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Abstract (publication only)

Influence of subinhibitory concentrations of phenyl lactic acid and its synergistic activity with antibiotics on Pseudomonas aeruginosa resistant strains

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The phenyl lactic acid (PLA) is a soluble antimicrobial compound, known to be produced by Lactobacillus spp. probiotic strains, which is not acting by acid pH. In this study, we have investigated the influence of subinhibitory concentrations (sIC) of PLA, on the growth, viability and efflux pumps activity of Pseudomonas aeruginosa MDR strains, acting alone or in association with cefepime (FEP). Materials and methods: Ten clinical P. aeruginosa strains were co-cultivated with sIC of PLA (1mg/ml) in Mueller Hinton broth medium (MHB) for 24 h and then the antimicrobial susceptibility pattern of the respective strains was performed using disk diffusion standard method. Two of the ten strains exhibited an increased susceptibility to FEP. These strains were further co-cultivated for another 24 h in MHB in the presence of sIC of PLA and different concentrations of FEP (MIC, 2xMIC, 3xMIC). The influence of PLA on efflux pumps activity and cell viability was investigated by the viable cell counts (VCCs) method and flow cytometry. For flow cytometry 1 ml aliquots was pelleted, washed twice with PBS and stained with 1 µg /ml propidium iodide (for viability) and ethidium bromide (for efflux pump activity). The fluorescence was evaluated using FL2 and FL3 channels. Results: The sIC of PLA exhibited an inhibitory effect on the growth of all tested strains, proved by the decreased VCCs values. In case of FEP resistant strains, the co-cultivation with sIC of PLA induced the increase of growth inhibition zone from 6 to 12 mm. The PLA at sIC in association with FEP induced the aggregation of bacterial strains in hydrophobic and hard to disperse and homogenize clusters, as compared to the standard conditions. An increased fluorescence of ethidium bromide registered in FL-2 indicating the inhibition of efflux pumps was observed for bacterial cultures co-cultivated with sIC of PLA, this signal being even more increased when the PLA acted concomitantly with FEP, with an intensity proportional with FEP concentration. In case of the synergic activity of PLA and FEP an increased FL-3 signal suggesting a cellular membrane damage, was observed. Conclusion: The obtained results suggest that PLA exhibits its antimicrobial effect by multiple pathways, including the inhibition of growth rate and the down-modulation of efflux pump activity, being a promising candidate to be used in association with different antibiotics in the anti-infective therapy.