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Antimicrobial resistance and genetic relatedness among *Escherichia coli* isolates across the animal-human-wildlife interface in Austria

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BACKGROUND

Surveillance of antibiotic resistant bacteria (ARB) following One Health guidelines is needed to combat antimicrobial resistance (AMR). Here we aimed at assessing the genetic relatedness, the phenotypic resistance and antimicrobial resistance genes (ARGs) in *E. coli* across the human-animal-wildlife interface.

METHODS

In 2020 we collected samples from eight interconnected environmental compartments (soil, pig manure, feed, wild animals, field drainage, river, groundwater, wastewater) at the Hydrological Open Air Laboratory (HOAL) located in Lower Austria. 49 *E. coli* isolates were obtained. Antimicrobial susceptibility testing was performed by Etest including critical and high priority antimicrobials. Isolates were characterized by Whole Genome Sequence (WGS)-based typing including core genome multilocus sequence typing (cgMLST) and identification of antimicrobial resistance genes (ARGs).

RESULTS

E. coli isolates showed high genetic diversity (36 STs detected). Three cgMLST clusters were formed by wastewater isolates (ST58, ST216, ST357-clusters) and one by wild animals isolates (ST212) (**fig.1**). The ST216-cluster included isolates from different collection dates.

Etest confirmed all cluster-related isolates as susceptible while in singletons and pseudoclusters we confirmed resistances to tetracycline (6/49), ampicillin (5/49), streptomycin (5/49), ciprofloxacin (3/49), moxifloxacin (3/49), trimethoprim-sulfamethoxazol (3/49), cefotaxime (1/49), amoxicillin-clavulanate (1/49), cefepime (1/49), azithromycin (1/49), erythromycin (1/49) and gentamicin (1/49). Neither colistin nor carbapenem-resistant isolates were detected.

Isolates from drainage, wastewater, soil and manure, harboring *aac(3)-IId*, *aadA2, aadA5*, *aph(3")-Ib* and/or *aph(6)-Id* genes, were resistant to aminoglycosides (**fig.2**). Drainage, wastewater and soil isolates carrying bla_{TEM-1} were resistant to ampicillin. Tetracyclin resistance mediated by *tet(A)* or *tet(B)* genes was found in wastewater, drainage, manure and feed isolates. Two ST345 drainage and one ST1193 isolates from wastewater were confirmed as multidrug-resistant, including 2nd and 4th generation fluoroquinolone resistance conferred by *gyrA* and *parC* point mutations and trimethoprim-sulfamethoxazol resistance, associated to *dfrA12/dfrA17* and *sul1/sul2* presence. The ST1193 was resistant to 3rd and 4th generation cephalosporins via $bla_{CTX-M-55}$, to macrolides due to *mphA* gene and to disinfectants via *qacEdelta1*.

CONCLUSION

Although no exchange of resistant or susceptible clones was observed between compartments, indistinguishable susceptible clones were detected at different time-points and overall, several potentially mobilisable ARGs were found. Increasing the number of investigated isolates might help when performing monitoring of ARGs and ARB in the environment in order to detect dissemination of resistant clones across environmental ecosystems and prevent their further spread.

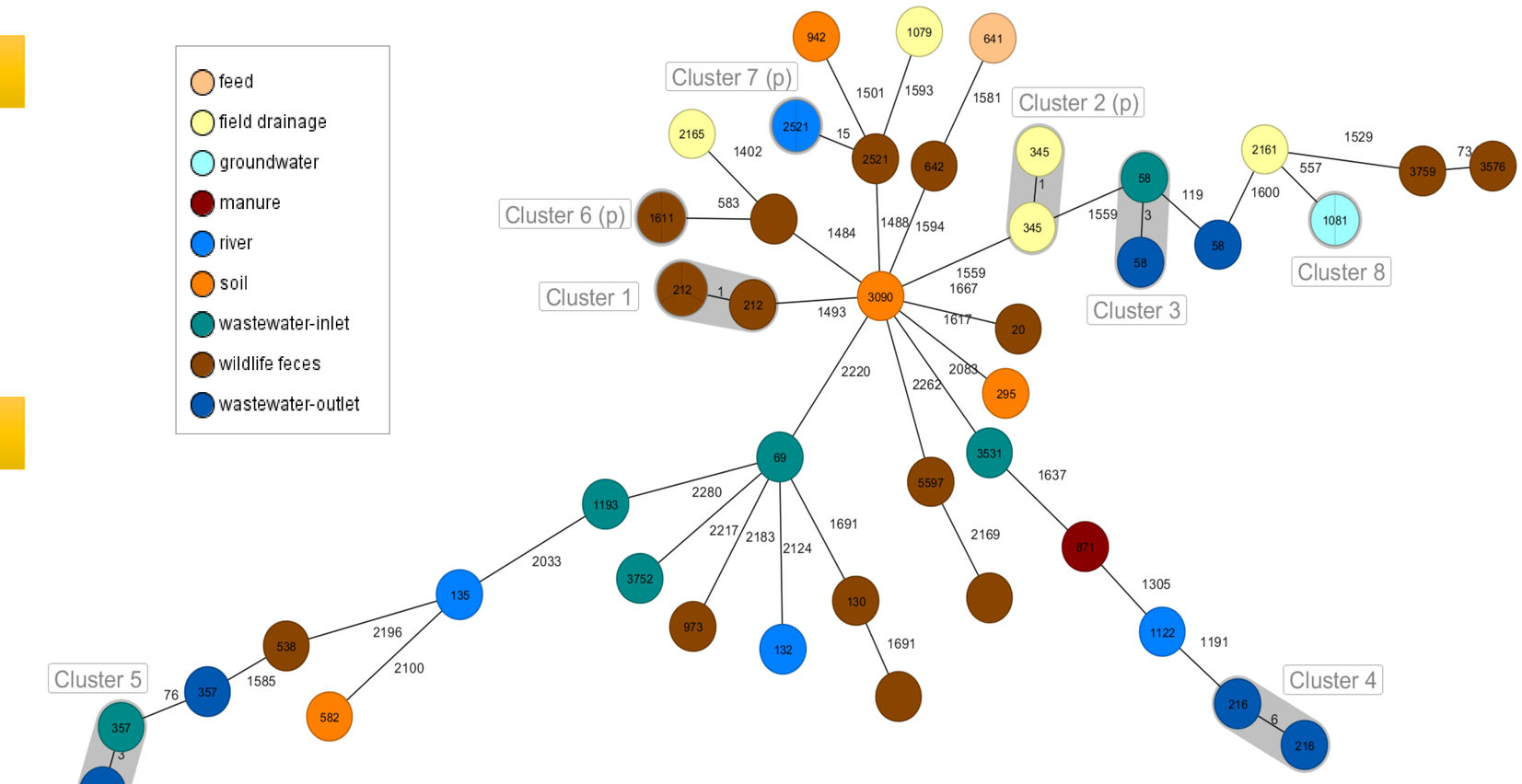

Figure 1. Minimum spanning tree displaying the genetic relatedness between the *E. coli* isolates based on their core genomes. Clusters and pseudoclusters (p) are highlighted in grey. On the connection lines between the isolates, the number of allelic differences is shown. Colors identify the compartment from which each isolate was gained. The applied cgMLST cluster threshold is 10 alleles. Three wildlife isolates had an unknown ST.

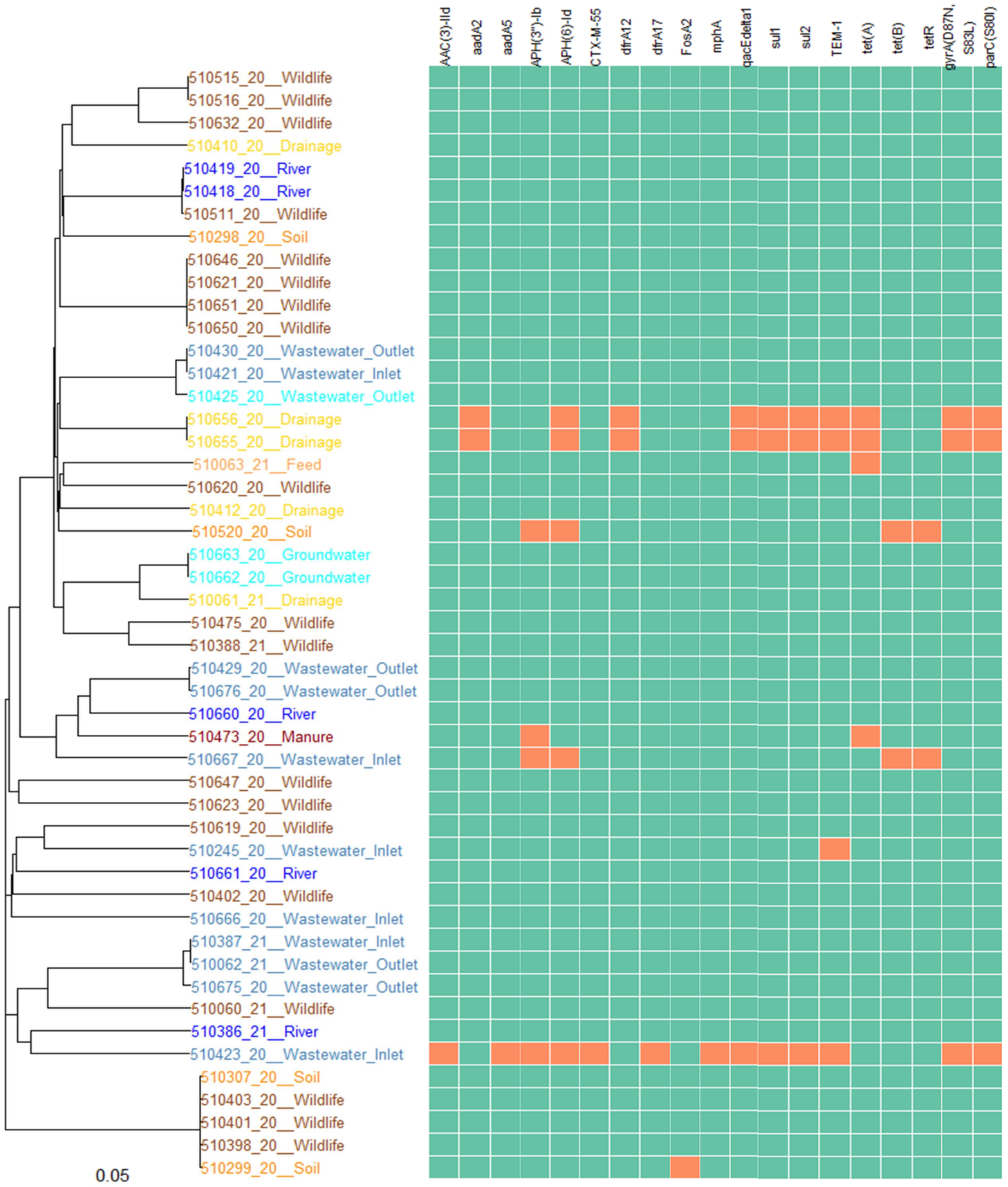

Figure 2. Heatmap showing the presence (orange) or absence (green) of ARGs found in each *E.coli* isolate. The neighbor-joining tree on the left side is based on the cgMLST. Isolate references are colored by compartment.

ACKNOWLEDGEMENTS

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