

EFFECT OF CHLORHEXIDINE AND PHENOXYETHANOL, ALONE AND IN COMBINATION, ON LEAKAGE FROM GRAM-NEGATIVE BACTERIA

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Chlorhexidine diacetate (CHA) and phenoxyethanol (POE) are membrane-active biocides. We describe their effects alone and in combination on leakage from strains of *Escherichia coli* and *Pseudomonas aeruginosa* (Table 1). Bacterial suspensions (final density 1mg dry wt./ml) in sterile water were treated at 20°C. K⁺ leakage was determined by a potassium ion-selective electrode, Rb⁺ by a radioactive method, and pentose against D-ribose as standard.

K⁺ leakage was induced readily by low, sublethal concentrations of CHA and increased as the concentration increased up to a maximum at 100µg/ml. The greatest amount of K⁺ leakage was observed with *Ps.aeruginosa* strains. Leakage was rapid, ca. 80% of total leakage occurring within 10 min. Mutant strains with depleted outer membrane lipopolysaccharide (LPS) were rather more sensitive than parent strains although minimum inhibitory concentrations (MICs) of CHA determined in broth were very similar. K⁺ leakage from POE-treated cells was also concentration-dependent, levelling off at ca. 1% v/v; leakage was rapid, with most taking place within 10 min, and mutant strains were rather more susceptible. A combination of CHA (100µg/ml) and POE (1% v/v) increased both the rate and extent of K⁺ leakage, with maximum effect after a contact period of 1 min. Rb⁺ leakage was induced readily by sublethal concentrations of CHA (10µg/ml) or POE (0.3%). Higher CHA concentrations had a rapid effect with maximal leakage occurring within 3 min. Only very high concentrations of POE (2%) induced rapid leakage. Little difference was observed between wild type strains and their envelope mutants. Combinations of CHA (100µg/ml) and POE (1%) increased the rate and extent of Rb⁺ leakage. CHA induced a diphasic pattern of pentose leakage with a maximal effect at 100 µg/ml. No such response was seen with POE (0 - 2%). Leakage was maximal within 10 min for either biocide used alone, and mutant strains were slightly more sensitive than the corresponding parents. A combination of CHA (100 µg/ml) plus POE (1%) induced very rapid pentose leakage which reached a maximum value within 1 min. Enhanced activity is thus achieved by use of appropriate combinations of CHA and POE, but there is little correlation between leakage and MIC values.

Table 1. Biocide-induced leakage from Gram-negative bacteria

Biocide	Leaked material		
	K ⁺	Rb ⁺	Pentose
CHA	Concn. dependent, max. 10 min	Concn. dependent, max. 3 min at high concn.	Diphasic pattern, max. 10 min
POE	Concn.dependent, max. 10 min	High concns. rapid in 3 min; lower 10-30 min	Concn.dependent, max. 10 min
CHA + POE	Max. 1 min	Max. 1 min	Max. 1 min