Antimicrobial Effect of $ZnSO_4$ on Multiple Drug Resistant *Pseudomonas aeruginosa* Strains

*Mohammad Dadook [1], Sedigheh Mehrabian [1], Saeed Irian [2]**

[1]*Faculty of Biological Sciences, Islamic Azad University, Tehran North Branch, Tehran, IR Iran*
[2]*Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, IR Iran*

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ABSTRACT

***Background*:** Due to the importance of the metal zinc as an essential biological element with known toxic properties, and considering the widespread secondary infections caused by *Pseudomonas aeruginosa* strains with resistance to antibiotics and disinfectants, the present study aimed at exploring the antimicrobial properties of the metal zinc in both clinical and environmental multiple drug resistant (MDR) strains of *P. aeruginosa*. MDR in *P. aeruginosa* is defined as the resistance to several antibiotics.
Methods: Bacterial strains were cultured in cetrimide agar medium at 37 °C for 48 h. Strains were identified both morphologically and biochemically. MIC values of the metal zinc were reported using the broth microdilution method.
Results: A total of 84 strains were isolated and identified. MIC values for sensitive and MDR strains were reported at 20 and 130 ppm, respectively.
Conclusions: It was concluded that the metal zinc at concentrations greater than 130 ppm will have an antimicrobial effect on all sensitive and MDR strains of *P. aeruginosa*.

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* Corresponding Author: Saeed Irian, phD., Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, IR Iran. *Tel*: +9821-88329220, *Email*: irian@khu.ac.ir.

Introduction

Pseudomonas aeruginosa is an aerobic, gram negative, opportunistic pathogen possessing lipopolysaccharides, polar flagella and pili (1, 2). This bacterium has a widespread distribution, acts a disease causing agent in patients with immune deficiency, neutropaenia, burns and those with conter, and is the most popular infectious agent of the respiratory system, urinal and gastrointestinal tracts, the CNS, bone and cartilage tissues, skin and the soft tissue, bacteremia, toxic septum and ear and eye infections (3, 4, 5). Treating patients infected with *S. aeruginosa*, in particular multiple drug resistant (MDR strains), has proved cumbersome (6). Following inappropriate experimental treatments, sensitive strains have turned resistant to antibiotics through either induction of enzymes with antibiotic inactivating activity or mutation in genes encoding outer membrane porins, or plasmid transfer. Production of β-lactamase is one of the most important mechanisms through which bacteria gain resistance, and the number of bacteria acquiring, through mutations, the ability to produce β-lactamase is on the rise (7, 8). Heavy metals are a group of elements with an atomic mass greater than 40 grams and a specific weight of more than 5 g/cm (9). They frequently find their way into soil through environmental contaminants including industry related atmospheric contaminations, uncontrolled use of pesticides and both municipal and industrial swage systems in a nonreturnable fashion (10). Unlike organic contaminants, which can be converted into nontoxic material, metals are intrinsically stable in nature (11). A number of these heavy metals including zinc (Zn+2) are among important elements of the biological systems, as they serve essential roles in the maintenance of the 3D structure of many proteins (12). The metal zinc as a micronutrient is considered an essential element in the growth and development of plants, however, when present in greater amounts, metabolic disorders eventually leading to growth inhibition may follow in both plants and microorganisms (13). One of the major mechanisms by which heavy metals, including zinc, cause toxicity is through the generation of free radicals and oxidative stress (14). Due to the importance of *P. aeruginosa* in health related infections and their resistance to antibiotics, the present investigation aimed at revealing the antimicrobial properties of $ZnSO_4$ in sensitive and MDR strains of *P.aeruginosa.*

Material and Method

Clinical and soil sampling

To isolate and purify *P. aeruginosa* strains, both clinical, prepared from patients' wounds, and soil samples were transferred to the laboratory under sterile conditions. Ten serial dilutions of 10-1-10-10, prepared from all samples, were plated on cetrimide culture medium in duplicates and incubated at 37 ℃ for 48 hours.

Strain identification

Bacterial colonies were examined both macroscopically and microscopically. Strain identification was performed using Gram staining, tests for oxidase, catalase, pigmentation, OF, citrate, urease, arginine dehydrolase, lysine and ornithine decarboxylase, reaction in TSI environment and growth at 42 ℃ (15).

Sensitivity to antibiotics

Bacterial suspensions with a turbidity of half a McFarland ($1.5x10^8$) were prepared according to CLSI (The Clinical and Laboratory Standards Institute). Sterile swaps of bacterial suspensions were then streaked on Mueller Hinton agar media.

Antibiotic discs, purchased from Mast Diagnostics (UK, Mast group Ltd), were used for Disc diffusion agar (Kirby bauer) method for strain sensitivity to the antibiotics pipercillin (100 µg), ciprofloxacin (5 µg) amikacin (30 µg) and ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), imipenem (10 µg) and meropenem (10 µg) (16). Following incubation at 37 ℃for 18-24 hours, zone of inhibition was measured for each disc. Antibiotic sensitivity was determined according to the CLSI table.

Sensitivity to different concentrations of zinc sulfate

Bacterial strain sensitivity to zinc was performed by both disc and broth microdilution methods. In the Disc method, bacterial suspensions with a turbidity of half a McFarland (1.5x108 CFU/ml) were initially prepared from fresh bacterial cultures. Using a sterile swap, these suspensions were transferred to Mueller Hinton agar plates, prior to overlaying the plates with sterile blank disks soaked in solutions of different zinc concentrations. These plates were then incubated at 37 ℃ for 24 h prior to being subjected to measurements of the zone of inhibition. In the broth microdilution method, tubes containing 1 ml of sterile LB broth medium and 10-500 ppm of zinc (serially diluted from a stock of 1000 ppm) (17) were initially prepared, followed by inoculation with 100 µl of bacterial suspension with a turbidity of half a McFarland prior to incubation at 37 ℃ for 24 h.

Result

Sampling and identification

A total of 84 strains of *P. aeruginosa* were isolated from both clinical (34 strains) and soil (50 strains) samples, and identified through biochemical tests. Of these, a total of 21 strains were MDR of which 19 were from clinical and 2 were from soil samples (Table 1).

Strain sensitivity to zinc sulfate

The Disk method revealed a minimum zinc concentration of 125 ppm required for the appearance of the zone of inhibition for the antibiotic sensitive strains, while that of the MDR strains was 500 ppm (Table 2). In the broth microdilution method, results of the turbidity assay for bacterial growth revealed MIC values of 20 ppm and 130 ppm for sensitive and MDR strains, respectively (Table 3).

Table 1. The antibiogram of MDR strains of *Pseudomonas aeruginosa*

Antibiotics	Resistant strains (NO.)	Resistant strains from clinical samples (NO.)	Resistant strains from soil samples (NO.)
Resistant strains	21	19	2
Amikacin	15	15	0
Ciprofloxacin	10	10	0
Pipercillin	13	13	0
Ceftriaxone	21	19	2
Ceftazidime	20	19	1
Cefepime	20	19	1
Imipenem	21	19	2
Meropenem	21	19	2

Discussion

Health related problems associated with infections by *P. aeruginosa* have been well documented, while dealing with resistance to antibiotics has been a challenging task. The present investigation aimed at revealing the antibacterial properties of the metal zinc in the form of ZnSO4. Of the examined samples, a total of 84 *P. aeruginosa* were isolated from both clinical and soil samples in cetrimide agar medium, and identified by both phenotypic and biochemical examination.

Table 2. Zinc sensitivity assay by the Disc method

Average diameter of zone of inhibition measured by Disc method			
Strain	**Concentration (ppm)**	**Disk (mm)**	**Well (mm)**
Sensitive	125	15	15
Resistant	500	13	13

MDR strains of *P. aeruginosa* demonstrated a greater resistance to the metal zinc than did the sensitive strains, and a ZnSO4 concentration more than 130 ppm showed an antibacterial property and inhibited the growth of both sensitive and MDR strains of *P. aeruginosa*. In a separate set of experiments, however, 80 ppm nanoZnO treatment of bacteria in the sewage system did not have any effect on their survival, while 100 ppm and 1000 ppm of the nanoparticle caused 36% and 80% reduction in the population of the bacteria, respectively (24). Despite major differences between the results obtained by this study and ours, it is very likely that such differences may originate from differences in the location of sampling as well as the nature of the zinc compound used in the two studies.

In conclusion, the present investigation demonstrated that of the sampled strains of *P. aeruginosa*, all were sensitive to the metal zinc, and that zinc concentrations greater than 130 ppm have antibacterial effect on all the examined strains. Due to the increasing number of MDR strains of *P. aeruginosa*, appropriate concentration of the metal zinc (ZnSO4) is suggested as a potential antibacterial agent. Despite its important role in the biochemical structure of the bacteria, too much zinc is toxic to the survival of microorganisms (18). A 30 ppm of the metal zinc was reported to be lethal to the survival of sensitive bacteria (19), while a short term treatment with increasing concentrations in soil of the metal zinc reduces bacterial population in a linear fashion (20). A MIC value of 1 mg/ml of the metal zinc has been reported for intestinal bacterial population (21).

Table 3. MIC values of the metal zinc for sensitive and MDR strains of *Pseudomonas aeruginosa*

Zinc growth inhibitory concentration (ppm)	Strain	Sample
130	Resistant	Clinical
20	Sensitive	Clinical and Soil

A reduction in the activity of soil bacteria due to a 2 mmol zinc treatment has been reported by Babich *et al* (22). While in a separate pot experiment, soil bacteria were sensitive to concentrations greater than 50 ppm of the metal zinc (23).

Conflict of interest

None declared conflicts of interest.

References

1. Driscoll JA, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs* 2007; **67**(3): 351-368.
2. Brooks GF, Butel JS, Morse SA. Jawet Melnick and Adelberg's Medical Microbiology. London: Lang Basic Science 2004; pp: 262-267.
3. Akhabue E, Synnestvedt M, Weiner M G, *et al*. Cefepime Resistant *Pseudomonas aeruginosa*. *Emerg Infect Dis* 2011; **17**(6): 1037-43.
4. Aoki S, Hirakata Y, Kondoh A, *et al*. Virulence of metallo-beta-lactamase-producing *Pseudomonas aeruginosa In vitro*. *Antimicrob Agents Chemother* 2004; **48** (5): 1876-1878.
5. Doring G, Pier GB. Vaccines and immunotherapy against *Pseudomonas aeruginosa*. *Vaccine* 2008; **26**: 1011-1024.
6. Maleknezhad P, Aligholi M, Moosavi S. Study of *Pseudomonas aeruginosa* resistance to Penicillins, Cephalosporins and Aminoglycosides. *The Univ Med J* 1998; **56** (4): 23-29.
7. Church D, Elsayed S, Reid O, *et al*. *Burn wound infections*. *Clin Microbiol Rev* 2006; **19**: 403-34.
8. Japoni A, Alborzi A, Kalani M, *et al*. Susceptibility patterns and cross resistance of antibiotics against *Pseudomonas aeruginosa* isolated from burn patients in the south of Iran. *Burns* 2006; **32**(3): 343-7.
9. Canli M, Alti G. The relationships between heavymetal (Cd, Cr, Cu, Fe, Pb, Zn) levels and size of six Mediterranean Fish species. *Environpollut* 2003; **121**: 129-136.
10. Chen X, Hu S, Shen C, *et al*. Interaction of Pseudomonas putida CZ1 with clays and ability of the composite to immobilize copper and zincfrom solution. *Bio resours technol* 2009; **100** (1):330-337.
11. Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. *Ecotoxicol Environ Saf* 2000; **45**(3): 198-207.
12. Khan F, Patoare Y, Karim P, *et al*. Effect of magnesium and zinc on antimicrobial activities of some antibiotics. *Pak J Pharm Sci* 2005; **18**(4): 57-61.
13. Ashraf R, Adam T. Effect of heavy metals on soil microbial community and Mung beans seed germination, *Pak J Bot* 2007; **39** (2): 626-636.
14. Nies DH. Microbial heavy-metal resistance. *Appl Microbial Biotechnol* 1999; **51**: 730-750.
15. Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott's *Diagnostic Microbiol*. 12th ed Missouri. Mosby. 2007; 340-50.
16. Washington JA, Anhatt JP. Antimicrobial susceptibility tests of aerobic and facultative lyan aerobic bacteria. In: Laboratory procedures in Clinical Microbiology. 2nd ed USA. Spinger. 1985; 67-85.
17. Harris DC. Quantitstive Chemical Analysis, Eighth Edition. 2010; ISBN-13: 978-1-4292-1815-3.
18. Meesungnoen O, Nakbanpote W, Thiwthong R, *et al*. Zinc and Cadmium Resistance Mechanism of *Pseudomonas aeruginosa* PDM Zn CD2003. *Res J Biol Sci* 2012; **7**(1): 4-13.
19. Shakibaie MR, Khosravan A, Frahmand A, *et al*. Application of metal resistant bacteria by mutational enhancement technique for bioremediation of copper and zinc from industrial wastes. *IrJ Environ Health Sci Eng* 2008; **5**(4): 251-256.
20. Rajapaksha RMCP, Tobor-kaplon MA, Baath E. Metal toxicity affects fungal and bacterial activities in soil differently. *Appl Environ Microbiol* 2004; **70**: 2966-2973.

21. Faiz U, Butt T, Satti L, *et al*. Efficacy of zinc as an antibacterial agent against enteric bacterial pathogens. *J Ayub Med Coll Abbottabad* 2011; **23**(2): 18-21.
22. Babich H, Stotzky G. Heavy metal toxicity to microbe-mediated ecologic processes: a review and potential application to regulatory policies. *Environ Res* 1985; **36** (1): 111-137.
23. Cevik N, Karaca A. Effect of Cadmium, Zinc, Copper and Fluoranthene on Soil Bacteria. *Ankara Dept Soil Sci* 2003; **6**: 1-14.
24. Malakootian M, Toolabi A. Determining and Comparing the Effect of Nanoparticle CuO, TiO2 and ZnO in Removing Gram Positive and Negative Bacteria from Wastewater Iran. *J Yazd Univ Health* 2011; **29**(3): 1-11.