Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVII

Isolation and molecular characterization of linezolid-resistant enterococci of human, animal, and environmental origin.

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INTRODUCTION

Oxazolidinones, linezolid (LZD) and tedizolid (TZD), are the last resort antibiotics used in human medicine to treat severe infections due to Gram-positives bacteria, including vancomycin-resistant enterococci. This antibiotic class inhibits bacterial protein synthesis by binding to the peptidyltransferase centre of the 50S ribosomal subunit.

Besides chromosomal mutations affecting the ribosomal structure, LZD resistance can either be mediated by acquisition of resistance determinants: optrA, poxtA and cfr genes. These genes are commonly associated with mobile genetic elements which facilitate their dissemination by horizontal transfer [1].

Enterococci are regarded as human opportunistic bacteria and continue to be important nosocomial pathogens worldwide. Enterococci, released in large amounts into the environment with the feces, can be found in different niches (soil, foods of animal origin, vegetables, and water).

More recently, enterococci have been also proposed for monitoring antibiotic resistance in food animals. Its spread in many natural habitats and its flexibility to respond to varying environmental conditions make the *Enterococcus* genus a central hub for the acquisition and dissemination of resistance genes among Gram-positive bacteria [2].

It is common knowledge that antibiotic resistance develops more rapidly through the misuse and overuse of antimicrobial drugs and that much antibiotic use is linked to animal production to prevent and/or cure infections. Florfenicol (FFC), which is extensively used in livestock, could promote the spread of LZD resistance genes, as an effect of co-selection mechanisms, with serious consequences for human health [3].

The aim of project will be to investigate possible reservoirs of LZD resistance genes both in animal and environmental setting.

METHODS

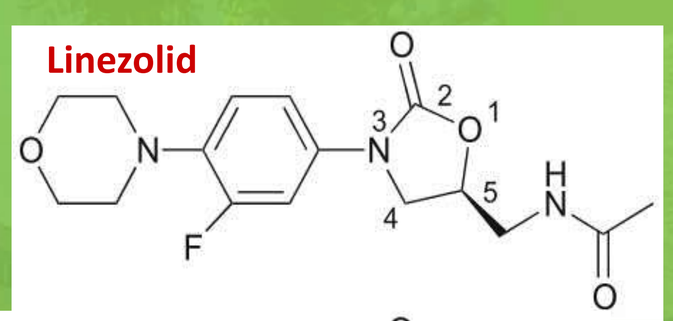
Bacterial strains: In collaboration with the "Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche", sampling will be carried out in order to obtain a collection of FFC-resistant enterococci of animal and environmental origin. Furthermore, the Infectious Diseases Clinic (Marche Polytechnic University) will be collect enterococci with reduced susceptibility or resistance to LZD from inpatients.

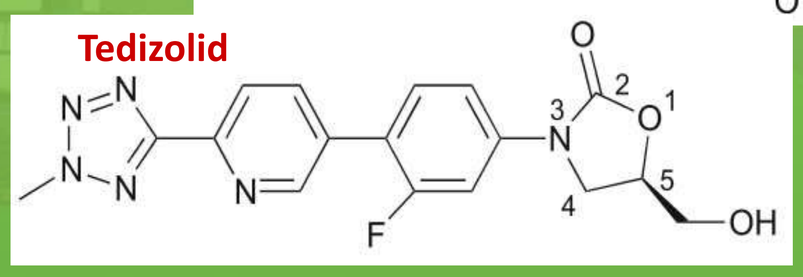
Genotypic and phenotypic characterization: enterococci will be screened by PCR for the presence of *cfr, optrA* and *poxtA* genes. Isolates will be tested for their susceptibility to FFC, chloramphenicol, LZD, TDZ, tetracycline and vancomycin by standard broth microdilution assay.

Location of LZD resistance genes: Then gene location will be determined by hybridization assays using suitable probes for each gene.

Characterization of the genetic environments of oxazolidinone resistance genes: All the isolates will be subjected to WGS analysis by Illumina approach.

Transfer experiments: enterococci carrying LZD resistance genes will be used as donors in filter mating experiments. E. *faecium* 64/3 and E. *faecalis* JH2-2 will be used as recipients.





EXPECTED RESULTS

The selective pressure exerted by FFC may have led to widespread of LZD resistance genes in animal and environmental setting. The currently worrisome situation in humans may be the tip of the iceberg of a more widespread phenomenon. This research will help to clarify the role of animal and environmental enterococci as a source of LZD resistance genes transferable to major human pathogens. This research, expected to provide data on the dynamics of onset and spread of LZD resistance in non-clinical enterococci, will be crucial to explain the role of certain reservoirs in whose absence resistances would probably fail to emerge in human pathogens.

REFERENCES:

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