Water **2015**, *7*, 3878-3905; doi:10.3390/w7073878

OPEN ACCESS

water

ISSN 2073-4441
www.mdpi.com/journal/water

Article

Review of Risk Status of Groundwater Supply Wells by Tracing the Source of Coliform Contamination

Nara Somaratne * and Gary Hallas

South Australian Water Corporation, 250 Victoria Square, Adelaide, SA 5000, Australia;
E-Mail: gary.hallas@sawater.com.au

* Author to whom correspondence should be addressed; E-Mail: nara.somaratne@sawater.com.au; Tel.: +61-8-7424-2379; Fax: +61-8-7003-2379.

Academic Editor: David Polya

Received: 22 December 2014 / Accepted: 3 July 2015 / Published: 14 July 2015

Abstract: Coliform source tracking was undertaken on 48 water sources of which 42 are potable in 26 water supply systems spread across South Australia. The water sources in the study vary from unprotected springs in creek beds to deep confined aquifers. The frequency analysis of historical coliform detections indicate that aquifer types, depth to water and casing depth are important considerations; whilst maintaining well integrity and the presence of low permeable clay layers above the production zone are the dominant parameters for minimizing coliform contamination of water supply wells. However, in karst and fractured rock aquifers, pathways for coliform transport exist, as evidenced in the >200 MPN/100 mL level of coliform detection. Data indicate that there is no compelling evidence to support the contention that the wells identified as low risk are contaminated through geological strata and clay barriers. However, data strongly supports the suggestion that coliform detection from sample taps and wellheads stem from the surrounding groundwater and soil-plant sources as a result of failed well integrity, or potentially from coliform bacteria that can persist within biofilms formed on well casings, screens, pump columns and pumps. Coliform sub-typing results show that most coliform bacteria detected in town water supply wells are associated with the soil-water-plant system and are ubiquitous in the environment: *Citrobacter* spp. (65%), *Enterobacter* spp. (63%), *Pantoea* spp. (17%), *Serratia* spp. (19%), *Klebsiella* spp. (34%), and *Pseudomonas* spp. (10%). Overall, 70% of wells harbor detectable thermotolerant coliforms (TTC) with potentially 36% of species of animal origin, including *Escherichia coli* species found in 12% of wells.

Keywords: risk assessment; groundwater risk assessment model (GRAM); coliforms; risk management; town water supply; coliform source tracking

1. Introduction

The security of a town water supply requires prudent management of water resources toward a desired level of drinking water quality. Identification of pollution sources and pathways is vitally important to implementing appropriate mitigation strategies that minimize risks to source water. In drinking water sources, the presence of faecal bacteria is considered a health hazard, as they may indicate the presence of human viruses, or parasites *Giardia* or *Cryptosporidia* [1]. In a groundwater risk assessment study [2] using a multi-barrier analysis approach, risk levels for town water supply wells compare favorably with respect to coliform detection. Out of 144 town water supply wells in South Australia, 142 wells recorded detection of coliform ranging from 1% of frequency in low risk confined aquifers to 87% frequency of detection in a high risk unconfined aquifer in karst limestone. Moreover, *Escherichia coli (E. coli)* had been detected, albeit at low frequency and low counts, in shallow wells. Coliform detection in some of the low risk, confined and semi-confined deep wells had been a concern for water quality management.

Data on coliform detections in confined aquifers are rare in the literature, as confined aquifers are overlain by low-permeability aquitards that are commonly assumed to protect underlying aquifers from microbial contaminants. Borchardt *et al.* [3] report human pathogenic viruses in well water from a deep sandstone aquifer confined by a regionally extensive shale aquitard. According to Borchardt *et al.* [3], hydrogeologic conditions support rapid porous media transport of viruses through the upper sandstone aquifer to the top of the aquitard, 61 m below ground surface. Natural fractures in the shale aquitard are one possible virus transport pathway through the aquitard; however, cross-connecting wells, or imperfect grout seals along well casings also may be involved [3]. Powell *et al.* [4] report regular detections of sewage-derived bacteria and viruses to depths of 60 m in unconfined sandstone and to a depth of 91 m in confined sandstone aquifers. Similar to Borchardt *et al.* [3], Powell *et al.* [4] highlight the vulnerability of sandstone aquifers to microbial contamination, but do not report on the role played by the condition of the well or its integrity.

Faecal pollution of water sources from warm-blooded animals including humans results in public health risks due to possible exposure to a wide range of pathogenic bacteria, viruses and protozoa [5,6]. In addition, enteric viruses are often the suspected cause of waterborne disease outbreaks [7]. Opisa *et al.* [8] report that *E. coli* were detected in 100% of samples taken from unprotected, and 92.6% of samples from protected public water supply wells. In a study of Staradumskyte and Paulauskas [9], contamination with coliform bacteria was discovered in 72.9% of investigated dug wells, with *E. coli* found in 54.8%, and intestinal *Enterococci* in 56.2%. Citing Blackburn *et al.* [10], Hynds *et al.* [11] note that diseases caused by waterborne pathogens continue to be a leading cause of illness in United States (US), and waterborne disease surveillance in the US indicates that approximately 74% of confirmed waterborne disease outbreaks occurring during the period of 2001–2002 were attributed to groundwater sources [10].

The presence of bacteria from animal sources is problematic in water supplies. For example, one of the problems with *Cryptosporidium* oocysts is they are resistant to harsh environmental conditions and remain ineffective for several months [12,13], posing a major problem for the water industry [14]. Khaldi *et al.* [15] show *Cryptosporidia oocysts* and/or *Giardia cysts* are transported from sinkhole to spring and well suggesting that oocysts are subject to storage and remobilization in karst conduits. Karst aquifers are known to be generally more vulnerable to contamination than aquifers with fractured or inter-granular porosity. Microbial pathogens can easily enter karst aquifers through thin soils and the epikarst or via sinkholes [16]. Thus, parasitic protozoa represent an insignificant threat to groundwater in general, except for groundwater directly connected to surface water and groundwater in karst environments.

In this study, we examine the risk status of selected water sources by identifying coliform sub-group and potential pathways. Aquifer pathways from hazard to receptor may exist due to the hydrogeological setting in a particular area, including soil and strata types, depth to groundwater and type of well construction and maintenance. This study builds on the development of a groundwater risk assessment model (GRAM) [2] and involves identifying the sub-type of coliform indicator bacteria present in the water sources ranging from low risk to high risk wells. The sub-typing of bacterial strains recovered from water supply wells constructed in different climate and geologic settings with different maintenance history is a valuable tool for identification of contamination sources and pathways. Thus, the risk status of groundwater supplies is evaluated to protect water sources by taking corrective measures in managing the hazard source and pathways.

2. Study Groundwater Systems

Coliform source tracking was undertaken on 48 water sources (out of 144), of which 42 are potable, in 26 water supply systems spread across South Australia (Figure 1). Non-potable springs in the northern region were used for comparison. The groundwater systems are located in four regions: Eyre, Northern, Outer Metro and South East and are described by Somaratne *et al.* [2]. Selected water sources for the coliform sub-typing study are given below in Table 1. The sample water sources were selected to represent different land use, aquifer type, and well construction and modelled GRAM risk level [2]. For completeness, a general description of the area is provided in this paper.

The Eyre region has seven water supply systems in limestone aquifers. Two systems (Uley South and Streaky Bay water supply aquifer-Robinson lens) are in karstic limestone. The average annual rainfall and pan evaporation varies from 550 and 1500 mm respectively at Port Lincoln and 375 and 2200 mm, respectively, at Streaky Bay. Land use is predominantly broad acre cropping and sheep grazing. Low salinity groundwater occurs in the areas known as fresh water lenses, in the saturated limestone surrounded by either dry limestone or brackish water zones. Soils in the region are characterized as shallow, calcareous and overlaying calcrete or limestone. The water supply systems Uley South, Uley Wanilla, Coffin Bay and Robinson lens are in water reserves whilst the Lincoln Basin water supply system is in a national park [2].

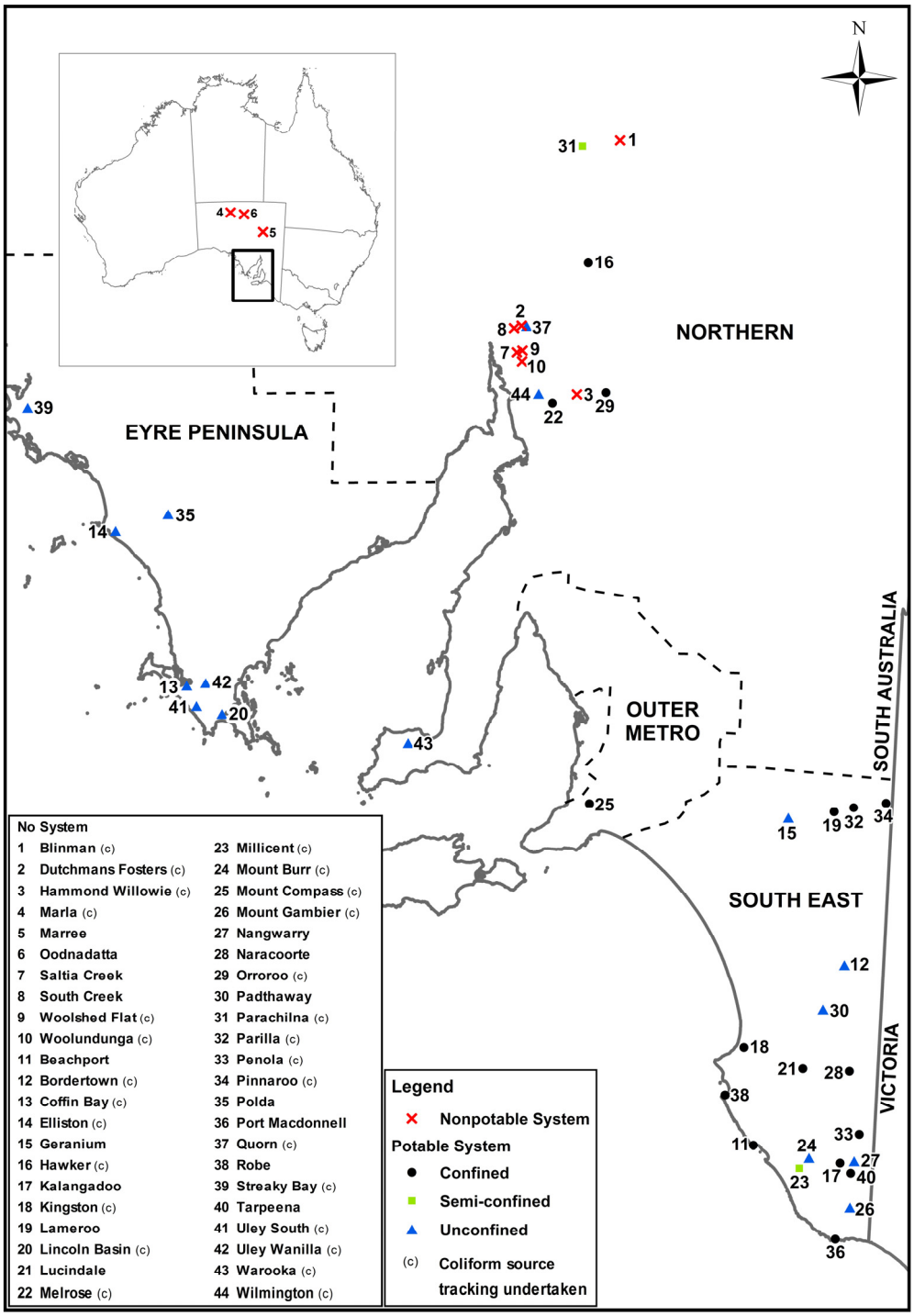


Figure 1. Location of non-metropolitan groundwater supply systems in South Australia (after Somaratne *et al.* [2]).

In the Northern region, there are 17 water supply systems including four springs. Except for the Para Wurlie basin and Parachilna, the other groundwater water supply systems extract from fractured rock/sandstone aquifers or the deep confined aquifer of the Great Artesian Basin. The climate in the area is typically characterized by hot dry summers and cool winters, with the highest rainfall occurring from May to September. Average annual rainfall is 447 mm and average annual pan evaporation is 1400 mm. The far north of the Northern region comprises arid lands and is bounded by the Simpson Desert.

Table 1. Water source data and GRAM [2] risk levels.

Water Source [a]	Source Type [b]	Geology [c]	Casing Type and Year [d]	Annulus Seal	Land Use [e]	GRAM Risk Level
Fosters Creek *	Creek	Fractured Rock	Not applicable	Not applicable	Farmland	High
Hammond-Coonatta Spring *	Spring	Fractured Rock	Not applicable	Not applicable	Farmland	High
Woolshed Flat spring*	Spring	Fractured Rock	Not applicable	Not applicable	Grazing land	High
Blinman Mine	Mine	Fractured Rock	Not applicable	Not applicable	Reserve	High
Wilmington Mine	Mine	Fractured Rock	Not applicable	Not applicable	National park	High
Streaky Bay Trench 1	UC	karst LS	Not applicable	Not applicable	Water reserve	High
Streaky Bay Trench 2	UC	karst LS	Not applicable	Not applicable	Water reserve	High
Bordertown TWS 8	UC	LS	Steel, 1982	No	Grazing land	Moderate
Bordertown TWS 10	UC	LS	PVC, 2011	No	Grazing land	Moderate
Millicent TWS 1	UC	LS	Steel, 1968	No	Grazing land	Moderate
Mt Gambier TWS 9	C	Sand	FRP, 1996	From 0 to 183 m	Township	Low
Mount Burr TWS 5	UC	LS	PVC, 2012	From 0 to 120 m	Forest reserve	Low
Parilla TWS 4	C	LS	PVC, 2007	From 0 to 89.5 m	Township	Low
Penola TWS 7	C	Sand	PVC, 2011	From 0 to 140 m	Township	Low
Pinaroo TWS 4	C	LS	Steel, 1971	Unknown	Township	Low
Kingston TWS 12	C	Sand	FRP, 1991	From 0 to 58 m	Road reserve	Low
Blinman TWS 1	UC	Fractured Rock	PVC, 1996	From 0 to 3 m	Reserve	High
Hawker TWS 1	C	Fractured Rock	Steel, 1963	No	Grazing-sheep	Low
Melrose TWS 5	C	Sandstone-	FRP, 1991	From 0 to 87.5 m	Winter cropping	Low
Orroroo TWS 7	C	Sandstone-	PVC, 2001	From 0 to 113.5 m	Winter cropping	Low
Parachilna TWS 1	SC	Sandy-clay	PVC, 2005	From 0 to 5 m	Road reserve	Moderate
Warooka TWS 2	UC	LS	Steel, 1962	Unknown	Winter cropping	High
Willowie TWS 1	SC	Fractured Rock	PVC Rehab. in 2012	No	Creek reserve	Moderate
Wilmington TWS 3	SC	Fractured Rock	PVC, 2009	From 0 to 48 m	National park	Low
Wilmington TWS 2	SC	Fractured Rock	PVC, 1999	From 0 to 2 m	National park	Moderate
Coffin Bay TWS 7	UC	LS	PVC, 2012	From 0 to 24 m	Water reserve	Moderate

Table 1. *Cont.*

Water Source [a]	Source Type [b]	Geology [c]	Casing Type and Year [d]	Annulus Seal	Land Use [e]	GRAM Risk Level
Elliston TWS 3	UC	LS	PVC, 1999	From 72.5 to 75 m	Winter cropping	Moderate
Lincoln Basin Well A	UC	LS	Steel, 1957	No	National park	High
Lincoln Basin Well B	UC	LS	Steel, 1959	No	National park	High
Lincoln Basin Well O	UC	LS	Steel, 1971	Unknown	National park	High
Lincoln Basin Well M	UC	LS	Steel, 1976	Unknown	National park	High
Lincoln Basin Well J	UC	LS	Steel, 1959	No	National park	High
Uley South TWS 1	UC	karst LS	Steel, 1964	Unknown	Water reserve	High
Uley South TWS 2	UC	karst LS	Steel, 1974	Unknown	Water reserve	High
Uley South TWS 3	UC	karst LS	Steel, 1974	Unknown	Water reserve	High
Uley South TWS 5	UC	karst LS	Steel, 1975	Unknown	Water reserve	High
Uley South TWS 7	UC	karst LS	Steel, 1975	Unknown	Water reserve	High
Uley South TWS 8	UC	karst LS	Steel, 1969	Unknown	Water reserve	High
Uley South TWS 10	UC	karst LS	PVC, 1999	2 m from surface	Water reserve	Moderate
Uley South TWS 11	UC	karst LS	PVC, 1999	Unknown	Water reserve	Moderate
Uley South TWS 14	UC	karst LS	PVC, 1999	1 m from surface	Water reserve	Moderate
Uley South TWS 15	UC	karst LS	PVC, 1999	1 m from surface	Water reserve	Moderate
Uley South TWS 16	UC	karst LS	PVC, 1999	1.5 m from surface	Water reserve	Moderate
Uley Wanilla TWS 1	UC	LS	Steel, 1948	No	Water reserve	High
Uley Wanilla TWS 2	UC	LS	Steel, 1946	No	Water reserve	High
Uley Wanilla TWS 7	UC	LS	FRP, 1990	From 12 m to 15 m	Water reserve	Moderate
Uley Wanilla TWS 8	UC	LS	FRP, 1989	From 0 to 0.5 m	Water reserve	Moderate
Mt Compass TWS 1	C	Sand	FRP, 1996	From 0 to 40 m	Road reserve	Low

Notes: [a].* Surface water sources; Fosters Creek is the water source for the Dutchmans Fosters system; TWS = Town Water Supply; e.g., Mt. Compass TWS 1 is Mt Compass water supply system town water supply well number 1. [b] UC = unconfined aquifer; SC = semi-confined aquifer; C = confined aquifer; [c] LS = Limestone; [d] PVC = Polyvinyl chloride, FRP = Fibre-reinforced plastic; [e] Winter cropping = Winter cropping and low-density livestock grazing in summer.

The Outer Metro region has a one wellfield at Mount Compass. The climate near Mount Compass is characterised by hot, dry summers and cool, wet winters with average rainfall of 840 mm per year. The region features irrigated horticulture (mainly berries, vegetables and olives) and cattle grazing. The township consists of semi-rural and residential areas. Two semi-confined water supply wells are located in a road reserve near the town [2]. The South East region has 19 water supply systems. The climate of the South East region is characterised by cool, wet winters and hot, dry summers. Average annual rainfall varies considerably within the region, from approximately 750 mm in the south near Mount Gambier, to 250 mm in the north of the region around Pinnaroo. Potential annual evapotranspiration increases from ~1400 mm in the south to ~1800 mm in the north. The dominant land use is dryland cropping and livestock grazing. There is some irrigated cropping, which includes pasture for dairy, wine grapes, lucerne, potatoes and cereals. Commercial forestry is a significant industry in the southern part of the South East (SE) region, with both softwood and hardwood plantations. Except in Kingston SE, Millicent and Bordertown, all water supply wellfields are located within townships [2]. In Mount Gambier, water supply system is the Blue Lake, a volcanic crater, but coliform source tracking was undertaken in confined aquifer water supply wells that are used for emergency water supply.

3. Methods

3.1. Sampling Strategy

Based on the previous study of Somaratne *et al.* [2], water sources were selected to represent a range of risk levels and availability of historical coliform detection data. Water sources (Table 1) were divided into several categories, according to the level of exposure to potential bacterial contamination sources, (surface water, trenches, mines in fractured rock aquifers, water supply wells in karst aquifers, wells of good to poor condition in limestone aquifers, wells in fractured rock aquifers with either shallow corroded casings or pressure cemented annulus and deep casings, semi-confined and confined aquifers with production zones below a clay layer). This allowed historical data that were available by routing monitoring, of coliform detection frequency to be presented under each category, and to undertake coliform sub-typing to identify species present in the different source water categories. The overall sampling strategy was to obtain representative water samples from each of the water source in each category.

3.2. Sample Collection

Both potable and non-potable water sources were routinely monitored for bacteria. Sampling taps were installed in the town water supply wells so as to minimise contamination of the samples during collection. The sample tap was flushed for at least 3–5 min to ensure that the sample will be representative of the water in the well, and then sterilised by flaming with a gas burner until steam issued from the nozzle of the tap. Pre- and post- flush samples were collected in order to detect any coliform bacteria within the sample taps or within the well-head. Samples were collected in sterile polypropylene bottles dosed with sodium thiosulphate according to AS/NZS (Australian/New Zealand Standard) 5667.11:1998 [17] and AS/NZ 2031:2001 [18], transported on ice, and analysed within 6 h of collection.

3.3. Method of Analysis

Coliform identification involves a traditional process, Analytical Profile Index (API) biochemical analysis, to identify bacteria detected at the genus and species levels at the various water sources [19,20]. Samples and dilutions were added to Colilert®-18 sterile vessels, and Colilert®-18 powders were aseptically added and mixed by shaking until completely dissolved. The vessel was aseptically emptied into a Quanti-Tray® and heat-sealed according to the manufacturer's instructions, then incubated at 35 °C for 18 h. The Quanti-Tray® was inspected for yellow wells indicating the presence of coliforms, and under long wave UV (366 nm) light for fluorescent wells indicative of *E. coli*. Estimated counts were determined using most probable number tables supplied by the manufacturer. Up to 10 positive (yellow) wells of the Colilert®-18 vessels were aseptically pierced and the bacterial suspension struck out onto CHROMagar [21,22], plates were incubated for 24 h at 37 °C according to manufacturers' instructions, before being examined.

Both typical and atypical colonies were investigated. Typical and atypical colonies were subcultured to Cysteine Lactose Electrolyte Deficient (CLED) agar with andrade indicator and incubated at 35 °C for 24 h. Pure isolates were then identified using API20E, from a pure isolate that has been re-struck from the selective CLED agar onto blood agar unless otherwise indicated. The CHROMagar plates were photographed using the digital colony counter to show the types of morphologies present in the sample matrices. Up to five morphologically distinct isolates were selected for identification and re-struck onto blood agar to confirm organism purity. A single isolated colony was selected from each blood agar plate and a suspension made in physiological saline (5 mL). This suspension was used to inoculate an API20E strip, which was incubated at 35 °C for 24 h. The API reactions were read back according to the manufacturer's instructions. API20E is a standardised biochemical system for the identification of *Enterobacteriaceae* and other non-fastidious, Gram negative bacilli [23].

The API20E strip [20] is composed of 20 micro-tubes containing dehydrated substrates. These are inoculated with a bacterial suspension which reconstitutes the media. After incubation for 18–24 h at 36 °C, bacterial metabolism produces colour changes which are either spontaneous or revealed by the addition of reagents. The reactions are converted into a seven-digit code. The code is then entered into an online database, apiweb™ identification software [20] and identification is produced, usually as genus and species. CHROMagar Orientation Medium is a non-selective medium designed for isolation, direct identification, differentiation and enumeration of pathogens. The agar contains proprietary chromogens; these colourless compounds are composed of a substrate (targeting a specific bacterial enzyme) and a chromophore. Enzymatic activity of the target organism results in release of the chromophore. In unconjugated form, the chromophore exhibits its distinctive colour and forms a precipitate [23].

Enterobacteriaceae strains were arranged into a weighted assortment correlating to the frequency with which they might be found in an environmental laboratory, the API20E correctly identified 71 (87.7%) at 24 h and 78 (96.3%) at 48 h. Reliability of genus was decided at the 90% confidence interval and species at the 95% confidence interval for determine consistent identification.

The coliform species identified in this study was collected during the sampling period from September 2013 to September 2014, with 2–10 routine sampling rounds. In addition one round of

sampling was carried out for the pre- and post- flushing of the water supply wells, to detect the presence of coliform in the wellhead. Hence, this study provides a snap-shot view of the coliform species detected.

4. Results and Discussion

There are many potential sources of bacterial pollution in a catchment; identification of genera and species provides a means to determine whether particular coliform sources are originated from soil, water and plant systems, or introduced by way of animal faeces. Data presented are related to total species detected at each sampling round at each water source and are not necessarily indicative of the general status of a particular groundwater source.

4.1. General Description of Detected Coliform Bacteria—Genera and Species

Survival of coliform bacteria in soil depends on soil characteristics; warm moist conditions are ideal for maximum survival [24]. Bacterial survival may be low in well aerated sandy soils, but if the depth to groundwater is low and water flow rate is high, bacterial contamination of groundwater may be substantial [24]. Thus, shallow karstic aquifers, and water sources that have direct contact with moist soils such as dug wells and trenches are prone to high bacterial contamination.

Total coliform bacteria that are able to ferment lactose at 44–45 °C are known as thermotolerant coliforms (TTC) [11]. TTC (faecal) bacteria, is a sub-group of the total coliform group because they can grow at higher temperatures and are found only in the faecal waste of warm-blooded animals. *E. coli* is one of the six species of faecal coliform bacteria found in animal and human waste [25]. In most waters, the predominant genus is *Escherichia*, but some types of *Citrobacter*, *Klebsiella*, *Hafnia*, *Proteus*, *Morganella* and *Enterobacter* are also thermotolerant. Sigler and Bauder [26] posit that, if a water sample is positive for total coliform but negative for *E. coli*, it is nevertheless important to determine from where the bacteria entered the system, even though the main purpose of quantifying coliform bacteria is to detect faecal pollution and thus the possible presence of faecal pathogens [27]. Coliform bacteria, other than *E. coli,* may originate from a multitude of sources including soil, decaying vegetation, industrial processes and effluent [27]. Bacteria from different pollution sources may consist of essentially the same species but with different strains predominating [28].

Bacterial colonies are identified by enzymatic activity specific to the genus or species. The coliforms identified in this study were recorded and an interpretation was made of the most probable source [29]. The usefulness of total coliforms as an indicator of faecal contamination depends on the extent to which the bacteria species found are of faecal origin. All members of the total coliform group detected in this study are given in Table 2. *Acinetobacter* species are considered to be ubiquitous in nature given that they can be recovered from almost all soil and surface water samples [30]. They survive on both moist and dry surfaces. In drinking water, they have been shown to aggregate bacteria that otherwise do not form aggregates [30].

Aeromonas spp. are Gram-negative rods of the family *Vibrionaceae*. They are normal water inhabitants and are part of the regular flora of animals [31]. *Burkholderia* comprises more than 60 species, and present in water sources *Burkholderia* suggest that each group might represent a different genus [32]. *Buttiauxella* species, a new genus of the *Enterobacteriaceae*, isolated from

surface water, soil, intestine of snails and some human samples and intestinal tracts of trout [32,33]. *Citrobacter* is a genus of Gram-negative coliform bacteria in the *Enterobacteriaceae family* [34]. The species *C. amalonaticus*, *C. koseri*, and *C. freundii* can use citrate as a sole carbon source. These bacteria can be found almost everywhere in soil, water, wastewater, and in the human intestine [34]. *Enterobacter* is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria of the family *Enterobacteriaceae*, commonly found in soil and water. The genus *Enterobacter* is a member of the coliform group of bacteria with some are belong to the TTC group but some members do not as does *E. coli*, because it is incapable of growth at 44.5 °C in the presence of bile salts [35].

Faecal streptococci generally occur in the digestive systems of humans and other warm-blooded animals and are used as indicators for water pollution [36]. *Enterococci* are a subgroup within the faecal *streptococcus* group. *Enterococci* are typically more human-specific than the larger faecal *streptococcus* group. *E. coli* is a Gram-negative facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms [37]. Since *E. coli* is released into the environment through deposition of faecal material, this bacterium is widely used as an indicator of faecal contamination of waterways [38]. The genus *Hafnia*, a member of the family *Enterobacteriaceae*, consists of Gram-negative bacteria which are occasionally implicated in both intestinal and extraintestinal infections in humans, despite the fact that the genus currently contains only a single species (*H. alvei*), commonly found in humans, animals and birds as well as soil, water and sewage [39].

Klebsiella spp. are Gram-negative, nonmotile, usually encapsulated rod-shaped bacteria, belonging to the family *Enterobacteriaceae*. *Klebsiella* spp. occur worldwide, particularly in tropical and subtropical regions, and are ubiquitous, including forest environments, vegetation soil, water, and mucosal membranes of host species. Specifically identified sources for some *Klebsiella* spp. are: *K. pneumoniae*—humans, horses, bovines, raptors, and common in all Australian mammals; *K. oxytoca*—humans, mammals (ringtail possums, gliders, and bats) throughout Australia, and insects.

K. variicola—humans and plants [40]. *Kluyvera* is a small, flagellated, motile Gram-negative bacillus that clearly belongs to the family *Enterobacteriaceae*. *Kluyvera* is present in the environment as free-living organisms in water, soil, sewage, hospital sinks, and food products of animal origin [41].

Morganella morganii is a species of Gram-negative bacteria. *M. morganii* has a commensal relationship within the intestinal tracts of humans, mammals, and reptiles as normal flora [42]. The enterobacterial genus *Pantoea* comprises 19 species of Gram-negative, yellow or beige pigmented, motile rods. Members of this genus have been isolated from a wide range of environments including soil, water, dust, dairy products, meat, fish, insects, humans and animals. Most frequently they are associated with a broad range of plant hosts, as non-pathogenic endophytes or epiphytes, colonizing leaves, stems and roots [43]. The genus *Providencia* consists of five species: *Providencia alcalifaciens*, *Providencia heimbachae*, *Providencia rettgeri*, *Providencia rustigianii*, and *Providencia stuartii. Providencia rettgeri* is a Gram negative bacterium that is commonly found in both water and land environments; and *Providencia stuartii* is a Gram negative bacterium that is commonly found in soil, water, and sewage [42].

Table 2. Coliform species detected in the water sources sampled in this study, together with potential origins [9,30–49].

Identification Code	Genus	Species	Summary of Potential Origins
Aci (bau, cal)	Acinetobacter (Aci)	baumannii/calcoacetius	Environment, soil [30]
Aer (sp)	Aeromonas (Aer)	*Aeromonas* spp.	Normal water inhabitant [31]
Bur (sp)	Burkholderria (Bur)	*Burkholderria* spp.	Soil and water bacteria [32]
But (sp)	Buttiauxella (But)	*Buttiauxella* spp.	Surface water and soil, intestine of snails [32,33]
Cit (sp)	Citrobacter (Cit)	*Citobacter* spp.	Mainly found in soil, water sewage, and also in intestinal tract of animals and humans [34]
Cit (fre)		freundii	
Cit (bra)		braaki	
Cit (kos)		koseri/amalonaticus	
Cit (you)		youngae	
Ent (sp)	Enterobacter (Ent)	*Enterobacter* spp.	Commonly found in soil and water [35]
Ent (aer)		Aerogenes	
Ent (clo)		Cloacae	
Ent (asb)		Asburiae	
Ent (can)		cancerogenus	
Ent (ger)		gergorviae	
Ent (amn)		amnigenus 2	
E (col 1)	Escherichia (E)	coli 1	Commonly found in intestine of warm-blooded animals [37] and released into environment through faecal material [38]
E (vul)		vulneris	
FS (sp)	Faecal Streptococci (FS)	Faecal Streptococci	Found in digestive systems of humans and warm-blooded animals [36]
Haf (sp)	Hafnia (Haf)	*Hafnia* spp.	Found in humans, animals, birds, soil, water and sewage [39]
Haf (alv 1)		alvei 1	
K (sp)	Klebsiella (K)	*Klebsiella* spp.	Ubiquitous in forest, vegetation, soil, and water environment. Species oxycota and pneumonia are enteric bacteria [40]
K (oxy)		oxytoca	
K (pne)		pneumonia ssp ozaenae	
Klu (sp)	Kluvera (Klu)	*Kluvera* spp.	Found in water, soil, and sewage [41]

Table 2. *Cont.*

Identification Code	Genus	Species	Summary of Potential Origins
M (mor)	Morganella (M)	morganii	Intestines of humans, mammals, reptiles [42]
P (sp)	Pantoea (P)	*Pantoea* spp.	In the environment, found in soil, water, dust, dairy product, meat, fish, insects, humans and animals. Found in association with plants, leaves, stems and roots [43]
P(sp2)		Pantoea spp 2	
P (sp3)		Pantoea spp 3	
P (sp4)		Pantoea spp 4	
Pro (sp)	Providencia (Pro)	*Providencia* spp.	Found in water and land environment [42]
Pro (ret)		rettgeri	
Pse (sp)	Pseudomonas (Pse)	*Pseudomonas* spp.	Ubiquitous in the environment. Humans, animals, contaminated water and soil [9]
Pse (aer)		aeruginosa	
Prt (sp)	Proteus (Prt)	*Proteus* spp.	Part of the human intestinal flora, animals & birds [44]
Rao (sp)	Raoultella (Rao)	*Raoultella* spp.	An environmental bacteria [45]
Rao (orn)		ornithinolytica	
Ser (sp)	Serratia (Ser)	*Serratia* spp.	Ubiquitous in soil, water, and plant surfaces with preference for damp conditions [46]
Ser (mar)		marcescens	
Ser (odo)		odorifera 2	
Ser (fic)		ficaria	
Ser (fon)		fonticola	
Ser (liq)		liquefaciens	
Ser (odo)		odorifera 2	
Ser (rub)		rubideae	
Ste (sp)	Stenottrophomonas (Ste)	*Stenottrophomonas* spp.	Common soil organisms to opportunistic human pathogen [48]
She (put)	Shewanella (She)	Shewanella putrefaciens group	Widely distributed in nature (soil and water) [47]
Vib (sp)	Vibrio (Vib)	*Vibrio* spp.	Typically found abundantly in aquatic habitat also in salt water [49]

The Genus *Pseudomonas* of the *Pseudomonadaceae* family are motile Gram-negative aerobic bacteria plump-shaped rods, with polar flagella. *Pseudomonas* spp. are ubiquitous in the environment; found in humans, animals and plants, contaminated soil and water. *Pseudomans* can survive for months on dry surfaces [9]. *Proteus* spp. are part of the human intestinal flora and found in humans, animals, birds, and fish. *Proteus* spp. are widespread within the environment, including soil, water, and sewerage [44]. *Raoultella planticola* is a Gram negative, aerobic, non-motile bacillillus primarily considered to be environmental bacteria. *Raoultella planticola* is an aquatic, botanical and soil organism that does not typically cause invasive infections in humans [45]. *Serratia* spp. are chemoorganotrophic, facultative anaerobic bacteria with low nutritional requirements, and belong to the *Enterobacteriaceae* family and are ubiquitous in soil, water, and plant surfaces. *S. marcescens* produces a biofilm, with unique cellular and structural differentiation characteristics to those of the standard biofilms produced by *Pseudomonas aeruginosa* and *E. coli.* The latter bacteria produce biofilms, which consist only of microcolonies of undifferentiated cells [33]. *S. marcescens* may survive from three days to two months on dry, inanimate surfaces, and five weeks on dry floor. Due to its abundant presence in the environment, and its preference for damp conditions, *S. marcescens* is commonly found in moist environments [46]. *Shewanella putrefaciens* is a bacteria that is found mainly in marine environments [47]. It is a Gram-negative bacteria. *Stenotrophomonas* is also a genus of Gram-negative bacteria widely distributed in nature, with soil and water being the natural habitat [48]. Vibrio species are typically found abundantly in aquatic habitats and in salt water [49].

Overall, the data suggest that a wide variety of coliform of species originating from various sources, are present in the groundwater supplies sampled. The most widely detected species are ubiquitous in soil-water-plant environments: *Citrobacter* (65%), *Enterobacter* (63%), *Pantoea* spp. (17%), *Serratia* (19%), *Klebsiella* (34%) and *Pseudomonas* spp. (10%). Even though *Escherichia* species were found to be present in 12% of wells, several other coliform species detected can be of potentially feacal origin, including: *Hafnia* spp. (12%) *Faecal Streptococci* (3%) and *Morganella morganii* (3%).

In the GRAM groundwater risk assessment model [2], barriers to contaminants are considered throughout, from hazard to receptor. The pathway component describes the likelihood of contact with, or transport to a receptor—the water supply well. For exposure to occur, a source of contamination or contaminated media must exist along with transport from the source to a point where exposure could occur. Therefore, understanding the potential sources causing risk and the exposure pathway forms an integral part of the assessment of the risk status of groundwater.

4.2. Potential Pathways for Coliforms

GRAM considers two types of pathways from hazard sources to receptor [2]. The first is strata vulnerability, indicating physical characteristics of the aquifer and its susceptibility to land use. The second pathway to ensure provision of adequate protection from seepage of contaminants, is the degree of well integrity, including of the well collar, casing and sealing of the annular space against physical damage. The bacteria could enter groundwater and water supply wells through many interacting variables related to land use, soil types, depth to water, types of geologic strata and method of well construction. Since springs, dug wells and trenches are in direct contact with soil-plant

systems, they are prone to high bacterial contamination as evidenced in coliform detection frequency data given in Table 3.

The pathway for any contaminant describes the likelihood of contact with, or transport to a water source. The coliform detection frequency data in Table 3 were assigned to 11 different exposure categories (Table 4). Different categories indicate different degrees of exposure of water sources to a source of coliform or contaminated media. In this regard, two basic factors are considered to determine the vulnerability; the level of hydraulic inaccessibility of the saturated zone of the aquifer or production zone of the water supply well which minimize contact with the land surface and upper soil and strata in the aquifer; and, the bacterial attenuation capacity of the strata overlying the saturated zone of the aquifer or production zone of the water supply well [2].

The number of wells available for each well construction category varied from one to nine, and therefore rigorous statistical analysis proved difficult. However, apart from the recently constructed water wells, the large sample size (25–275) of the historical data (Table 3) enabled comparison of water source categories based on the frequency of coliform detection. The results show that the frequency of coliform detection appears to be influenced (Table 4) by aquifer type, geological strata (karst or fractured rock), well construction (shallow or deep casing, sealing of the annulus) and degree of well integrity (corroded or damaged well casing).

Trenches are similar to dug-wells, as their walls are unsealed and provide contact with the surrounding sub-surface soil-water-plant root environment. Unconfined karst aquifers are known to be generally more vulnerable to contamination than fractured or intergranular porosity aquifers and are classified as high vulnerability strata. Microbial pathogens can easily enter karst aquifers through the thin soils and the epikarst or via sinkholes, as in the Uley South basin (Figure 2).

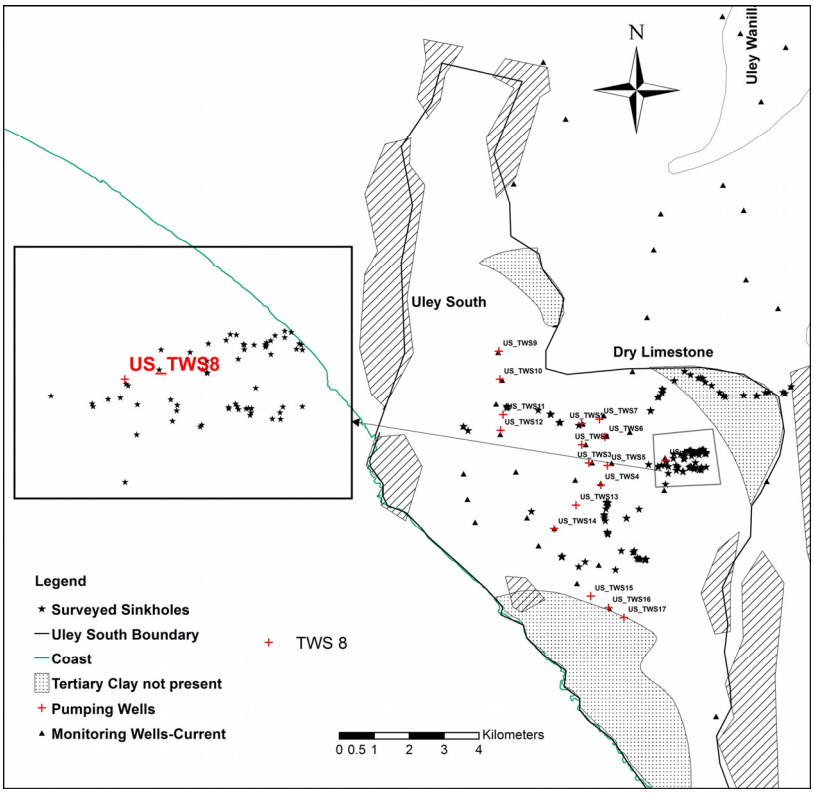


Figure 2. Sinkholes and town water supply wells in Uley South basin.

Table 3. Historical coliform data and 2013/2014 data with detected species in water sources (Historical data source: South Australian Water Corporation).

Water Source	Historical Detection Frequency (%) and Quantification (MPN/100 mL) of Coliforms Including *E. coli*						Species Detected in 2013/2014 Identification Codes (Abbreviations) From Table 2		Potential Coliform Origin
	Year of First Sample	Sample Size	(MPN/100 mL)	Coliforms Maximum MPN/100 mL	*E. coli* Detections	*E. coli* Maximum MPN/100 mL	Species	(MPN/100 mL)	
Fosters Creek	2009	15	60%	520	13%	48	Cit (bra), Ent (sp), K (oxy, pne), Ser (mar), Bur(sp)	580	Env/groundwater/biofilm
Hammond-Coonatta Spring	2005	22	95%	37,000	82%	2000	Cit (sp), Ent (clo), E (col 1), Prt (sp), Ser (mar), She (put), Vib (sp)	2400–55,000	Animal and groundwater
Woolshed Flat spring	2008	16	100%	10,000	94%	16	Aer (sp), Cit (sp, bra), Ent (sp, clo), K (oxy), P (sp3), Pro (sp,ret), Prt(sp), But(sp), Rao(orn), Ser (fon), She (put), Vib (sp)	11,000–16,000	Animal/Env/dirty water contamination
Blinman Mine	2006	25	8%	11	0%	0	Cit (bra)	1	More data required
Wilmington Mine	2005	115	72%	180	3%	2	Cit (fre), Ent (sp, can), K (sp), M (mor), P (sp), Ser (mar, fon), Ste (sp)	1–410	Animal/groundwater/biofilm
Streaky Bay Trench 1	1985	210	90%	730	47%	140	Cit (fre, bra), Ent (sp), She (put)	870	Animal/soil-plant/groundwater contamination
Streaky Bay Trench 2	1985	197	96%	1600	59%	200	Cit (sp, bra), Ent (clo), Ser (mar),She (put)	210	Animal/soil-plant/groundwater contamination
Bordertown TWS 8	1985	124	8%	390	2%	390	Aci (bau, cal), Ent (aer, clo)	1	More data required
Bordertown TWS 10	2012	4	3%	0	0%	0	Aci (bau, cal), Ent (aer, clo)	1600	Soil contamination
Millicent TWS 5	1998	135	8%	>200	0%	0	Cit (fre, bra), K (oxy)	3–5	Env., soil-plant, biofilms, plant roots
Mt Gambier TWS 9	1985	145	14%	>2400	0%	0	Cit (sp,fre), Ent(sp, asb), K (sp), P (sp2)	14–120	Animal, Env/soil/plant
Mount Burr TWS 5	2013	4	0%	0	0%	0	Cit (sp)	1	More data required
Parilla TWS 4	2008	45	4%	1	0%	0	Ser (mar)	1	More data required

Table 3. *Cont.*

Water Source	Historical Detection Frequency (%) and Quantification (MPN/100 mL) of Coliforms Including *E. coli*						Species Detected in 2013/2014 Identification Codes (Abbreviations) From Table 2		Potential Coliform Origin
	Year of First Sample	Sample Size	(MPN/100 mL)	Coliforms Maximum MPN/100 mL	*E. coli* Detections	*E. coli* Maximum MPN/100 mL	Species	(MPN/100 mL)	
Penola TWS 7	2012	5	0%	0	0%	0	Ent (can), K (sp)	1–4	Env/soil/plant
Pinaroo TWS 4	1985	265	21%	>200	2%	45	Cit (bra, kos)	5	More data required
Kingston TWS 12	1994	82	3%	2	0%	0	E (vul, her), P (sp3),Pse (sp)	10	Animal/soil, water, plant
Blinman TWS 1	2006	25	40%	1000	8%	3	Ent (sp), Haf (sp), P (sp4)	2–34	Animal/groundwater
Hawker TWS 1	1998	203	24%	>200	0%	0	Cit (sp), Ent (clo, can)	1–21	Env/soil/plant
Melrose TWS 5	2000	110	14%	70	0%	0	Cit (sp), Ent (sp, can), Ser (sp)	31–1000	Animal/Env/plant
Orroroo TWS 7	2004	58	33%	980	10%	920	Ent (sp,clo)	1–2	Env/soil/plant
Parachilna TWS 1	2006	275	33%	>200	2%	170	Cit (sp), Ent (clo), E (col 1), Pse (sp, aer)	1–3	Env/biofilm/dirty water
Warooka TWS 2	1996	114	46%	56	3%	2	Cit (kos), Ent (can), Haf (sp), Rao(sp)	6	Warm blooded animals
Willowie TWS 1	2005	30	93%	14000	30%	14	Cit (sp), Ent (clo), P (sp4), Prt (sp), Ser (mar, odo, fic), She (put), Vib (sp)	22	Animal and groundwater
Wilmington TWS 3	2009	34	8%	>200	0%	0	Haf (sp)	730	Warm blooded animal
Wilmington TWS 2	2005	115	46%	180	5%	25	Ent (sp, can, ger), E (col 1), P (sp2), Ser (fic, liq)	1–5	Animal and groundwater, soil/plant contamination
Coffin Bay TWS 7	2012	4	0%	0	0%	0	Ent (clo, amn), K (oxy)	1	Biofilm likely, more data required
Elliston TWS 3	1999	104	13%	>200	1%	1	Cit (sp), Ent (sp)	6	Env/soil/plant; more data required
Lincoln Basin Well A	2006	34	47%	56	3%	1	Cit (sp, bra, you), K (pne)	1–34	Env/soil/plant
Lincoln Basin Well B	2006	34	38%	59	0%	0	Cit (bra, kos, you), She (put)	1–5	Env/soil/plant/groundwater
Lincoln Basin Well O	2006	57	82%	1700	0%	0	Cit (bra), Ent (amn), K (pne)	38	Biofilm likely,animal/env
Lincoln Basin Well M	2006	35	71%	>200	17%	170	Cit (bra, you), Ent (aer), E (col 1), FS (sp), Pse (sp), Rao (sp), Ste (rub)	1–>2400	Animal and groundwater
Lincoln Basin Well J	2006	48	52%	1400	0%	0	Cit (sp, bra, you)	14	Env/soil/plant

Table 3. *Cont.*

Water Source	Historical Detection Frequency (%) and Quantification (MPN/100 mL) of Coliforms Including *E. coli*						Species Detected in 2013/2014 Identification Codes (Abbreviations) From Table 2		Potential Coliform Origin
	Year of First Sample	Sample Size	(MPN/100 mL)	Coliforms Maximum MPN/100 mL	*E. coli* Detections	*E. coli* Maximum MPN/100 mL	Species	(MPN/100 mL)	
Uley South TWS 1	2006	38	71%	78	0%	0	Cit (sp, fre, bra, you), Ent (sp, clo, can, amn), Haf (sp), K (sp, oxy), Klu (sp), Pse (sp)	1–2400	Animal and possible plant roots
Uley South TWS 2	2006	48	87%	>200	0%	0	Cit (bra), Haf (alv1), K (oxy)	2–7	Env/biofilm/warm blooded animals
Uley South TWS 3	2007	39	44%	200	2%	1	K (pne)	3	Env/biofilms
Uley South TWS 5	2006	33	18%	770	0%	0	Ent (sp, clo, amn), Ser (sp)	2–5	Env/groundwater
Uley South TWS 7	2006	44	25%	980	0%	0	Cit (sp)	1	More data required
Uley South TWS 8	2006	42	45%	>200	2%	1	Cit (sp, bra, you), Ent (clo), Haf (sp), P (sp), Ser (sp, mar, liq)	4–>2400	Env/groundwater/soil/plant
Uley South TWS 10	2006	32	22%	5	0%	0	Cit (sp, bra,), Ent (amn), K (oxy)	3–7	Env/biofilm
Uley South TWS 11	2006	35	3%	1	0%	0	Ent (clo, amn), Ser (sp)	1	Env/groundwater
Uley South TWS 14	2006	35	17%	48	0%	0	Cit (bra), Ent(amn), P(sp4), Pse (sp), Ser (rub)	2–6	Animal and groundwater
Uley South TWS 15	2006	45	33%	>200	4%	3	Cit (bra, you), Ent (clo, amn), K (oxy)	1–200	Animal/groundwater/biofilm
Uley South TWS 16	2006	39	23%	10	0%	0	Cit (you), Ent (clo), E (col 1), K (oxy)	>2400	Animal/groundwater/biofilm
Uley Wanilla TWS 1	2007	43	65%	>200	4%	2	Cit (sp, bra, you), Ent (clo), Ser (liq)	1–23	Animal/groundwater/soil/plant
Uley Wanila TWS 2	2007	41	27%	14	0%	0	Cit (sp), Ent (clo), K (sp)	2–3	Env/soil/plant
Uley Wanilla TWS 7	2007	38	13%	>200	0%	0	Cit (sp)	4	Env/soil/plant
Uley Wanilla TWS 8	2007	31	24%	11	0%	0	Ent (clo), K (oxy)	1–4	Env/biofilm/soil/plant
Mt. Compass TWS 1	2006	88	13%	77	0%	0	Cit (sp), Ent (sp, clo), E (vul)	11	Animal and Env contamination

Table 4. Water source category and historical coliform detection frequency.

Category	Water Sources	Number of Water Sources in Category	Historical Coliform Detection Frequency	
			Mean	Range
Surface water	Fosters creek, Hammond Coonatta spring, Woolshed flat spring	3	85%	60%–100%
Trenches in limestone aquifer	Streaky Bay Trench 1 and Trench 2	2	93%	90%–96%
Mines in fractured rock aquifer	Blinman and Wilmington mines	2	40%	8%–72%
Wells in limestone aquifer with corroded casings	Lincoln basin Wells A, B, O, M, J, Warooka TWS 2, Bordertown TWS 8	7	49%	38%–82%
Wells in semiconfined aquifers with shallow corroded casings in fractured rock aquifer	Blinman TWS 1, Hawker TWS 1, Willowie TWS 1, Wilmington TWS 2	4	50%	24%–93%
Wells in karst aquifer with depth to water <15m	Uley South TWS 1, 2, 3, 5, 7, 8, 10, 11, 14	9	37%	3%–87%
Wells in karst aquifer with depth to water >15m	Uley South TWS 15 and 16; Uley Wanilla TWS 1, 2, 7, 8	6	31%	13%–65%
Wells in deep sandstone or limestone aquifers	Parachilna TWS 1, Elliston TWS 3	2	23%	13%–33%
Wells in confined aquifers with pressure cemented annulus and production zone below a clay layer	Mt Gambier TWS 9, Parilla TWS 4, Penola TWS 7, Pinnaroo TWS 4, Kingston TWS 12, Mt Compass TWS 1, Melrose TWS 5, Orroroo TWS 7	8	12%	0%–33%
Wells in semiconfined aquifers with pressure cemented annulus in fractured rock aquifer	Wilmington TWS 3	1	8%	8%
Wells in limestone aquifer with deep casing and sealed annulus but the production has a large cavity (with possible link to sinkholes)	Bordertown TWS 10	1	3%	3%
Wells in limestone aquifer with deep casing and sealed annulus	Coffin Bay TWS 7, Mt Burr TWS 5, Millicent TWS 5	3	0%	0%

In the Uley South basin, eight town water supply wells (TWS 1–8) were constructed with steel casing, in the 1964–1975 period (Table 1) and at the time of sampling were believed to be at least partially corroded. In addition, the well annuli were sealed to a maximum of 2 m below ground, leaving much of each well in contact with surrounding aquifer material. The frequency of coliform detection in each well varies depending on the degree of contact with surface water runoff and the karstic nature of the aquifer. TWS 2 had the maximum historical coliform detection, with 87% frequency (Table 3).

Fractured rock aquifers are also susceptible to contamination and are considered a high vulnerability class, but to a lesser degree than karst aquifers [16]. Wilmington town water supply is sourced from an abandoned copper mine, and two wells (TWS 2 and TWS 3) are located in an unconfined fractured rock aquifer recharged from a steep rocky catchment subject to livestock grazing. The mine is similar to an uncased dug well, with high potential contaminant exposure through tree roots and insects. Similar to the Wilmington mine, TWS 2 has a high frequency of coliform detections (Table 3). The well is located on the bank of an ephemeral creek (Figure 3), and drilled to a depth of 75 m with an openhole production zone between 15 and 75 m. The PVC casing depth is limited to 12 m with only the upper 2 m of the annulus being cemented; and hence is considered prone to contamination. Despite the well annulus of TWS 3 being pressure cemented to the full casing depth of 53.7 m, it has infrequent low level detections of coliforms (Table 3). Drawdown response in TWS 2 during the pumping test conducted at TWS 3 shows that the wells are connected through the fracture network that share the same aquifer. TWS 3 is located about 50 m down-gradient from the mine, hence it is possible that the three water sources are linked and share recharge water from the ephemeral creek.

(**a**) (**b**) (**c**)

Figure 3. Mine, Wilmington TWS 2, and TWS 3 wells. (**a**): Mine entrance; (**b**): TWS 2; (**c**): TWS 3.

Willowie TWS 1 is a non-potable water supply source, in a fractured rock aquifer with a historical coliform detection of 93% and *E. coli* detection of 30% frequency (Table 3). The well is located at the edge of an ephemeral creek surrounded by cropping and grazing lands.

The well was tested for integrity and the shallow steel casing was found to be completely corroded and thus rehabilitated with PVC casing in 2012. Despite this, coliform detections continue, albeit at low counts (Figure 4), possibly due to high vulnerability of the fractured rock aquifer and the grazing landuse.

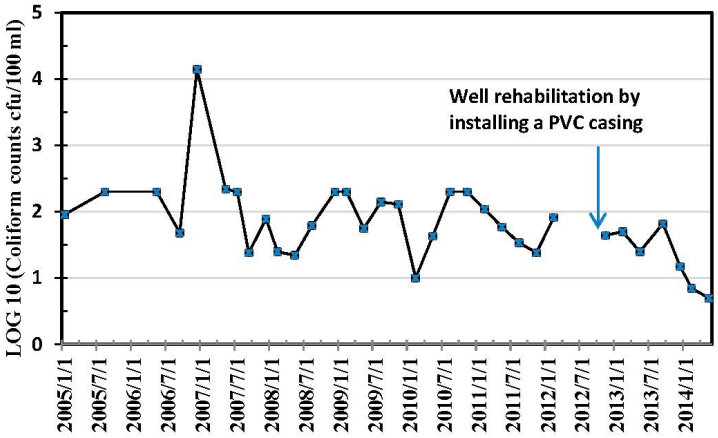
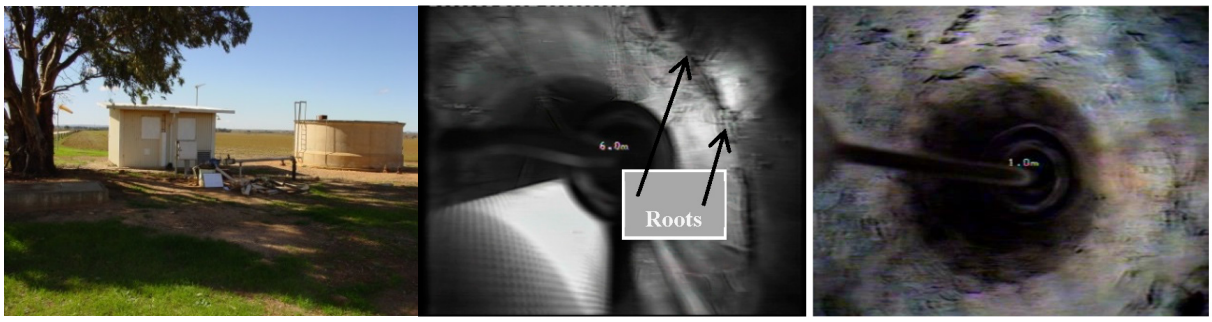


Figure 4. Coliform bacteria detection in Willowie TWS 1 from 2005 to 2014.

Damage to well casings can provide a pathway for contaminants to enter water supply wells, even in low vulnerability aquifers. Melrose TWS 5 is in a semi-confined sandstone aquifer with a production zone set at 57.5–97.5 m depth. The well is cased with FRP, and the full casing depth (57.5 m) of the well annulus is pressure cemented. Due to the poor annulus seal and joining of the casing, roots have penetrated from a nearby tree (Figure 5a,b). As a result, the well had a coliform detection frequency of 14%. The species detected, *Citrobacter* spp. and *Enterobacter* spp. are commonly found in the environment and *Serratia* spp. are ubiquitous in soil, water, and plant surfaces and can form biofilms.



(**a**) (**b**) (**c**)

Figure 5. Failed well casings—pathways for contaminants; (**a**) tree in the vicinity of Melrose TWS 5; (**b**) roots penetration through opening between PVC casing joints-Melrose TWS 5; (**c**) steel casing corrosion and lamination in Lincoln basin.

Even though the primary mechanism of recharge is infiltrated water through the soil surface (diffuse recharge), high coliform detections were found in Lincoln basin wells and Warooka TWS 2. Well integrity testing and camera views revealed that all steel cased wells in Lincoln basin were in poor condition with severe corrosion (Figure 5c). As the well annuli are presumed not to be sealed (a historical well construction practice), the 38%–82% frequency of coliform detection found in Lincoln basin wells, and 42% frequency from Warooka TWS 2 well are attributable to unsealed well head and corroded casing. The lack of sealed annuli and failed steel casings expose the well, making direct contact with the surrounding soil-water-plant system, thus providing a direct pathway for contaminants in surface water runoff.

With little or no possibility of coliform bacteria, particularly of faecal origin, entering into the confined aquifer through the aquitard or sealed annulus, another possible pathway for the detected bacteria is through the well head or sample taps. This was tested by re-sampling of wells for: (a) first flush sample (pre-flush) that may provide total coliform and *E. coli* that resides within the sample tap and well head; (b) after standard flushing time, normally three well volumes to obtain aquifer water (post-flush). During both pre- and post-flush sampling, sample taps were not sterilised. The result show that 82% of wells show pre-flush coliform of 1–22 MPN/100 mL, and 40% of wells contains post-flush coliform of 1–3 MPN/100 mL (Table 5). The exception was the Uley South TWS 2 where 200 MPN/100 mL of *Klebsiella oxytoca* was detected in pre-flush sampling and 18 MPN/100 mL of *Klebsiella oxytoca* found in a post-flush sample. This indicates that bacteria have the potential to reside within the sample tap or well head and even to form biofilms. Dislodgement of biofilms may be one possible reason for infrequent detection of high counts of coliform bacteria.

Table 5. Pre- and post- flush coliform detection.

Well Name	Sampling Round	Coliform Count (MPN/100 mL) and Species			
		Pre-Flush	Species	Post Flush	Species
Uley South TWS 16	1	5	K (oxy),C (bra), Ent (amn), Rao (sp)	0	-
	2	22	Cit (you), Cit (bra), Pse (sp)	2	Ent (amn), Cit (you)
Uley South TWS 15	1	5	Cit(bra)	0	-
	2	0	-	3	Cit (bra)
Mt Gambier TWS 9	1	1	K (oxy)	0	-
Wilmigton Mine	1	2	Pse (sp), Ser (mar)	0	-
Wilmington TWS 2	1	1	Ser (fic)	1	Ser (fic)
Uley Wannila TWS 1	1	1	Ent (clo)	0	-
	2	1	Ent (clo)	0	-
Uley Wannila TWS 2	1	0	-	1	Ent (amn)
Uley South TWS 1	1	4	Cit (you), Cit (bra), M(mor)	-	-
Uley South TWS 1	2	22	Cit (you), Cit (bra), K(oxy), Pse (sp)	2	Ent (amn),Cit (you)
Uley South TWS 2	1	200	K(oxy)	18	K(oxy)
Mt Compass TWS 1	1	1	Ser (sp)	0	-
Lincoln Basin Well A	1	12	Haf (alv 1), Rao (sp),Cit (bra)	3	Rao (sp), Ser (sp)
Lincoln Basin Well B	1	3	Cit (bra), Ent (amn)	0	-
Lincoln basin well O	1	3	Ent (amn)	2	Ent (amn)

Pinaroo TWS 4 is a steel cased confined aquifer well with an historical coliform detection frequency of 21%. A well integrity test indicated that the well casing is corroded. Confined aquifer wells, Parilla TWS 4 and Kingston TWS 12, are PVC cased wells with pressure cemented annulus to full casing depths. Both however, feature low levels of coliform detection (4% frequency of 1 MPN/100 mL in Parilla TWS 4 and (3% frequency of 1–2 MPN/100 mL in Kingston TWS 12). This is possibly associated with incomplete sterilisation of sampling taps prior to collection of water samples.

Parachilna TWS 1 well is constructed in deep unconfined sandstone aquifer in a semi-arid area (Figure 6a). The production zone of the well is at 66.5–72.5 m, with only the upper 5 m of annulus of the PVC casing pressure cemented. Depth to water is about 60 m. The well shows coliform bacteria at

33% frequency and *E. coli* at 2% frequency. The species detected (*Citrobacter*, *Enterobacter* and *Pseudomonas*) are environmental bacteria. They can grow on the inside surface areas of polyvinyl chloride pipes [9]. Similar to the Parachilna TWS 1, historically high coliform detections are found in Orroroo TWS 7 (Figure 6b), a confined aquifer well, with a production zone at 114.5–118 m and pressure cemented annulus to the full casing depth. As at first step towards further investigation, this well is planned for integrity testing. The production zone of Mount Gambier TWS 9 is at 238–250 m depth (Figure 6c). The annulus of the casing is pressure cemented to a depth of 183 m. Intermittent historical detection (in 2002, 2004, 2008) of coliform in Mount Gambier TWS 9 (Table 2) may be associated with well maintenance activities carried out in the past (personnel communication with operational staff). The well is constructed in a deep confined aquifer and the production zone is separated from the upper unconfined aquifer by two aquitards (Table 1), these should provide adequate barriers against environmental contamination.

(**a**) (**b**) (**c**)

Figure 6. Deep unconfined and confined aquifer wells (**a**) Parachilna TWS 1; (**b**) Orroroo TWS 7; (**c**) Mount Gambier TWS 7.

The well head of the Parachilna TWS 1 is constructed in a surface level manhole with a top-cover. In a similar well-head construction in shallow wells, Hynds *et al.* [11] report significant association with TTC presence, depending on the separation distance of manhole cover to well casing cap with uncontaminated wells having an average 98 mm clearance, and wells with TTC presence having a mean clearance of 62 mm. Hynds *et al.* study reviewed very shallow wells (mean measured depth less than five metres) in a semi intensive livestock grazing environment. Citing Savageau [50], Gordon [51] suggests that the typical *E. coli* cell spends, on average, half of its life in the external environment [51,52] and notes that fate and survival rate in the external environment are poorly understood.

Data indicate that there is no compelling evidence to support the contention that the wells identified as low risk using GRAM [2] are contaminated with coliform bacteria originating from the land surface and transported to production zone through soil and geological strata. However, the data strongly supports the suggestion that coliform detections from the sample taps and wellheads stem from the surrounding groundwater and soil-plant sources as a result of failed well integrity or even possible incomplete sterilization of the sample taps. In addition, coliform bacteria can persist within biofilms formed on well casings, screens, pump columns, and pumps. Disturbances during pumping can cause the biofilms to dislodge, releasing the coliform bacteria [53].

4.3. Options for Minimizing Risk of Water Well Contamination by Coliform Bacteria

In agricultural settings, grazing animals often come in close proximity to wellheads. As such, all members of the coliform bacteria family, and the parasites *Giardia* and *Cryptosporidia* which occur in the soil-water-plant environment and in animal manure can be present. Data indicate a relationship between well integrity and coliform detection, with wells with poor integrity showing high counts at higher frequencies and *vice-versa*. A number of international studies report similar findings [11,54]. The importance of setting a production zone of the well below an impermeable layer and maintaining well integrity is illustrated by Somaratne *et al.* [2] in the Millicent town water supply well No. 5 (TWS 5). The depth to water in the shallow unconfined Gambier Limestone unit is 2–3 m. The upper aquifer unit is calcaranite, having 1 m of silty soil cover. This results in the upper aquifer unit having high vulnerability to contamination. However, setting the production zone below a stiff clay aquitard of over 20 m thick results in the vulnerability index being reduced to a negligible level. The land use is predominantly livestock grazing and a single tree near a waterhole adjacent to the TWS 5 acts as a shelter for the animals. The waterhole receives runoff from the surrounding land and scour water from the well. The upper part of the aquifer is known to be polluted, having nitrate (as NO_3) levels exceeding 60 mg/L [2]. The TWS 5 well was constructed in 1967. In 2009, coliform bacteria were detected at a frequency of 80% (Figure 7). However, the production zone of TWS 5 has been set below the impermeable clay aquitard layer 80 m from the ground surface (Figure 8).

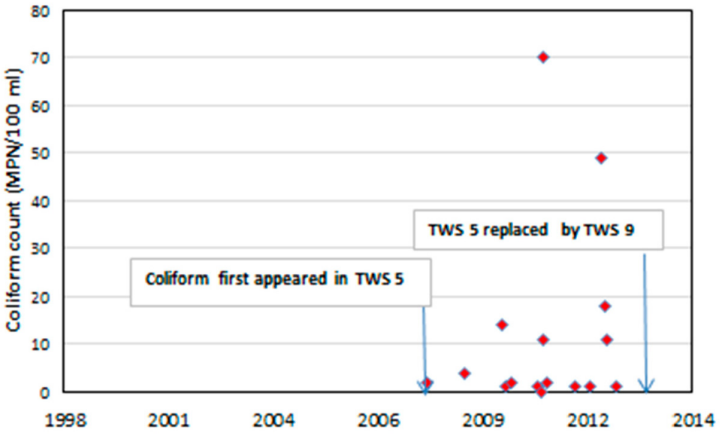


Figure 7. Coliform detection in Millicent TWS 5.

The test confirmed that the casing was corroded through in several locations. The annulus of the well had not been pressure cemented, and this combined with the failed casing made a pathway of contact with the polluted upper part of the aquifer. A replacement well (TWS 9) with PVC casing and pressure cemented annulus was constructed at the site, and is currently supplying coliform free water, despite the fact that the adjacent waterhole is hydraulically connected to the upper aquifer. According to the GRAM analysis, this action reduced the risk level from High to Medium. The risk level could be further reduced to "Low" by removing the hazard source (removing water ponding and fencing to prevent animal entering). This indicates the importance of sound well design, construction, and maintenance (condition of well/wellhead, presence of scour water ponding, presence of effective

sanitary seal around well head, fencing and diverting surface runoff away from well head), in potentially reducing the risk level and water supply source contamination.

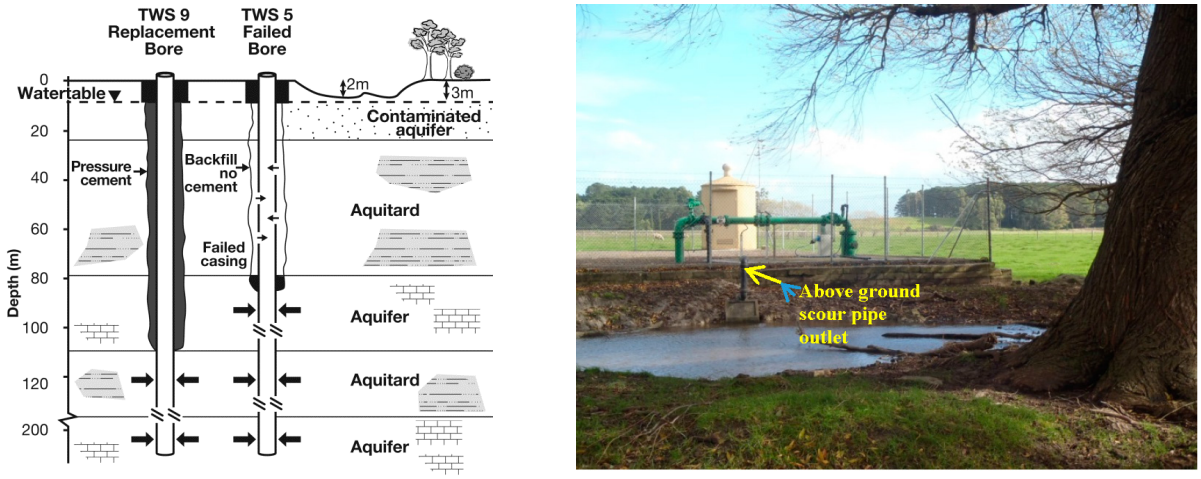


Figure 8. Millicent water supply wells TWS 5 and TWS 9 [2]. (**a**) schematic diagram; (**b**) view of the waterhole and TWS 9.

5. Conclusions

Overall, in the study area, 70% of sampled town water supply wells detected TTCs with potentially 36% of species being of animal origin. *E. coli* were found in 12% of wells (excluding surface water sources, mines and trenches). Although *E. coli* is probably the best indicator available for pathogenic enterobacteria and as such remains a useful tool for water quality monitoring [55], coliform sub-typing provides additional information on the potential source of coliforms and the presence of feacal origin coliforms, even if *E. coli* have not been detected.

High levels of coliform in wells can be due to poor design/construction and maintenance (corrosion, shallow casings, unsealed annuli). This indicates contaminant pathways exit to the production zone of the well making contact between upper soil layers and tree roots through fractures and solution features. A number of design/construction characteristics are found to be important in reducing risk factors, with well head finish, casing depth, cementing and sealing the annulus, sealing casing joints, and setting the production zone below low permeable layers all being important.

Coliform bacteria can persist within biofilms formed within the well by naturally occurring groundwater microorganism. Disturbances during pumping or well maintenance can cause the biofilm to dislodge, releasing the coliform bacteria. In addition, well construction defects such as insufficient well casing depth, improper sealing of the annulus, corroded and cracked casing and poor well seals or caps can allow surface water or insects to carry coliform bacteria into the well.

In a compromised water supply well where coliform sources are unknown or poorly understood, Microbial Source Tracking (MST) techniques [28,51] provide an opportunity to analyse water samples in a way that identifies the source of fecal bacteria in the sample. This can help identifying whether the source is human *versus* animal or it can sometimes involve identifying the source down to the species (e.g., cow, dog, kangaroo) or eliminating insignificant sources of fecal bacteria.

Acknowledgments

Two anonymous reviewers and the academic editor David Polya are thanked for useful comments which helped to improve the original manuscript. The followings are also thanked: Thorsten Mosisch and Loraine Bulbeck for facilitating the project; Scott Kraft and Jodie Gunn for arranging pre- and post-flush water sampling; Glyn Ashman for review of the manuscript.

Author Contributions

The study was designed and conceived by Nara Somaratne as part of a town water supply wellfield protection program. Nara Somaratne also analysed risk levels, hydrogeology, conducted the literature survey on coliform species, and determined the application of the project outcome to improving well design and protection of water sources. Nara Somaratne wrote the first draft of the paper. Gary Hallas contributed to microbiology aspects of the paper providing testing methodology, sub-typing, fingerprinting, further literature on species and identification of the most probable source origins. Both authors contributed to revisions of the original manuscript equally.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Robertson, L.J.; Edberg, S.C. Natural protection of spring and well drinking water against surface microbial contamination. *Crit. Rev. Microbiol.* **1997**, *23*, 143–178.
2. Somaratne, N.; Zulfic, H.; Ashman, A.; Vial, H.; Swaffer, B.; Frizenschaf, J. Groundwater risk assessment model (GRAM): Groundwater risk assessment model for wellfield protection. *Water* **2013**, *5*, 1419–1439.
3. Borchardt, M.A.; Bradbury, K.R.; Gotkowitz, M.B.; Cherry, J.A.; Parker, B.L. Human enteric viruses in groundwater from a confined bedrock aquifer. *Environ. Sci. Technol.* **2007**, *41*, 6606–6612.
4. Powell, K.L.; Taylor, R.G.; Cronin, A.A.; Barrett, M.K.; Pedley, S.; Sellwood, J.; Trowsdale, S.A.; Lemer, D.N. Microbial contamination of two urban sandstone aquifers in the UK. *Water Res.* **2003**, *37*, 339–352.
5. Baker, K.H.; Herson, D.S. Detection and occurrences of indicator organisms and pathogens. *Wat. Env. Res.* **1999**, *37*, 909–913.
6. United States Environmental Protection Agency (USEPA). *Wastewater Technology Fact Sheet—Bacterial Source Tracking*; EPA 832-F-02-010; USEPA: Washington, DC, USA, 2002.
7. United States Environmental Protection Agency (USEPA). *National Primary Drinking Water Regulations: Groundwater Rules*; Federal Register 71, No. 65574–65660; USEPA: Washington, DC, USA, 2006.

8. Opisa, S.; Odire, M.R.; Jura, W.G.Z.O.; Karanja, D.M.S.; Mwinzi, P.N.M. Feacal contamination of public water sources in informal settlements of Kisumu city, Western Kenya. In Proceedings of the International Conference on Hydrology and Groundwater Expo, San Antonio, TX, USA, 10–12 September 2012.
9. Staradumskyte, D.; Paulauskas, A. Non-Fermantative Gram-negative bacteria in drinking water. *J. Water Resour. Prot.* **2014**, *6*, 114–119.
10. Blackburn, B.G.; Craun, G.F.; Yoder, J.S.; Hill, V.; Calderon, R.L.; Chen, N.; Lee, S.H.; Levy, D.A.; Beach, M.J. Surveillance for water-borne disease outbreaks associated with drinking water: United States, 2001–2002, *MMWR Surveill. Summ.* **2004**, *53*, 23–45.
11. Hynds, P.D.; Misstear, B.D.; Gill, L.W. Development of a microbial contamination susceptibility model for private domestic groundwater sources. *Water Resour. Res.* **2012**, *48*, doi:10.1029/2012WR012492.
12. Betancourt, W.Q.; Rose, J.B. Drinking water treatment processes for removal of *Crptosporidium* and *Giardia*. *Vet. Parasitol.* **2004**, *126*, 219–234.
13. Fayer, R.; Gasbarre, L.; Pasquali, P.; Canals, A.; Almeria, S.; Zarlenga, D. *Cryptosporidium* parvum infection in bovine neonates:dynamic clinical, parasitic and immunologic patterns. *Int. J. Parasitol.* **1998**, *28*, 49–56.
14. Carpenter, C.; Fayer, R.; Trout, J.; Beach, M.J. Chlorine disinfection of recreational water for Cryptosporidium parvum. *Emerg. Infect. Dis.* **1999**, *5*, 579–584.
15. Khaldi, S.; Ratajczak, M.; Gargala, G.; Fournier, M.; Berthe, T.; Favennec, L.; Dupont, J.P. Intensive exploitation of a karst aquifer leads to *Cryptosporidium* water supply contamination. *Water Res.* **2011**, *45*, 2906–2914.
16. Goeppert, N.; Goldscheider, N. Transport and variability of faecal bacteria in carbonate conglomerate aquifers. *Groundwater* **2011**, *49*, 77–84.
17. *Water Quality Sampling-Part II: Guidance on Sampling of Groundwaters*; Australian/New Zealand Standard AS/NZS 5667.11.1998; Standard Australia: Sydney, Australia, 1998.
18. *Selection of Containers and Preservation of Water Samples for Microbiological Analysis*; Australia Standard AS/NZS 2031-2001; Standard Australia: Sydney, Australia, 2001.
19. O'Hara, C.M.; Rhoden, D.L.; Miller, J.M. Reevaluation of the API 20E identification system *versus* conventional biochemical for identification of members of the family *Enterobacteriaceae*: A new look at an old product. *J. Clin. Microbiol.* **1992**, *30*, 123–125.
20. Aldridge, K.E.; Hodges, R.L. Correlation studies of entero-set 20, API20E and conventional media systems for Enterobacteriaceae identification. *J. Clin. Microbiol.* **1981**, *13*, 120–125.
21. Merlino, J.; Siarakas, S.; Robertson, G.J.; Funnell, G.R.; Gottlieb, T.; Bradbury, R. Evaluation of CHROMagar Orientation for differentiation of GRAM-negative bacilli and *Enterococcus* species. *J. Clin. Microviol.* **1996**, *34*, 1788–1793.
22. Hengstler, K.A.; Hammann, R.; Fahr, A.M. Evaluation of BBL CHROMagar orientation medium for detection and presumptive identification of urinary tract pathogens. *J. Clin. Mictrobiol.* **1997**, *35*, 2773–2777.
23. Hallas, G.; Giglio, S.; Capurso, V.; Monis, P.T.; Grooby, W.L. Evaluation of chromogenic technologies for use in Australian potable water. *J. Appl. Microbiol.* **2008**, *105*, 1138–1149.

24. Wellowner.org: Informing Consumers about Groundwater and Water Wells. Available online: http://wellowner.org (accessed on 13 February 2013).
25. Bureau of Environmental Health, Ohio Department of Health. Total and Faecal Coliform bacteria. Available online: http://www.odh.ohio.gov/~/media/ODH/ASSETS/Files/eh/HAS/coliform.ashx (accessed on 12 May 2014).
26. Sigler, A.; Bauder, J. Well-Educated: Total *Coliform* and *E. coli* Bacteria. Northern Plains & Mountains Regional Water Program, Montana State University. Available online: http://waterquality.montana.edu (accessed on 9 July 2015).
27. Kuhn, I.; Allestam, G.; Stensrom, T.A.; Mollby, R. Biochemical fingerprinting of water coliform bacteria, a new method for measuring phenotypic diversity and for comparing different bacterial populations. *Appl. Environ. Microbiol.* **1991**, *57*, 3171–3177.
28. Kuhn, I.; Allestam, G.; Engdahl, M.; Stenstrom, T.A. Biochemical fingerprinting of coliform bacteria populations—Comparisons between polluted river water and factory effluents. *Water Sci. Technol.* **1997**, *35*, 343–350.
29. Hallas, G. *Coliform Indicator Bacteria*; *Coliform Source Tracking Project*; South Australian Water Corporation: Adelaide, Australia, 2014, unpublished.
30. Baumann, P.; Doudoroff, M.; Stanier, R.Y. A study of the *Moraxella* group II. Oxidative-negative species (genus *Acinetobacter*). *J. Bacteriol.* **1968**, *95*, 1520–1541.
31. Merino, S.; Rubires, X.; Tomas, J.M. Emerging pathogens: *Aeromonas* spp. *Int. J. Food Microbiol.* **1995**, *28*, 157–168.
32. Estrada-de los Santos, P.; Vinuesa, P.; Martinez-Aguilar, L.; Hirsch, A.M.; Caballero-Mellado, J. Phylogenetic analysis of *Burkholderia Species* by multisequence analysis. *Curr. Microbiol.* **2013**, *67*, 51–60.
33. Sproer, C.; Mendrock, U.; Swiderski, J.; Lang, E.; Stackebrandt, E. The phylogenetic position of Serratia, Buttiauxella and some other genera of the family Enterobacteriaceae. *Int. J. Syst. Bacteteriol.* **1999**, *49*, 1433–1438.
34. Lipsky, B.A.; Hook, E.W., III; Smith, A.A. Citrobacter infections in humans: Experience at the Seattle Veterans Administration Medical Center and review of the literature. *Rev. Infect. Dis.* **1980**, *2*, 746–760.
35. Cabral, J.P.S. Water microbiology: Bacterial pathogens and water. *Int. J. Environ. Res. Public Health* **2010**, *7*, 3657–3703.
36. LENNTECH Water Treatment Solutions. Faecal Bacteria. Available online: http://www.lenntech.com/faecal-bacteria.htm (accessed on 7 May 2014).
37. Singleton, P. *Bacteria in Biology, Biotechnology and Medicine*, 5th ed.; Willey: Hoboken, NJ, USA, 1999; pp. 444–454.
38. Ishii, S.; Sadowsky, M.J. Escherichia coli in the environment: Implication for water quality and human health. *Microbes Environ.* **2008**, *23*, 101–108.
39. Ianda, J.M.; Abbott, S.L. The *Genus Hafnia*: From soup to nuts. *Clin. Microbiol. Rev.* **2006**, *19*, 12–18.
40. Public Health Agency of Canada. *Klebsiella* Spp., Pathogen Safety Data Sheet-Infectious Substances. Available online: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/klebsiella-eng.php (accessed on 28 April 2014).

41. Sarria, J.C.; Vidal, A.M.; Kimbrough R.C., III. Infections caused by *Kluyvera* Species in humans. *Clin. Infect. Dis.* **2001**, *33*, doi:10.1086/322686.
42. Maayer, P.D.; Chan, W.Y.; Blom, J.; Venter, S.N.; Duffy, B; Smits, T.H.M.; Coutinho, T.A. The large universal Pantoea plasmid LPP-1 plays a major role in biological and ecological diversification. *BMC Genomics* **2012**, *13*, doi:10.1186/1471-2164-13-625.
43. O'Hara, C.M.; Brenner, F.W.; Miller, J.M. Classification, identification, and clinical significance of *Proteus*, *Providencia* and *Morganella*. *Clin. Microbiol. Rev.* **2000**, *13*, 534–546.
44. Public Health Agency of Canada. *Proteuss* Spp., Pathogen Safety Data Sheet-Infectious Substances. Available online: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/proteus-eng.php (accessed on 28 April 2014).
45. Nada, B.; Areej, M. *Raoultella planticola*, a central venous line exit site infection. *J. Taibah Univ. Med. Sci.* **2014**, *9*, 158–160.
46. Public Health Agency of Canada. *Serratia* Spp., Pathogen Safety Data Sheet-Infectious Substances. Available online: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/serratia-spp-eng.php (accessed on 28 April 2014).
47. Sharma, K.K.; Kalawat, U. Emerging infections: Shewanella—A series of five cases. *J. Lab. Phys.* **2010**, *2*, 61–65.
48. Palleroni, N.J; Bradbury, J.F. Stenotrophomonas, a new bacteria genus for Xanthomonas maltophilia (Hugh 1980) Swings *et al.* 1983. *Int. J. Syst. Evol. Microbiol.* **1993**, *43*, 606–609.
49. Thompson, F.L.; Gevers, D.; Thompson, C.C.; Dawyndt, P.; Naser, S.; Hoste, B.; Munn, C.B.; Swings, J. Phylogeny and molecular identification of vibrios on the basis of multilocus sequence analysis. *Appl. Environ. Microbiol.* **2005**, *7*, 5107–5115.
50. Savageau, M.A. *Escherichia Coli* habitats, cell types and molecular mechanisms of gene control. *Am. Nat.* **1983**, *122*, 732–744.
51. Gordon, D.M. Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contaminations. *Microbiology* **2001**, *147*, 1079–1085.
52. Brennan, F.P.; O'Flaherty, V.; Kramers, G.; Grant, J.; Richards, K.G. Long-term persistence and leaching of *Escherichia coli* in temperate maritime soils. *Appl. Environ. Microbiol.* **2010**, *76*, 1449–1455.
53. Michigan Department of Environmental Quality. Coliform Bacteria and Well Water Sampling, Fact Sheet, Office of Drinking Water & Municipal Assistance, Environmental Health Section. Available online: http://www.michigan.gov/documents/deq/deq-wd-gws-wcu-coliformbactiwell watersampling_270604_7.pdf (accessed on 5 May 2014).
54. Howard, G.; Pedley, S.; Barrett, M.; Nalubega, M.; Johal, K. Risk factors contributing to microbiological contamination of shallow groundwater in Kampala. *Water Res.* **2003**, *37*, 3421–3429.
55. Kitts, C.; Shaffner, A.; Samadpour, M.; Reyburn, I. *Feacal Contamination Source Tracking by Ribotype Fingerprints of Environmental E. coli from the Coachella Valley Stormwater Channel*. *Final Report*; State Water Resources Control Board: Sacremento, CA, USA, 2004.