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A simple method for the screening of efflux pump activity in Multi-Drug Resistant Gram-negative bacteria

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The over-expression of efflux pumps (EPs) in Gram-negative bacteria has been widely associated with phenotypic Multi-Drug Resistance (MDR). These EPs extrude antibiotics (AB) prior to reaching their targets. To detect the activity of EPs and to reverse MDR, EPs inhibitors (EPIs) may be used. A simple method was developed to screen EPs in 30 *Escherichia coli* and 3 *Salmonella* MDR clinical isolates. The strains were assayed for reduction or reversal of resistance to tetracycline (TET) and fluoroquinolones. Four EPIs were tested: Phe-Arg-napthylamide (PAN), thioridazine (TZ), chlorpromazine and carbonyl cyanide m-chlorophenylhydrazone (CCCP). Broth cultures were grown in 24-well plates to which AB discs were added, in the presence or absence of the EPI (½ the MIC). After 16 h at 37 °C, plates were examined for absent (reversal), partial (reduction) or full (no effect) growth. From the *E. coli* strains, 4 showed reduction or complete reversal of resistance to TET and OFX and 1 showed complete reversal of resistance to TET and OFX with PAN (MIC reduction from 480 to 15 mg/L and 80 to 2.5 mg/L, respectively). Another strain showed complete reversal of resistance to TET with PAN. In 2 other strains reduction of resistance to TET was observed with TZ and PAN (MIC reductions from 240 to 30 and 15 mg/L, respectively). The EPIs were also shown to reduce or reverse resistance of *Salmonella* strains. This simple method can be used to screen the activity of EPs of MDR bacteria.

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Characterization of reduced susceptibility to biocides and quinolones in a *Staphylococcus aureus* strain exposed to ethidium bromide

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Efflux pumps (EPs) of *Staphylococcus aureus* reduce susceptibility to antibiotics (AB) and biocides. Ethidium bromide (EB) has been identified as a substrate for several EPs of over 20 bacterial species, including *S. aureus*. Exposure of *S. aureus* ATCC25923 to increasing concentrations of EB increased the MIC of EB from 6.25 to 200 mg/L. This EB adapted strain demonstrated increased resistance to compounds known as substrates for EPs, in particular biocides and quinolones. Among the several EP inhibitors tested, thioridazine, chlorpromazine and reserpine were the most effective in reversing induced resistance of the adapted culture to the compounds tested. Whereas the presence of genes coding for the *S. aureus* EPs *norA*, *mdeA* and *mepA* was confirmed by PCR, the ones corresponding to EPs *Qac*A and *Smr* were not detected in the isogenic strains. Sequencing the QRDR region in *grlA* detected none of the mutations usually associated with quinolones resistance in *S. aureus*. RT-PCR analysis indicate that *norA* and *mepA* are being over-expressed in the adapted culture, whereas no over-expression was detected for *mdeA*. These results indicate that the over-expression of EPs resulting from exposure of *S. aureus* strains to quaternary compounds such as EB increases their resistance to other biocides and quinolones.