

GENETIC CHARACTERISATION OF MULTI-DRUG RESISTANT ENTEROBACTERIACEAE ISOLATED FROM WASTEWATER FROM IRISH FARMS.

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INTRODUCTION

The role of Enterobacteriaceae, a group of Gram-negative bacteria frequently exhibiting extended antimicrobial resistance (AMR), in the transmission of resistance genes to other bacterial species present in the same environment warrants investigation. Concerns over resistance to 3rd generation cephalosporins represent a significant issue as AMR is increasing in hospitals and communities in Europe leading to severe infection and/or death. The World Health Organization categorised carbapenem resistant and ESBL-producing Enterobacteriaceae as critical pathogens due to their impact on human health and the urgency for new antibiotics, with fluoroquinolone resistant Enterobacteriaceae falling under the high priority category. The role of the environment in the transmission of AMR is a key area in tackling AMR in both humans and animals and this is in line with the One Health approach. To gain a better understanding of the dissemination of significant resistance determinants, namely ESBL-encoding, *ampC* and *qnr* genes, this study assessed the prevalence and diversity of AMR genes in Enterobacteriaceae isolated from wastewater from different farms in Ireland by Whole Genome Sequencing (WGS).

METHODOLOGY

Eighty-two Enterobacteriaceae (*E. coli* n=79, and one of each *Citrobacter gillenii*, *Enterobacter cloacae* and *Klebsiella pneumoniae*) sourced from farm wastewater were selected on the basis of their phenotypic AMR pattern that was obtained by MIC determination: chromosomal fluoroquinolone (CF) (37), ESBL (22), *ampC* (7), ESBL & PMQR (4), CF & *ampC* (4), unknown phenotype (4), CF & ESBL/*ampC* (3) and ESBL & *ampC* (1). DNA was extracted using the MagNA pure (Roche Diagnostics), sample libraries were prepared using the Illumina prep kit and sequencing was performed on a MiSeq platform (Illumina) using v3 chemistry as 300-cycle paired-end runs. The generated FastQ files were assembled using the BioNumerics software and its integrated calculation engine and, using the genotyping plugin tools within BioNumerics, AMR genes and point mutations were identified, and results compared to the phenotypic results.

RESULTS

- 37 CF isolates had double mutations in the chromosomal *gyrA* gene (codons 83 and 87), 33 had mutations in *parC* (codon 80) and 9 had double mutations in *parC* (positions 56 and 80).
- 22 ESBLs carried CTX-M-14 or CTX-M-15.
- 7 *ampC* isolates were quite varied: 4 were CMY-2 positive, one carried CTX-M-14 and 2 carried TEM-1 genes. Another isolate was TEM-1B positive and had a mutation in the promotor. The *E. cloacae* isolate had ACT-14, TEM-1B and SHV-12, *qnrB2* and the recently described *mcr-9* colistin resistant gene.
- 4 ESBL & PMQR isolates were all *qnrS1* positive. Three carried CTX-M-15 and one carried CTX-M-65.
- 4 CF & *ampC* isolates had double mutations in *gyrA* and *parC*; 2 had a mutation in their *ampC* β -lactamase promotor and the other 2 were CMY-2 positive.
- Among the 4 isolates of unknown phenotype, 3 had mutations in the promotor, one was also SHV-12, TEM-1C and *qnrS1* positive and 3 were also TEM-1B positive.
- 3 CF & ESBL/*ampC* isolates had double mutations in *gyrA* and a mutation in *parC*. In addition, one carried CTX-M-14.
- 37 plasmid-mediated ESBL/*ampC* isolates were identified, with 33 different plasmid replicon types, the most prevalent being IncFII.

DISCUSSION

The presence of AMR genes corresponded, for the most part, to the phenotypic results. 28 of 30 presumptive ESBL Enterobacteriaceae genomes possessed at least one CTX-M type β -lactamase, with *bla*CTX-M-14, *bla*CTX-M-15 and *bla*CTX-M-65 identified in 18, 9 and 1 isolate(s) respectively. In addition, one presumptive *ampC* was also CTX-M-14 positive. CTX-M-14 and CTX-M-15 are the most frequently isolated CTX-M variants from humans in Europe and these results show their spread in the environment.

High levels of fluoroquinolone resistance require the presence of multiple mutations in *gyrA* and/or *parC* and these were found among the 44 CF isolates. In conclusion, Enterobacteriaceae from wastewater from Irish farms carry a wide variety of ESBL/*ampC* and quinolone resistant genes and may pose a threat to public health. Detailed knowledge on the specific contribution of different environmental sources is currently lacking and warrants further investigation. Understanding the epidemiology of the highly transmissible mobile genetic elements of Enterobacteriaceae and the AMR genes is therefore important for effective screening and farm/environment management.