
Use of flow cytometry and FACS to isolate antibiotic resistant *Salmonella enterica* sv. Typhimurium following exposure to biocides

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Abstract

Biocides are commonly used for the inhibition of bacterial growth in diverse situations such as farming, food production and hospitals. Unlike antibiotics, biocides frequently function by interacting with and inhibiting multiple bacterial targets and pathways and thus have multiple modes of killing (Russell, 2003). However, recent concerns have been raised over the development of multiply drug resistant bacteria upon exposure to biocides at sub-inhibitory concentrations (Karatzas et al., 2008). In many cases, antimicrobial compounds such as biocides are pumped out of bacteria by overexpressed efflux pumps, potentially leading to resistance to a broad spectrum of biocides and antibiotics (Blair & Piddock, 2009). This is of particular concern as biocides accumulate in the environment, potentially generating a reservoir of multidrug resistant bacteria. We used flow cytometry to analyse the physiological response of *Salmonella enterica* sv. Typhimurium to four commonly-used biocides at both sub-inhibitory and working concentrations. We then used FACS to isolate bacteria that were able to survive in the presence of biocide. Sorting from low concentrations of biocide resulted in bacteria that were not significantly different to the starting strain in terms of biocide or antibiotic resistance, suggesting inherent heterogeneity in the culture gave rise to increased survival of some bacteria. Bacteria isolated from working concentrations of biocide were more resistant to a range of antibiotics, although not to biocides. The molecular basis of their acquired resistance was identified. Given that small numbers of drug-resistant bacteria can give rise to large populations over time, and the prevalence of multiply drug-resistant bacteria in clinical settings, the usefulness of single-cell techniques in the study of multidrug resistance is highlighted. References Blair JM, Piddock LJ (2009) Structure, function and inhibition of RND efflux pumps in Gram-negative bacteria: an update. *Curr Opin Microbiol* 12: 512–519. Karatzas KA et al. (2008) Phenotypic and proteomic characterisation of multiply antibiotic-resistant variants of *Salmonella enterica* serovar Typhimurium selected

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Keywords: Salmonella, Antibiotic resistance, Biocides, FACS