Supplementary material

Bioinformatic analysis methodology

The nucleotide sequences of the blaOXA-23 and blaOXA-24/40 genes were retrieved from the GenBank database (https://www.ncbi.nlm.nih.gov/), ensuring access to curated and publicly available genetic information. The downloaded sequences were subsequently processed using DNAMAN software version 4.0, which was employed to translate the sequences into amino acids and to identify the corresponding open reading frames (ORFs), allowing accurate determination of the functional coding regions.

Multiple sequence alignment was performed to identify conserved regions among the analyzed sequences. Highly conserved aligned regions with more than 90% sequence identity were selected and used for downstream evolutionary analyses. Phylogenetic analyses were conducted using MEGA software version 6.0 (Kumar et al., 2016), enabling the evaluation of evolutionary relationships among the blaOXA variants.

Functional domains of interest within both proteins were identified using the Conserved Domain Database (CDD) (Derbyshire et al., 2015), providing insights into structurally and functionally relevant regions associated with β-lactamase activity. Additionally, amino acid substitutions resulting from synonymous and non-synonymous mutations were identified and analyzed, with the aim of assessing their potential effects on protein structure and function. These variations were further examined using Network software version 4.6 (Bandelt et al., 1999), which facilitated the analysis of genetic diversity and phylogenetic relationships.

Phylogenetic trees were reconstructed using the Minimum Evolution method, and the robustness of the inferred clusters was evaluated through 1,000 bootstrap replicates, ensuring statistical support for the observed groupings. Amino acid sequence alignments were also performed using the Alignment Explorer/CLUSTALW tool, implemented within the Molecular Evolutionary Genetics Analysis software package (MEGA version 11), to improve alignment accuracy and consistency.

The tertiary structures of the blaOXA-23 and blaOXA-24/40 proteins were predicted using the SWISS-MODEL server (Biasini et al., 2014). Model evaluation was based on sequence identity percentages, Global Model Quality Estimation (GMQE) values, and Qualitative Model Energy Analysis (QMEAN) scores. For comparative modeling, β-lactamase-oxacillin (4f94.1.A), β-lactamase-imipenem (7rp9.1.A), β-lactamase-ertapenem (7rpe.1.A), and β-lactamase-doripenem (3pae.2.A) were included as reference structures, all of which are available in the SWISS-MODEL Repository.

Finally, phylogenetic dendrograms and tertiary structure models generated by MEGA and SWISS-MODEL were exported and subsequently edited and visually enhanced using Paint and CorelDRAW software, improving figure resolution, layout consistency, and overall graphical quality for publication purposes.