Broad-spectrum analysis of beta-lactam resistant *Granulicatella adiacens*: from Penicillin-Binding Proteins mutational and antibiotic affinity profile to cell morphology and peptidoglycan composition

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Beta-lactam resistance in Gram-positive bacteria is mainly driven by penicillin-binding proteins (PBPs) alterations. They can also adapt their cell wall after antibiotic exposure. *Granulicatella adiacens* infections are typically treated with beta-lactam antibiotics but their resistance is increasing. This work aims to characterize *G. adiacens* PBPs and their mutations' role in beta-lactam resistance and evaluate peptidoglycan alteration determined by antibiotic stress.

Beta-lactam activity, synergy, and PBP binding were compared between IS48 clinical isolate and *G. adiacens* ATCC 49175. Affinity to ceftriaxone, ceftobiprole, and ampicillin was assessed with BocillinFL. PBP homology and mutations were analysed by sequence alignment and WGS. PBP1A was cloned and expressed in *E. coli* BL21(DE3) into pET24 and purified. Growth curves were performed in supplemented BHI with D-amino acids probes to analyse cell morphology. Peptidoglycan composition was studied *via* LC-MS after antibiotic exposure to ceftobiprole, ceftriaxone, ampicillin (1/2-fold MIC).

Normal diplococci morphology emerged only in BHI supplemented with blood and L-cysteine. IS48 was not susceptible to penicillin and ampicillin, resistant to ceftriaxone, with higher ceftobiprole MIC than the control strain. Ampicillin *plus* ceftobiprole/ceftriaxone were synergistic. Five PBPs were identified. Most IS48 PBPs carried mutations near catalytic motifs. PBP2B and PBP2 exhibited consistently low acylation levels, whereas PBP1A and PBP2A demonstrated significant inhibition. PBP1B reached IC_{50} only by double treatments in the ATCC strain and by ceftriaxone and ampicillin/ceftriaxone in IS48.

The mutations observed may affect the drug binding, impacting on the resistance phenotype. However, beta-lactam treatment may be effective due the inhibition of key bifunctional PBPs (PBP1A, PBP2A).