1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan, all in English)

Project title:

Presence and spread of microorganisms and their antibiotic resistance genes from cemeteries to the natural environment.

2. Project goals: This study will focus on investigate cemeteries soils and waters as potential environmental reservoirs of antibiotic resistant bacteria as these organisms could represent a potential health threat through the contamination of groundwater and fresh waters (lakes and rivers).

2.1. Outline:

There is a growing concern regarding the global increase in antibiotic-resistant microorganisms. These antibiotic-resistant organisms have a major impact on public health as they have caused an increase in therapeutic failures [1], hospitalisation [2] and death rates [3]. There has also been a remarkable re-emergence of neglected diseases, such as extremely drug-resistant tuberculosis [4]. Although some bacteria are resistant to a single antibiotic, multi-drug-resistant organisms have developed simultaneous resistance to several antibiotics [5,6]. To face this challenge, several combined therapies have been developed with different spectrums of activity [7,8]. Unfortunately, resistance to many combined therapy formulations has also been reported [9,10]. Although antibiotic resistance can be intrinsic, a strong association between human activities and the occurrence of acquired antibiotic resistance has been observed in the environment. Activities, such as the discharge of untreated or poorly treated sewage into receiving water bodies [11], use of sewage sludge in agriculture [12] and use of animal faeces as fertiliser [13], have been linked to the increased detection of antibiotics and antibiotic-resistant bacteria in the environment. Despite their effectiveness in removing bacteria from wastewater, wastewater treatment works, for example, are unable to completely remove antibiotics from wastewater, thus resulting in the discharge of these metabolically active compounds into the environment [14]. It has been suggested that microbial exposure to these sub-lethal doses of antibiotics leads to the development of

resistance to the antibiotics in bacteria [15,16]. Another public health concern associated with environmental pollution due to human activities is the introduction of pathogens into the environment. These pathogenic organisms, which include bacteria, viruses, fungi and protozoa [17], have been proven to be involved in many human and animal diseases. One inevitable phase of every living being is death. In the human population, death is usually accompanied by diverse funeral practices and rituals depending on the community involved [24]. Although it has been reported that some communities practise building or settlement burials (usually to keep them close to their loved ones), most burials are carried out in cemeteries, which are specific areas dedicated for the purpose [25]. As such, cemeteries are regarded as historical monuments where people usually go to remember their lost loved ones [26]. The burial of human remains in cemeteries is said to facilitate the decomposition of the corpse without posing a danger to public health. However, it is believed that cemeteries could represent a health risk. In particular, this risk is greatest in grave diggers due to injuries or contamination of wounds during digging of the graves [27]. The potential contribution of burial in cemeteries as a human activity to the presence of drug-resistant microbial pathogens in the environment has not been given full attention. Thus, this project is conducted to investigate cemeteries soils and waters as potential environmental reservoirs of antibiotic resistant bacteria as these organisms could represent a potential health threat through the contamination of groundwater.

2.2. Work plan

Year 1:

1. Review of the literature on the subject of research and preparation of a review publication.
2. Selecting at least 5 cemeteries meeting the project requirements (location on a hill near surface waters: lake, river).
3. Establishing cooperation with funeral homes operating in selected cemeteries.
4. Obtaining approval for sampling and setting piezometers from the institution managing the cemetery.
5. Sampling from at least one cemetery and at least 5 graves and groundwater from piezometers
6. Microbiological analysis of the collected samples and analysis of the presence of antibiotic resistance genes to selected antibiotics.

Year 2:

1. Sampling from 2 consecutive cemeteries and at least 5 graves and groundwater from piezometers
2. Microbiological analysis of the collected samples and analysis of the presence of

antibiotic resistance genes to selected antibiotics.
3. Presentation of the results of the research carried out at at least one scientific conference.
4. Preparation of publications based on the obtained research results.

Year 3:
1. Sampling from 2 consecutive cemeteries and at least 5 graves and groundwater from piezometers
2. Microbiological analysis of the collected samples and analysis of the presence of antibiotic resistance genes to selected antibiotics.
3. Presentation of the results of the research carried out at at least two scientific conferences.
4. Preparation of publications based on the obtained research results.

Year 4:
1. Possible additional or repeated sampling and testing.
2. Preparation of the next publication.
3. Preparation of a doctoral dissertation.
4. Conducting the defense of the doctorate.

2.3. Literature

1. Borel, N.; Leonard, C.; Slade, J.; Schoborg, R.V. Chlamydial antibiotic resistance and treatment failure in veterinary and human medicine. Curr. Clin. Microbiol. Rep. **2016**, 3, 10–18.
2. Mauldin, P.D.; Salgado, C.D.; Hansen, I.S.; Durup, D.T.; Bosso, J.A. Attributable hospital cost and length of stay associated with healthcare-associated infections caused by antibiotic-resistant gram-negative bacteria. Antimicrob. Agents Chemother. **2010**, 54, 109–115.
3. De Kraker, M.E.A.; Stewardson, A.J.; Harbarth, S. Will 10 million people die a year due to antimicrobial resistance by 2050? PLoS Med. **2016**, 13, e1002184.
4. Alanis, A.J. Resistance to antibiotics: Are we in the post-antibiotic era? Arch. Med. Res. **2005**, 36, 697–705.
5. Adesoji, A.T.; Ogunjobi, A.A.; Olatoye, I.O.; Douglas, D.R. Prevalence of tetracycline resistance genes among multi-drug resistant bacteria from selected water distribution systems in southwestern Nigeria. Ann. Clin. Microbiol. Antimicrob. **2015**, 14, 35.
6. Ferreira, A.M.; de Andrade, D.; Rigotti, M.A.; de Almeida, M.T.G. Methicillin-resistant Staphylococcus aureus on surfaces of an intensive care unit. Acta Paul. Enferm. **2011**, 24, 453–458.
7. Worthington, R.J.; Melander, C. Combination approaches to combat multi-drug resistant bacteria. Trends Biotechnol. **2014**, 31, 177–184.
8. Fischbach, M.A. Combination therapies for combating antimicrobial resistance. Curr. Opin. Microbiol. **2011**, 14, 519–523.
9. Stapleton, P.; Wu, P.J.; King, A.; Shannon, K.; French, G.; Phillips, I. Incidence and mechanisms of resistance to the combination of amoxicillin and clavulanic acid in Escherichia coli. Antimicrob. Agents Chemother. **1995**,

39, 2478–2483.
10. Rahnama, M.S.;Wagenvoort, J.H.T.; van der Linden, C.J. Amoxicillin/Clavulanate (Augmentin®) resistant Escherichia coli in bacterial peritonitis after abdominal surgery—Clinical outcome in ICU patients. Neth. J. Med. **2009**, 67, 173–176.
11. Xu, J.; Xu, Y.;Wang, H.; Guo, C.; Qiu, H.; He, Y.; Zhang, Y.; Li, X.; Meng,W. Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river. Chemosphere **2015**, 119, 1379–1385.
12. Lau, C.H.F.; Li, B.; Zhang, T.; Tien, Y.C.; Scott, A.; Murray, R.; Sabourin, L.; Lapen, D.R.; Duenk, P.; Topp, E. Impact of pre-application treatment on municipal sludge composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest. Sci. Total Environ. **2017**, 587–588, 214–222.
13. Tien, Y.C.; Li, B.; Zhang, T.; Scott, A.; Murray, R.; Sabourin, L.; Marti, R.; Topp, E. Impact of dairy manure pre-application treatment on manure composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest. Sci. Total Environ. **2017**, 581–582, 32–39.
14. Sinthuchai, D.; Boontanon, S.K.; Boontanon, N.; Polprasert, C. Evaluation of removal efficiency of human antibiotics in wastewater treatment plants in Bangkok, Thailand. Water Sci. Technol. **2016**, 73, 182–191.
15. Kohanski, M.A.; DePristo, M.A.; Collins, J.J. Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. Mol. Cell **2010**, 37, 311–320. Antibiotics **2018**, 7, 73 12 of 14
16. Jørgensen, K.M.; Wassermann, T.; Jensen, P.Ø.; Hengzuang, W.; Molin, S.; Høiby, N.; Ciofu, O. Sublethal ciprofloxacin treatment leads to rapid development of high-level ciprofloxacin resistance during long-term experimental evolution of Pseudomonas aeruginosa. Antimicrob. Agents Chemother. **2013**, 57, 4215–4221.
17. Abraham, W.-R. Megacities as sources for pathogenic bacteria in rivers and their fate downstream. Int. J. Microbiol. **2011**, 2011, 798292.
18. Oliveira, B.; Quinteiro, P.; Caetano, C.; Nadais, H.; Arroja, L.; Ferreira da Silva, E.; Senos Matias, M. Burial grounds' impact on groundwater and public health: An overview. Water Environ. J. **2013**, 27, 99–106.
19. Sofield, C.M. Living with the dead: Human burials in anglo-saxon settlement contexts. Archaeol. J. **2015**, 172, 351–388.
20. Uslu, A.; Bari, E.; Erdo, E. Ecological concerns over cemeteries. Afr. J. Agric. Res. **2009**, 4, 1505–1511.
21. Całkosiński, I.; Płoneczka-Janeczko, K.; Ostapska, M.; Dudek, K.; Gamian, A.; Rypuła, K. Microbiological analysis of necrosols collected from urban cemeteries in Poland. BioMed Res. Int. **2015**, 2015, 169573.

2.4. Required initial knowledge and skills of the PhD candidate

Completed master's studies in the field of biological sciences. Master's thesis in the field of microbiology. Motivation for scientific work, awareness of the need to publish research results and participate in scientific conferences.

2.5. Expected development of the PhD candidate's knowledge and skills

1. Wide knowledge in microbiology
2. Wide knowledge in antibiotic resistans
3. Practical skills in modern techniques used in microbiology
4. Practice in international scientific contacts