ESBL-*E. coli* in Scottish drinking water sources

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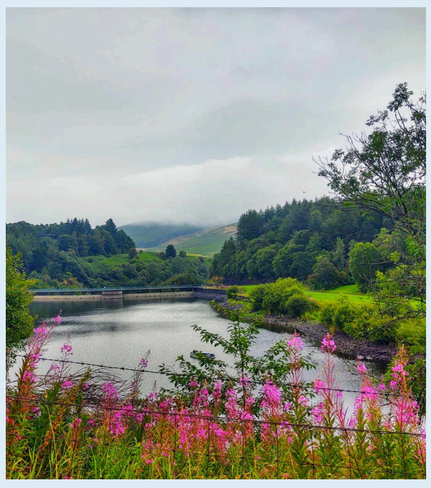
Background & Objectives

Antibiotic-resistant bacterial infections caused 1.27 million deaths in 2019 (1). Particularly extended-spectrum beta-lactamase-(ESBL)-producing *E. coli* contribute to AMR deaths, and have been classed a as WHO critical priority pathogen (2). They have been detected across all One Health compartments, including in bathing water in Scotland. However, there is no data on ESBL-*E. coli* in Scottish drinking water sources at present.

This study investigated the prevalence of ESBL-*E. coli* in drinking water sources in catchments with different pollution sources to identify risk factors as a baseline for interventions. A selection of ESBL-*E. coli* isolates were investigated using whole-genome sequencing (WGS) to determine their likely sources and their pathogenic potential, and to understand resistance mechanisms in a local context.

Methods



Loch Drunkie (drinking water source)

- Samples were collected from surface water (3 rivers, 4 reservoirs) & raw water influent from drinking water treatment works (WTW; n=23) (Fig. 1)
- Final water samples were collected from 3 treatment works post-treatment
- Surface/raw water faecal pollution risks were based on catchment land use data from Scottish Water (wildlife, livestock, agriculture, human waste)
- ESBL-*E. coli* were recovered using membrane filtration and incubation on TBX agar with 4mg/L cefotaxime (5)
- 28 ESBL-*E. coli* from 8 sites were analysed using hybrid WGS (MicrobesNG, Birmingham)
- Sequences were analysed using the Center for Genomic Epidemiology's tools and EnteroBase (6)

Results

1. Prevalence of ESBL-*E. coli*

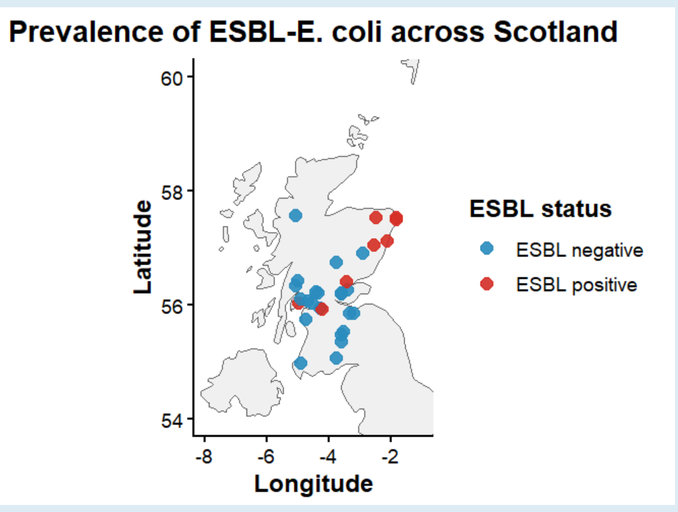


Fig. 1. Sampling sites with/without ESBL-*E. coli*. One WTW and its source were both sampled and shown as one location on figure (ESBL-positive).

- ESBL-E. coli were recovered at 8/30 sampling sites (Fig. 1)
- The proportion of *E. coli* that were putative ESBL-producers was low at 0.6%, but one site had 10.5%
- ESBL-*E. coli* presence was sig. associated with total *E. coli* levels, and with the total faecal pollution risk in the catchment
- The densities of wastewater treatment plants, combined sewer overflows, and septic tanks in the catchment were sig. associated with ESBL-*E. coli* presence
- Final water analysed on 3 occasions (from the WTWs with the highest *E. coli* levels in the raw water and positive for ESBL-*E. coli*) were negative for ESBL-*E. coli* and total *E. coli*
- 28 ESBL-*E. coli* from 8 sites, representing different phylogroups or REP types within sites (7), were selected for hybrid WGS

2. Resistance amongst ESBL-*E. coli*

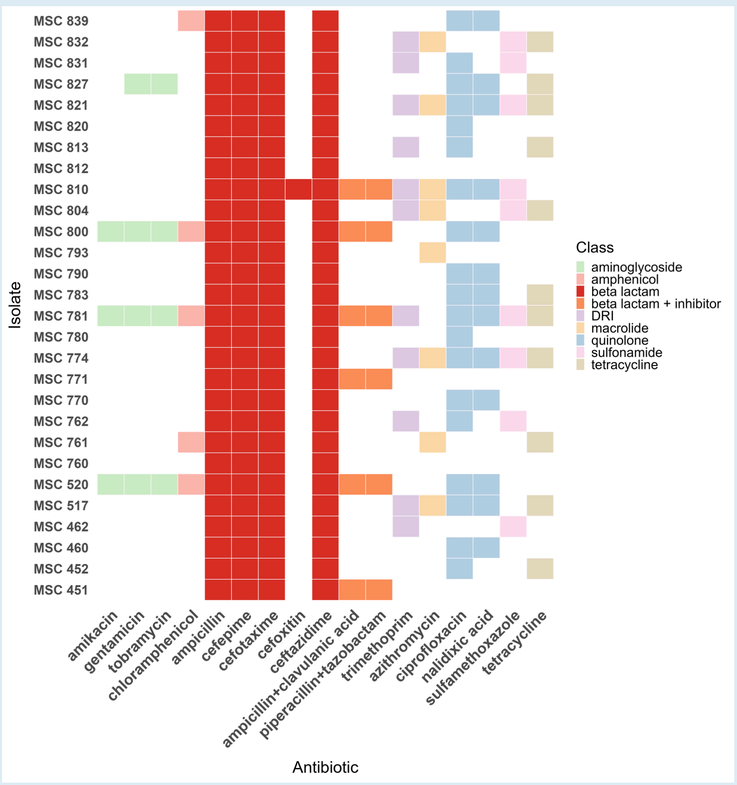


Fig. 2. Antibiotic -resistance profile of 28 ESBL-*E. coli* from Scottish drinking water sources; determined using ResFinder; DRI - dihydrofolate reductase inhibitor

- 17/28 isolates were multidrug-resistant (Fig. 2); they were isolated from 7 sites
- MSC781 was resistant to 15 antibiotics (6 classes)
- 19/28 isolates were resistant to ciprofloxacin and 11/28 to tetracycline
- ESBL genes included *bla*CTX-M-15 (n=22), *bla*CTX-M-27 (n=4), *bla*CTX-M-55 (n=1), and *bla*CTX-M-65 (n=1) (Fig. 3)
- 15/28 ESBL genes were located on the chromosome and 13/28 on plasmids
- These plasmids included phage-plasmids (IncFIB(H89-PhagePlasmid); n=2), Incl1 (n=2), IncY (n=1), IncFII (n=1), and IncFII-multireplicon plasmids (n=7)

3. Phylogeny of ESBL-*E. coli*

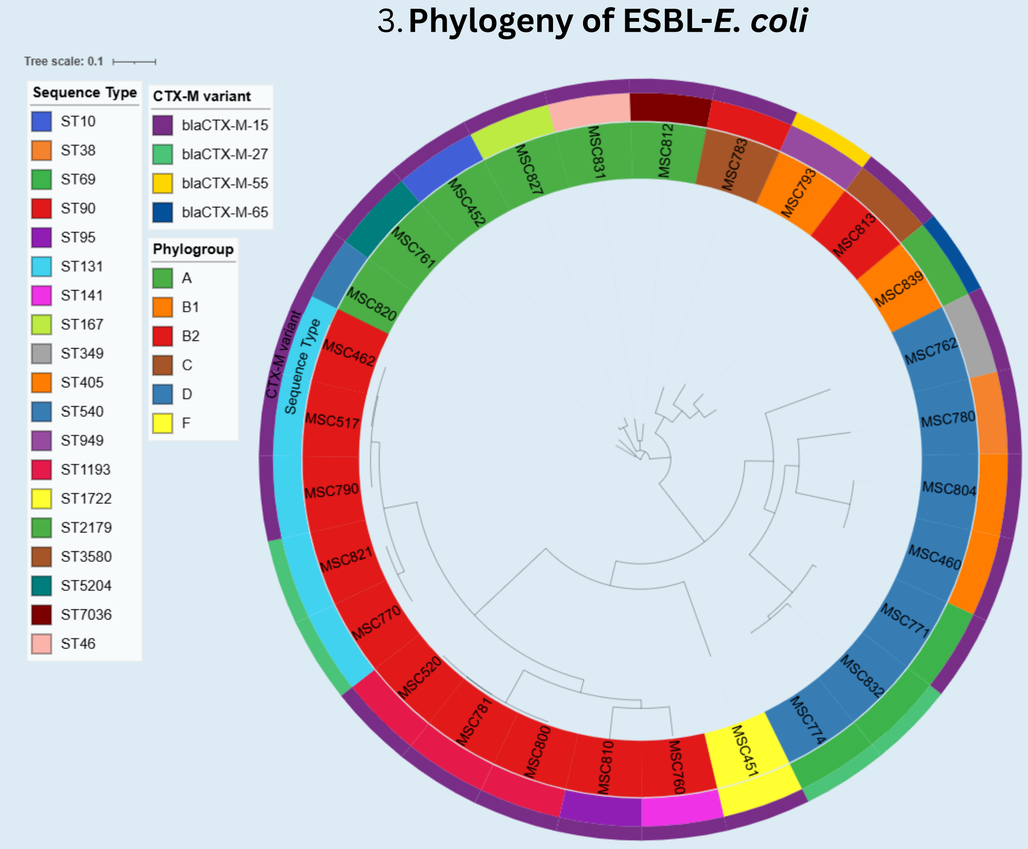


Fig. 3. Core-genome phylogenetic tree of 28 ESBL-*E. coli* from Scottish drinking water sources; phylogroups (inner ring), STs (middle ring), and ESBL-genes (outer ring) colour-coded. Fig. made in iTOL. MSC793 & MSC821 were novel STs (ST949 & ST131 closest STs in MLSTFinder)

- 11/28 isolates were phylogroup B2 and 7/28 were D (Fig. 3)
- ST131 dominated with 4/28 isolates, followed by ST1193 (n=3), ST69 (n=3), and ST405 (n=2)
- Two isolates (MSC793 & MSC821) were assigned a novel ST by EnteroBase (ST155 and ST131 clonal complex respectively)
- Clinically relevant phylogroups & ExPEC-associated STs were not restricted to specific sites (phylogroup B2 was detected at 7 sites and ST131 clonal complex at 4 sites)

Conclusions

The prevalence of ESBL-*E. coli* was low in drinking water sources and no bacteria were recovered from final waters. However, clinically relevant STs such as ST131 were detected in drinking water sources and these STs are amongst the globally leading ExPEC STs, mostly associated with UTIs and bacteraemia (3). The presence of strains with pathogenic potential and clinically-relevant AMR-profiles in drinking water sources may add to the AMR burden in a One Health context and calls for routine AMR monitoring.

While transmission of AMR pathogens to humans via treated drinking water is unlikely, bacteria or antibiotic resistance genes may be taken up via leisure activities (e.g. bathing), and further disseminated via wildlife, livestock, pets or other usage of surface waters (e.g. crop irrigation) (4).

Statistically significant associations between human wastewater sources and ESBL-*E. coli* in drinking water sources were found, highlighting the need to develop targeted intervention strategies that reduce AMR across One Health sectors.

Acknowledgements

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References