6th World Congress and Expo on **Applied Microbiology**
&
8th Edition of International Conference on **Antibiotics, Antimicrobials & Resistance**
&
12th International Conference on **Allergy & Immunology**
October 21-22, 2019 Rome, Italy

Establishing standard operating procedures (SOP) for disk diffusion quorum sensing inhibition assay

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Many studies report the high prevalence of multidrug resistant (MDR) bacterial pathogens. Because they are difficult and expensive to treat, these represent a growing public health concern, associated with increased mortality. Bacterial cells communicate with surrounding cells using diffuse signaling molecules (autoinducers), forming multicellular communities. This cell-cell communication, termed quorum sensing (QS) alters the expression of various bacterial genes, resulting in phenotypic changes (e.g., antibiotic resistance, biofilm, virulence factors) depending on the density of the cell population. QS-inhibiting compounds represent a potential therapeutic alternative to antibiotics, as they do not exert selection pressure on microorganisms. The most frequently used, inexpensive *in vitro* screening method for QS-inhibitory compounds is disk diffusion; however, the reproducibility of this method has not yet been characterized. The aim of the study was to optimize the characteristics of disk diffusion QS-inhibition assay. The growth and pigmentation characteristics of several QS-indicator and signal molecule-producing strains were evaluated using various liquid and solid culture media, inoculum size, incubation time and temperature. In addition, the QS-inhibitory activity of 30 non-antibiotic pharmaceutical compounds is evaluated. The antibacterial activity of the compounds was also investigated by broth microdilution method according to CLSI standards. For optimal reproducibility, the ideal experimental conditions were defined as follows: diameters of QS-inhibition were measured after 48 hours incubation at room temperature on modified Luria-Bertani (LB*) agar, supplemented with 2 g/L glucose and a microelement stock solution. Metamizole sodium, 5-fluorouracil, cisplatin, methotrexate, bleomycin and phenothiazines had concentration-dependent QS-inhibitory activity on the bacterial strains examined. We report the standardization of a cheap and high-throughput method for screening of QS-inhibitory activity. Thousands of drugs are marketed for human therapeutic purposes with different chemical structures and mechanisms of action. These compounds can be considered as potential sources of QS inhibitory agents, as their tolerability *in vivo* has already been verified.

Biography

Mario Gajdacs has completed his Graduation as a Pharmacist and has completed his PhD in Medical Microbiology in the Doctoral School of Interdisciplinary Medicine at the University of Szeged. After spending time in the clinical microbiology laboratory, he enrolled post-doctoral studies and specialized pharmacist-studies in the Institute of Pharmacodynamics and Biopharmacy, where he is currently working as an Assistant Lecturer. He has broad interdisciplinary experience in medical microbiology and the pharmacology/use of antimicrobial drugs. His research interests include the development and screening of novel compounds with antibacterial and anticancer properties, problem perception and development of antibiotic resistance by the public and various healthcare professionals and the driving forces behind the non-prudent use of antimicrobial drugs. He has published 18 papers in reputed journals.

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