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Antimicrobial Efficacy of Some Antibiotics Combinations Tested against Some Molecular Characterized Multiresistant Staphylococcus Clinical Isolates, in Egypt

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Abstract : The resistance of staphylococci to various antibiotics has become a major concern for health care professionals. The efficacy of the combinations of selected glycopeptides (vancomycin and teicoplanin) with gentamicin or rifampicin, as well as that of gentamicin/rifampicin combination, was studied against selected pathogenic staphylococcus isolated from Egypt. The molecular distribution of genes conferring resistance to these four antibiotics was detected among tested clinical isolates. Antibiotic combinations were studied using the checkerboard technique and the time-kill assay (in both the stationary and log phases). Induction of resistance to glycopeptides in staphylococci was tried in the absence and presence of diclofenac sodium as inducer. Transmission electron microscopy was used to study the effect of glycopeptides on the ultrastructure of the cell wall of staphylococci. Attempts were made to cure gentamicin resistance plasmids and to study the transfer of these plasmids by conjugation. Trials for the transformation of the successfully isolated gentamicin resistance plasmid to competent cells were carried out. The detection of genes conferring resistance to the tested antibiotics was performed using the polymerase chain reaction. The studied antibiotic combinations proved their efficacy, especially when tested during the log phase. Induction of resistance to glycopeptides in staphylococci was more promising in presence of diclofenac sodium, compared to its absence. Transmission electron microscopy revealed the thickening of bacterial cell wall in staphylococcus clinical isolates due to the presence of tested glycopeptides. Curing of gentamicin resistance plasmids was only successful in 2 out of 9 tested isolates, with a curing rate of 1 percent for each. Both isolates, when used as donors in conjugation experiments, yielded promising conjugation frequencies ranging between 5.4 X 10-2 and 7.48 X 10-2 colony forming unit/donor cells. Plasmid isolation was only successful in one out of the two tested isolates. However, low transformation efficiency (59.7 transformants/microgram plasmid DNA) of such plasmids was obtained. Negative regulators of autolysis, such as arlR, lytR and lrgB, as well as cell-wall associated genes, such as pbp4 and/or pbp2, were detected in staphylococcus isolates with reduced susceptibility to the tested glycopeptides. Concerning rifampicin resistance genes, rpoBstaph was detected in 75 percent of the tested staphylococcus isolates. It could be concluded that in vitro studies emphasized the usefulness of the combination of vancomycin or teicoplanin with gentamicin or rifampicin, as well as that of gentamicin with rifampicin, against staphylococci showing varying resistance patterns. However, further in vivo studies are required to ensure the safety and efficacy of such combinations. Diclofenac sodium can act as an inducer of resistance to glycopeptides in staphylococci. Cell-wall thickness is a major contributor to such resistance among them. Gentamicin resistance in these strains could be chromosomally or plasmid mediated. Multiple mutations in the rpoB gene could mediate staphylococcus resistance to rifampicin.

Keywords : glycopeptides, combinations, induction, diclofenac, transmission electron microscopy, polymerase chain reaction
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