05 - RISK FACTOR ANALYSIS OF *ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA* OCCURRENCE IN PRIVATE DOMESTIC GROUNDWATER SUPPLIES IN THE REPUBLIC OF IRELAND

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Abstract
Groundwater is the primary drinking water source for approximately 2.2 billion people worldwide and over 750,000 in the Republic of Ireland (RoI). Hence, it is critical to ensure that groundwater resources are protected and of acceptable drinking quality. However, poor maintenance, inappropriate well location and a historical lack of regulation has led to many instances of groundwater contamination and associated public health issues. For example, verotoxigenic *Escherichia coli* (VTEC) enteritis, a serious infectious disease for which Ireland currently reports the highest incidence rates in Europe, has been associated with private domestic wells. Accordingly, the current study aims to elucidate external factors associated with microbial contamination of private groundwater supplies by examining two distinct bacterial species. In total, 132 private wells distributed across twenty-one administrative counties in the RoI were surveyed and sampled during Autumn 2019. Samples were tested for the presence of *Escherichia coli* (a faecal indicator bacteria) and *Pseudomonas aeruginosa* (a pathogenic bacteria that, unlike *E. coli*, is both soil and water resident). Local anthropogenic, hydrogeological and meteorological parameters were also recorded, with non-parametric bivariate modelling employed to identify risk factors. Findings highlight the prevalence of bacterial contamination in Irish groundwater supplies, with 35 (26.5%) and 8 (6%) wells testing positive for *E. coli* and *P. aeruginosa*, respectively. *E. coli* positive wells were located across all groundwater vulnerability categories, while *P. aeruginosa* were only found in regions of high (H=6.5%) and extreme (E=6.5%; X=14.3%) vulnerability, however neither *E. coli* ($p = 0.842$) or *P. aeruginosa* ($p = 0.198$) were statistically associated with local groundwater vulnerability. Risk factor analyses point to supply type (i.e. borehole versus hand-dug), and short-term antecedent rainfall (i.e. 10 days) as being significantly associated with contamination with both bacterial species. Infrastructural properties, specifically absence of protective casing ($p = 0.013$) and reduced lining clearance above well chamber floor ($p = 0.041$) were also associated with *P. aeruginosa* and E. *coli* occurrence, respectively. These findings seem to indicate that *E. coli* contamination takes place primarily above ground, while *P. aeruginosa* enters groundwater predominantly via subsurface ingress. Current study results show that microbial presence within Irish groundwater supplies is a persistent issue, and highlight how risk factor analyses can facilitate a better understanding of microbial ingress mechanisms. From a mitigation standpoint, modelling results also elucidate the source protective features which must be prioritized in future intervention strategies to prevent contamination.

1. INTRODUCTION
The presence of potentially pathogenic microorganisms within groundwater supplies is a persistent public health concern for over 2 billion people reliant on groundwater as a drinking water source globally (Murphy *et al.*, 2017). It is estimated that up to 59.4 million yearly cases of acute gastrointestinal infection worldwide are linked to the consumption of contaminated groundwater

(Murphy *et al.*, 2017). The risk of human exposure to pathogens through contaminated groundwater and subsequent onset of illness is especially common amongst private (unregulated) well users, where instances of inappropriate location, construction, and maintenance are not uncommon (Hynds *et al.*, 2013). A recent study in the Republic of Ireland (RoI) found that confirmed cases of verotoxigenic *Escherichia coli* (VTEC) enteritis, a serious infectious disease for which Ireland frequently reports the highest incidence rate in Europe, is significantly associated with private well usage (OR 18.727, $p < 0.001$; ÓhAiseadha *et al.*, 2017). Similarly, Risebro *et al.* (2012) showed that the incidence of infectious intestinal diseases among people served by private supplies in England was significantly higher than within the population as a whole. In light of these public health concerns relating to groundwater-reliant sub-populations, it is crucial to identify the principal factors facilitating ingress of microbial contaminants to these supplies, so that they can be targeted and managed to safeguard public health.

Accordingly, the current research examined private wells across the RoI and employed risk factor analyses to identify the components significantly linked with presence of two bacterial species, namely, *E. coli* and *P. aeruginosa*. The former is a well-known faecal indicator that primarily inhabits the gastrointestinal tract of warm-blooded animals (Savageau, 1983) and may, in specific cases, be pathogenic (e.g. VTEC; Kuntz and Kuntz, 1999). Conversely, *P. aeruginosa* is a bacterial pathogen that can cause an array of systemic infections (e.g. urinary, respiratory, and gastrointestinal), and resides in moist environments such as soil and water (Bodey *et al.*, 1983). Identification of these two distinct bacteria, originated from different sources, across spatially diverse samples may be used to provide information on both surface and subsurface ingress mechanisms. Thus, providing a more holistic assessment of microbial contamination of groundwater resources, with the primary goal of providing recommendations for the promotion of source protection measures.

2. METHODS

2.1 Sample collection and analysis

The study was undertaken in the RoI, where over 15% of the population (>750,000 people) rely on private groundwater supplies, most of which are exempt from complying with the European Drinking Water Directive 98/83/EC (EU 2018). Private well owners across the country were recruited to participate in the study via social media and institutional contact lists, with no spatial biases present. Sampling took place from the 8th of September to the 3rd of November 2019 and involved collection of untreated water from outdoor taps into 120-ml sterile vessels containing sodium thiosulphate for the neutralisation of residual chlorine. A minimum volume of 2L was allowed to run prior to sample collection to ensure water was not taken from the distribution system. All samples were transported on ice and bacterial enumeration was performed within 6h. The most probable number (MPN) of *E. coli* and *P. aeruginosa* were enumerated via standard ISO approved (ISO, 1998) commercial kits (Colilert and Pseudalert, IDEXX Laboratories Inc., Westbrook, Maine) using 51-"well" quanti-trays. Results were read following 24h incubation at 37 °C by counting the number of positive quanti-tray "wells" and recording the associated MPN.

2.2 Risk factors

Geo-referenced hydrogeological and antecedent weather data, and on-site assessments were used to identify potential risk factors related to each studied supply. On-site assessments were used to identify and record source characteristics and setback distances/gradient variations between sampled supplies and potential sources of contamination (e.g. septic tank, animal grazing fields, slurry sheds, roads) adjacent to it (i.e. within 100m). Hydrogeological parameters such as superficial bedrock geology, aquifer type, vulnerability category, etc. were extracted from Geological Survey of Ireland (GSI) geospatial mapping resources (GSI, 2020). Geo-referenced hydrogeological data were assigned to each supply based on recorded GPS coordinates. Supply coordinates were also used to identify nearest synoptic weather stations and (antecedent) rainfall information were extracted from Met Eireann's open-source database (Met Eireann, 2020).

2.3 Statistical analyses

Non-parametric bivariate techniques were employed in this study to identify potential risk factors statistically associated with presence of *E. coli* and *P. aeruginosa* within private groundwater supplies. Briefly, Mann-Whitney U and Chi-square tests were employed to identify categorical associations between dependant and independent variables. Independent variables (i.e. risk factors) comprise all supply-specific, site-specific and meteorological data collected, with presence/absence of *E. coli* and *P. aeruginosa* used as binary dependent variables. All statistical analyses were undertaken using SPSS Version 24.

3. RESULTS

In total, 132 private household wells were included in the current study, situated in 21 of the 26 administrative counties in RoI. Counties Cork (29.5%) Limerick (7.6%), and Wicklow (7.6%) were the most represented in this study, with poorly productive bedrock aquifers underlying the majority of studied sites (81.8%, n=108). Furthermore, 40.1% of wells were located in areas where groundwater vulnerability is classified as High (H=53), followed by regions of Extreme (37.1%; E=29 and X=20), Moderate (17.4%; M=23) and Low (5.3%; L=7) groundwater vulnerability classifications.

All wells in this study were situated in rural areas, with 90.1% (n=119) and 88.6% (n=117) located within 100 meters of a domestic wastewater treatment system (DWWTS; e.g. septic tanks) and livestock grazing fields, respectively. The supplies were predominantly drilled (88.6%; n=117), with hand-dug wells accounting for 11.4% (n=15) of studied wells.

3.1 Microbial contamination

Of the 132 studied groundwater supplies, 27.3% (n=36) showed evidence of microbial contamination (see Figure 1), with 26.5% (n=35) and 6% (n=8) testing positive for *E. coli* and *P. aeruginosa*, respectively; 5.3% (n=7) tested positive for both. Microbial contamination was more frequent within hand-dug wells, where *E. coli* and *P. aeruginosa* presence was found in 53% (n=8) and 20% (n=3) of supplies, respectively. Regarding local hydrogeological characteristics, *E. coli* positive wells were located across all groundwater vulnerability categories, while *P. aeruginosa* were only found in regions of High (H=2/53) and Extreme (E=4/29; X=2/20) vulnerability.

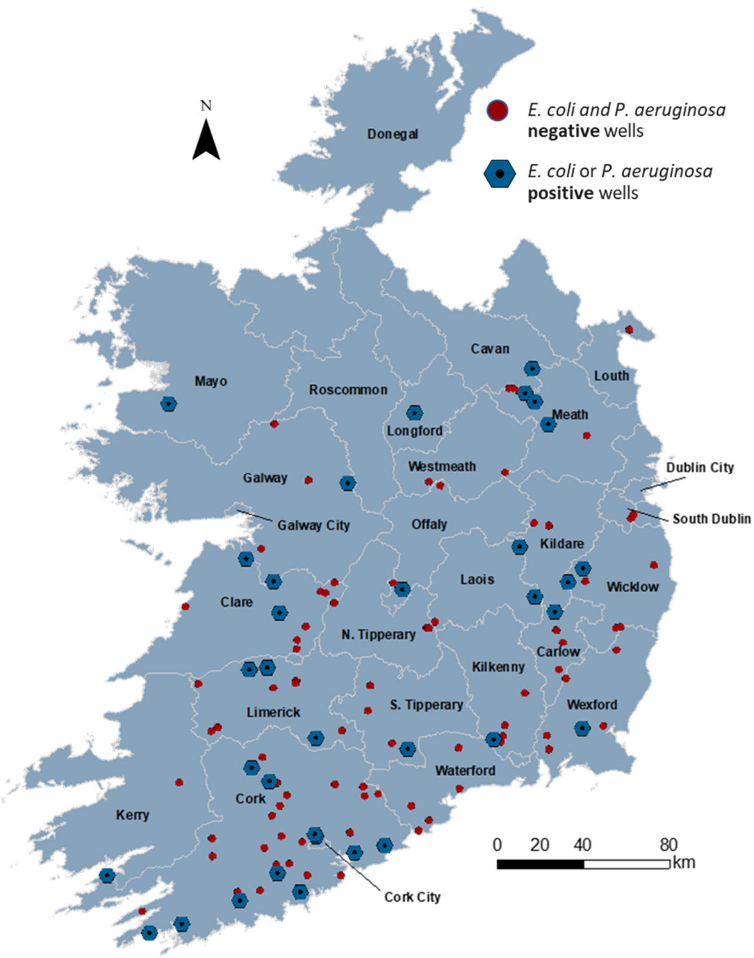

Figure 1: *Map of the Republic of Ireland showing all sampling locations and their microbial test results (i.e. positive/negative for Escherichia coli and Pseudomonas aeruginosa presence)*

3.2 Risk factor analyses

As shown (Table 1), neither the presence of *E. coli* (p = 0.842) nor *P. aeruginosa* (p = 0.198) were statistically associated with local groundwater vulnerability. In fact, non-significant results were observed in relation to all hydrogeological parameters analysed (i.e. aquifer type, aquifer importance and recharge coefficient). Similarly, local land-use (i.e. DWWTS and livestock density) and proximity to potential sources of contamination were not significantly associated with presence of either bacterial species.

Notably, the incidence of both *E. coli* and *P. aeruginosa* were significantly associated with supply type (i.e. higher among hand-dug wells). Moreover, absence of protective casing was significantly associated with *P. aeruginosa* occurrence (p = 0.013), and reduced lining clearance above well chamber floor was significantly associated with *E. coli*- positive results (p = 0.041). No significance was found involving other infrastructural properties, such as presence of protective well cover and cap, or location of wellhead finish (e.g. bellow, at, or above ground) with presence of either studied species.

Of all the meteorological risk factors analysed (i.e. 0 to 180-day antecedent rainfalls), presence of *P. aeruginosa* species within studied groundwater supplies were significantly associated with antecedent rainfall immediately prior to sample collection (i.e. 24h, 48h and 5-day antecedent rainfall; p = 0.002, 0.018, and 0.029, respectively), and 10-day antecedent precipitation was significantly associated with contamination with both bacterial species (p = 0.050 for *E. coli* and p = 0.012 for *P. aeruginosa*).

Table 1: *Risk factor analyses results*

Risk factors	**N**	***E coli***		***P aeruginosa***	
		test statistics	***p***	**test statistics**	***p***
Supply-specific data					
Supply type [a]	132	**6.247**	**0.012***	**5.776**	**0.016***
Presence of protective cover [a]	126	0.010	0.918	1.397	0.237
Presence of protective cap [a]	125	0.453	0.501	1.355	0.244
Presence of protective casing [a]	121	0.483	0.487	**6.200**	**0.013***
Wellhead finish [a]	129	4.866	0.088 [§]	4.012	0.135
Clearance above chamber floor (cm) [b]	126	**1195.0**	**0.041***	327.0	0.143
Setback distance to household DWWTS (m) [b]	132	1402.5	0.128	461.0	0.738
Gradient difference to household DWWTS [a]	132	0.362	0.948	4.423	0.219
Setback distance to nearest livestock (m) [b]	132	1605.0	0.633	446.0	0.633
Gradient difference to nearest livestock [a]	118	2.340	0.310	1.789	0.409
Site-specific data					
Aquifer type [a]	132	2.282	0.516	1.472	0.689
Aquifer importance [a]	132	3.519	0.172	1.449	0.484
Groundwater vulnerability [a]	132	1.412	0.842	6.013	0.198
Recharge coefficient [b]	132	1626.5	0.692	330.5	0.088 [§]
Livestock density [b]	132	1573.5	0.523	472.0	0.819
DWWTS density [b]	132	1456.5	0.214	407.0	0.396
Water table (m) [b]	110	1139.0	0.570	300	0.214
Weather and climate related data					
day precipitation data (mm) [b]	132	1527.5	0.378	325.5	0.102
24 h precipitation data (mm) [b]	132	1418.5	0.148	**171.5**	**0.002***
48 h precipitation data (mm) [b]	132	1460.5	0.221	**247.5**	**0.018***
5-day precipitation data (mm) [b]	132	1491.0	0.287	**267.0**	**0.029***
10-day precipitation data (mm) [b]	132	**1318.0**	**0.050***	**233.5**	**0.012***
30-day precipitation data (mm) [b]	132	1623.0	0.701	450.5	0.664
60-day precipitation data (mm) [b]	132	1456.0	0.213	379.5	0.266
90-day precipitation data (mm) [b]	132	1594.0	0.594	430.5	0.532
180-day precipitation data (mm) [b]	132	1601.5	0.621	387.5	0.301

[a] Chi-square test employed, [b] Mann-Whitney test employed, * Statistically significant ($p<0.05$), [§] Approaching significance ($0.05<p<0.10$)

4. DISCUSSION

The presented study sought to examine current levels of microbial contamination and the factors statistically associated with their presence within private (household) wells in the RoI. Results identified relatively high levels of bacterial prevalence, with *E. coli* and *P. aeruginosa* found in 26.5% and 6% of the 132 studied supplies, respectively. Yet, even higher prevalence has been reported in previous RoI-based studies. A similar cross-sectional study of 262 private wells in RoI, for example, found a mean *E. coli* occurrence rate of 29.4% over a 2-year sampling period from 2008-2010 (Hynds *et al.*, 2014). The national Environmental Protection Agency (EPA) groundwater monitoring programme also reported higher prevalence of *E. coli*, observed in 43% of locations monitored in 2017 and 42% in 2016 (EPA, 2018a; 2018b). Moreover, in a study that employed repeat sampling, as many as 58.4% of sampled wells in the Mid-west of the country were reported to be *E. coli*-positive at least once (O'Dwyer *et al.*, 2014). Winter samples, for example, may have had higher detection rates, as rainfall has been shown to increase the susceptibility of groundwater supply contamination with *E. coli* in the country (Hynds *et al.*, 2012; O'Dwyer *et al.*, 2014). To the author's knowledge this is the first RoI-based study which has examined presence of *P. aeruginosa* in groundwater supplies.

As both bacterial species included in this study have the potential to cause human infection, their presence within private wells also corroborate findings from other national and international studies that link groundwater consumption to the development of waterborne illnesses (ÓhAiseadha *et al.*, 2017; Murphy *et al.*, 2017; Chique *et al.*, 2020). In that context, private household wells may be at a particularly high risk of contamination with these pathogenic microorganisms, as they are often poorly constructed (e.g. lack basic protective features) and inappropriately located (e.g. at close proximity to sources of faecal contamination such as septic tanks and husbandry practices) (Hynds et al. 2013). To prevent instances of groundwater contamination with (pathogenic) microorganisms and the subsequent onset of waterborne illnesses amongst its many consumers, it is important to identify the principal factors driving their occurrence so that they may be targeted. In this study, the authors employed simple binary risk factor analysis to achieve this. Using this method, results show, for example, that both *E. coli* and *P. aeruginosa* presence in groundwater are significantly associated with supply type (i.e. higher within hand-dug wells), which is concurrent with findings from other microbial contamination studies in RoI (Hynds *et al.*, 2014; O'Dwyer et al., 2018). It is widely acknowledged that hand-dug groundwater supplies, with their simpler design features and shallower depths, are more conducive to both direct contamination ingress at the wellhead, and ingress via preferential flow paths and infiltration (Howard *et al.*, 2003; Swistock and Sharpe, 2005; Gonzales, 2008). It is also worth noting that, despite lack of official statistics, these are thought to account for up to 10% of all private (household) wells in RoI (Hynds *et al.*, 2012); in the current study they represented 11.4% of included supplies.

Risk factor analyses results exhibited no significant association between hydrogeological setting and groundwater contamination with either *E. coli* or *P. aeruginosa*. However, it is worth noting that *P. aeruginosa* were only found in areas where groundwater vulnerability was classified as H, E and X. It is thought that lack of significance in this instance may have been due to prohibitive sample numbers (i.e. *P. aeruginosa* were only found in 8 locations). Observing the meteorological risk factors, analyses results also show that presence of both species within sampled locations were significantly associated with very short-term antecedent rainfall (i.e. 10-day rainfall), and in the case of *P. aeruginosa* immediately antecedent precipitation (i.e. 24h, 48h and 5-day rainfall) was also significant.

In the case of *E. coli,* reduced liner clearance above well chamber floor was significantly associated with instances of contamination (p=0.041). This finding combined with the significant impact of short-term antecedent precipitation (i.e. 10-day rainfall; p= 0.050) as well as lack of significance relating it with local groundwater vulnerability potentially indicates that *E. coli* contamination is occurring predominantly via short-circuiting mechanisms. Conversely, *P. aeruginosa* contamination was significantly associated with absence of protective casing of the well (p=0.013). This finding, in addition to the significant results linking very short-term antecedent rainfall to instances of *P. aeruginosa* contamination and the fact that these only occurred in areas of High/Extreme groundwater vulnerability, gives rise to the assumption that recent mobilisation of surface contaminants and infiltration along preferential pathways may be the principal mechanism through which it enters groundwater the supplies. However, as *P. aeruginosa* also reside in the soil interpretation is complex and requires additional data. It is also important to acknowledge that the mechanistic inferences provided here have been based on temporally limited data (i.e. samples collected over 2 months) utilising a simplistic modelling technique, and as such, may not account for the inherent complexities associated with all groundwater contamination mechanisms. However, study findings are still valuable as they can be used to inform more detailed studies which look at microorganism ingress into groundwater supplies.

5. CONCLUSION

Study results show that microbial presence within Irish groundwater supplies is a persistent issue, with 27% of wells studied found to be colonised with potentially pathogenic bacteria (*E. coli* and/or *P. aeruginosa*, Figure 1). The more simplistic analysis employed in this study (i.e. binary risk factor analyses) precludes the definite identification of *E. coli* and *P. aeruginosa* groundwater ingress mechanisms. However, as shown in reported results, this approach is still valuable in indicating potential avenues for future research as well as identifying important well construction features that should be improved to prevent microbial contamination at source, namely, presence of protective casing and wellhead elevation above chamber floor.

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