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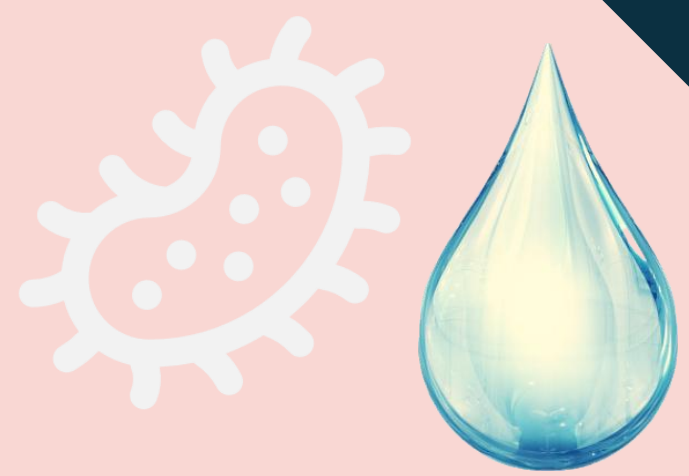
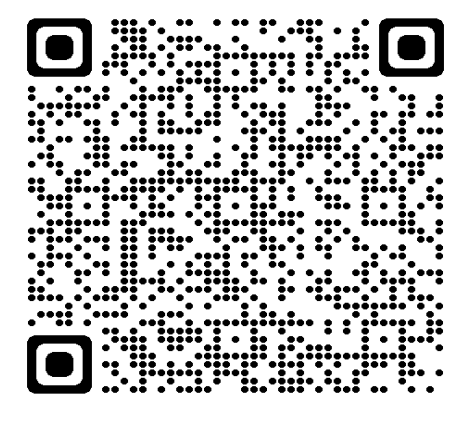
# Occurrence and persistence of multi-drug resistant Enterobacterales isolated from urban, industrial and surface water in Monastir, Tunisia

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## Introduction

- Antimicrobial resistance (AMR) is among the top ten threats to global health (1).
- The One Health approach of antimicrobial resistance highlighted the role of the aquatic environment as a reservoir and a major vector for the spread of resistance genes (ARG) and resistant bacteria (ARB), especially due to anthropogenic activities (Fig 1),
- Resistance to extended-spectrum cephalosporins (ESC) conferred by extended-spectrum beta-lactamases (ESBLs) in *E. coli* has been proposed as the marker of the AMR burden in cross-sectoral approaches (2).

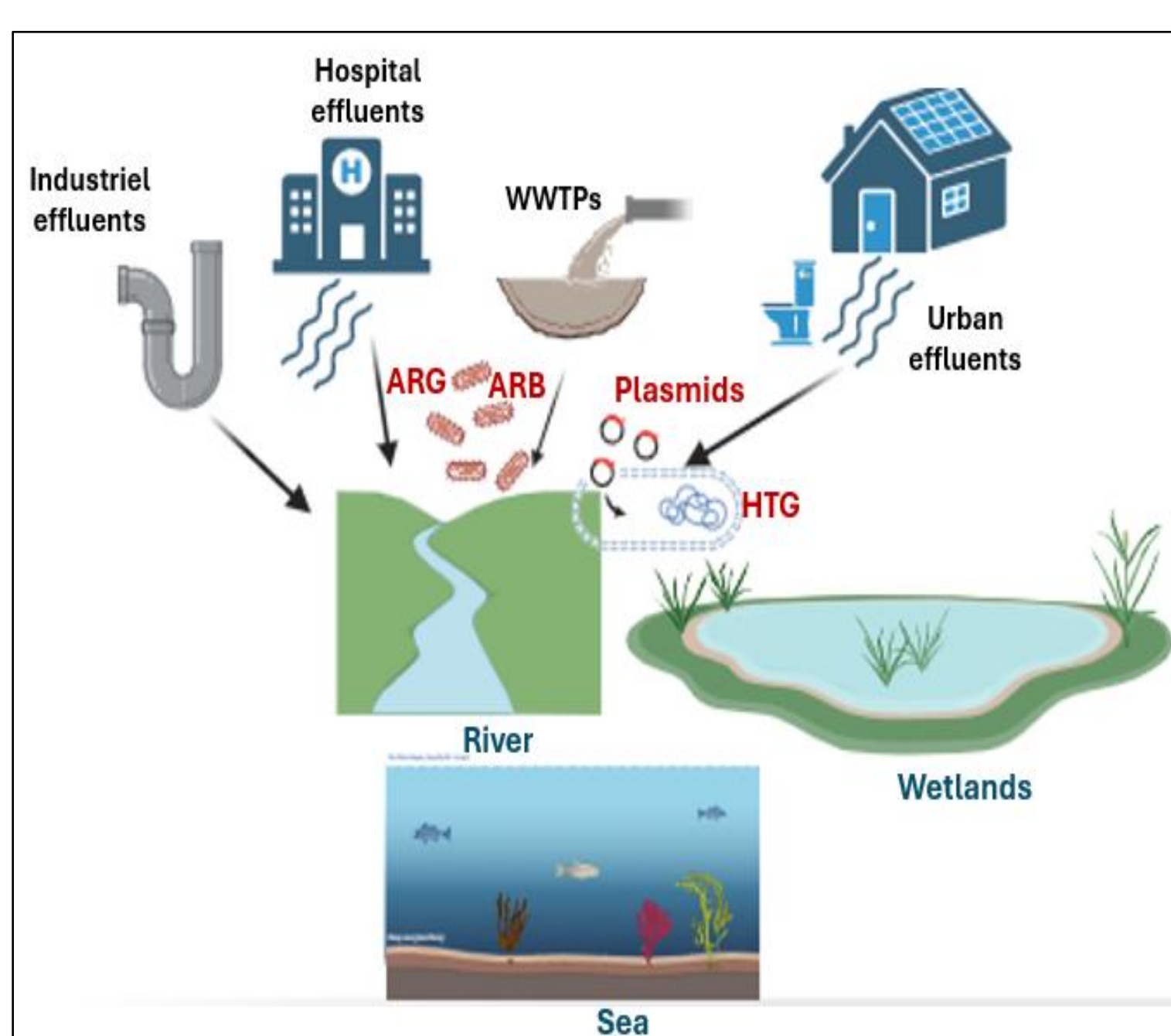


Fig 1. Dissemination of ARGs and ARBs in the aquatic environment, mainly through horizontal transfer genes (HTG), due to various anthropogenic activities. (created by Biorender.com)

## Aim of the study

Our aim was to evaluate the dissemination of ESC- and CP-R Enterobacterales in wastewater, surface water and seawater that are subjected to official water quality monitoring in Monastir, Tunisia, and to characterize the collected isolates using phenotypic, genotypic and genomic methods.

## Methodology

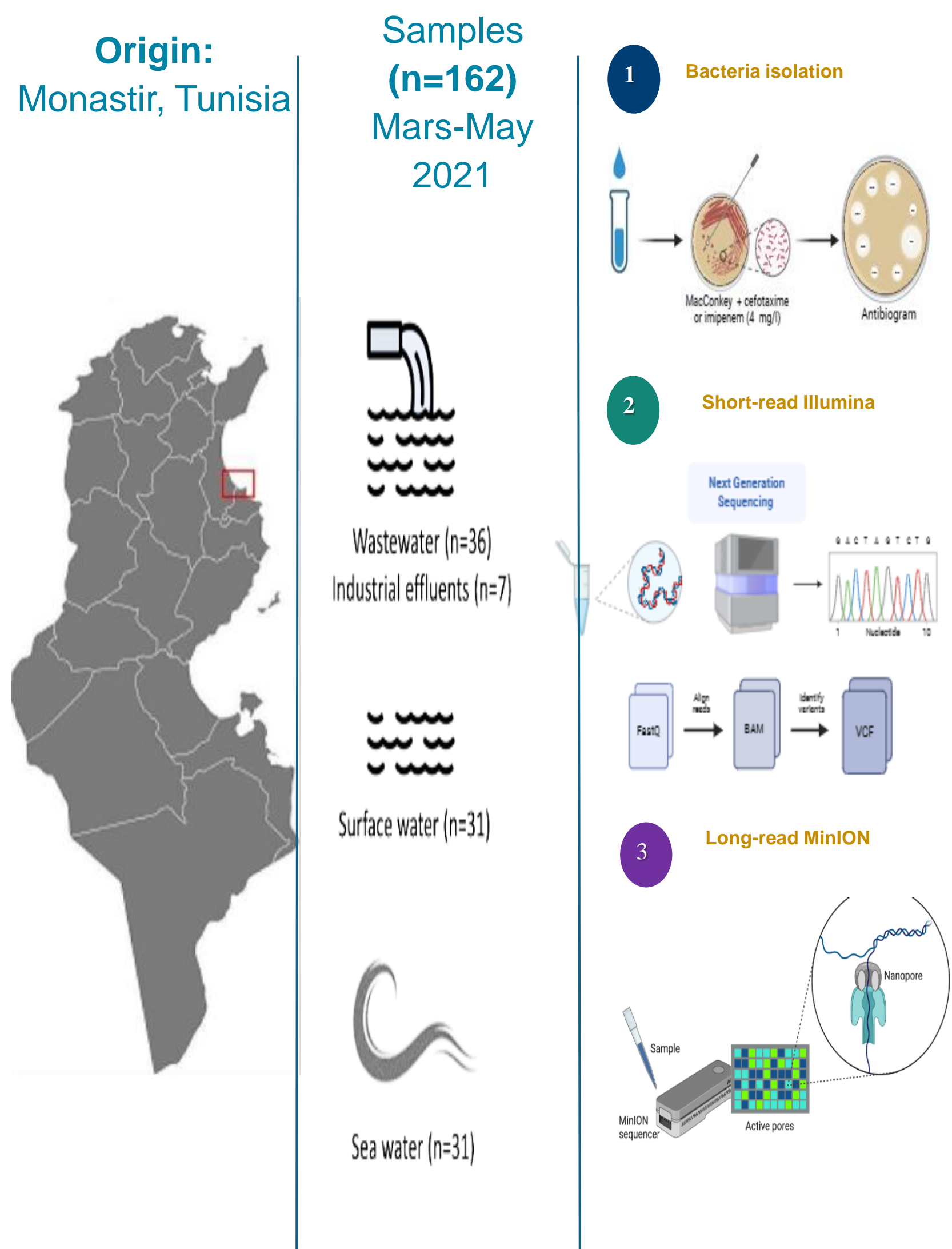


Fig 2. Origin, type of water samples and the applied methodology in this study

## Results

### 1 Genetic diversity of ESC-R Enterobacterales in water

- 31 water samples (19.1 %, 31/162) were contaminated with ESC-R Enterobacterales:
  - ✓ WWTPs (25/36, 69.4 %)
  - ✓ industrial effluents (21/43, 48.8 %)
  - ✓ surface water (10/31, 32.3 %)
- No ESC-R Enterobacterales was isolated from the sea water samples.
- The 39 resistant isolates were identified as:
  - ✓ 27 *Escherichia coli* (27/39, 69.3 %)
  - ✓ 10 *Klebsiella pneumoniae* (25.6 %)
  - ✓ 2 *Citrobacter freundii* (5.1 %)
- High genetic diversity, with 13 STs over *E. coli* and 6 STs over *K. pneumoniae* isolates, including the high pandemic clone *E. coli* ST131-fimH30
- The most prevalent gene was *bla*<sub>CTX-M-15</sub>, presented in 22 isolates (56.4 %, identified in 10 *E. coli*, 10 *K. pneumoniae* and the two *C. freundii*).
- *bla*<sub>CTX-M-55</sub> (n = 8), *bla*<sub>CTX-M-3</sub> (n = 7) and *bla*<sub>CTX-M-1</sub> (n = 2) were detected only in *E. coli* isolates

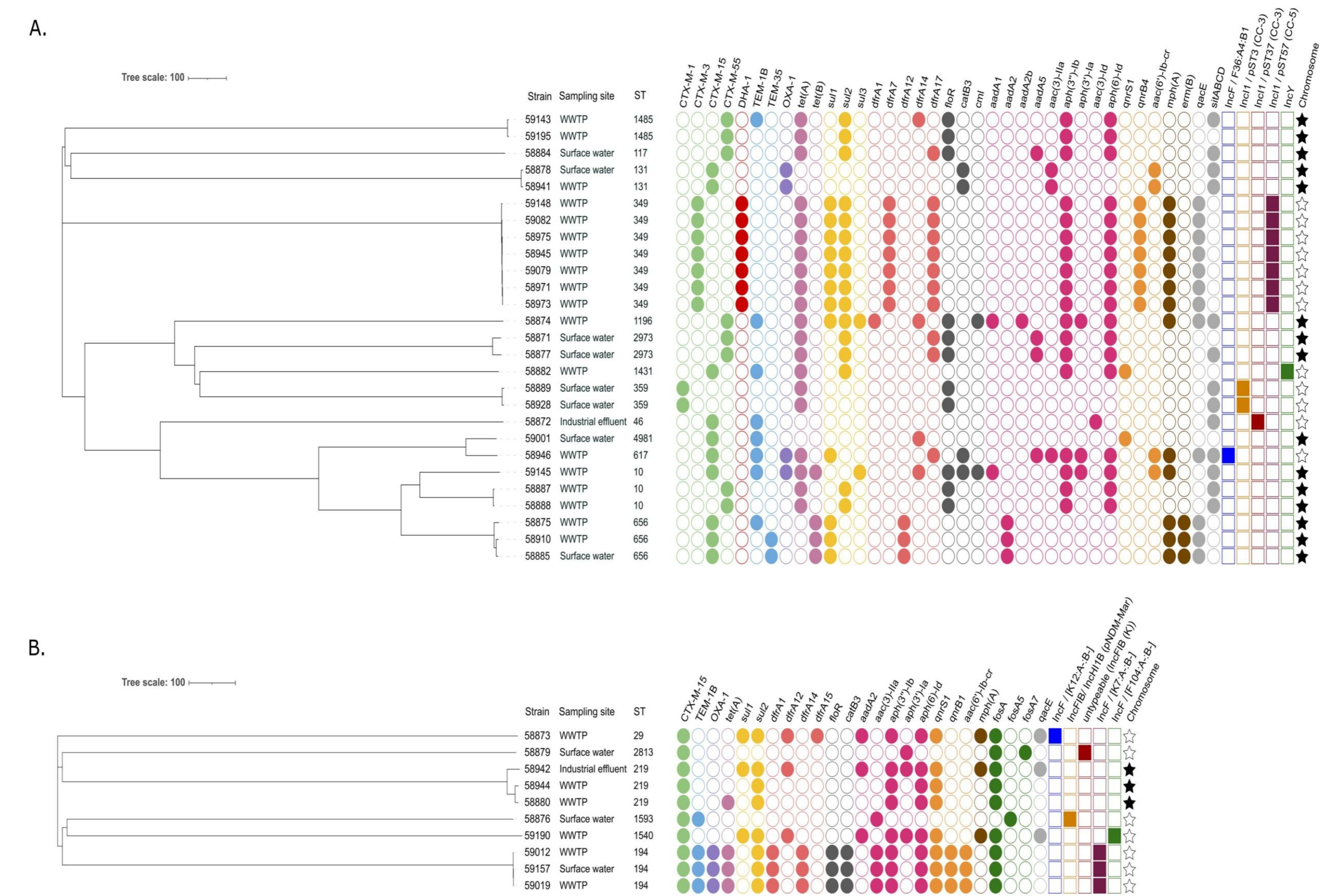


Fig 3. cgMLST-based phylogeny and molecular characterization of *E. coli* (A) and *K. pneumoniae* (B) isolates

### 2 Chromosomal carriage of bla<sub>CTX-M</sub> genes

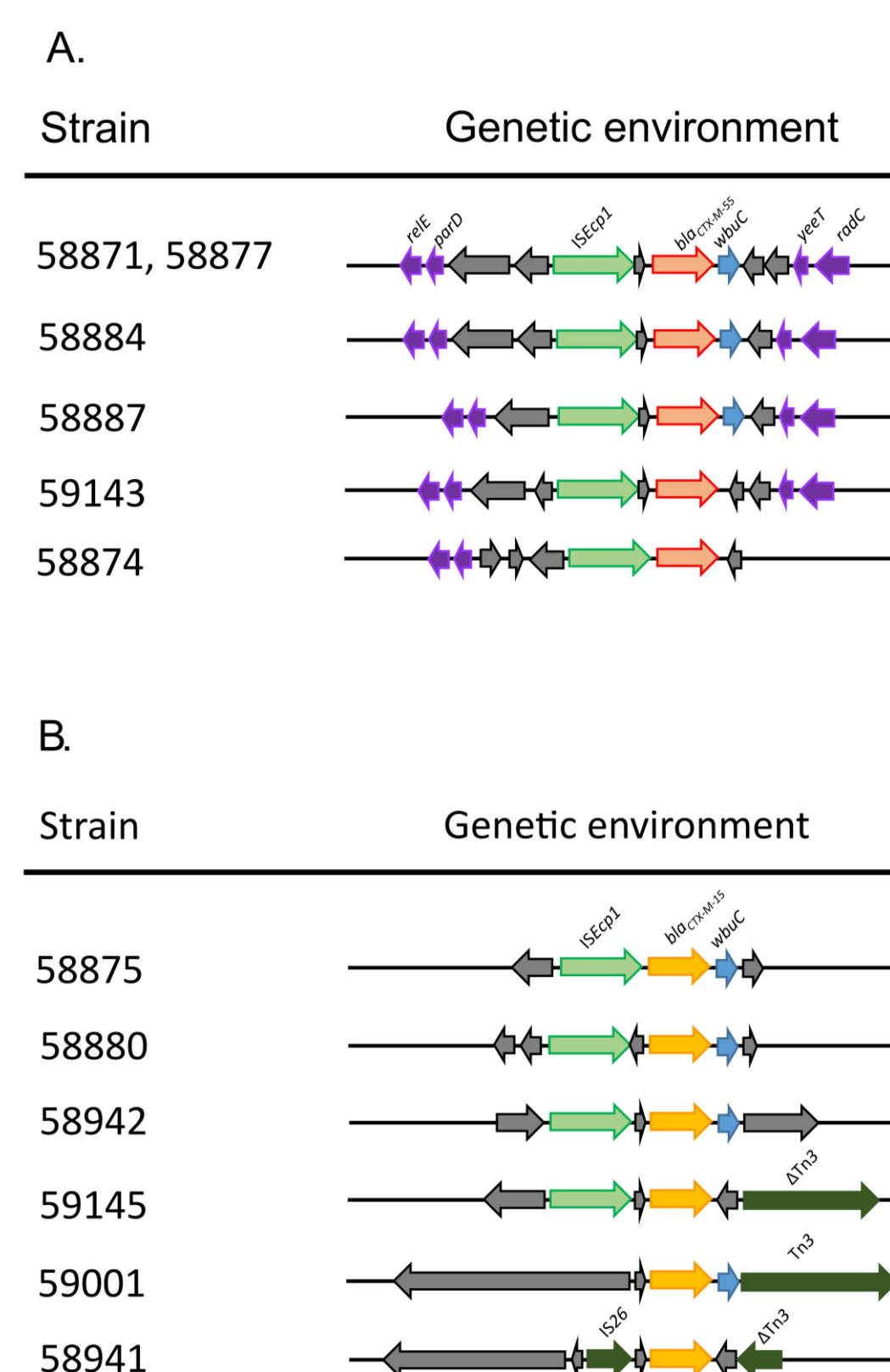


Fig 4. Schematic representation of the chromosomal genetic environment of *bla*<sub>CTX-M-55</sub> (A) and *bla*<sub>CTX-M-15</sub> (B) genes.

- All *bla*<sub>CTX-M-55</sub> genes in *E. coli* isolates chromosomally-located, most of them preceded by the ISEcp1 element and followed by the wbuC gene (Fig. 4).
- Among the 11 chromosomally-encoded *bla*<sub>CTX-M-15</sub> genes, the typical ISEcp1-*bla*<sub>CTX-M-15</sub>-orf477 was identified in seven isolate (Fig. 4).

## Conclusion

- Our study proved the occurrence and persistence of ESBL-producing Enterobacterales in urban and industrial wastewater as well as surface water in Monastir.
- No typical human-associated pathogens originating from hospitals could be identified, apart from the widely spread ST131.
- No detection of carbapenem resistance isolates
- All but one sample were considered as clean by the official quality monitoring that are not designed to detect AMR, this underlines the importance of including total coliform counts and ESBL counts, determined by bacterial growth on selective plates, in the major improvement of official water quality monitoring in Tunisia.

### 3 Plasmid carriage of bla<sub>CTX-M</sub> genes

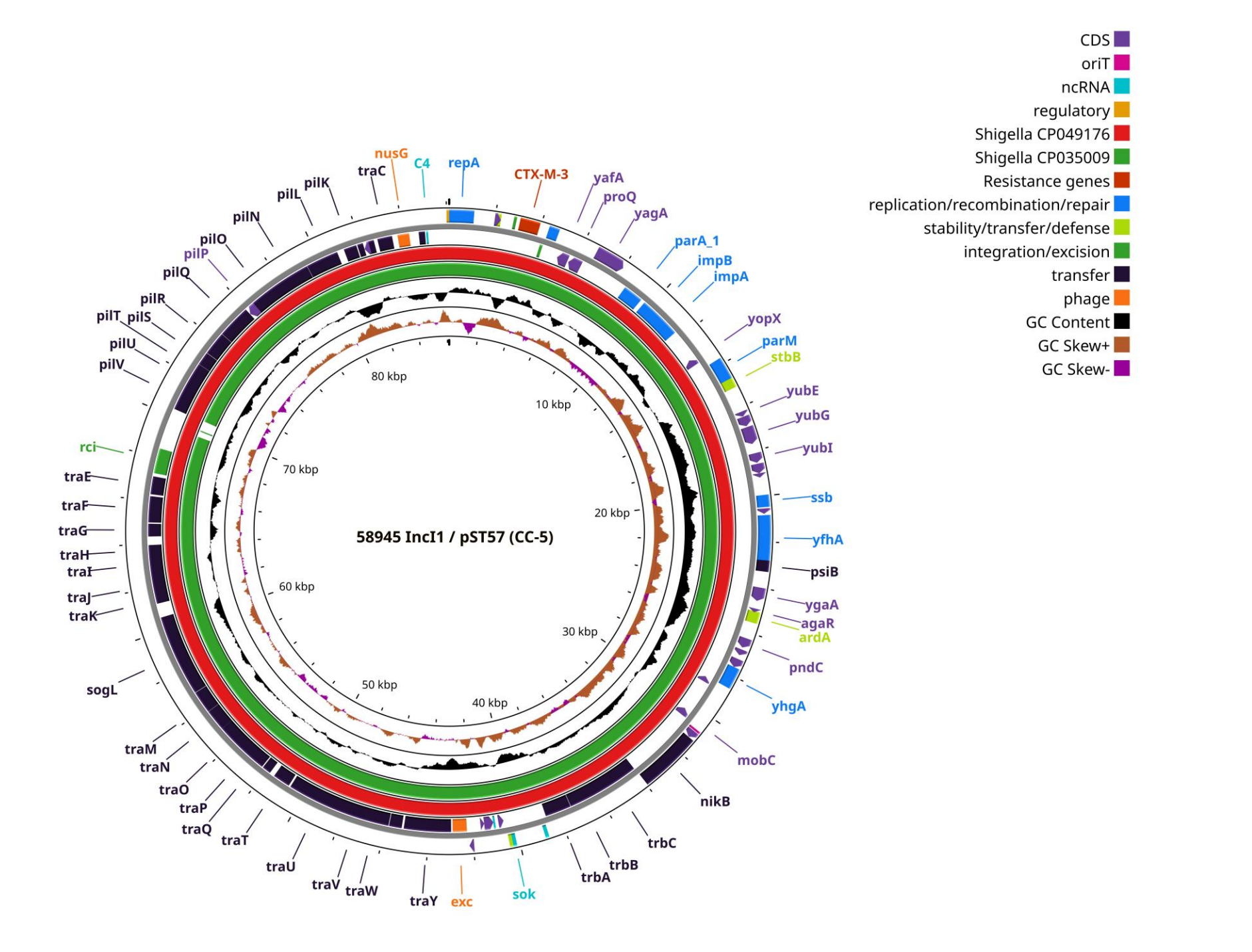


Fig 5. Visualization of the Inc1/pST57 plasmid carrying the *bla*<sub>CTX-M-3</sub> gene and comparison with the highly similar CP049176 plasmid isolated in Switzerland.

- The Inc1/pST57 plasmid was found in the seven clonal ST349 *E. coli* and only harboured one resistance gene (*bla*<sub>CTX-M-3</sub>)
- IncF plasmids carried the *bla*<sub>CTX-M-15</sub> gene in *K. pneumoniae* (n = 6), *E. coli* (n = 1) and *C. freundii* (n = 1) isolates
- Hybrid IncFIB/IncHI1 plasmid carried *bla*<sub>CTX-M-15</sub> in one ST1593 *K. pneumoniae*
- IncY plasmid carried the *bla*<sub>CTX-M-15</sub> gene in one *E. coli* isolate

## References

- 1, World Health Organization. (2019). Monitoring and evaluation of the global action plan on antimicrobial resistance: framework and recommended indicators
- 2, World Health Organization. (2021). WHO integrated global surveillance on ESBL-producing *E. coli* using a "One Health" approach: implementation and opportunities. World Health Organization.

## Acknowledgements

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