

Identification of alternative therapeutic compounds with antimicrobial action

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Introduction

Infections caused by bacteria can become a challenge when it comes to their treatment, especially in the case of those with multiple mechanisms of resistance. With the discovery of antibiotics, life expectancy has increased significantly, but yet their abuse has led to resistance mechanisms that are difficult to combat.

The doctoral thesis focuses on one of the microbial strains commonly found in infections, *Pseudomonas aeruginosa*, the choice being justified by the particular relevance of this bacterium in the infections caused but also the multiple resistance mechanisms found in the genetic baggage of the bacteria.

Several aspects relating to: their resistance profile and the genetic mechanisms underlying resistance, the epidemiological implications especially in the case of multiresistant bacteria. Finally, the thesis presents the influence of essential oils with antimicrobial potential on strains with increased resistance.

Study 1- Epidemiology, Evolution of Antimicrobial Profile and Genomic Footprint of *Pseudomonas aeruginosa* before and during COVID-19: Transition from Resistance to Susceptibility

The study was carried out on isolated strains of *P. aeruginosa* in the period 2017-2022 with several perspectives being analysed. First, research was done following the prevalence of strains, the impact of the COVID-19 pandemic on prevalence, their distribution in the sections of the Mureș County Clinical Hospital (MCCH) and their antibiotic resistance. Secondly, the genomic analysis of strains was studied, using molecular techniques (ERIC-PCR) aimed at identifying possible microbial clusters or strains with high similarity.

Materials and methods

Information collected from the WHONET database, on departments, types of pathological products, species and antibiotic resistance profile involving 1994 strains of *Pseudomonas* spp. isolated over a period of 6 years in MCCH were studied. Of 50 multiresistant isolates, genomic analysis was carried out through genetic tests (ERIC-PCR) to prove potential clonal distribution.

Results

A number of 1994 strains of *Pseudomonas* spp. were isolated in the period 2017-2022, of which *P. aeruginosa* was the most common species, 97.39% (n = 1942) with a higher incidence in the year 2017 (n=538), in the section of dermatology, the main pathological product being purulent secretion. A decrease in harvest rate was observed in 2020 due to COVID-19 restrictions. As for the strength profile, there are some changes. The susceptibility of *P. aeruginosa* to carbapenems, piperacillin-tazobactam and amikacin underwent changes before and during COVID-19. The molecular footprint showed three groups of *P. aeruginosa*, including strains with 80-99% similarity.

Study 2- Discovery of Resistance Mechanisms in Clinical Extended Resistant *Pseudomonas aeruginosa* Isolates: Perspectives from Genetic Expression and Phenotypic Tests

The aim of the study was to describe the activity of Mex Efflux Pumps in Multiresistant Clinical Isolate (MDR) of *P. aeruginosa* and to compare the tests to identify carbapenem resistance with the end-point PCR method.

Materials and Methods

Sixty strains of *P. aeruginosa* MDR have been analyzed for the detection of carbapenemase by diffusion method, carbapeneme inactivation method and modified Hodge test. The genetic method (end-point PCR) was used to detect 7 carbapenemase genes (*bla*_{KPC}, *bla*_{OXA48-like}, *bla*_{NDM}, *bla*_{GES-2}, *bla*_{SPM}, *bla*_{IMP}, *bla*_{VIM}) and *mcr-1* for colistin resistance. The expression of the *mexA*, *mexB*, *mexC*, *mexE* și *mexX* genes corresponding to the four main efflux pumps was also evaluated.

Results

Out of the total test strains, 71.66% have at least one carbapenemase gene, *bla*_{GES-2} being the most frequent (63.3%). Compared to the PCR test, the accuracy of the phenotypic tests did not exceed 25% for *P. aeruginosa*.

Efflux pump genes were present in all strains except one. In 85% of the isolates, overexpression of *mexA*, *mexB* and especially *mexC* was detected. Previous treatment with ceftriaxone increased *mexC* activity by more than 160 times.

Study 3- The Antibacterial Effect of 16 Essential Oils and Modulation of the Genetic Expression of Mex Efflux Pumps on Multiresistant Clinical Isolates of *Pseudomonas aeruginosa*: Is Cinnamon a Good Fighter?

The aim of the study was to describe the antimicrobial activity of 16 common essential oils (EO) on clinical isolates of *P. aeruginosa* MDR, including the determination of effects on the gene expression of Mex efflux pumps.

Materials and methods

A number of 72 clinical isolates of *P. aeruginosa* collected between 2020-2022 have been analyzed for antimicrobial activity to EO using the Kirby-Bauer method to identify oils with antimicrobial potential. The minimum inhibitory concentration (MIC) was subsequently determined for EO that showed antibacterial activity after diffusion method screening. Positive and negative controls strains were also used to validate the method. Because cinnamon essential oil showed the best antimicrobial activity, it was subsequently used to evaluate its influence on the expression of genes in *mex A*, *B*, *C*, *E* and *X* efflux pumps using real-time RT-PCR

Results

Cinnamon EO inhibited all strains of *P. aeruginosa*, followed by thyme (37,5%, n = 27) and lavender (12,5%, N = 9). The other EO were less efficient. CMI showed that cinnamon, at a concentration of 0.05% v/v, inhibited all *P. aeruginosa* MDR isolates. The essential oils of thyme, turmeric, mint, basil and lavender showed varying results, most of them having activity at concentrations higher than 12.5% v/v.

By studying the activity of cinnamon EO on mex efflux pumps, it was found that *mexA* and *mexB* (66,5%) were generally underexpressed. The remarkable results obtained using very low concentrations of cinnamon EO with 100% antimicrobial activity against clinical isolates of *P. aeruginosa* MDR, XDR, PDR, supplemented by severe alteration of the messenger RNA system, support its potential to be used as an adjuvant treatment with impact on therapeutic results.