indicator parameters for microbiological analysis, according to the Austrian drinking water ordinance part C.(8)

Indicator	Limit	Unit
Colony forming units at 22°C (CFU 22)	100	/ml
Colony forming units at 37°C (CFU 37)	20	/ml
Coliform bacteria	0	/100ml
Clostridium perfringens (and spores)	0	/100ml
Pseudomonas aeruginosa	0	/100ml

Table 3: Microbiological indicators (not disinfected water); from the Austrian Drinking Water Ordinance (8): p.11

Disinfected water has to be analysed directly after disinfection of the water tap in order to test the effectiveness of the performed action. Limits for colony forming units (CFU) are reduced to 10/ml and coliform bacteria, *Clostridium perfringens*, and *Pseudomonas aeruginosa* should not appear in a volume of 250ml water. (8)

1.2.3 Comparison of Austria's and India's Physical-Chemical Water Standards

The following chart (Table 4) compares Austria's and India's standards and indicator parameter limits.

Indicator	Unit	Austria's limit	India's limit / *
Odour	-	without abnormal changes	agreeable
Taste	-	without abnormal changes	agreeable
Turbidity	-	without abnormal changes, 1 NTU**	1 / 5 NTU
Temperature	° Celsius	25 or without abnormal changes	-
Colour (436nm)	m^{-1}	0.5 or without abnormal changes	-
pH - value	pH- Units	≥ 6.5 and ≤ 9.5	≥ 6.5 and ≤ 8.5
Oxidizability	mg/l O2	5.0	-
TOC***	-	without abnormal changes	-

Electric conductivity	µS cm^{-1} at 20°C	2500	-
Aluminium	mg/l	0.2	0.03 / 0.2
Ammonium	mg/l	0.5	0.5
Chloride	mg/l	200	250 / 1000
Iron	mg/l	0.2	0.3
Manganese	mg/l	0.05	0.1 / 0.3
Nitrate	mg/l	50	45
Nitrite	mg/l	0.1	-
Sodium	mg/l	200	-
Sulphate	mg/l	250	200 / 400

Table 4: Comparison of chemical and physical indicator parameters – from the Austrian Drinking Water Ordinance (8)**: p.10+11and the Indian Standards for Drinking Water** (7)**: p.1+2**

* Permissible limit in absence of alternate source (if existing written after a slash, i.e.: manganese: 0.1 / 0.3)

**NTU = Nephelometric Turbidity Unit

*** TOC = Total organic carbon

1.3 Bacteria Used as Indicators for Water Quality

In the following, the three bacterial species or subgroups that can be used as indicators for water quality are described, including *E. coli*, the total coliform bacteria and *Enterococci*.

1.3.1 Escherichia coli

E. coli are part of human and animal microbiota but when found in water, *E. coli* provides evidence of faecal contamination. (5) *E. coli* is furthermore used as a reliable indicator for contamination with Salmonella spp. in general (3), and large outbreaks of typhoid fever are often due to faecal-contaminated drinking water. (10) It is important to know that *E. coli* is not a reliable indicator for contamination with *Vibrio cholerae* and two serotypes (*V. chlolerae* O1 and non-O1) have been found in waters free from *E. coli*.(9)

1.3.2 Total Coliform Bacteria and Thermotolerant Coliform Bacteria

Total coliform bacteria are a group of gram-negative bacteria that can grow in the presence of bile salts, ferment lactose (within 24 hours at 35- 37° Celsius) and produce β-galactosidase, as well as acid and aldehyde. Their ability to survive and grow in water makes the total coliform bacteria unusable for the indication of resent contamination with faeces. As described below, *E. coli* is the main indicator for faecal contamination. Nonetheless, total coliform bacteria are used as an indicator for the cleanliness of a water distribution system. (5)

Thermotolerant coliforms include, for the most part, *Escherichia*, but also *Citrobacter*, *Klebsiella* and *Enterobacter*. They are able to ferment lactose at 44 – 45° Celsius. *E.coli* can be identified by its abilities to produce indole from tryptophan and producing β-glucuronidase. (5) The production of β-glucuronidase enables the point-of-care-system Petrifilm™ (3M) to detect *E. coli* (10). The function of the Petrifilm™ will be introduced below.

1.3.3 Enterococci

As *E. coli, Enterococci* are a part of the physiological gut microbiota in humans and animals. Classified as streptococci group D, they can be held responsible for urinary tract infections, wound infections and are partly cause of an increasing number of nosocomial infections in hospitals. *Enterococci* in water samples are a sign of faecal contamination, because of their role as intestinal commensal. *E. faecalis* and *E. faecium* are of great relevance for human medicine. Other species are *E. raffinosus, E. casseliflavus, E. avium*or *E. munditii. Enterococci* are more resistant to chlorination than *E. coli* and therefore are as parameter additionally used when the raw water is of very low quality. *Enterococci* survive longer than *E. coli* in water, hence they indicate past contaminations.(9)

1.3.4 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative rod. Despite its role as a human pathogen microorganism, *Pseudomonas aeruginosa* is also used to check water quality. *Pseudomonas sp.* (species) can be found in the soil and in surface water. In sweet waters it survives similarly as long as *E. coli* and coliform bacteria.(9)

1.4 Health Issues Due to Low Drinking Water Quality

The SDG 3 'Ensure healthy lives and promote well-being for all at all ages' (p. 4) sees low quality drinking water and a lack of hygiene services as a major factor of risk concerning the mortality due to infections. This is especially the case in southern and central Asia, as well as in Sub-Saharan-Africa. (11)

In 2002, Prüss et al.(12) estimated the total disease burden in DALYs (disability-adjusted life years) related to water, sanitation and hygiene worldwide with 5.7%. Furthermore, 4% of all deaths are associable to water, sanitation and hygiene issues.(12)

Access to improved water sources lowers the under-five mortality rate, as Cheng et al. published in 2012.(13) Concluding their findings, the recommendation towards an improvement of water and sanitation access to achieve the millennium development goals (MDG) four and five, which are to improve child and maternal health, is made.(13)

The most frequent infectious disease epidemics transmitted through drinking water are cholera, typhoid fever and parasite infections.(9)

1.4.1 Bacteria Leading to Infectious Diseases when Contaminating Water

The human gastrointestinal tract is host to a wide number of bacterial species, mainly the Enterobacteriaceae. Nonetheless, pathogen species, for example Salmonella*, Yersinia, Shigella,* and pathogen variants of *E. coli* can enter this system mostly by oral ingestion and lead to infections. Figure 1 shows where these Enterobacteriaceae target the human gut. (9)

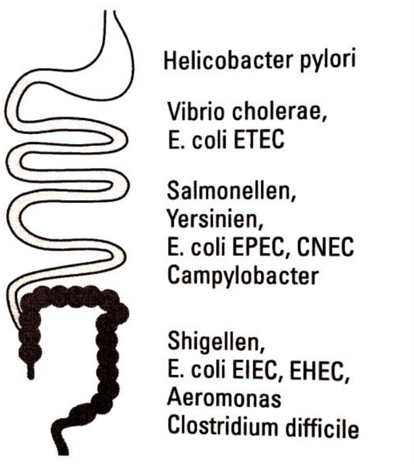


Figure 1: Pathogens and where they target the GI-tract, according to Heesemann, cited in Höll (9): p. 320

Another transmission pathway for bacteria is the inhalation of aerosols, especially *Legionella* or Mycobacteria can infect the human respiratory system.(5)

1.4.1.1 Pathogenic Strains of **E. coli**

Several enteropathogenic strains of *E. coli* have been identified. The severity of the clinical symptoms covers a wide range from mild to potentially fatal. The most common pathogenic strain is probably the enterohemorrhagic *E. coli* (EHEC), but there are even more:

- Enterohemorrhagic *E. coli* (EHEC) causes mild to highly bloody diarrhoea which in 2 - 7 % of the cases leads to the life-threatening haemolytic uremic syndrome (acute kidney failure, haemolytic anaemia). EHEC has a very high infectivity. 100 organisms can cause an infection. EHEC strains are commonly found in cattle, sheep, goats, pigs and chicken. (5)
  To point out the possible severity of infections with EHEC, an example in which the water system was the source of pathogens is given: In May 2000 an outbreak with EHEC serotype *(E. coli* O157:H7) and in coincidence with *Campylobacter jejuni* took place in Walkerton, a small town in Canada, associable with faeces contaminated water. Due to a lack of continuous chlorination and turbidity monitoring, the pathogens were able to enter one of the Walkerton's wells and sooner causing seven deaths and over 2000 infections.(14)

- Enterotoxigenic *E. coli* (ETEC) produces enterotoxins (both heat-labile and heat-stable) that lead to diarrhoea, being one of the most important reasons for diarrhoea in developing countries(5) and is endemic in warm climate zones.(9)

- Enteropathogenic *E. coli* (EPEC) causes non-bloody diarrhoea and is also mostly present in developing countries.(5) EPEC forms colonies adhering to the epithelial cells of the bowl, destroying the microvilli and interfering with the cells signal transduction.(9)

- Enteroinvasive *E. coli* (EIEC) strains have been known to invade the cells of the colon. Invasion of epithelial cells that leads to diarrhoea with blood and mucous.(9)

Furthermore, there is the Enteroaggressive *E. coli* (EAEC) and the diffusely adherent *E. coli* (DAEC). The WHO states in its guidelines, that 'conventional testings for *E. coli* (or, alternatively thermotolerant coliform bacteria) provides an appropriate indicator for the enteropathogenic serotypes in drinking water.'(5: p241)

1.4.1.2 **Shigella**

Shigella are gram-negative rods that are specifically found in humans and primates. They are subdivided in four species: *S. dysenteriae, S. boydii, S. flexneri,* and *S. sonnei*. The latter two named are endemic in Europe. (9) All *Shigella spp*. (species pluralis) lead to bacterial dysenteria, causing over 2 million infections and 600 000 deaths every year, mainly in developing countries. Because *Shigella spp.* are not stable in water and can only be found in humans and primates, they are an indicator for recent contamination with human (or primate) faeces.(5)

1.4.1.3 Salmonella

Salmonella (usually not written in italics) are gram-negative, facultative anaerobic bacteria. The subspecies S. enterica causes enteritis and diarrhoea and /or typhoid fever (S. typhi and paratyphi). (6) Non-typhoidal salmonella (NTS) are rising in prevalence and cause, especially in patients with compromised immune systems severe and invasive salmonellosis. (7)

One disease caused by salmonella is typhoid fever. More precisely, it is caused by Salmonella entericaserovar Typhi. Symptoms of the typhoid fever are, as mentioned in the name, fever, as well as chills, headaches, general malaise, muscle aches, constipation and sometimes emesis. In severe cases or without treatment confusion, perforation of the intestines or death may occur. (8) The incidence for South Asia is with more than 100 cases per 100 000 person-years the highest in comparison to the rest of the world. John et al. (15) state that the prevalence (laboratory-confirmed) in India is estimated to be about 9.7% in patients with fever. Since the 1950s, a decline in prevalence is noticeable. Nevertheless, typhoid fever remains a problem as especially children suffer from the disease. Commonly described factors of risk are poor sanitation, a lack of hygiene practices, low social-economic status and a lack of adequate-quality drinking water. (9)

Gandra et al. (16) detected Salmonella sp. as the most frequent gram-negative bacteria isolated in blood cultures across India.

1.4.1.4 **Vibrio cholerae**

Vibrio cholerae is a gram-negative rod with a flagellum and the ability of producing a mucinase that helps the bacteria penetrating the mucous membrane of the small bowel. Infections with *Vibrio cholerae* lead to watery diarrhoea with a loss of fluids up to 25 litres per day. At the end of the twentieth century, the lethality rate for intestinal *Vibrio cholerae* infections was 15.2 % and the frequency of transmission through drinking water was 83.3 %.(9)

1.5 Problems Due to Chemical or Physical Aberrations in Water

If chemical substances appear in drinking water they show adverse health effects only after years and years of exposure. There are also a number of chemicals substances and parameters, which are of no health concern but may have an effect on the acceptability of the water, for example, chloride, iron, manganese, pH and hardness of the water. Only elevated nitrate can lead to immediate problems, as described below. (5)

1.5.1 Methaemoglobinaemia

Methaemoglobin is being formed when haemoglobin meets nitrite. The methaemoglobin binds oxygen and does not further release it. As a consequence, the oxygen transport is blocked. When the methaemoglobin level exceeds 10% of all the haemoglobin in a human body, cyanosis will occur. In infants, this symptom is called "blue baby syndrome". This is a great risk for bottle-fed infants, especially in developing countries. Studies showed that methaemoglobinaemia does not occur in infants when the nitrate level did not exceed 50 mg per litre. (5)

1.6 India and the Study Area Tamil Nadu

The following will describe the situation in India, starting with general information and going into the details of the study area, everything with regard to water quality and accessibility. Furthermore, an overview about antimicrobial resistances of bacteria in India is given.

1.6.1 General Information about India

1.6.1.1 Population in India

India is the second most populous country in the world, counting 1,281,935,911 inhabitants. The median age is 27.9 years. The demographic development in India leads towards a high density population. Increasing population being one reason, medical care in India is challenging. There are only 0.73 physicians available per 1000 population. (17) In comparison, Austria counts 5.15 physicians per 1000 population.(18)

If basic drinking water service is defined as water accessible within less than 30 min collection time (3) , only 88 % of the Indians have access to basic drinking water service. (19) Additionally, this water source has often to be improved, which means it has to be protected from, mainly, faecal contamination from outside. This term was established by the *Joint Monitoring Program for Water Supply and Sanitation* of the WHO and UNICEF.(20)

1.6.1.2 Geography and Climate in India

As by area the eighth largest country in the world India shares borders with Bangladesh, Burma, Bhutan, China, Nepal and Pakistan. Huge parts of India are surrounded by water, more precisely by the Bay of Bengal in the east and the Arabian Sea in the west. The capital is New Delhi. (17) India counts 28 states and 7 union territories. (21)

The northern parts of the country have a continental, more moderate climate with low temperatures in the winter. Oppositely, southern India is coined by a hot and tropical climate. During this time, the monsoons streak India bringing a lot of rain, coming from the ocean and carried by trade winds. (22)

1.6.2 India's State Tamil Nadu

Tamil Nadu is localized in the South-East of India.(23) The capital is Chennai, inhabited by over 72 million people (more specific 72,147,030) and the spoken language is Tamil.(24) Speaking of climate, Tamil Nadu is not getting as much rain from the south-west monsoon as the other states due to the Western Ghats, a mountain region, holding of clouds from the west.(25)

In Tamil Nadu, agriculture and food production are the main occupation. Plantations and forests, as well as the production of bio-fertilizers are branches of the local economy. Further industries are the automobile and railway coach production, tanning and cotton.(24) Tamil Nadu holds 3 % of the water resources in proportion to the whole country India. 2 400 000 hectares of land are irrigated with surface water. The Tamil Nadu Government formulated some key challenges in 2017. On the one hand the reduction of the groundwater extraction, which is beyond recharge capacity, is necessary. On the other hand, the pollution of water by fertilizer usage, the industry, and municipal wastewater needs to be limited. (25)

1.6.3 Bacterial Resistances in India

The occurrence of multiresistant bacteria is an emerging problem worldwide. Kantele et al. identified South Asia as a high risk region for acquiring extended-spectrum betalactamase-producing *Enterobacteriaceae* (ESBL-E) when travelling.(26) From 2008 to 2014, Gandra et al. conducted a study across India collecting blood samples for cultures that showed resistance rates of *E. coli* to fluorchinolones and cephalosporines of the third generation up to 80%. Also resistance to carbapenems was found in *Pseudomonas aeruginosa* (49%), in *Klebsiella pneumoniae* (41.4 – 56.6%) and *E. coli* (7.8 to 11.5%). (16)

In a study, which was conducted in New Delhi, Lamba et al. found a significant correlation between faecal coliforms and antibiotic resistant bacteria in the effluents of the city's sewage treatment plants. (27) In a further study, the same research team found a significant correlation between the levels of faecal coliforms and carbapenem-resistant Enterobacteriaceae in hospital waste waters and implied that those resistances originate from hospitalised patients, treated with antibiotics.(28)

2 Material and Methods

Aim of the study was to answer the question, if a point-of-care-testing with Petrifilms™ is a reliable way to test the water quality in health care centres without access to microbiological laboratories. The information and material for this thesis came, on the one hand, directly from India, where data about the water system and the water quality (up to November 2017) have been surveyed and the samples have been collected and partially analysed (point-of-care-testing). On the other hand, the results (physical, chemical and bacteriological analysis) from the water laboratory of the Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine in Graz, Austria, and literature research provided additional information. The study was conducted at the S.F.S. Health Centre in Kakkaveri which is a small village in rural India, about 43 km away from the city Salem in the district Salem in Tamil Nadu. The S.F.S. Health Centre in Kakkaveri is a small hospital with 17 rooms. There are between two and six beds in every room. There is a sink with a water tap installed in every room. Available services in Kakkaveri hospital are spread from general medicine and surgery to maternity and child care. Furthermore, X-ray, ECG (electrocardiography) and ultrasound scans are accessible. The hospital pays special attention to HIV (human immunodeficiency virus), leprosy, tuberculosis and STI (sexually transmitted infections)-patients in care and rehabilitation.(29) According to the hospital management, Kakkaveri hospital provides health care for about 2170 patients per year in the inpatient sector and 12.576 in the out-patient sector.(30) The S.F.S. Health Centre is part of the Doctor Typhagne Memorial Charitable trust.(29)

2.1 Water Sampling

At first, an investigation and documentation (through photographs, see appendix) of the water supply system was conducted. As a next step, points for water sampling were determined, following the relevant points of the water circulation through the hospital. The collection of the water samples was conducted using the following materials:

- 5 sterile bottles for chemical and physical analysis (each 0.5l)
- 15 bottles for bacteriological analysis (each 0.25l)
- 30 point-of-care-testing: Petrifilm™ *E. coli* and coliform count (ECC) plates; 3M

Additionally, swabs and dip slides were used to get sample material from difficult to access places, such as the inner parts and filters of water taps. The usual analysis in a water laboratory does not include susceptibility testing. Also for this purpose, the swabs and dip slides have been chosen as method. Another benefit of the swabs and dip slides is that they are easily transportable.

- 12 dip slides: Envirocheck® dip slides; Merck
- 12 cotton swabs: Amies Gel Agar Medium Transport Swabs ®; COPAN Diagnostics

In addition to the above-named materials, sterile syringes (2ml) and sterile pipettes (3ml), a gas lighter, a permanent marker, gloves, paper and pencils (for documentation) were needed. Before the collection started, all materials have been numbered and assigned to a sampling place (numbers 1 to 15). The order of actions at each of the 15 sampling place was as following:

- Photographical documentation (Appendix)
- Documentation of date and time of sampling (
- Table 5)
- Heating of the tap with the gas lighter (Figure 2)
- Filling of the bottle for chemical analysis
- Filling of the bottle for bacteriological analysis
- Inoculation of the Petrifilms™
- Cotton swab from inside the water tap
- Dip slide from the opening of the water tap

Figure 2: Heating of a water tap, according to the guidelines – Photographer: Angelika Schirnhofer

On the next page Figure 3 gives a brief overview over the used materials and methods of evaluation. In Figure 4 the collected samples are shown.

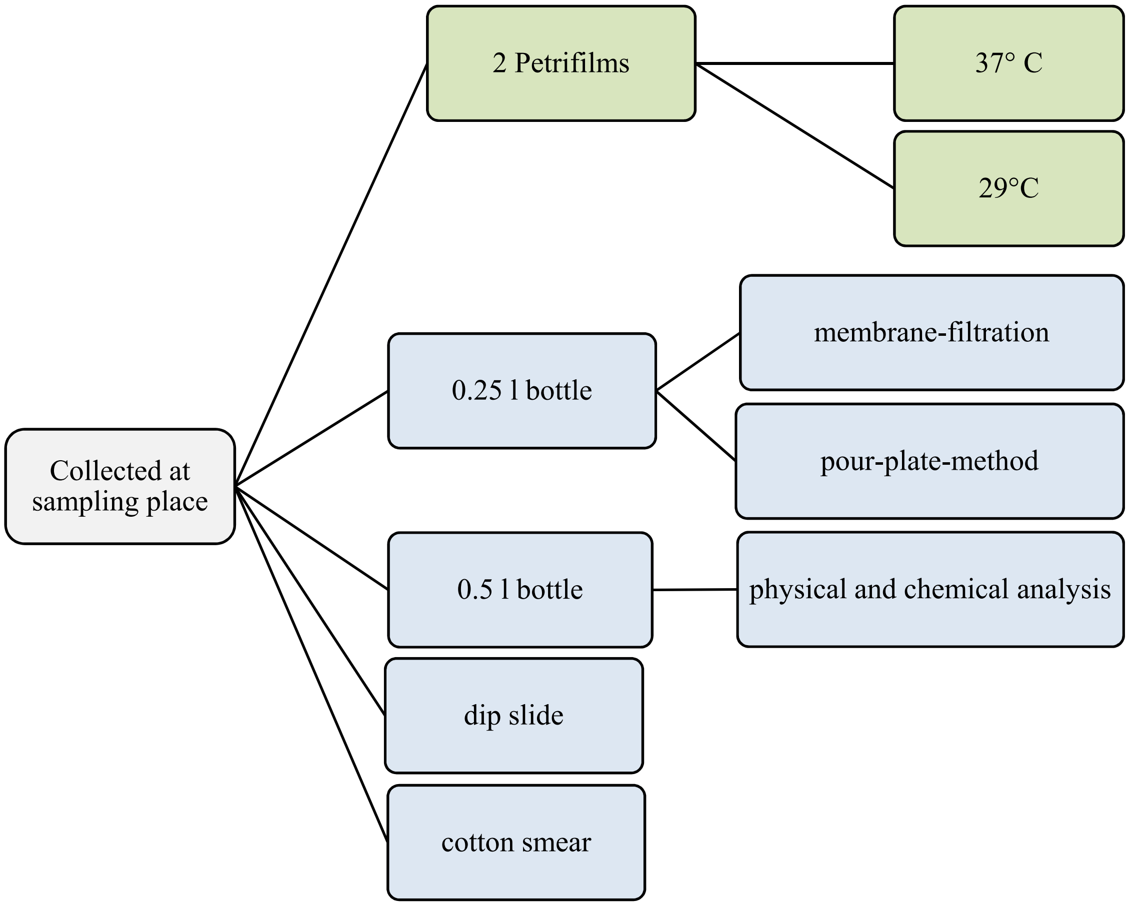


Figure 3: Overview water sampling and methods

Methods in green coloured boxes were performed in Kakkaveri, whereas blue coloured boxes mark collected samples, which were analysed in the water laboratory in Graz.

Figure 4: The collected samples: Left front Petrifilms™ behind are the dip slides; right front filled bottles and right behind transport containers filled with the cotton swabs – Photographer: Angelika Schirnhofer

2.2 Point-of-Care-Testing of Water Samples in Kakkaveri, India

To use it as a point-of-care-testing “3M™ Petrifilm™ *E. coli*/Coliform Count Plates” have been brought along to India. The Petrifilms™ contains a violet red bile agar as a nutrient medium and a colour indicator (tetrazolium) to facilitate the counting of the colonies that had formed. Also a water-soluble gelling agent is added to ensure the transportability of the plates. The coliform bacteria are gram-negative and ferment lactose and hence produce acid and gas. Furthermore, the Petrifilms™ contain an indicator for glucuronidase activity, which is necessary for testing for *E.coli*. (10)

The Petrifilms™ need to be inoculated with 1ml of a water sample and depending to the chosen method, have to be incubated for 24-48 hours. In total, there are five methods for the evaluation of the Petrifilms™ available. They all require different incubation periods and temperatures. (31) For the usage in the India-project in November we chose to incubate the Petrifilms™ for 24 hours at 37° Celsius for the following two reasons:

1) Striving for the best comparability of the samples, the Petrifilms™ and the samples for the water laboratory (bottles, dip slides and cotton swabs) have been collected at the same day. The samples that ought to be brought back to Graz, Austria must not be stored for longer periods of time. The minimal possible storage time was 24 hours, because the shortest period of incubation for the Petrifilms™ is 24 hours.

2) Additionally, the incubator for the Petrifilms™ (provided from the Institute for Pathology) was located in the bigger city Salem, where the team of the GHD spent the last day before flying back to Austria. This incubator had a maximum temperature of 37° Celsius.

The chosen method of evaluation was the AOAC-method (short for “association of official agricultural chemists / association of official analytical chemists”). This method allows the detection for coliform bacteria after an incubation period of 24 hours. For the detection of *E. coli* 48 hours of incubation would have been necessary. For this reason only the coliform bacteria colonies have been registered. (31) The AOAC-method (991.14) can be used for counting coliform bacteria colonies after 24 hours (±2h) at 35° Celsius (± 1°C).(31)

Because of the fact that sample incubators are not accessible everywhere in rural India, two groups of Petrifilm™ samples have been formed (Figure 5). The aim was to find out the function of the Petrifilms™ at room temperature in comparison with the recommended incubation temperature. The measured room temperature during the incubation period was stable at 29° (± 1°) Celsius.

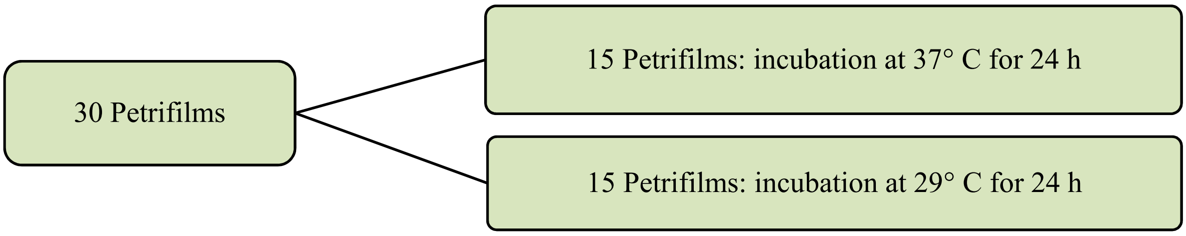


Figure 5: Petrifilm™ incubation times, temperatures and methods of evaluation

2.3 Analysis of the Collected Water Samples

Back in Austria, all collected samples were further analysed at the Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz. At the water laboratory a bacteriological as well as a physical and chemical analysis were performed. The bacteriological testing involved the pour-plate method and the membrane filtration method from the water, collected in the sterile 0.25l bottles. At the bacteriological laboratory the cotton swabs and dip slides were analysed.

2.3.1 Bacteriological Testing at the Water Laboratory

2.3.1.1 Pour-Plate Method

According to the EN ISO 8199:2007 three plates for each sample have been inoculated with the water samples brought from India (Figure 6):

- 1 ml at 22 ± 2° Celsius
- 0.1ml at 22 ± 2° Celsius
- 1ml at 36 ± 2° Celsius

The 45 sterile plates have been accurately labelled before the tests started. After the water sample is pipette onto the sterile plates, 15ml of yeast extract agar is poured onto the samples and carefully mixed with it.(32) The yeast extract agar has been prepared as stated in ISO 6222 and had a temperature of 45° Celsius when it was poured onto the samples.(33)Then, when the agar has solidified itself, the samples are incubated.(32) The 22° Celsius samples have been incubated for 68 hours(±4 h) and the 36° Celsius samples for 44 hours(±4h).(33) After the incubation period, the colonies are counted.

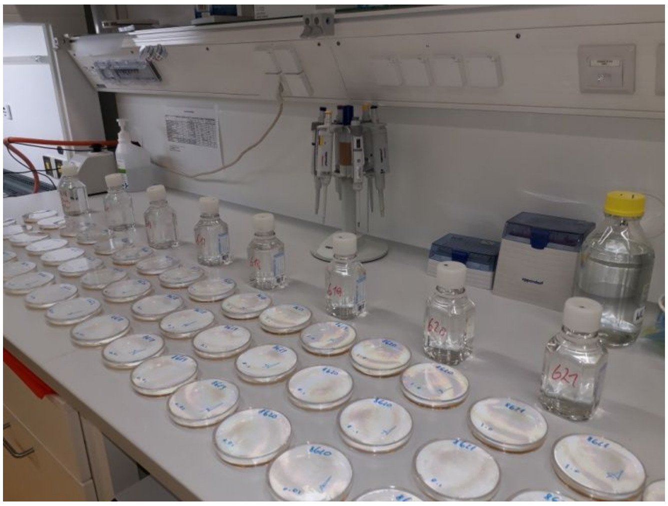


Figure 6: Pour-plate method at the water laboratory, Medical University of Graz – Photographer: Sabine Platzer

2.3.1.2 Membrane Filtration

The membrane filtration method (Figure 7) is defined in EN ISO 8199:2007 (German version). It is a suitable method for water samples with few particles or colloid materials. For this reason, the method can be used to analyse drinking water. The water sample in a defined volume (at least 10ml) is filled into a funnel and then it is filtered through a sterile membrane by negative pressure (about 70kPa). The membrane has pores sized 0.45µm. Bacteria bigger than the pores cannot go through and hence stay in the membrane.(32)

As a next step, the membrane with the filtered bacteria is put onto selective plates and is incubated. The plates used for the Indian water samples were:

- "Chromocult® Coliform Agar (ISO)" for *E. coli* and coliform bacteria
- "Slanetz and Bartley Agar" for *Enterococci* with an incubation at 36° Celsius (± 2° C) and reading after 44 hours (±4 h)

- "Pseudomonas Agar Base" + "Pseudomonas C-N Sel. Suppl." for *Pseudomonas aeruginosa*

The colonies must be counted immediately after the incubation period and for exact characterisation confirmatory tests might be necessary.

Figure 7: Membrane filtration method for water samples at the water laboratory, Medical University of Graz – Photographer: Sabine Platzer

2.3.2 Analysis of the Cotton Swabs and Dip Slides

Cultivation of the swabs was performed using thioglycolate, blood- and Mac Conkey Agar, (Becton Dickinson). All plates were incubated for 48 h at 37° Celsius, followed by a visual evaluation of the colonies.

The dip slides were incubated for 48 hours at 37° Celsius. After the incubation period, a visual evaluation and a semiquantitative colony counting were performed. The results of the semiquantitative colony count were classified in (+) (very sporadic), + (sporadic), ++ (moderate), and +++ (plentiful). After that colonies were transmitted on Müller Hinton agar plates (Bio Merieux) for further identification.

2.3.2.1 Identification of Bacterial Species and Resistance Testing

The identification of the bacteria was performed using mass spectrometry (Matrix-assisted laser desorption time-of-flight mass spectrometry(MALDI-TOF MS from Bio Merieux)). An antimicrobial susceptibility testing (AST) was done using an agar diffusion test. The resistances have been interpreted according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).(34)

2.4 Physical-Chemical Analysis of Water Samples

A physical and chemical testing was also performed in the water laboratory. For that the water collected in the 0.5 l bottles has been used. The water was analysed according to the following standards (35):

- Colour (436 nm): DIN EN ISO 7887
- pH-value: DIN 10523
- Electric conductivity: EN 27888
- Total hardness: DIN 38406-3
- Carbon hardness: EN ISO 9963-1
- Iron: DIN 38406-1
- Manganese: DIN 38406-33
- Ammonium: DIN 38406-5
- Nitrite: EN 26777
- Nitrate, Chloride and Sulphate: EN ISO 10304-1
- TOC: DIN EN ISO-1484

2.5 Literature Research

Literature research started in September 2017. The information used for this thesis came, on the one hand, from reference books about water quality, from documents provided from the WHO (for example the JMP WASH documents) and governmental documents, for example, the drinking water ordinances from Austria and India have been used. On the other hand thorough search on "PubMed.gov" and “scholar.google.at” was conducted. The Diagnostic and Research Institute for Hygiene, Microbiology and Environmental Medicine in Graz provided access to the ISO standards and several books about water quality and related topics.

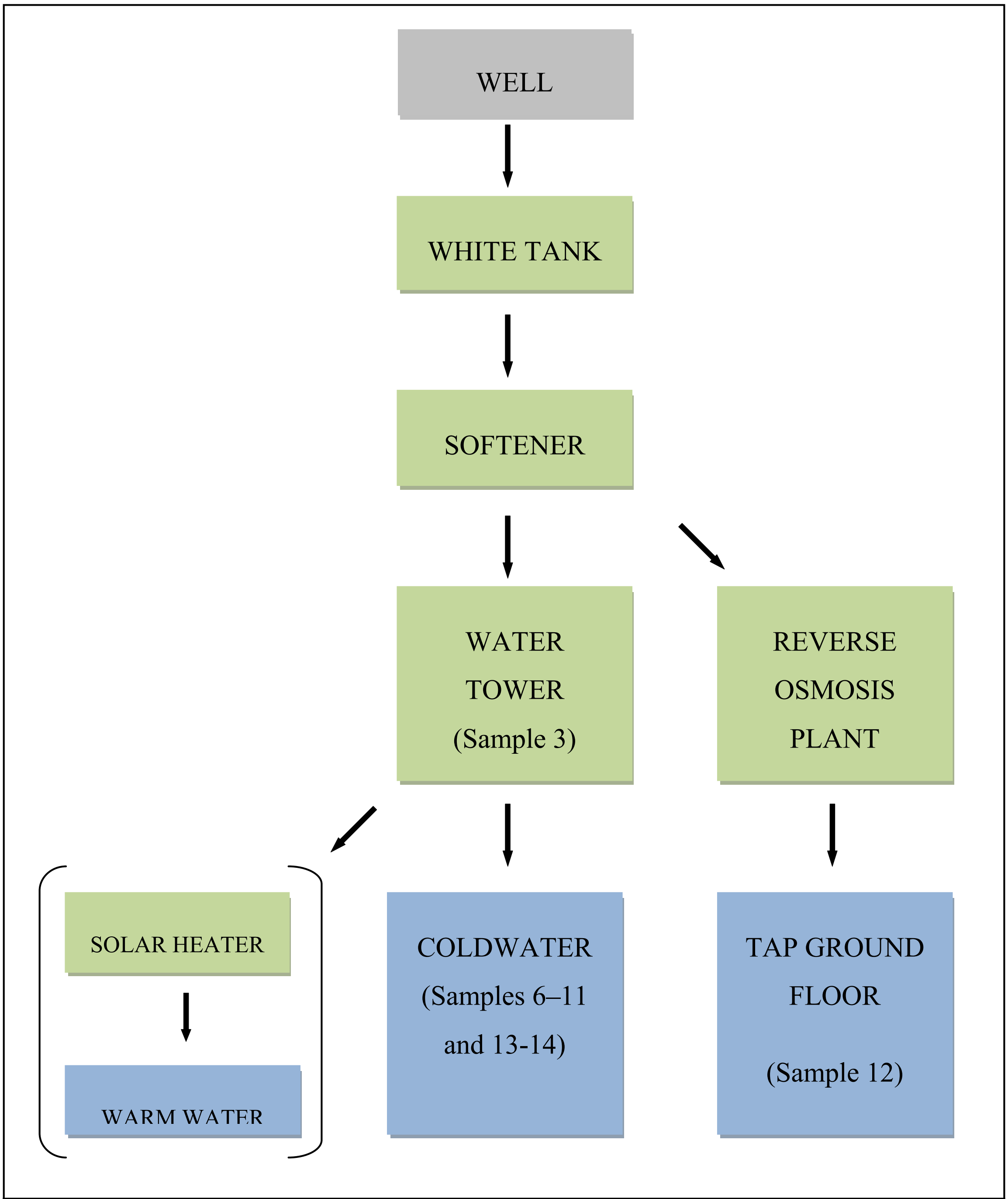
3 Collection Procedure and Documentation

In the following, the water system and the sampling points at Kakkaveri hospital are described and the protocol of collection is given.

3.1 Water Sampling Points and the Water System in Kakkaveri

Before the samples were collected, the relevant sampling points had to be identified. The local responsible employee was asked about the way of the water through the pipe system and a plan was created. This plan is shown in Figure 8. Resulting in 15 sampling points, photographs have been taken to document the current condition of the sampling places when the water was collected in November 2017.

The hospital in Kakkaveri gets all its water from a well located on the premises. As it is shown in Figure 8 the well is connected to a water tank (white tank, sample number 2) which can be found on the roof of the hospital. From there the water is pumped through a water softener. The softened water then takes different directions. The majority of it is saved in a big water tank located in a tower of the hospital (sample number 3). Another part of the water is undergoing further processing and therefore enters the water purification plant, which is working with reverse osmosis (R.O.-plant). The purified water from the R.O.-plant is first piped into the metallic tank (sample number 4) and later to one tap on the ground floor of the hospital (sample number 12).A small part is also led from the tower into the solar heater.



Grey:	behind the main building of the hospital; well (Sample 1)
Green:	on the hospital roof
Blue:	in the building; water taps

Figure 8: Overview water system Kakkaveri, India

3.2 Water Testing Routinely Performed in Kakkaveri

The responsible person for the water of Kakkaveri hospital does a weekly check of the total dissolved solids (TDS). Therefore he uses a device named TDS-3 (HM Digital). Those checks are important to verify the function of the R.O.-plant membrane. If TDS exceeds a certain limit, the membrane needs to be changed. The written documentation of the values in the orange notebook (Figure 9) goes back to February 2016. The device uses the measurement unit parts per million (ppm). 1 ppm is approximately 1 mg/l.(36) The WHO claims that there are no health-based guidelines for the TDS set and that water with a TDS value less than 1000 mg/l is known to be palatable. Above that value, the water might not be acceptable for human taste. (5)

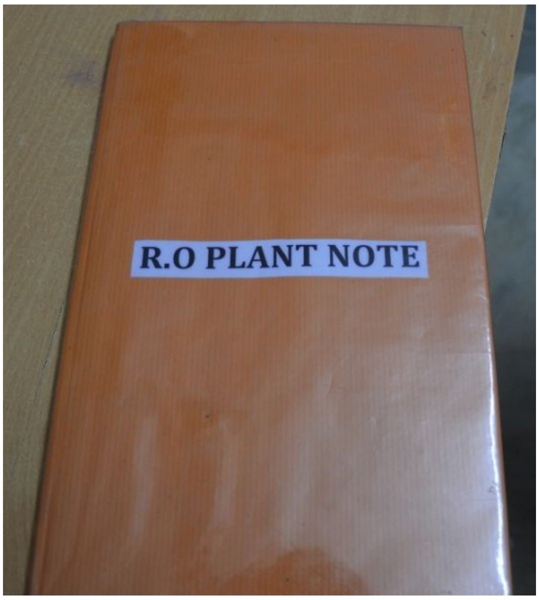


Figure 9: Notebook with TDS values – Photographer: Isabella Dicker

3.3 Collection of the Water Samples in Kakkaveri

All the water samples have been collected on the 10th November 2017. The exact times are recorded in the protocol. In every of the 15 locations five to six samples for different analytical methods have been taken (

Table 5).

No.	Sampling Place	Time	Chemical and Physical Testing	Bacteriological Testing	Petrifilm I	Petrifilm II	Cotton swab	Dip slide
1	Well	8:41 AM	X	X	X	X		
2	White tank on the roof	9:03 AM		X	X	X		
3	Water in the tower	9:31 AM	X	X	X	X		
4	Metallic tank	9:10 AM		X	X	X	X	X
5	Solar heater	9:17 AM		X	X	X	X	X
6	Water tap operating theatre (OT)	9:54 AM		X	X	X	X	X
7	Water tap intensive care unit (ICU)	10:07 AM		X	X	X	X	X
8	Water tap room 4, 1st floor	10:10 AM		X	X	X	X	X
9	Water tap in the bathroom of room 4, 1st floor	10:19 AM		X	X	X	X	X
10	Water tap room D, ground floor	10:30 AM		X	X	X	X	X
11	Water tap room C, ground floor	10:48 AM	X	X	X	X	X	X
12	Water tap with reverse-osmosis-plant water, ground floor	10:57 AM	X	X	X	X	X	X
13	Water tank 1st floor	11:08 AM		X	X	X	X	X
14	Water tank ground floor	11:15 AM		X	X	X	X	X
15	Oxygen-humidifier, pow and OT	11:23 AM		X	X	X	X	X

Table 5: Protocol of the collection of water samples in Kakkaveri, India

3.4 Limitations of the Study

When critically observing the used methods it needs to be mentioned that in this study the number of samples was limited, due to the weight limitations on flights. Furthermore, there was no sample incubator available in the rural village of Kakkaveri. Also the team spent only one day and one night in Salem, where the used sample incubator was located (at the Institute of Pathology), resulting in a limitation of time and an incubation period of 24 hours (instead of 48 hours). Despite all accuracy and diligence when taking the samples, there is always a small potential of iatrogenic contamination. The samples that have been brought back to Austria for laboratory analysis additionally might have suffered from the journey and therefore showed lower/maybe higher values as they would have when tested immediately after collection. Summarizing all that points, the results have to be considered as a snapshot of a specific situation. To provide a steady picture of the situation concerning water quality, regular check-ups are needed.

4 Results

In the following the results of the bacteriological and chemical analyses of the water samples are presented. The data are given in tables and diagrams, as well as in written form. The interpretation of the results was done in accordance with India's drinking water quality standards. If there was no Indian reference value given, the Austrian drinking water ordinance from 2017 was consulted. A comparison of the limits is given on page 20. The team did not change or alter neither the water distribution system nor the process of the water treatment and interference has been avoided. It is necessary to state that all results may only be seen as a snapshot and an actual state analysis.

4.1 Results Bacteriological Analysis at Water Laboratory, Graz

The routine microbiological testing performed at the water laboratory includes colony forming units (CFU) at 22 and 37° Celsius, as well as the growth of the bacteria *E. coli, Enterococci* and *Pseudomonas aeruginosa* and coliform bacteria. In every sample, the CFU at 22° Celsius and at 37° Celsius have grown above the recommended maxima (100.0%; 15/15). Especially the water tower (sample 3), the tap water in room R4 (sample 9), the tap water in room C (sample 11) and water tanks' water (sample 13) remarkably outreached the limitations with values >100 000 CFU/ml (Figure 10). Indicating faecal contamination, *E. coli* was found in 1/15 (6.6%) samples (sample 1: wells water). *Enterococci* were also present in 1/15 (6.6%) samples, which was in the bathroom of room R4 (sample 9).

Pseudomonas aeruginosa (Figure 11) was found in 11/15 (73.3%) samples. Growth was found in the samples 1-3, 5-11 and 13. No growth of *P. aeruginosa* was found in the R.O.-plant treated water (samples 4, 12, 14), the tap water from room D (sample 10) and the water from the oxygen humidifier (sample 15), which is usually filled with distilled water. Coliform bacteria were found in 10/15 samples (66.6%), including the samples 2, 3, 5-9, 11, 13 and 15. Coliform bacteria were not found in the R.O.-plant water samples (4, 12, and 14). There was also no contamination with coliform bacteria in the white water tank (sample 2) and the tap water from room D (sample 10).Table 6 summarises all the bacteriological results. As there is no standard for colony forming units, *Enterococci* and

Pseudomonas aeruginosa given in the Indian Standard for drinking water (5), Austria's drinking water ordinance has been used.

No.	CFU 22	CFU 37	*E. coli*	*Entero-cocci*	*Pseudomonas aeruginosa*	Coliform bacteria
1	**4100**	**19000**	**170**	0	**40**	**210**
2	**350**	**7300**	0	0	**50**	0
3	**>100000**	**>100000**	0	0	**26000**	**4300**
4	**5900**	**15000**	0	0	0	0
5	**37200**	**24000**	0	0	**1200**	**12**
6	**8600**	**8800**	0	0	**7000**	**4**
7	**13000**	**10000**	0	0	**120**	**8**
8	**14400**	**6000**	0	0	**11000**	**130**
9	**>100000**	**>100000**	0	2	**26000**	**220**
10	**18000**	**30000**	0	0	**200**	0
11	**>100000**	**27000**	0	0	**2900**	**240**
12	**12000**	**24000**	0	0	0	0
13	**>100000**	**>100000**	0	0	**6**	**200**
14	**8200**	**1800**	0	0	0	0
15	**5800**	**5300**	0	0	0	**46**

Table 6: Results of the bacteriological testing of the water samples from Kakkaveri, tested at the water laboratory;

CFU = colony forming units/ml

Bold: elevated values

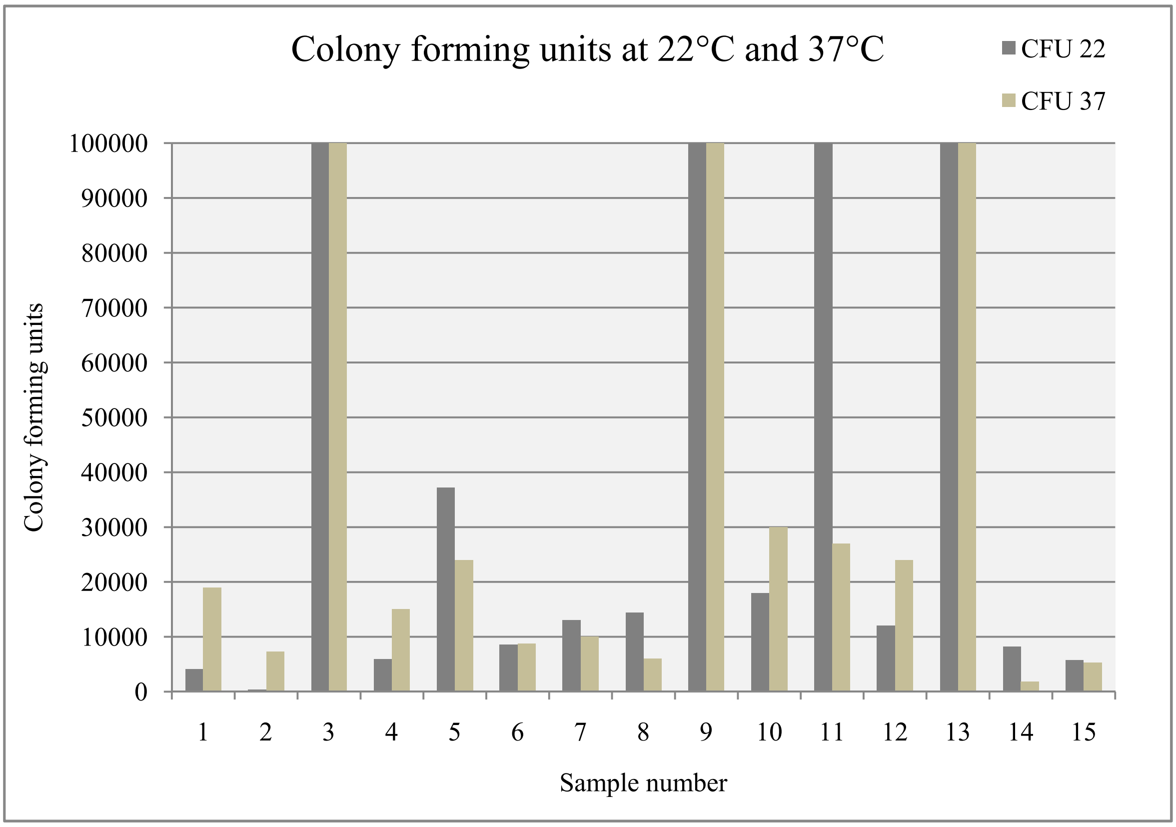


Figure 10: Results colony forming units of the water samples from Kakkaveri analysed at the water laboratory

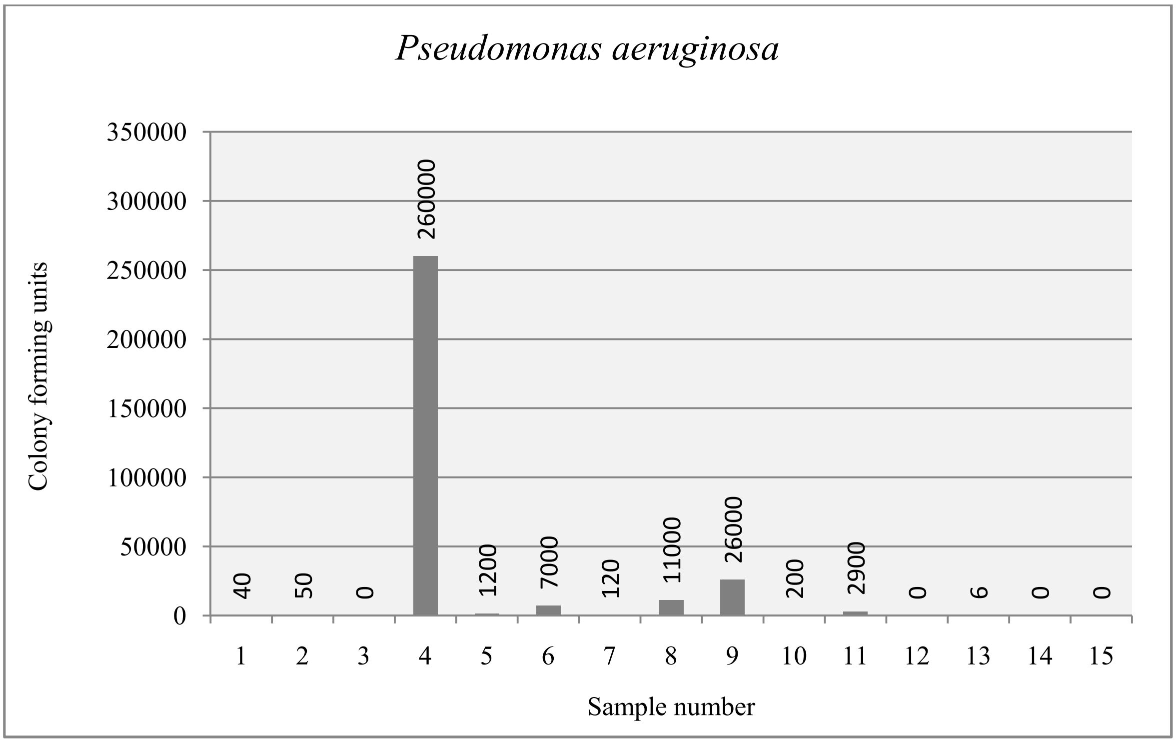


Figure 11: Results *Pseudomonas aeruginosa* of the water samples from Kakkaveri analysed at the water laboratory

4.1.1 Results Cotton Swabs and Dip Slides

Microorganisms found on the cotton swabs samples were *Pseudomonas aeruginosa* (10/12; 83.3%), *Enterobacter sp.* (7/12; 58.3%), *Klebsiella pneumoniae*(3/12; 25.0%), *Stenotrophomonas maltophilia* (3/12; 25.0%), aerobic spore-forming bacteria (3/12; 25.0%), *Neisseria sp*. (1/12; 8.3 %) and *Micrococcus sp*.(1/12; 8.3 %).

The found microorganisms on the dip slides included *Pseudomonas aeruginosa* (11/12; 91.6%), *Klebsiella pneumoniae* (3/12; 25.0%), *Micrococcus sp.* (2/12; 16.6%), aerobic spore-forming bacteria (4/12; 33.3%), mould (2/12; 16.6%), *Aspergillus sp.* (1/12; 8.3 %) and *Candida sp*. (1/12; 8.3 %).

4.1.2 Results Bacterial Resistances

All of the bacteria found on the cotton swabs and dip slides showed normal susceptibility to the tested antibiotics, respectively all demonstrated their natural resistance pattern. None of the Enterobacteriaceae was therefore an extended spectrum β-lactamase producer, nor was resistance to carbapenems found.

4.2 Results Point-of-Care-Testing Using Petrifilms™

A total number of 30 Petrifilms™ has been evaluated. Two groups with 15 Petrifilms™ each have been formed. We took two samples from each sampling place. The Petrifilms™ of group I were incubated at 37° Celsius for 24 hours. Group II was left in a room at 29 ±1° Celsius for 24 hours (room temperature). As mentioned in chapter 2.2, the Petrifilms™ have been analysed according to the manufacturer guideline using the AOAC method. The results are depicted and compared to the laboratory results in Table 7.

4.3 Results Point-of-Care-Testing Petrifilms™ Compared to Results Routine Microbiological Laboratory

In comparison to the results for coliform bacteria tested in the water laboratory, the Petrifilms™ of group I do match in 66.6 % (10/15), group II in 53.3 % (8/15).Because the sample volume of the Point-of-care-testing is 100 times smaller than the sample volume used in the laboratory, this percentage does not include the laboratory results <100 CFU. The laboratory values >100 CFU /100ml are written bold in the table below. From six measurements >100 CFU five are also registered with the Petrifilms™.

In two cases (sample 9 and 11) the Petrifilms™ of group I did not show bacteria (13.3%; 2/15) even when the laboratory found >100 CFU /100 ml. Sample 7 showed a lot more bacteria in the Petrifilm™ sample, than in the water laboratory and is thus classified as false positive. Table 7 depicts the percentages of the matching results of every Petrifilm™ sample incubated at 37° C.

No.	Laboratory confirmed Coliform bacteria [CFU/100ml]	[CFU/1ml]*	Petrifilms™ 37°Coliformbacteria [CFU/1ml]	Matching percentage [%]
1	**210**	**2.10**	1	47.62 % (1/2.10)
2	0	0.00	0	100.00 % (0 / 0.00)
3	**4300**	**43.00**	1	2.33 % (1/43.00)
4	0	0.00	0	100.00 % (0 / 0.00)
5	12	0.12	0	0.00 % (0/0.12)
6	4	0.04	0	0.00 % (0 / 0.04)
7	8	0.08	2	** 2500.00 % (2 / 0.08)
8	**130**	**1.30**	1	76.92 % (1 / 1.30)
9	**220**	**2.20**	0	0.00 % (0 / 2.20)
10	0	0.00	0	100.00 % (0 / 0.00)
11	**240**	**2.40**	0	0.00 % (0 / 2.40)
12	0	0.00	0	10.00 % (0 / 0.00)
13	**200**	**20.00**	1	5.00 % (1 / 20.00)
14	0	0.00	0	100.00 % (0 / 0.00)
15	**46**	**4.60**	0	0.00 % (0 / 4.60)

Table 7: Comparison of coliform bacteria laboratory confirmed and detected with Petrifilms™ 37°C (Group I);

CFU = colony forming units

Bold = detectable values (>100 CFU /100ml)

***calculated colony forming units (divided by 100)**

****false positive result**

The same situation occurred in some samples of group II, incubated at 29 ° Celsius, where the samples 3, 8 and 9 gave false negative results (20.0%; 3/15), when compared to the results of the microbiological laboratory. The detailed matching percentages for every Petrifilm™ sample incubated at 29° C are listed in the following Table (Table 8).

	Laboratory confirmed Coliform bacteria		Petrifilms™ 29°C Coliform bacteria	Matching percentage
No.	[CFU/100ml]	[CFU/1ml]*	[CFU/1ml]	[%]
1	**210**	**2.10**	2	95.24 % (2 / 2.10)
2	0	0.00	0	100.00 % (0 / 0.00)
3	**4300**	**43.00**	0	0.00 % (0 / 43.00)
4	0	0.00	0	100.00 % (0 / 0.00)
5	12	0.12	0	0.00% (0 / 0.12)
6	4	0.04	0	0.00% (0 / 0.04)
7	8	0.08	0	0.00% (0 / 0.08)
8	**130**	**1.30**	0	0.00% (0 / 1.30)
9	**220**	**2.20**	0	0.00% (0 / 2.20)
10	0	0.00	0	100.00 % (0 / 0.00)
11	**240**	**2.40**	1	41.67 % (1 / 2.40)
12	0	0.00	0	100.00 % (0 / 0.00)
13	**200**	**20.00**	2	10.00 % (2 / 20.00)
14	0	0.00	0	100.00 % (0 / 0.00)
15	**46**	**4.60**	0	0.00% (0 / 4.60)

Table 8: Comparison of coliform bacteria laboratory confirmed and detected with Petrifilms™ 29°C (Group II);

CFU = colony forming units

Bold = detectable values (>100 CFU /100ml)

* **calculated colony forming units (divided by 100)**

4.4 Results Chemical Testing

The chemical analysis does not show any health concern of the drinking water. The total hardness of the water was elevated excessively throughout the water samples, except the R.O.-plant's water. The R.O.-plant water sample (sample 12) showed only one parameter out of the limitations: the pH-value. Table 9 presents all elevated values (written bold).

No.	Colour [/m]	pH at 20°	TH °dH	EC [µS/cm]	Iron [mg/l]	Nitrate [mg/l]	Chloride [mg/l]	Sulphate [mg/l]
1	**1.2/m**	⊥*	**47.7**	**2971**	**0.29**	**41.5**	**579.4**	**286.5**
3	**0.6/m**	⊥	**65.4**	**3499**	⊥	**41.5**	**755.7**	**329.7**
11	**0.7/m**	⊥	**66.3**	**3510**	⊥	⊥	**753.6**	**342.0**
12	⊥	**5.93**	0.9	⊥	⊥	⊥	⊥	⊥

Table 9: Results of chemical analysis of the water samples of Kakkaveri, India; in bold elevated laboratory results

TH = total hardness

EC = electric conductivity at 20° C

*** Normal; not elevated values**

With two exceptions (sample 1 and 11) the results for nitrate and nitrate are within the recommended limitations. The results are depicted in the following diagram (

Figure 12).

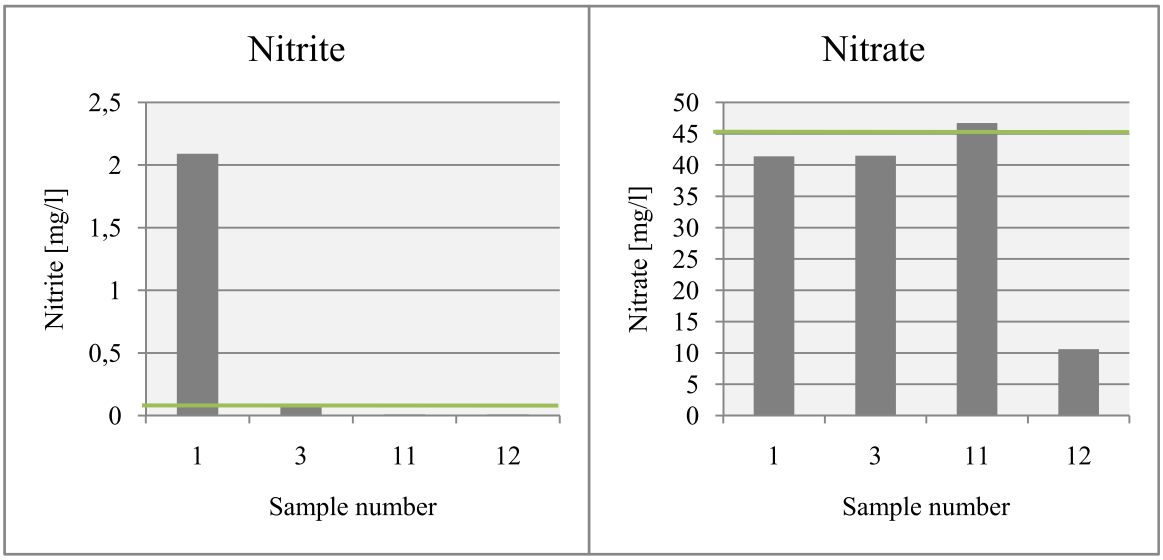


Figure 12: Nitrite and Nitrate levels; the green line for Nitrite represents the Austria's standard (0.1 mg/l), for Nitrate it represents India's standard (45 mg/l)

5 Discussion

The regular verification of a countries drinking water is essential for detection of aberrations in its quality, especially when it comes to regions with low infrastructure.

For the year 2012, Prüss-Ustün et al. did a retrospective analysis including 145 low and middle income countries concerning the disease burden from inadequate water, sanitation and hygiene (WASH). 1.5% of the global disease burden in 2012 has been attributable to inadequate WASH (in total numbers 842 000 deaths). In the age group under five years 5.5% of the global disease burden was associated with those factors, meaning that 361 000 deaths could have been prevented. (37) Worldwide, 663 million people use unimproved water sources. (1) Having access to an improved source of drinking water does still not grant that the water is free from faecal contamination. (38) This issue is accompanied by the increasing occurrence of antimicrobial resistances worldwide. (2) The sustainable development goals are defined to change different aspects of the world. As more than 2 billion people have no access to safe drinking water, the sustainable development goal number six aims towards improvement in that area. (1)

The hospital in Kakkaveri has some of these infrastructural difficulties concerning the targets named in the sustainable development goal number six. The health care centre does not meet all the listed criteria, which are improved source, located on the premises, available when needed and free from faecal contamination. (3) Due to the bacterial contamination we found, the last point could not be fulfilled. Only if all four requirements are met, the water is considered safely managed. It is important for the local responsible to manage the achievement of all four criteria for safely managed drinking water. (39)

5.1 Discussion of the Bacteriological Results, Water Laboratory Graz

When speaking about the whole water system in Kakkaveri, the first contamination of the water supply starts already from the well on. Results primarily may reflect external contamination due to rainwater and faeces polluted water from the surrounding area running into the well. The well is apparently not impervious to contaminations from outside. Furthermore, the water in the water tower showed the highest rates of contamination, whereas later in the water system the R.O.-plant reduces the bacterial load.

Bacteria as *E. coli* and *Pseudomonas aeruginosa* must not be present in a 100 ml water sample.(7, 8) The following discusses the results for each species separately.

In the laboratory analysis, *E. coli* was only found in the well's water. This might be due to the fact that allochthonous (introduced) microorganisms are exponentially decreasing over time, for example *E. coli* is eliminated by three logarithmic levels in 100 days.(9) With 175 CFU the number of the CFU`s is obviously above the limit, but in conjunction with possible elimination over time, it can be explained, that the downstream samples are free from *E. coli.*

The presence of *Pseudomonas aeruginosa* in the majority of the samples can be explained by the fact, that *P. aeruginosa* produces polysaccharides that allow them to form a biofilm.(41) It is important to check the oxygen humidifier, because *P. aeruginosa* can grow also in distilled water.(9) Fortunately in this study this was not the case when investigating such samples.

The CFU values were elevated throughout all the water samples of Kakkaveri but the results of the water samples taken after the R.O.-plant showed at least the positive effect of the R.O.-plant. Four samples (3, 9, 11, and 13) with values > 100 000 were standing out in particular. These four exceedingly high results need a closer observation.

- Starting with sample 3, the water in the tower is heated by the sun all day long which creates ideal conditions for bacterial growth. A shade providing roof might reduce the warming of the water, and might be one of the ideas for the improvement of the water system in Kakkaveri.
- The taps of the rooms 4 (sample 9) and C (sample 11) where heated with the gas lighter as required. One theory to explain the extremely elevated CFU values in this study is that both of the taps were contaminated with *Pseudomonas aeruginosa.* As stated before, these bacteria form biofilms and are also able to populate water taps.(9)
- The water tanks, standing on the floors of the hospital and providing the drinking water for the patients are filled with R.O.-plant processed water and it was

supposed that the water of the water tanks should show similar results, but was not the case in one of two tested samples of the tanks. An approach for cleaning and disinfecting the tanks as recommended from Yukon Health and Society Services (42) is strongly suggested for the water tanks in Kakkaveri. The cleaning and disinfecting of water holding tanks includes scrubbing the empty tank, disinfecting it with household bleach (for 2 h) and rinsing it thoroughly. After this procedure, the water tank is ready to be refilled with potable water. (42)

Concerning the resistance patterns in the found bacteria contrary to Sahoo et al. (40), who found *E. coli* resistance to various antimicrobial substances in non-coastal regions in India, the susceptibility testing in this study did not show any (multi-) resistant pathogens in the water samples of S.F.S Health Centre in Kakkaveri. Because of the high appearance of resistances in drinking water in India (40), this was a very important and positive analysis.

5.2 Discussion of the Results of the Point-of-Care-Testing Petrifilms™ and a Routine Microbiological Laboratory

Routine microbiological laboratory analysis of water is not accessible everywhere around the world. The question and aim of this study was to compare laboratory results of a routine water laboratory to the Point-of-care-testing system Petrifilms™.

Concerning the results of the Point-of-care-testing system Petrifilms™, it must be taken into consideration that the sample volume for the Petrifilm™ is with 1ml rather smaller than the 100ml sized sample the water laboratory uses for routine testing. To make allowance to this fact the laboratory results were divided by 100. Laboratory measured values less than 100 CFU/ml hence do not appear on the Petrifilms™. Also the limited possibility of having an incubator was taken into account. Usually the Petrifilms™ are incubated at 37°C.One idea was to use room temperature, which is very high in India, for incubation of the samples, but the results of the Petrifilms™ did not fulfil the expectations. It is thus on the one hand recommended to use an incubator and follow the instruction of the manufacturer. And it has to be considered that only definitively high numbers of CFU`s are detectable; with 66.6 % (4/6) matching samples in group I and 50.0% (3/6) in group II, the Petrifilms™ we got nearly the same results as Vail et al. (43)but while he stated that the Petrifilms may be used as a screening method with the advantage of being low in costs and easy to use, we would not support such a statement.

5.3 Discussion of the Physical-Chemical Analysis

Physical and chemical-wise, the water in Kakkaveri does not show health concerns. We detected high nitrite values and the presence of minerals like iron, manganese, sulphate and chloride. Those substances are characteristic for groundwater (6), and therefore it is conclusive that the water in Kakkaveri is ground water. The levels of sulphate, chloride and the electric conductivity were also elevated, meaning that the water of the hospital is corrosive. (4) After processing through the R.O.-plant those markers were normalized.

One problem, which was addressed by the Indian colleagues, was the numerous necessity of changing the membranes for the R.O.-plant. This could be explained by the exceedingly high values of total hardness in the water. Discussing that problem with an Austrian expert for water sanitation (Co. Mittendrein, Graz, Austria) it was suggested to test the water three times a day for elevated values of total hardness. If this parameter reaches or exceeds 6°dH, a regeneration of the water has to be performed.

Furthermore, it is important to look at the nitrite and nitrate levels, which are fundamental when it comes to feeding infants.(5, 41) The water from the well showed elevated nitrate levels, but after a while above the surface, when the water gets in contact with oxygen, the nitrite oxidizes to nitrate. (6) Throughout our investigations this chemical reaction was observable with high nitrite levels in the well but later on increasing nitrate levels in the samples downstream the well. When it comes to preparing infants meals, especially the water from the R.O.-plant with no nitrite and very low nitrate can be recommended for usage.

5.4 Future Implications

Taking everything into account, practical recommendations and implications can be drawn. First of all, it is strongly recommended to check the well's water and further important point of the water system (water tanks, R.O.-plant treated water) routinely for bacteriological contamination. Secondly, a structural change of the wells entrance is most likely to improve the situation. Structural conditions need to include, that the well's lining should be higher than the ground surrounding it, so no contamination can take place at this early point of a water supply system. Also all plants growing around the well ought to be removed. The massive growth of bacteria found in the tower could be eliminated by water disinfection on a regular and routinely base. Additionally, protection against incoming rain water is recommended as well as to build a sunscreen above the tower. For future investigations, it would be interesting, if restructuring of the well leads to lower bacterial contamination from outside. Another step towards stable high-quality water would be the disinfection of the water in the tower, because at that point of the distribution system massive growth of bacteria takes place. Lastly, the control of the water's total hardness is important to keep the R.O.-plant membranes clear and as a result of the clear membranes, the water quality high.

6 Afterword

Safe drinking water is essential for every aspect of life and all efforts to achieve this can save lives.(1) In the hospital of Kakkaveri patients affected by leprosy, as well as patients positive for HIV or tuberculosis are treated. Salesians Sisters, headed by Sr. Francina built up the health centre and are responsible for the medical attendance of the patients as well as for all aspects of hygiene and sanitation. Within the cooperation with Global Health and Development at the Medical University of Graz it was possible to test the reliability of a Point-of-care-testing-system for drinking water. Further the idea of the study was to investigate the whole water supply system of Kakkaveri Hospital to identify potential critical points and to find technical solutions if necessary. The work in India has opened our minds, meaning that sometimes even small changes can make a big difference.

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8 Appendix: Photographs Sampling Sites

Figure 13: Sample 1: well – Photographer: Angelika Schirnhofer

Figure 14: Sample 2: white tank on the roof – Photographer: Angelika Schirnhofer

Figure 15: Sample 3: water tower – Photographer: Angelika Schirnhofer

Figure16: Sample 4: metallic tank – Photographer: Angelika Schirnhofer

Figure17: Sample 5: solar heater – Photographer: Angelika Schirnhofer

Figure 18: Sample 6: water tap operating theatre, first floor – Photographer: Isabella Dicker

Figure 19: Sample 7: water tap ICU, first floor – Photographer: Angelika Schirnhofer

Figure 20: Sample 8: water tap room 4, first floor – Photographer: Angelika Schirnhofer

Figure 21: Sample 9: water tap in the bathroom of room 4, first floor – Photographer: Angelika Schirnhofer

Figure 22: Sample 10: water tap room D, ground floor – Photographer: Angelika Schirnhofer

Figure 23: Sample 11: water tap room C, ground floor – Photographer: Angelika Schirnhofer

Figure 24: Sample 12: water tap with R.O.-plant water, ground floor – Photographer: Angelika Schirnhofer

Figure 25: Sample 13: water tank, first floor – Photographer: Angelika Schirnhofer

Figure 26: Sample 14: water tank, ground floor – Photographer: Angelika Schirnhofer

Figure 27: Sample 15: oxygen-humidifier, pow and operating theatre, ground floor and first floor – Photographer: Angelika Schirnhofer