

Isolation of a single *Serratia fonticola* strain from interstage samples in a drinking water treatment plant: Genomic insights and floc-shielding effects during chlorination

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ABSTRACT

Ensuring microbial safety in drinking water remains a challenge, particularly for ageing infrastructure and complex treatment processes. Because water treatment works sit at the interface between source-water ecosystem integrity and public-health protection, failures in particle control also have broader environmental-safety implications. Here, we investigated the repeated detection of *Serratia fonticola* (a coliform) across multiple stages of a water treatment works (WTW). Sixty-seven isolates, obtained from raw, interstage, and final waters, were analysed via whole-genome sequencing. Phylogenomic and average nucleotide identity analyses (>99.94%) confirmed a single, near-clonal strain that is adapted to the WTW and is genetically distinct from raw-water isolates. Variant calling revealed minimal differences, suggesting the organism's repeated detection was not caused by multiple sources but rather a single persistence variant. The variant carried antimicrobial resistance genes, including a FONA-family β -lactamase and bla_{SFDC}, together with plasmid-encoded toxin-antitoxin systems; however, none of the isolates possessed known high-level chlorine-resistance genes. Flow cytometry-based disinfection assays demonstrated that free-floating *S. fonticola* was rapidly inactivated by modest chlorine doses, achieving a > 3-log reduction with a Ct of around 10 mg·min L⁻¹. However, floc-associated cells showed markedly higher resilience, requiring a Ct of approximately 100 mg·min L⁻¹ to achieve a ~2.5-log reduction, indicating strong physicochemical protection of the organism within flocs. These findings suggest that physical shielding, rather than intrinsic chlorine tolerance, facilitates *S. fonticola* survival through the treatment process. The invariable presence of a specific plasmid in nearly all of the WTW isolates suggests that it may confer traits enhancing resilience to chlorine and promote survival within biofilms. Optimising filtration and minimising floc carry-over, alongside strategies to remove biofilm, may therefore be key to preventing final-water detections of this opportunistic organism.

1. Introduction

Ensuring the provision of safe drinking water remains a continuing challenge for water utilities, from managing the formation of harmful disinfection by-products and emerging pollutants to tackling the difficulties posed by ageing infrastructure (World Health Organization (WHO), 2017). Drinking water supply systems in the UK, for instance, are often characterised as being founded on a relatively old asset base, including the drinking water distribution system (pipes, storage

facilities etc), and the drinking water treatment works (WTW). This can introduce additional complexities in maintaining reliable water quality and meeting regulatory standards (Drinking Water Inspectorate (DWI), (2020)). Because WTWs connect source-water ecosystem integrity to final-water quality, inadequate control of particle-associated microorganisms has implications for environmental safety as well as regulatory compliance. These networks can be vulnerable to leaks, biofilm development, and contaminant intrusion, which can undermine the ability of conventional treatment to deliver safe drinking water (Flemming et al.,

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2002; Islam et al., 2015; Speight, 2015). Consequently, controlling even low-risk opportunistic organisms remains essential for protecting public health and meeting regulatory requirements (Ding et al., 2019). One such opportunistic organism that has attracted attention is *Serratia fonticola*, a coliform increasingly detected in drinking water.

Serratia fonticola is a Gram-negative, facultatively anaerobic rod in the family Yersiniaceae (order Enterobacterales, class Gammaproteobacteria). Ubiquitously distributed in aquatic habitats (including drinking water), soil, avian faeces, animals, and plants, it often enters water supplies via animal–water contact, the decomposition of organic matter, or from proliferation within distribution-system biofilms (Gavini et al., 1979; Müller, 1986; Devi et al., 2013; Tasić et al., 2013; Dong et al., 2021; Waegenaar et al., 2023). Although *S. fonticola* rarely causes disease in healthy individuals, it can act as an opportunistic pathogen in immunocompromised patients, eliciting skin, soft tissue, and urinary tract infections (Farmer et al., 1985; Julie et al., 2009; Aljorayid et al., 2016). Environmental, rather than faecal, sources predominate in its introduction to drinking water systems (Tasić et al., 2013).

The genomes of *S. fonticola* isolates have been sequenced, and at least 16 high-quality assemblies are publicly available (Chen and Walker, 2020; Lim et al., 2015). These genomes range from 4.99 Mb to 6.34 Mb (mean = 5.80 Mb; median = 5.79 Mb), share an average GC content of 53.7%, and typically contain seven 16S rRNA gene copies (Yoon et al., 2017a). Six of these genomes have been fully assembled (GCA_001006005.1, GCA_001514455.1, GCA_002588845.1, GCA_003668775.1, GCA_000638145.1, GCA_005489985.1)—the first corresponding to type strain DSM 4576. *S. fonticola* is not on the WHO list of priority pathogens and is seldom linked to clinical outbreaks. However, its recurrent detection in finished drinking water, together with the antibiotic-resistance genes found in some isolates, justifies closer investigation of its potential for self-preservation in water treatment systems, including signs of resistance to chlorine.

An *S. fonticola* isolate has been recovered multiple times from drinking-water networks. In a Flemish drinking water distribution system, the organism colonised mature biofilms (Waegenaar et al., 2023). A strain isolated from a capped spring on Mt Vlasina shared 98% 16S-rRNA identity with the reference strain, indicating an autochthonous origin (Tasić et al., 2013). A further “*S. fonticola*-like” isolate was reclassified as the novel species *Serratia aquatilis* sp. nov. after multilocus sequence analysis and low DNA–DNA hybridisation (Kämpfer and Glaeser, 2016). Drug-resistant *S. fonticola* strains have also been detected in Polish surface reservoirs, including carbapenem-resistant variants, showing their potential to disseminate antibiotic resistance in natural and urban waters (Rybak et al., 2021). Here, we investigate repeated recovery of *S. fonticola* at several points within a UK WTW, with intermittent detections in finished water. Possible root causes for these occurrence events include release of the organism from biofilms, the potential for some degree of chlorine tolerance, and/or protection of the organism in flocs carried through the WTWs. Although dedicated chlorine-resistance genes have not yet been confirmed in *S. fonticola*, related coliforms carry loci that enhance oxidant survival (Chen et al., 2021). Operational issues such as imperfect filtration, ageing infrastructure, and limited disinfectant penetration may further aid breakthrough. Flocs and biofilms can physically shield embedded bacteria from free chlorine, diminishing disinfection efficacy and allowing *S. fonticola* to persist (Kollu and Örmeci, 2012; Lee et al., 2018). Together, these biological and engineering factors likely explain the organism’s recurrence and pose an ongoing challenge for water utilities.

Previous work has shown that particle-associated microorganisms can be substantially more difficult to disinfect than free-floating cells because the surrounding matrix both slows oxidant transport and exerts its own disinfectant demand. In drinking-water systems, protection can arise within coagulation flocs, retained solids and biofilms, where disinfectant penetration becomes a coupled reaction-diffusion problem rather than a simple bulk-water exposure process (Chen and Stewart, 1996; Kollu and Örmeci, 2012; Lee et al., 2018). The magnitude of this

protection depends on floc size, structure, organic content and hydraulic history, so failures in coagulation, floc removal or filter performance can translate directly into microbiological risk.

Several strains of *S. fonticola* carry clinically important antimicrobial-resistance genes (Saavedra et al., 2003; Stock et al., 2003; Henriques et al., 2013; Fuentes-Castillo et al., 2021). Resistance to oxidative stressors such as chlorine often relies on molecular pathways that overlap with those conferring antibiotic resistance, so chlorination can promote the environmental co-selection of both traits (Tong et al., 2021). Against this backdrop, we investigated whether chlorine tolerance explains the recurrent detection of *S. fonticola* within the WTW. Specifically, we sought to determine whether a single clonal lineage is responsible for all detections across the plant, whether that lineage carries genomic features that could confer enhanced chlorine resilience, and whether it survives chlorination more readily than its planktonic counterparts under controlled conditions. We also evaluated the extent to which entrapment within coagulation flocs affords additional physical protection during disinfection. Together, these lines of enquiry were designed to clarify the biological and operational factors that enable this opportunistic coliform to persist through the WTW.

2. Materials and methods

2.1. Site information

A surface WTW in the UK receives raw water from three separate supplies (including an aqueduct pipeline and multiple reservoirs), which are gravity fed and blended at the inlet. Coagulant (alum) and poly-electrolyte are then added under rapid mixing to promote floc formation. The flocculated water travels through clarifiers and proceeds to rapid gravity filters (RGFs) for further particulate removal. An initial chlorine dose is applied before the water passes through manganese contactors. A second chlorine dose is then administered, followed by a long residence time in covered contact tanks that provide both disinfection contact time and supply buffering. This site supplies water to a population equivalent of ~700,000, translating to an approximate flow of 105,000 m³ per day.

2.1.1. Phylogenomic analysis

A phylogenomic analysis based on a concatenated alignment of 1000 single-copy core genes was conducted to compare the 67 *S. fonticola* isolates found in this study with six publicly available reference genomes (Fig. 1). As expected, all *S. fonticola* isolates formed a well-supported clade distinct from the *S. marcescens* outgroup (bootstrap ≥ 99%). Among the *S. fonticola* group, the seven raw-water isolates (R1, R2, R3, R4, R5, R6, R7; red font) resolved into several sub-branches, indicating moderate genetic diversity. In contrast, the 60 isolates recovered from the WTW (blue font) exhibited virtually no sequence divergence, forming a single polytomy at this resolution. These results indicate that a single strain disseminates through the WTW, from clarifiers to final water, and that this lineage is genetically distinct from most raw-water isolates. Raw-water isolates R6 and R7 fell inside the treatment-plant cluster, suggesting either occasional ingress of the plant lineage into the source water or recent divergence from a common ancestor. Together, these observations underscore the persistence of a single lineage within the works while the source water harbours a more heterogeneous *S. fonticola* community.

2.2. Isolates and naming conventions

A total of 67 isolates of *S. fonticola* were obtained from all key stages of the UK WTW, with the sampling occurring between May 2023 and July 2023 (Figure S1). Bacterial isolation was performed using 0.45 µm membrane filtration, followed by plating on membrane Lactose Glucuronide Agar (mLGA), a medium commonly used to enumerate presumptive coliforms based on characteristic colony morphologies

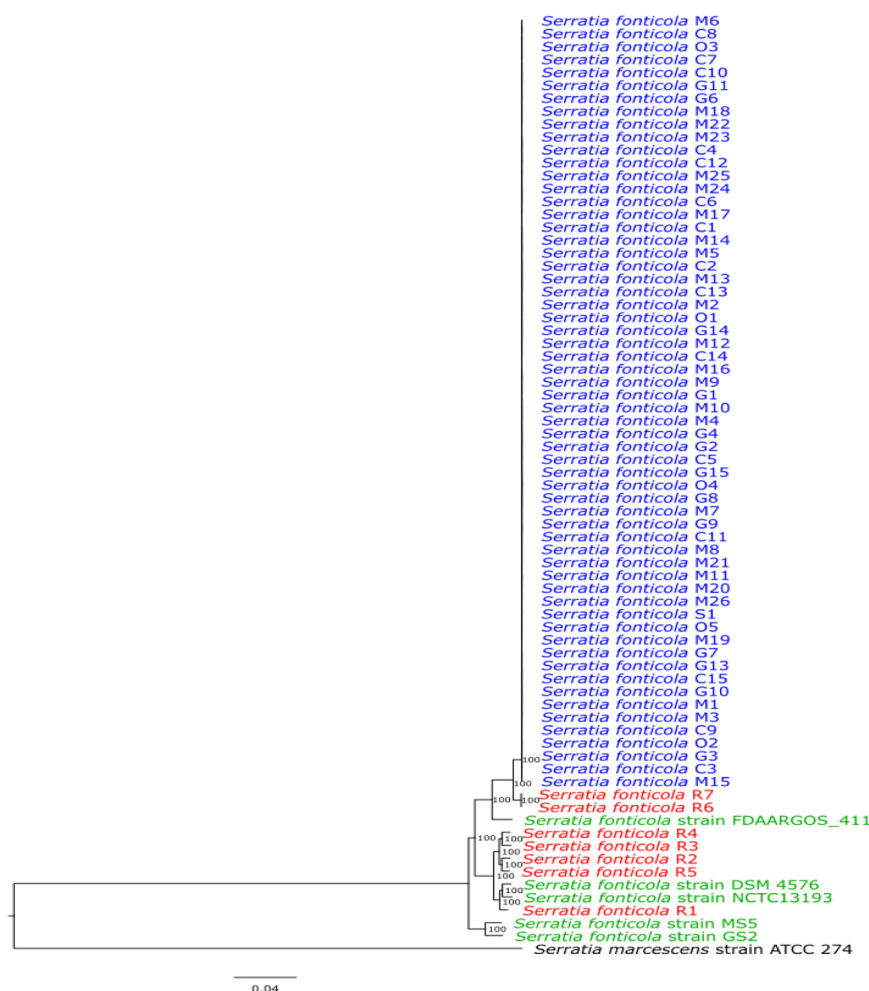


Fig. 1. Phylogenomic tree comparing *S. fonticola* isolates from a UK water treatment works (WTW; blue) and raw water sources (red), alongside reference *S. fonticola* strains (green). *S. marcescens* strain ATCC 274 was used as an outgroup. The analysis employed 1000 single-copy genes, including 361,810 amino acids and 1085,430 nucleotides per genome, to infer evolutionary relationships among the isolates.

(typically pink colonies). Plates were incubated at 30 °C for 4 h, then 37 °C for a further 14 h. Presumptive coliforms were streaked on Nutrient agar, incubated for 18–24 h at 37 °C, and identified as *S. fonticola* by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry (Bruker Microflex LT) using MBT Compass software (version 2.0). Frozen glycerol stocks and streaked agar plates were prepared for each isolate to enable long- and short-term storage. These stocks were subsequently used for whole-genome sequencing (WGS) and chlorine tolerance testing. The sample names of isolates and the corresponding treatment stage are listed in Table S1. In brief, the nomenclature is “R” for raw water, “S” for sedimentation (clarifiers), “G” for rapid gravity filters, “M” for manganese contactors, “C” for clean water/chlorine contact tanks, and “O” for the WTW outlet, with numbers assigned sequentially within each stage.

2.3. Genomic analysis

2.3.1. Whole-genome sequencing of the isolates

A total of 67 *S. fonticola* isolates from each stage of the WTW flow-sheet (Table S1) were sent for WGS to MicrobesNG (Birmingham, UK). Genomic DNA libraries were prepared using the Nextera XT Library Prep Kit (Illumina, San Diego, USA) with two key modifications: the input DNA was doubled, and the PCR elongation was extended to 45 s. DNA quantification and library preparation were automated on a Hamilton Microlab STAR system (Hamilton Bonaduz AG, Switzerland). Libraries were sequenced on an Illumina NovaSeq 6000 (250 bp paired-end

reads), yielding an average of 1036,842 reads per sample and 77.03x average coverage (Table S2). Adapter trimming was performed with Trimmomatic v0.30 (Bolger et al., 2014) using a sliding-window quality cutoff of Q15. SPAdes v3.7 (Bankevich et al., 2012) performed the de novo assembly, and Prokka v1.11 (Seemann, 2014) provided annotation. Detailed sequencing and assembly metrics are available in Table S2.

For three representative isolates (R1, R6, and O3), an enhanced sequencing strategy combined Illumina with Oxford Nanopore Technologies (ONT). The Illumina workflow was identical, while ONT libraries were prepared using the SQK-RBK114.96 kit (Oxford Nanopore Technologies, UK) with 200–400 ng of high-molecular-weight (HMW) DNA. Barcoded samples were pooled and loaded onto a FLO-MIN114 (R.10.4.1) flow cell in a GridION device (ONT, UK). Hybrid assemblies were generated with Unicycler v0.4.0 (Wick et al., 2017) using default parameters, followed by annotation with the same pipeline described above. Additional information on these assemblies is presented in Table S3, including average ONT coverage and average read length. All raw reads and assemblies have been deposited in the European Nucleotide Archive under project PRJEB102418.

2.3.2. Phylogenomic comparisons

For phylogenomic comparisons, amino acid and nucleotide sequences were extracted from conserved single-copy BV-BRC global Protein Families (PGFams) (Davis et al., 2016) to construct a comprehensive alignment and generate a phylogenetic tree. Both protein

(amino acid) and gene (nucleotide) sequences were retrieved for each chosen PGFam. MUSCLE v3.8 (Edgar, 2004) aligned the protein sequences, while the Codon align function of Biopython (Cock et al., 2009) was used for the corresponding nucleotide coding regions. The resulting concatenated alignment of all proteins and nucleotides was written to a PHYLIP-formatted file. A partitions file for RAxML (Stamatakis, 2014) specified four partitions: one for the protein alignment, and three additional partitions corresponding to the first, second, and third codon positions of the nucleotide data. Support values were computed using 100 iterations of the “Rapid” bootstrapping method (Stamatakis et al., 2008) in RAxML v8.2.11 under GTRCAT. The final analysis included 1000 genes, 361,810 amino acids, and 1085,430 nucleotides per genome. Given the large size of the concatenated alignment (>1.4 million characters), phylogenetic signal is expected to be strong and bootstrap support values typically stabilize rapidly in genome-scale phylogenomic analyses (Pattengale et al., 2010). The analysis encompassed 67 isolates from this study and 6 reference strains, including *Serratia marcescens* ATCC 274 to root the tree. All computations were performed on the BV-BRC platform (Olson et al., 2023). The resulting phylogeny was visualised in Archaeopteryx.js v2.1.1.a1.

2.3.3. Average nucleotide identity and variant calling analysis

Average nucleotide identity (ANI) was calculated for all 67 isolates using OrthoANIu v1.2 (Yoon et al., 2017b). Pairwise genomic comparisons were performed with the OrthoANI algorithm (Lee et al., 2016) to generate a matrix of ANI values for assessing intraspecies similarity using default parameters.

Variant calling was conducted using Snippy v4.6.0 (Seemann, 2015) with default parameters. The reference was the enhanced assembly of *S. fonticola* isolate O3, which was selected because it belonged to the dominant WTW lineage, was recovered at the outlet after chlorination, and sequencing yielded a complete hybrid assembly with a closed chromosome and circular plasmid. Its sequence was compared against the sequencing reads of the 59 closely related isolates identified by phylogenomic analysis and ANI. The minimum number of reads covering a site was set to 10, while the minimum proportion for variant evidence was 0.9. These stringent filters were chosen to capture fixed differences within a near-clonal haploid lineage while minimising false positives arising from sequencing error, mapping ambiguity or low-frequency within-culture variation. Summary statistics are reported in Table S4.

2.3.4. Plasmid annotation and presence in other isolates

The full-length circular plasmid from *S. fonticola* isolate O3 (167,946 bp) was additionally annotated via the PlasmidScope v1.2 web server (Li et al., 2025) using eggNOG-mapper v2 (Cantalapiedra et al., 2021). Manual inspection involved checking CDS boundaries and overlaps, resolving duplicate or truncated annotations, and grouping loci into functional categories relevant to persistence, including plasmid maintenance, adhesion/biofilm formation, secretion, and oxidative-stress response. The presence of this plasmid in each of the *S. fonticola* isolates was inspected using ncbi-blast+ version 2.12.0 +ds-3build1 (Camacho et al., 2009) with the enhanced assembly of the plasmid as query and each of the genomes of all other *S. fonticola* isolates as subjects. The summed length of all hits with > 99.9% identity was obtained for each genome and expressed both in bp and relative to the 167,946 bp plasmid length.

2.3.5. Proteome comparison

Predicted proteome comparisons were performed using the Sequence-based Comparison tool from RAST (Aziz et al., 2008), implemented via the BV-BRC platform (Olson et al., 2023). This tool calculates protein similarity using BLASTP (Boratyn et al., 2013), classifying each gene as unique, a unidirectional best hit, or a bidirectional best hit relative to the reference genome. The resulting whole-genome schematic was colour-coded by BLAST identity and visualised with Circos

(Krzywinski et al., 2009). The default parameters for BLASTP were used: a minimum coverage of 30%, a minimum identity of 10% and a BLAST E-value of 1e-5.

2.3.6. Antimicrobial resistance gene detection

AMRFinderPlus v3.12.8 (database v2024-01-31.1) (Feldgarden et al., 2021) was used to identify antimicrobial resistance genes and resistance-associated point mutations in the assembled genomes. Positive hits were initially screened at $\geq 60\%$ coverage and $\geq 70\%$ identity to capture potentially divergent environmental homologues. RESFinder v4.5.0 (Bortolaia et al., 2020) was also applied with the same parameters to cross-validate these results. Hits identified by either tool above the specified thresholds were manually reviewed, and only curated, cross-validated calls were retained in the final set of resistance determinants. These thresholds were therefore used as an inclusive first-pass screen rather than as uncurated end-point calls.

2.3.7. Detection of genes mediating chlorine resistance

A comprehensive HOCl-resistance gene set, established by Chen et al. (2021) from the ASKA library of *Escherichia coli* single-gene over-expression strains, was used to screen for potential chlorine-resistance determinants among the *S. fonticola* isolates. This set includes 114 genes that confer at least a twofold increase in NaOCl MIC (≥ 2 mM) relative to a non-insert control and an additional 23 genes that confer a threefold increase (≥ 3 mM). A custom bash script searched the annotated Prokka outputs of all 67 isolates for these strong-resistance genes. Additionally, the disinfectant database of ResFinder v4.5.0 (Bortolaia et al., 2020) was employed to detect any other HOCl-resistance genes outside the Chen et al. list, however, no hits were found using this strategy.

2.4. Chlorine tolerance testing

The chlorine tolerance of *S. fonticola* isolates was evaluated via flow cytometry for intact and total cells, adapted from Gillespie et al. (2014). Initial tests used 0.1 M phosphate-buffered saline (PBS), followed by experiments in pasteurised post-RGF water (72 °C, 30 min, then rapid cooling) to approximate operational conditions at the WTW (Pick et al., 2019). The water was filtered (0.22 µm) prior to use, reducing the indigenous microbial community by ~ 2.24 log₁₀ ($\sim 99.4\%$) for both steps. Chlorine demand was determined by dosing samples (0.1–1.4 mg/L Cl₂) and measuring free chlorine every 5 min for 25 min (DPD colourimetric method, Hach Pocket Colorimeter II). A 0.54 ± 0.06 mg/L Cl₂ reduction was noted within 25 min when initial concentrations were ≥ 0.5 mg/L Cl₂ (Figure S2). As the *S. fonticola* inactivation occurred mostly in the first minute, and matrix consumption was slower, 0.12 mg/L Cl₂ was added initially to offset this early demand. All chlorination was performed in AOC-free glassware (APHA-AWWA-WEF, 2012).

Isolate *S. fonticola* O1 was selected for chlorine tolerance and flocc-shielding trials because it belonged to the dominant WTW lineage and was recovered at the WTW outlet after chlorination, making it representative of the cells most relevant to disinfection breakthrough. *S. fonticola* O3 was tested in parallel as an independently cultured outlet isolate and because its complete hybrid assembly was used as the genomic reference. Cultures were grown in beef extract (3 g/L) and peptone (5 g/L) medium (DSMZ Medium 1) at 30 °C, refreshed 24 h before each run to ensure exponential growth. On testing days, the intact cell count (ICC) was determined by flow cytometry, fresh NaOCl (~ 200 mg/L Cl₂) was prepared, and the pH of post-RGF water adjusted. A concentrated culture (twice washed in PBS) was spiked into pasteurised post-RGF water to 1.3×10^6 cells/mL and stirred for 5 min. The target free-chlorine dose (0.20, 0.35, 0.50, 0.90, or 1.10 mg/L) was added, and 300 µL aliquots were collected each minute, immediately quenched (Na₂S₂O₃, 10 µL, 200 mM), and stained (SG+PI). After 15 min incubation at 30 °C, flow cytometry was conducted using fast setting of

66 $\mu\text{L}/\text{min}$ with 50 μL sample volumes, gating for the ICC as per Gatza et al. (2013).

2.5. Floc disinfection

To investigate whether floc breakthrough protects *S. fonticola* from disinfection, assays were adapted to assess *S. fonticola* in differently sized flocs formed following coagulation of the raw water. Water samples were collected in January 2024 from the same WTW and stored at 4 °C. To suppress the native microbiome, the raw water was twice-pasteurised (72 °C for 30 min, then ice-cooled for 30 min, repeated once, stored at 4 °C), as a single pasteurisation was insufficient to reduce the ICC to negligible levels. Filtration was omitted to preserve the natural particulate and organic matter necessary for subsequent flocculation. Pasteurization reduced the ICC by 0.98 log (from 1.85×10^5 – 1.21×10^5 events/mL) over 10 replicates, and after *S. fonticola* augmentation (1.3×10^6 cells/mL target), the native microbiome comprised ~9.2% of the total ICC.

The polyelectrolyte solution had a negligible chlorine demand (0.16 mg/L loss over 30 min), while the raw water exhibited a substantial demand, with break-point chlorination exceeding 14 mg/L Cl_2 for 30 min. For residuals of interest (0.5–10.0 mg/L Cl_2), separate ‘high’ (3–10 mg/L) and ‘low’ (0.5–3.0 mg/L) best-fit curves were generated to calculate the chlorine dose needed for a 30-min residual (Figure S3). These predicted doses were validated experimentally by measuring the chlorine residual every 2 min for 30 min.

Flocs were formed in double-pasteurised raw water using a jar tester, employing identical alum and polyelectrolyte doses as deployed at the WTW. Under rapid mixing (200 rpm, 1.5 min), pH was adjusted to 7.5 with NaOH, before adding 5.8 mg/L Al (alum) and 0.0–0.6 mg/L polyelectrolyte. To complete floc formation, the speed was reduced to 30 rpm for 15 min, followed immediately by disinfection tests (also at 30 rpm). Floc size varied by polyelectrolyte concentration and was measured following a similar protocol as Jarvis et al. (2004) using a Malvern Mastersizer 2000 and a variable-speed PB-900 jar tester with 76 × 25 mm flat paddle impellers. The median floc sizes generated under different polyelectrolyte doses are provided (Table S5).

To monitor disinfection, 10 mL samples were collected every 2 min from the reaction flasks using a wide-bore pipette to ensure a representative sample of flocs. Each sample was then transferred into a sterile centrifuge tube containing 200 μL of 1 M $\text{Na}_2\text{S}_2\text{O}_3$ to quench any remaining chlorine. Flocs were dispersed at 14 000 rpm for 30 s using an IKA T25 digital ULTRA-TURRAX (IKA, Staufen im Breisgau, Germany), under previously optimised conditions, to separate the organism from the flocculated material to allow release of the bacteria back into the water for measurement via FCM. Immediately afterward, 200 μL of SG+PI and incubated prior to flow cytometry enumeration as described above (Figure S4).

The LRVs were calculated using Eq. 1. Throughout this study, “dose” refers to the nominal free-chlorine concentration added at the start of the experiment, whereas Ct was calculated from the measured free-chlorine residual at each timepoint multiplied by the elapsed time ($\text{mg} \cdot \text{min L}^{-1}$).

$$\text{LRV} = \log_{10}(\text{baseline ICC} \div \text{ICC}) \quad (1)$$

3. Results and discussion

3.1. Comparative genomic and proteomic characterisation of isolate relatedness

A core-gene phylogeny based on 1000 single-copy genes placed all 67 isolates within a well-supported *S. fonticola* clade and showed that the 60 interstage/final-water isolates formed a tight cluster with minimal internal divergence (Fig. 1). Most raw-water isolates resolved on

separate branches, whereas R6 and R7 grouped nearest the WTW lineage, consistent with either recent divergence from a common ancestor or occasional exchange between source water and the works. Together, the phylogeny supports persistence of a single treatment-plant lineage alongside a more heterogeneous source-water *S. fonticola* community.

3.1.1. Intraspecies genetic diversity assessed via average nucleotide identity

To further resolve the genetic relationships among the 67 *S. fonticola* isolates, we calculated pair-wise ANI with OrthoANIu, which uses sliding-window BLASTn searches and corrects reciprocal-identity bias. Because ANI interrogates orthologous fragments that span most of the genome, it provides finer resolution than single-gene phylogenies. A total of 2211 pairwise comparisons were generated; the aligned sequence length per comparison ranged from 3.36 Mb to 5.87 Mb (Fig. 2). Species-level cut-offs of 95–96% ANI (Richter and Roselló-Móra, 2009; Arahal, 2014) were comfortably exceeded by all isolate comparisons (>95%), confirming their assignment to *S. fonticola*. For intraspecies delineation, we applied the genomovar boundary of $\geq 99.5\%$ ANI and the proposed strain boundary of $\geq 99.99\%$ ANI (Rodríguez-R et al., 2024).

Three distinct clusters were identified (Fig. 2a). Cluster a-1 (comparisons 1–1771) includes the 60 interstage and final-water isolates; their ANI values were all $\geq 99.94\%$ and many reached 99.999%, indicating a single clonal lineage. Cluster a-2 (comparisons 1772–1901) centres on ~98% ANI and links raw-water isolates R6 and R7 with the plant lineage while also inter-comparing R1–R5. Cluster a-3 (comparisons 1902–2211) lies at 95–96% ANI and separates R1–R5 from the treatment-plant strain. Minor tool-to-tool variation (Palmer et al., 2020) can shift absolute values by a few hundredths of a percent but does not alter these groupings. Together, the OrthoANIu data corroborate the core-gene phylogeny: a single *S. fonticola* lineage persists from clarification through chlorination to final water. This persistence underscores the need for high-resolution surveillance and process-level interventions, such as enhanced floc removal and studies of disinfectant penetration, to curtail its passage. Future work should focus on whether the reservoir resides in biofilms, in retained solids within rapid-gravity filters, or is replenished by episodic ingress from the catchment.

3.1.2. Genomic variation among WTW isolates

To complement the ANI results, Snippy v4.6.0 variant calling was performed, aligning Illumina reads from 59 treatment-plant isolates to a hybrid Illumina–Nanopore assembly of reference isolate *Serratia fonticola* O3, selected because it belonged to the dominant WTW lineage, was recovered at the outlet after chlorination, and yielded a complete hybrid assembly. The assembly comprises a 6,254,558 bp chromosome and a 167,946 bp circular plasmid. No polymorphisms were detected on the plasmid, indicating complete conservation of this replicon throughout the plant lineage and implying either recent acquisition or strong selective maintenance.

Across the 59 query genomes, 6.20–6.42 Mb (mean $\approx$ 6.32 Mb) aligned to the reference at an average depth of $88 \times$. Twenty-eight isolates were indistinguishable from O3, whereas the remainder carried one to five variants, yielding only 63 polymorphisms in total. All single-nucleotide polymorphisms mapped to eight chromosomal loci; no deletions were observed. Insertions were confined to three hotspots: a 13-bp tandem repeat at position 3 766 920 (20 isolates), an 11-bp repeat at 5 256 437 (8 isolates), and a 5-bp repeat at 6 212 344 (1 isolate). These loci fall within, or adjacent to, predicted membrane-transport and stress-response genes (Table S6), although the short indels are unlikely to confer large functional shifts.

The exceptionally low variant burden and the clustering of indels into repeat motifs confirm that the interstage and final-water isolates represent a single, near-clonal population persisting within the works. This genetic homogeneity supports the hypothesis of ongoing internal amplification of the organism, rather than repeated external seeding, underscoring the importance of locating and disrupting the in-plant

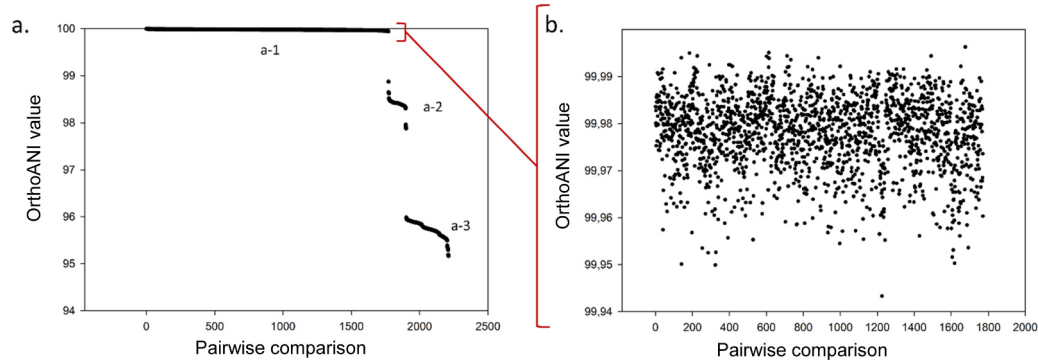


Fig. 2. Average nucleotide identity (ANI) matrix for the 67 *Serratia fonticola* genomes sequenced in this study and calculated with OrthoANIu (Yoon et al., 2017b). Panel (a) displays all 2211 pair-wise comparisons. Three clusters were evident: (a-1) comparisons among the 60 interstage and final-water isolates together with comparisons between raw-water isolates R6 and R7; (a-2) comparisons that involve R6 or R7 and the remaining raw-water isolates R1–R5 as well as comparisons within R1–R5; and (a-3) comparisons between R1–R5 and the interstage/final-water lineage. Panel (b) enlarges the dense a-1 cluster, highlighting ANI values $\geq 99.94\%$, which indicate a single near-clonal strain present through the treatment train.

reservoir.

3.1.3. Proteomic differences between raw water and treatment-stage isolates

Genomic DNA sequences from the isolates were annotated and translated into protein sequences; these predicted proteomes were then

clustered by BLASTP bit-scores and visualised in a Circos plot. In this representation, the fully assembled genome of *Serratia fonticola* isolate O3 (from the WTW outlet; track 1) serves as the reference ring for comparison against selected isolates obtained from raw water (tracks 2–6) and from successive treatment stages—rapid-gravity filter (G2), manganese contactor (M5), and clean-water/contact-tank isolates C3

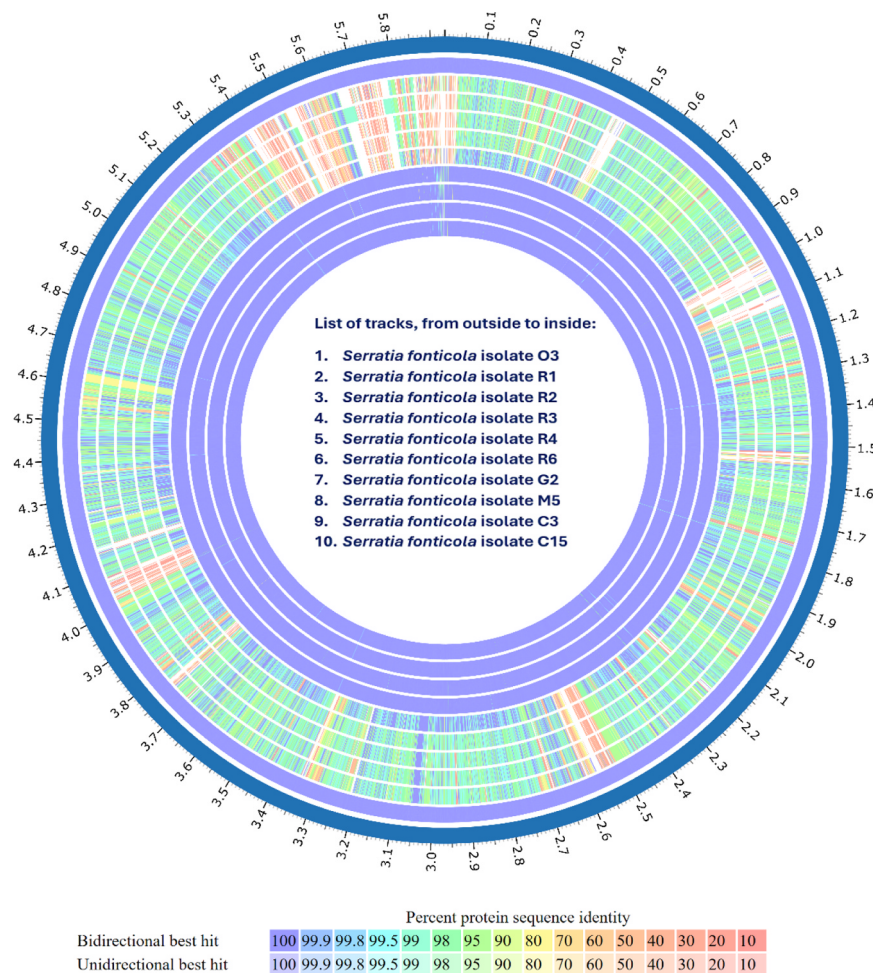


Fig. 3. Circos plot of predicted proteomes for selected *Serratia fonticola* isolates, with the fully assembled outlet strain O3 as the reference (track 1). Tracks 2–6 depict raw-water isolates (R1–R4 and R6) and tracks 7–10 correspond to rapid-gravity filter (G2), manganese contactor (M5), and clean-water/contact-tank isolates C3 and C15. Continuous coloured ribbons represent bidirectional best BLASTP hits, whereas gaps highlight proteins absent or highly diverged relative to O3, thereby visualising regions of sequence conservation and variation across the treatment train.

and C15—within the WTW (tracks 7–10).

Nearly identical predicted proteome patterns (continuous bands of reciprocal best hits) can be observed for isolates G2, M5, C3 and C15 relative to isolate O3 (Fig. 3), indicating functional equivalence across the plant lineage. In contrast, the raw-water isolates exhibit varying degrees of proteomic similarity and lack between 3% and 6% of the O3 protein complement, particularly membrane-associated transporters and secretion proteins. Of note, isolate R6 retains more than 98% of the O3 proteome, including all stress-response and envelope-biogenesis proteins, which is consistent with its higher genetic relatedness to isolate O3 compared with the other raw-water isolates analysed. These patterns suggest that while a single functional clone persists through the treatment train, a broader and more heterogeneous *S. fonticola* community exists in the source water. Proteins uniquely absent upstream but conserved in the plant lineage warrant further investigation for roles in chlorine tolerance and floc attachment.

3.2. FCM disinfection

We quantified the chlorine susceptibility of selected representative isolates across a range of operationally relevant doses. Isolate O1, which typifies all interstage and final-water isolates based on genomic relatedness, was subjected to triplicate dose-response assays, with isolate O3 serving as an independent validation strain. At both tested pH values (7.5 and 8.5), chlorine doses of ≥ 0.5 mg L⁻¹ Cl₂ effectively inactivated planktonic *Serratia fonticola* within 5 min; 90% reductions were achieved in less than 2.5 min at pH 7.5 and 3.8 min at pH 8.5 (Fig. 4). These kinetics correspond to Ct values of roughly 1–2 mg·min L⁻¹, calculated from measured free-chlorine residuals rather than the nominal applied dose, and are well below the 10 mg·min L⁻¹ benchmark often cited for primary disinfection in drinking water practice (Desiderio and Nibbering, 2010). Thus, the free-chlorine residual normally present in post-RGF water should be more than adequate for disinfection of free-living cells, even under modestly alkaline conditions.

3.2.1. Floc disinfection

Floc carry-over from RGFs can physically shield entrained microorganisms, including *S. fonticola*, from oxidant exposure and may therefore explain their survival into distribution. To test this mechanism we generated alum flocs in raw water across a gradient of synthetic polymer doses and characterised their stability with standard jar-tester shear cycles (Fig. 5a,b). Raising the velocity gradient from 30 to 200 rpm sheared the aggregates, shrinking the median diameter by 57–60%. For example, initial floc sizes of 256.4, 322.8, 455.3 and 469.1 μ m for increasing polymer dose from 0.1 to 0.6 mg/L (with 0.0, 0.2 and 0.4 mg/L used in the actual experiments below) reduced to 110.4, 138.8, 182.1 and 201.7 μ m, respectively, with the lowest floc size observed for the no-polymer control. This provided evidence that large, open structures can be easily disrupted under the turbulent conditions that might be typical as water passes through water treatment processes, including RGFs and operational backwash. This breakage provides a possible route for bacteria to be released back into the water. When gentle mixing was restored, re-flocculation recovered only 18.5% of the lost size at zero polymer. This was consistent with the limited regrowth and potential for polymer bonds to reform once flocs have been broken, as reported by Jarvis et al. (2004).

Repeating the test in raw water spiked to 10⁶ cells mL⁻¹ with *S. fonticola* produced similar mean trends (Fig. 5c,d) but the aggregates were appreciably more fragile. The standard deviation of median floc size increased by 113%, but the largest aggregates formed at 0.6 mg L⁻¹ polymer disintegrated completely under shear, so this condition was excluded from subsequent work. High bacterial biomass appears to interfere with polymer bridging and reduces floc strength, a finding consistent with surface-charge heterogeneity and steric hindrance models of flocculation.

For chlorine-inactivation experiments for bacteria incorporated into flocs, we selected four representative sizes: 100, 225, 350 and 475 μ m, which span the typical range of coagulation flocs and worst-case breakthrough from RGFs (Table S5). The 100 μ m condition represented breakage-derived flocs generated without polymer and is shown

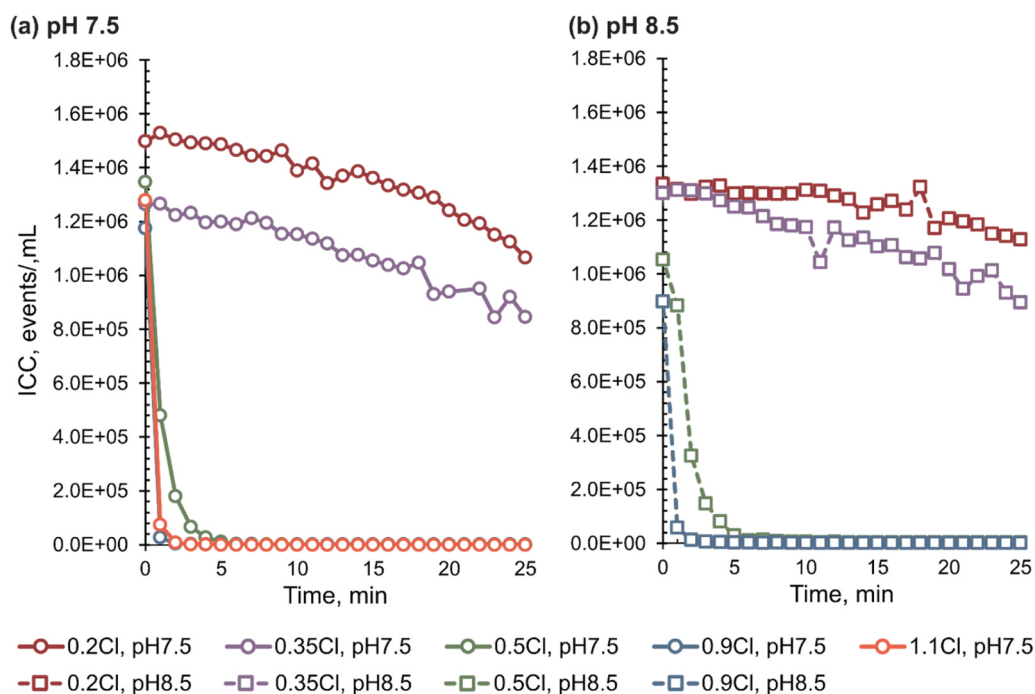


Fig. 4. Disinfection profiles of pasteurised post-RGF water spiked with *Serratia fonticola* isolate O1 (WTW; 1.3×10^6 cells/mL), at pH (a) 7.5 and (b) 8.5. Experiments were undertaken in 3 repeats and symbols represent the average of these experiments. Chlorine doses administered were 0.2 mg/L (red), 0.35 mg/L (purple), 0.5 mg/L (green), 0.9 mg/L (blue), and 1.1 mg/L (orange) free chlorine. Time represents minutes after chlorine addition. Error bars represent $\pm$ standard deviation of the average value and are smaller than the symbols.

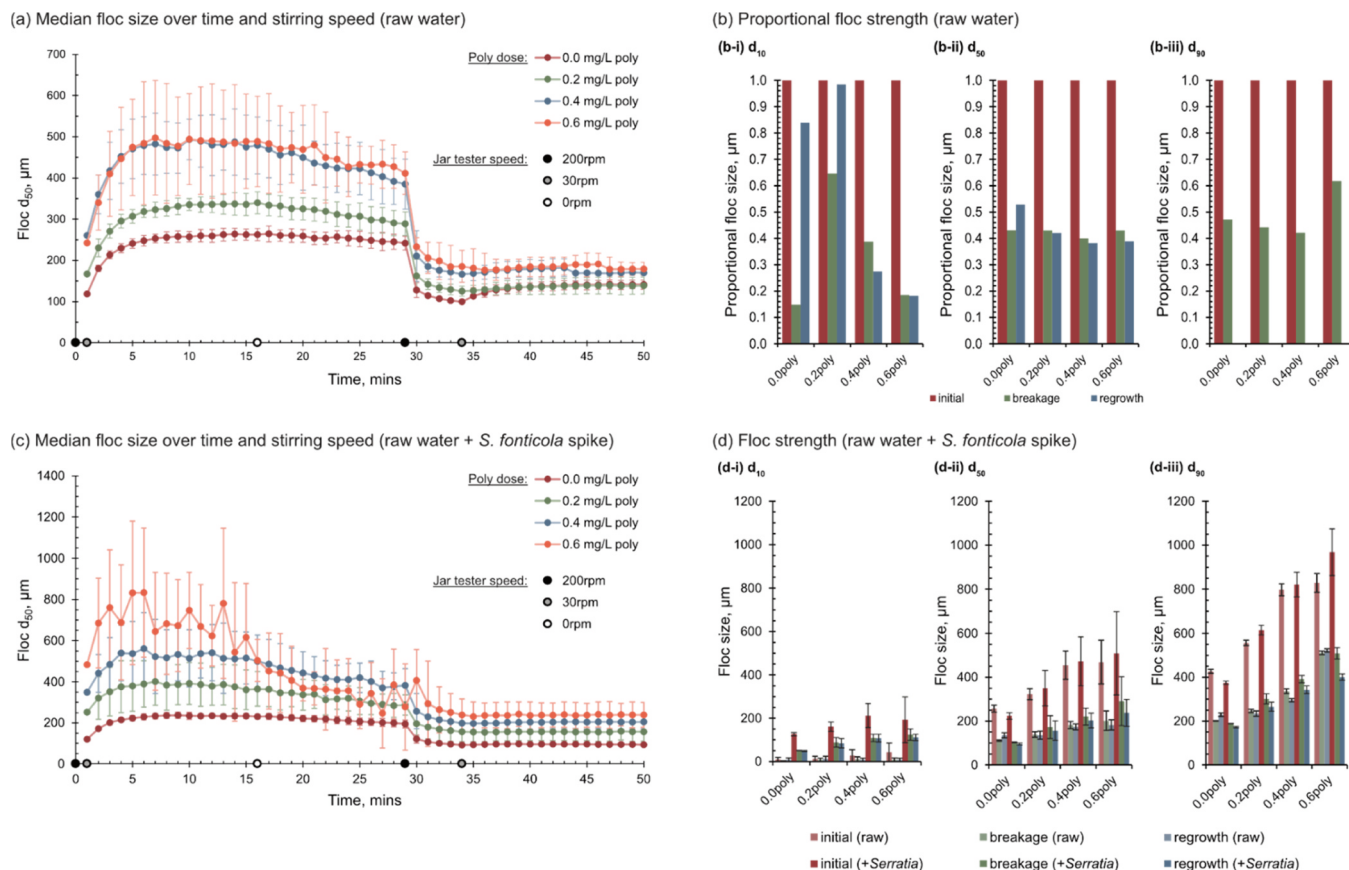


Fig. 5. (a) Floc formation and breakage in jar tests. (a,b) Median floc size (a) and floc strength (b) in raw water at different polyelectrolyte doses (0–0.6 mg L⁻¹). The jar test initially ran at 200 rpm for rapid mixing, then 30 rpm for gentle mixing. Floc strength was measured as the proportional drop in floc size under rapid stirring (breakage). (c,d) As in (a,b) but with raw water spiked with *Serratia fonticola*. Spiking *S. fonticola* slightly increased initial floc size and decreased floc strength. Error bars represent standard deviations from replicate measurements.

separately in Figure S4, whereas the main Ct comparison (Fig. 6) focuses on the three larger, more stable breakthrough-relevant classes. The bacterial load was intentionally elevated to stress the system, and although the larger size classes had broad distributions (Table S5), these floc sizes provide a conservative assessment of mass-transfer limitation because the diffusive path length for hypochlorous acid within a 475 μm floc approaches the penetration depth where internal chlorine demand can out-strip supply (Chen and Stewart, 1996). The results therefore bracket the operational envelope within which floc shielding is likely to dictate *S. fonticola* persistence.

Floc association dramatically attenuated the efficacy of chlorine disinfection. In post-RGF water, a Ct of 10 mg-min L⁻¹ reduced the planktonic intact-cell count by more than three orders of magnitude, yet the same bacterium embedded within coagulation flocs required roughly 100 mg-min L⁻¹ to achieve even a 2.4–2.7-log decline (Fig. 6). A ten-fold increase in exposure for comparable disinfection demonstrates that shielding arises from coupled transport and consumption of chlorine within the particulate matrix rather than from intrinsic resistance alone. Across the tested floc classes, including the 100 μm supplementary condition (Figure S4), Ct-LRV relationships overlapped substantially and no clear monotonic size effect was resolved within experimental variability. Because the larger floc bins had broad size distributions (Table S5), subtle size-dependent effects may have been masked; nevertheless, the data indicate a reaction-diffusion-limited shielding effect rather than a purely diffusion-limited response. Because the 10 mg-min L⁻¹ benchmark already exceeds many operational targets for secondary disinfection, the observed shielding highlights the practical necessity of minimising floc carry-over and suppressing biofilm regrowth in contact tanks (Lee et al., 2018).

3.3. Genes

3.3.1. Detection of the *HOCl*-defence gene repertoire

The cellular response to HOCl has not been characterised as thoroughly as that of other bactericidal agents (e.g., antibiotics), but several protective pathways are well established. Detoxifying enzymes such as catalases, peroxidases and superoxide dismutases are rapidly up-regulated upon HOCl exposure (Small et al., 2007; Wang et al., 2009; Bodet et al., 2012). Concurrently, DNA- and protein-repair systems, including Fe-S cluster reconstitution and methionine-sulfoxide reductases, are induced to reverse oxidative damage (Small et al., 2007; Wang et al., 2009; Melnyk et al., 2015). Cells also boost chaperone production (Groitl et al., 2017; Goemans and Collet, 2019) and remodel their envelopes, reducing porin density and increasing surface hydrophobicity, thereby restricting HOCl ingress (Morales et al., 2012; Salazar et al., 2013). Additional defences, such as holdase-type chaperones, elevated quorum sensing activity and an increased tendency to form biofilms, have likewise been documented under HOCl stress (Nizer et al., 2020).

To assess whether the 67 *Serratia fonticola* isolates possess analogous defence genes, we screened their genomes for loci previously linked to HOCl tolerance in related bacteria, notably *Escherichia coli*. Figure S5 displays the presence-absence matrix for these high-confidence resistance determinants. The interstage and final-water isolates, which form a single clonal lineage by phylogenomic criteria, share an identical HOCl-resistance gene complement, indicating that any enhanced chlorine resilience in the treatment plant is unlikely to stem from differential gene content within this group.

Collectively, the genomes encode a suite of HOCl-associated defence

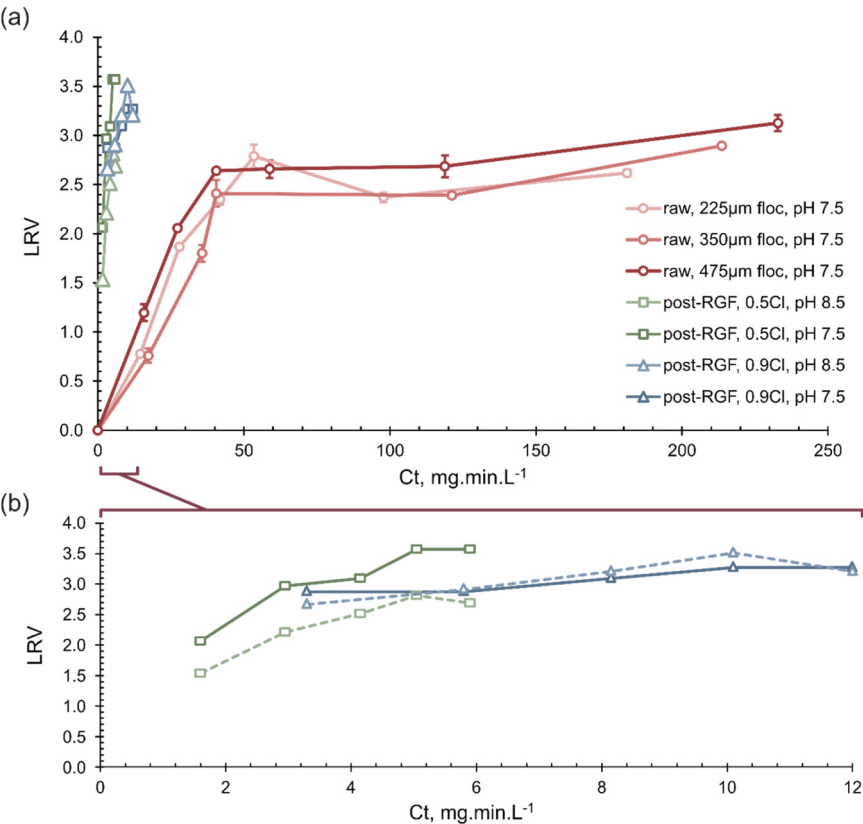


Fig. 6. Disinfection kinetics of *S. fonticola* in floc-bound vs. free-floating states. (a) Full Ct range and (b) magnified view of lower Ct values. Red curves indicate floc-bound *S. fonticola* in raw water (pH 7.5) for the larger breakthrough-relevant floc classes (225–475 µm), while green and blue curves show free-floating cells in post-RGF water (pH 7.5 or 8.5). Ct values were calculated from measured free-chlorine residuals at each sampling time rather than from the nominal applied dose; the separate 100 µm floc condition is shown in Fig. S4. Error bars represent standard deviations from triplicate disinfection experiments. Higher Ct values were required to achieve comparable log reductions in the floc-bound cells, highlighting the protective effect of flocs.

genes, but these loci are neither more numerous nor unique in the WTW lineage compared with raw water isolates. This suggests that gene content alone is unlikely to explain the organism’s persistence through chlorination and that any advantage will depend on how strongly these pathways are induced under HOCl exposure. Functional assays (e.g. RNAseq, proteomics or enzyme activity measurements under chlorine challenge) will therefore be required to determine whether HOCl-defence genes confer a measurable fitness benefit in the treatment plant.

A more plausible scenario that warrants further investigation is that heightened resistance arises from transcriptional up-regulation rather than differential gene carriage. RNA-seq analysis that compares chlorine-challenged cells with unchallenged controls would clarify whether the plant lineage overexpresses HOCl-defence genes. Parallel proteomic or enzyme-activity assays could confirm that up-regulation translates into greater detoxification capacity. Additional mechanisms

also warrant attention. *S. fonticola* readily forms or colonises biofilms, and HOCl itself can trigger biofilm development. Within a biofilm matrix, cells experience lower oxidant fluxes, altered redox potentials and enhanced collective defences, all of which increase survival compared with free-floating cells. Targeted experiments that quantify biofilm formation and measure chlorine penetration into these structures would determine whether biofilm shielding underlies the repeated detection of a single lineage in the WTW.

3.3.2. Antibiotic resistance gene analysis of *S. fonticola* isolates

To characterise the antibiotic-resistance repertoire of the collection, we screened each assembly with AMRFinderPlus and cross-validated the results with RESFinder (Table 1). AMRFinderPlus detected five core resistance loci present in all 67 genomes: glutaredoxin-dependent arsenate reductase (*arsC*), acid-resistance repetitive basic protein (*asr*),

Table 1
Antimicrobial resistance genes identified in each isolate via AMRFinderPlus. Identity % refers to the gene-sequence match percentage. FONA-family allele labels differed between AMRFinderPlus and RESFinder; tool-specific AMRFinderPlus calls are shown here, while all genomes were conservatively interpreted as carrying a FONA-family β-lactamase.

Isolate code	Identity %		<i>bla</i> FONA	<i>bla</i> _S FDC	<i>fie</i> F	<i>fos</i>	<i>ter</i> D	<i>bla</i> FONA-1	<i>bla</i> FONA-10
	<i>ars</i> C	<i>asr</i>							
R1	84.78	65.05	-	97.38	82.94	62.77	93.23	100	-
R2	83.33	66.99	-	97.38	82.94	-	93.23	-	100
R3	82.61	55.28	-	97.91	82.94	61.76	93.23	-	100
R4	84.06	66.02	-	98.17	82.94	62.04	93.23	-	100
R5	83.33	66.99	-	96.86	82.94	-	93.23	-	100
R6	84.78	66.99	99.66	96.07	82.61	62.04	93.75	-	-
R7	84.78	66.99	99.66	96.07	82.61	62.04	93.75	-	-
Interstage / Final	84.06	66.99	99.66	96.86	82.61	62.77	93.75	-	-

an SFDC-family class C β -lactamase (blaSFDC), a CDF-family cation-efflux transporter (fieF) and a tellurium-resistance membrane protein (terD). Every isolate also carried a FONA-family class A β -lactamase, although allele-level assignments differed between databases: AMR-FinderPlus resolved blaFONA, blaFONA-1 or blaFONA-10, whereas RESFinder labelled the corresponding conserved determinant blaFONA-6. We therefore refer conservatively to this locus as a FONA-family β -lactamase unless tool-specific allele calls are explicitly discussed. In addition, the two raw-water isolates R6 and R7 encoded a CMY-type AmpC β -lactamase (blaCMY-144), highlighting a modest but notable expansion of β -lactamase diversity in the source-water fraction.

Previous studies have documented antimicrobial-resistance determinants in *S. fonticola*, including the FONA-family class A β -lactamases found here. Chromosomal extended-spectrum β -lactamases (ESBLs) of the FONA lineage have been reported, and a novel allele, FONA-7, was recently described (Fuentes-Castillo et al., 2021; Tanimoto et al., 2021). Likewise, SFDC-family β -lactamases have been detected in other *S. fonticola* strains; for example, the AmpC enzyme SFDC-1 was recovered from a rabbit isolate (Dong et al., 2021). In contrast, the additional loci identified here, including: *terD*, *fieF* and *arsC*, confer resistance to metalloids such as tellurium, heavy metals including zinc, and arsenic, respectively. These genes primarily promote resistance to antibiotics and toxic metals, rather than to chlorine. Although some mechanisms can offer cross-protection against multiple stressors, none of the loci detected are linked to the efflux, membrane-modification or oxidant-detoxification pathways that underpin bacterial chlorine tolerance. We therefore cannot ascribe the persistence of *S. fonticola* to these genes without further evidence.

Nonetheless, the presence of multiple antibiotic-resistance genes is of concern because it implies that *S. fonticola* could be more likely to withstand antimicrobial therapy and act as a resistance reservoir in aquatic environments. Horizontal gene transfer between environmental and clinical bacteria could amplify this threat, potentially undermining both water treatment efficacy and public-health safeguards. Chlorine's broad, non-specific mode of action means that single genes rarely confer high-level resistance; however, a multigene resistance background can still reduce susceptibility and facilitate survival under sub-optimal disinfection.

Waterborne *S. fonticola* isolates have previously carried ESBLs that inactivate third-generation cephalosporins and genes that bestow resistance to fluoroquinolones, aminoglycosides and sulfonamides (Rybak et al., 2021). One environmental strain, UTAD54, even harboured the B2 metallo- β -lactamase Sfh-1, which grants carbapenem resistance (Saavedra et al., 2003). The resistance profile observed in our isolates is therefore consistent with the broader environmental literature and shows the need for continual surveillance of potential pathogens in drinking water systems. The absence of additional resistance genes in interstage samples suggests that current treatment steps are not actively selecting for further antibiotic-resistance acquisition, yet phenotypic assays will be required to confirm the clinical significance of the genotypes detected.

3.4. Plasmid-encoded determinants potentially influencing *S. fonticola* persistence

Analysis of the plasmid sequence from the *S. fonticola* O3 isolate revealed multiple genes whose functions suggest a role in stress tolerance or virulence. Type II toxin-antitoxin modules (e.g., *mazEF*, *parDE*) may stabilize plasmid inheritance and modulate cell viability under unfavourable conditions, including transient growth arrest that can increase oxidative-stress survival, while fimbrial and pilus assembly proteins could enhance biofilm formation and promote adhesion, potentially limiting the efficacy of chlorine disinfection. A complete Rcs phosphorelay system (sensor histidine kinase plus the response regulator *rcsB*) is present and is predicted to coordinate envelope-stress responses, exopolysaccharide synthesis and mature biofilm architecture.

Additional loci, such as an ABC transporter and a glutathione S-transferase, could assist in nutrient acquisition as well as detoxification of oxidative agents. Several genes, notably pore-forming hemolytic toxins, also point to possible virulence traits. Taken together, these plasmid-encoded features provide a mechanistic rationale for the enhanced persistence of the O3 lineage in chlorinated process water (Table S8). Future investigations should confirm these roles by pairing RNA-seq or qRT-PCR under chlorine challenge with targeted gene knockouts to resolve each locus's contribution to survival, biofilm formation and virulence.

3.5. Significance of interstage-restricted carriage of the plasmid

High-stringency BLAST mapping revealed that the 167,946 bp plasmid from *S. fonticola* isolate O3 is essentially absent from all seven raw-water genomes R1–R7 (0–17 kb aligned at $\geq 99.9\%$ identity) but recovered at near-full length in 59 of the 60 interstage/final-water genomes (168–179 kb equivalent aligned length). One manganese-contactor isolate (M6) showed a partial match of 73 kb (43.5% of the plasmid length), suggesting either partial carriage, plasmid rearrangement or assembly fragmentation (Table S7). This sharp bimodality indicates that the plasmid is acquired within the works and then vertically maintained, rather than being continually imported from the catchment. Its presence therefore acts as a molecular tag distinguishing the plant-adapted lineage that repeatedly reaches the chlorine-contact tanks and, on occasion, final water. As noted above, the plasmid carries several genes that could improve survival in flocs, biofilms and low-oxidant niches, even though canonical high-level HOCl-resistance genes are absent. These loci include adhesion factors, toxin-antitoxin modules and oxidative-stress enzymes, together forming a “toolkit” that may offset the energetic burden of plasmid carriage by enhancing persistence under sub-lethal chlorine exposure. Consequently, evidence points towards the bacterial detections in final water tracing back to the interstage reservoir of organisms rather than from raw-water ingress. Mitigation efforts should therefore focus on removal of the bacterial pool from the WTW, ensuring chlorine contact systems are designed appropriately, and minimising floc breakthrough, as free-living bacteria were easily disinfected. Pilot-scale trials that compare plasmid-bearing with plasmid-cured derivatives under realistic hydraulic and disinfectant conditions would clarify the plasmid's true contribution to in-plant survival.

4. Conclusion

Our results show that the *S. fonticola* repeatedly detected across various stages of a WTW can be traced to a single, near-clonal strain that propagates from clarification through to the final water outlet. Comparative genomic analyses confirmed that while the organism contains multiple antibiotic-resistance determinants and plasmid-encoded features, such as toxin-antitoxin systems, fimbrial assembly proteins and regulatory elements, we found limited evidence of chromosomal or plasmid loci previously linked to high-level chlorine tolerance. Instead, flow cytometry-based disinfection experiments indicate that physical shielding within flocs and biofilms, rather than intrinsic chemical resistance, is the dominant factor that allows *S. fonticola* to survive or re-emerge after treatment. Nevertheless, the fact that all 60 isolates from the WTW maintain the same specific plasmid indicates that the energetic cost of keeping it is outweighed by the benefits it provides, suggesting that plasmid-encoded adhesion and stress-response functions may indirectly enhance survival in chlorinated water.

These findings show the importance of optimising particle removal and limiting biofilm growth rather than merely increasing chlorine dosage, which may offer limited benefit if bacterial cells remain physically protected. Active monitoring and maintenance of rapid-gravity filters, along with improved coagulant management and operational vigilance, could significantly reduce the risk of *S. fonticola* and other

opportunistic organisms evading final disinfection. Overall, the study highlights that engineering controls and microbial ecology are both decisive in determining the effectiveness of drinking-water treatment.

CRediT authorship contribution statement

Luke Montgomery: Writing – review & editing, Project administration, Methodology. **Victor Castro Gutierrez:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Raoul Gave:** Writing – review & editing, Validation. **Benjamin Exton:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Peter Jarvis:** Writing – review & editing, Validation, Methodology. **Francis Hassard:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Jenny Banks:** Writing – review & editing, Project administration, Data curation. **Neil Whitehead:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data access statement

Raw reads and assemblies are available in the European Nucleotide Archive under project PRJEB102418. Additional operational/site data are available from the corresponding author on reasonable request, subject to a client confidentiality agreement

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2026.120147](https://doi.org/10.1016/j.ecoenv.2026.120147).

Data availability

The data that has been used is confidential.

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