“BABEŞ-BOLYAI” UNIVERSITY OF CLUJ-NAPOCA
FACULTY OF BIOLOGY AND GEOLOGY
DOCTORAL SCHOOL IN INTEGRATIVE BIOLOGY

Ph.D. Thesis

Carbapenem resistance in bacterial isolates and communities from municipal wastewaters

Summary

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Key words: antibiotics, antibiotic resistance, resistance genes, carbapenemases, carbapenems, wastewater, antimicrobial activity, bacterial community, wastewater treatment plant

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Introduction

This Ph.D. thesis pursued interdisciplinary methods for monitoring carbapenem resistance in different types of municipal wastewater. This work highlights the importance of monitoring the level of contamination with carbapenem-resistant bacteria and resistance genes (CRGs) in populations using municipal wastewater. The obtained results provide information on the structure and composition of the bacterial community, the abundance of specific carbapenem resistance genes, and the physicochemical parameters of the wastewater. In addition, were analysed possible indicators of wastewater pollution with CRGs.

The structure of the doctoral thesis consists of four main chapters. The first chapter contains literature data on antibiotic resistance and the spread of carbapenem resistance in the environment. The second chapter presents the materials and methods used in research activities, and the third chapter includes the presentation of the results obtained from the experiments within the thesis. Finally, chapter four describes the conclusions generated based on the results obtained.

Research purpose and objectives

The Ph.D. thesis aims to evaluate the possibilities of using municipal wastewater to monitor the abundance of CRGs and some carbapenemases-producing bacteria spread in the communities associated with the investigated wastewater treatment plants. The main objectives of the research activities were the epidemiological investigation of *Klebsiella pneumoniae* isolates and the analysis of microbial diversity and gene abundance for carbapenem resistance in municipal wastewater.

The research activities were carried out in collaboration with: the Environmental Microbiology Laboratory of the Cluj-Napoca Biological Research Institute (ICB Cluj), the Analytical Laboratory of Compania de Apa Someș SA and the Technical University of Dresden, Germany.

1. Literature

The emergence of carbapenem resistance among Gram-negative bacteria is a significant public health concern. These substances are considered last-line agents, and they are used to treat severe infections (Codjoe and Donkor, 2017). The presence of carbapenem-resistant bacteria and CRGs in municipal wastewater is both a sanitary concern and a threat to natural ecosystems. These contaminants are constantly released into the natural environment, particularly in wastewater (Mills and Lee, 2019; Alexander et al., 2020), and recent studies show that resistant bacteria from these ecosystems are transmitted to the population, having a significant impact on public health (Laurens et al., 2018). Despite these facts, no studies have been conducted to date that investigates the true potential of municipal wastewater for monitoring carbapenem resistance genes in relation to microbial diversity in a given ecosystem or in comparison to clinical data on this type of contamination. There are various methods for analyzing microbial diversity or measuring the CRGs abundance in a given habitat. These can be designed dependently or independently of the *in vitro* cultivation of investigated bacteria. Notably, only 1–10% of bacteria are culturable *in vitro*, and this aspect must be taken into consideration when creating an experimental concept (Rozen and Belkin, 2005; Vaz-Moreira et al., 2014). One of the most effective approaches to antimicrobial resistance is to combine several methods. For example, there are three main types of methods used in an epidemiological study of wastewater: *in vitro* cultivation of some bacteria, analysis of the abundance of various genes by the qPCR method, and metagenomic analysis (Manaia et al., 2018). These investigation methods are the basis of the experimental concept of this research.

2. Materials and methods

2.1. Sampling

Raw (influent) and treated (effluent) 24-h-composite wastewater samples were collected monthly for a year (2019–2020) from different wastewater treatment plants (WWTPs), located in the Cluj County, Romania. Epidemiological analysis of *Klebsiella pneumoniae* isolates was performed using wastewater samples from two wastewater treatment plants: S1, which processes approximately 115,000 m^3 of wastewater/24 h coming from 400,000 residents and the city's hospitals, and S2, which processes 864 m^3 of wastewater/24 h originating from 10,000 inhabitants, without having hospital wastewater input. Finally, the microbial diversity and CRGs abundance analysis was done using wastewater samples from three different WWTPs (S1, S2 and S3). S1 processes around 115,000 m^3 of wastewater/24 h from an average of 400,000 inhabitants, S2 receives water from around 20,000 people and can process 3,456 m^3/24 h, and S3 is treating 864 m^3/24 h, from an average of 10,000 inhabitants. Furthermore, besides water from the city, S1 receives wastewater from several hospitals, S2 collects water from a single hospital, and S3 has no hospital input (Teban-Man, et al., 2022).

After sampling, the water samples were processed in the chemical analysis laboratory of Compania de Apa Someș SA and the Environmental Microbiology Laboratory of the Biological Research Institute from Cluj-Napoca.

2.2. Epidemiological investigation of *Klebsiella pneumoniae* isolates

- Carbapenemase activity testing by the mCIM method (modified carbapenem inactivation method)
- Taxonomic identification of *Enterobacteriaceae* isolates
- Phylogenetic analysis of *Klebsiella* isolates
- Molecular investigation of carbapenemases diversity in *Klebsiella pneumoniae* isolates
- Phenotypic and molecular testing of hypervirulence in *Klebsiella pneumoniae* isolates
- Antibiotic susceptibility analysis
- MLST (Multilocus Sequence typing) analysis of *Klebsiella pneumoniae* isolates
- Statistical analyses

2.3. Microbial diversity and CRGs abundance analysis

- Quantification of 16S rRNA gene, using Real-time PCR, in order to determine the total bacterial abundance in the collected WWTP samples and for the normalization of CRG abundance data
- Quantification of several carbapenemase genes (*bla*KPC, *bla*NDM, *bla*OXA-48, *bla*VIM and *bla*IMP), using Real-time PCR method
- Microbial community diversity analysis (NGS sequencing)
- Statistical analyses

3. Results and discussions

3.1. Epidemiological investigation of *Klebsiella pneumoniae* isolates

After phenotypical analyses, 74 presumptive carbapenemase-producing *K. pneumoniae* strains were isolated from the influent (I) and effluent (E) of two wastewater treatment plants over a period of six months: S1—received input from multiple hospitals; S2—had no hospital input. A total of 52 isolates (70%) showed positive carbapenemase activity after the mCIM test, 41 (79%) from I and 11 (21%) from E. The 52 sequences derived from this study fell into the cluster of *Klebsiella pneumoniae sensu stricto* (Kp1), a common inhabitant of wastewater (Muller et al., 2018; Alexander et al., 2020). The molecular screening by polymerase chain reaction (PCR), targeting the presence of specific CRGs (*bla*KPC, *bla*OXA-48, *bla*NDM and *bla*VIM), showed that 33 (63%) out of the 52 isolates possess at least one CRG, while 19 (37%) most likely have different carbapenem hydrolysis determinants (Teban-Man et. al., 2021).

Samples from station S1, that receives hospital wastewaters, contained all types of investigated CRGs, in both influent and effluent. It is worth mentioning that all four tested CRGs were present in S2 isolates as well, even if no hospital input is associated, with the exception that only *bla*KPC was observed in the S2 effluent (Fig. 1) (Teban-Man et. al., 2021).

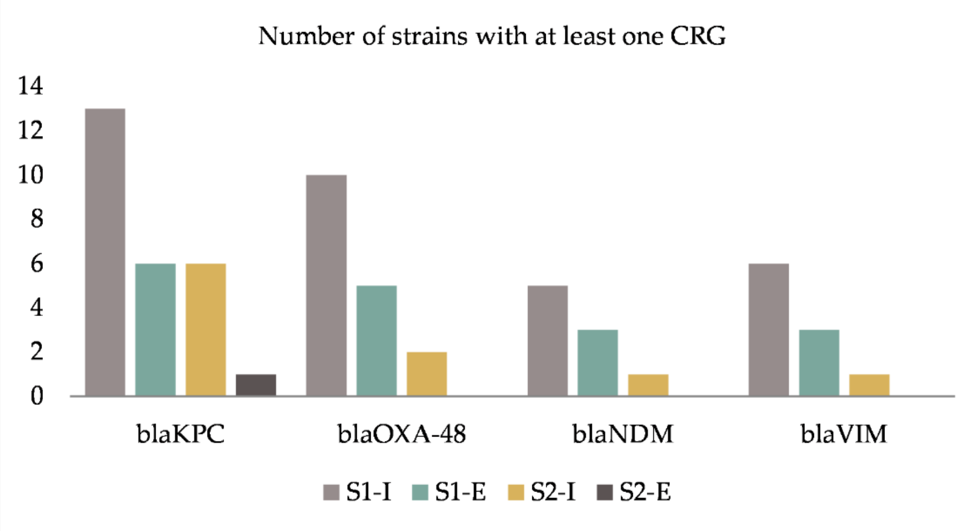

Fig. 1. The distribution of CRG types in *K. pneumoniae* isolates from the investigated wastewaters. S1, S2 - WWTPs, I-influent, E-effluent (Teban-Man et. al., 2021).

MLST analysis revealed seven types of STs in the 33 *K. pneumoniae* isolates. The most frequent clone was ST101 (14 isolates), followed by ST258 (eight isolates), observed in both untreated and treated wastewater. With a lower incidence (one-two isolates), ST525, ST744, ST2505, ST147, and ST418 were also identified (Teban-Man et al., 2021).

According to antibiotic susceptibility analysis, all *K. pneumoniae* isolates, from both influent and effluent wastewater, showed an MDR profile (resistance to at least three different antibiotics), in agreement with Magiorakos et al., 2012, due to resistance to the following classes of antibiotics: β-lactams (ampicillin-sulbactam, piperacillin-tazobactam), cephalosporins (cefuroxime, cefoxitin, cefotaxime, ceftazidime, ceftaroline), carbapenems (imipenem), monobactams (aztreonam) and fluoroquinolones (ciprofloxacin). A lower percentage of resistance was identified for aminoglycosides: 94% (31/33 isolates) amikacin and 67% (23/33 isolates) gentamicin; 94% (31/33 isolates) trimethoprim-sulfamethoxazole; 73% (24/33 isolates) tigecycline, 64% (21/33) chloramphenicol and 64% (21/33) tetracycline. It is important to note that all strains isolated from treated wastewater (E), regardless of the treatment plant, are 100% resistant to all tested antibiotics. These antibiotic-resistant bacteria will eventually reach surface waters, thus putting public health at risk (Teban-Man et al., 2021).

Finally, statistical analyses were performed to compare the relative frequencies of antibiotic resistance and CRGs (Fig. 2). Regarding the CRGs, *bla*KPC-2 and *bla*OXA-48 are the most usually found CRGs in *K. pneumoniae* isolated from the two wastewater types, while *bla*NDM-1 and *bla*NDM-6 are the least common. Thus, the wastewater treatment process helps with the reduction of antibiotic-resistant bacteria by selecting multidrug-resistant strains without affecting the CRG profile. Therefore, according to the Chi-square test (Fischer's exact test), the differences between CRGs frequencies are not statistically significant, regardless of the antibiotic resistance profile (Teban-Man et al., 2021).

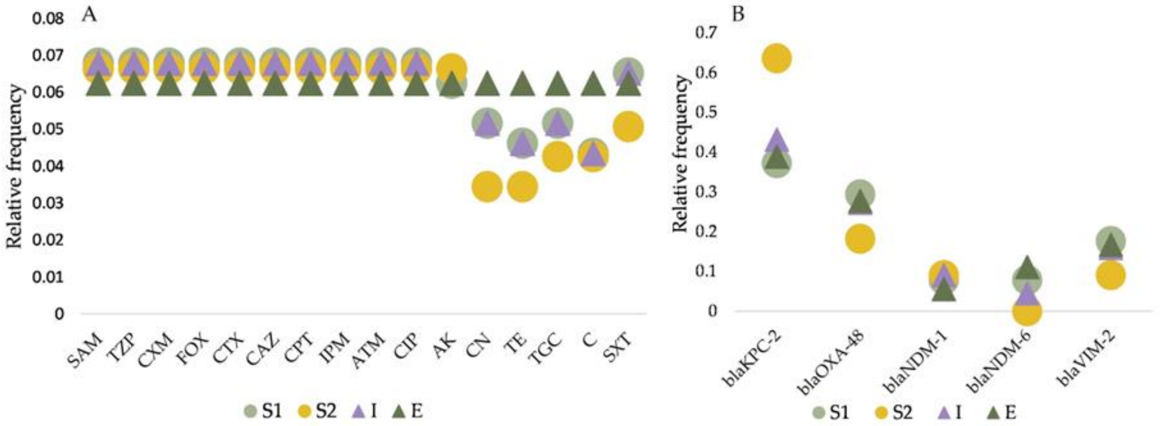

Fig. 2. Relative frequency of antibiotic resistance (A) and carbapanemase resistance genes (B) in *K. pneumoniae* Kp1 isolated from wastewaters. S1 = wastewaters with hospital input; S2 = wastewaters without hospital input; I = wastewater influent; E = wastewater effluent; SAM = ampicillin-sulbactam; TZP = piperacillin-tazobactam; CXM = cefuroxime; FOX = cefoxitin; CTX = cefotaxime; CAZ = ceftazidime; CPT = ceftaroline; IPM = imipenem; ATM = aztreonam; CIP = ciprofloxacin; AK = amikacin; CN = gentamycin; TE = tetracycline; TGC = tigecycline; C = chloramphenicol; SXT = trimethoprim-sulfamethoxazole (Teban-Man et. al., 2021).

3.2. Microbial diversity and CRGs abundance analysis

The bacterial diversity was assessed based on operational taxonomic unit (OTU) clustering and revealed a total of 7,138 OTUs, with little differences between bacterial communities from S1 and S2. Therefore, as both these WWTPs receive communal and hospital wastewaters, they were grouped in the hospital influent/effluent (H-I/H-E) categories. In contrast, S3 samples were classified as non-hospital influent/effluent (N-I/N-E), as this treatment plant does not receive hospital sewage. When groups and wastewater types (influent and effluent) were compared (ANOSIM test), either significant differences or differences with some similarities were observed. However, in the case of overall H versus N and H-I versus N-I, high levels of similarity were observed (R = 0.231, P, 0.01and R = 0.193, P, 0.05, respectively), emphasizing that the presence of hospital wastewaters in WWTPs had no significant influence on the overall microbial community composition, an observation previously also made by Sorgen et al. (2021). Also, the absolute and relative abundance of different groups showed an even distribution of CRGs among all the tested wastewater samples (NMDS analysis based on ANOSIM test) (Fig. 3). As in the case of microbial diversity, we can conclude that the presence of hospital sewage does not influence the abundance of CRGs in communal wastewaters (Teban-Man et. al., 2022).

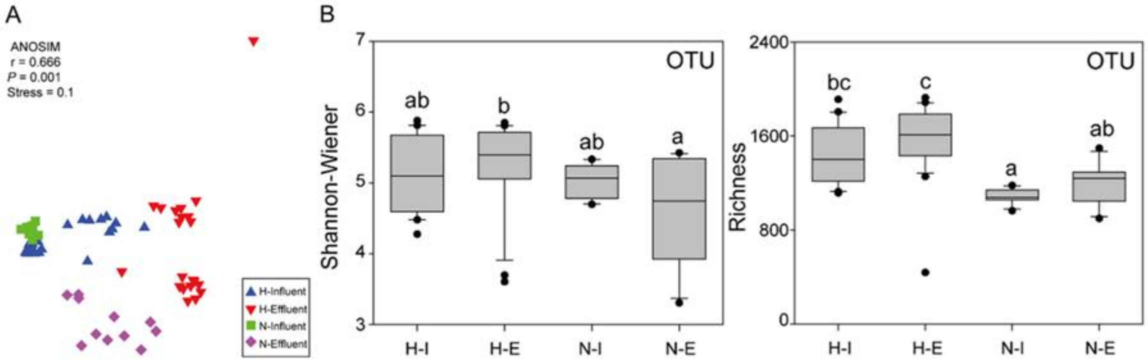

Fig. 3. (A) Nonmetric multidimensional scaling (NMDS) ordination of bacterial communities based on Bray-Curti's similarity. (B) The comparison of bacterialcommunity diversity indices for different groups. H-I, hospital influent; H-E, hospital effluent; N-I, non-hospital influent; N-E, non-hospital effluent. Different letters (a, b, c) above bars indicate significant difference at P, 0.05 based on nonparametric Kruskal-Wallis tests. (Teban-Man et. al., 2022).

The results of taxonomic analysis showed an average of 37 phyla, 78 classes, 201 orders, and 317 bacterial families commonly found in all wastewater samples. The main bacteria phyla (>1% of total reads) were *Proteobacteria* (29%), *Firmicutes* (21%), *Actinobacteria* (19%), *Patescibacteria* (9%), and *Bacteroidetes* (5%) (Fig. 4). Although these bacterial taxa were present in all the wastewater samples, some differences in terms of frequency among the investigated groups was observed. *Proteobacteria* and *Firmicutes* were more abundant in the N type sequencing libraries (40% and 27%, respectively) compared to H wastewater (24% and 17%, respectively). In the latter, *Actinobacteria*, *Patescibacteria*, and *Chloroflexi* were more prevalent (22%, 11%, and 5%, respectively) (Fig. 4). Overall, seasonal variation had a minimal impact on microbial diversity, except for a slight increase for

Proteobacteria and *Patescibacteria* during winter and *Actinobacteria* in the summer (Fig. 4) (Teban-Man et. al., 2022).

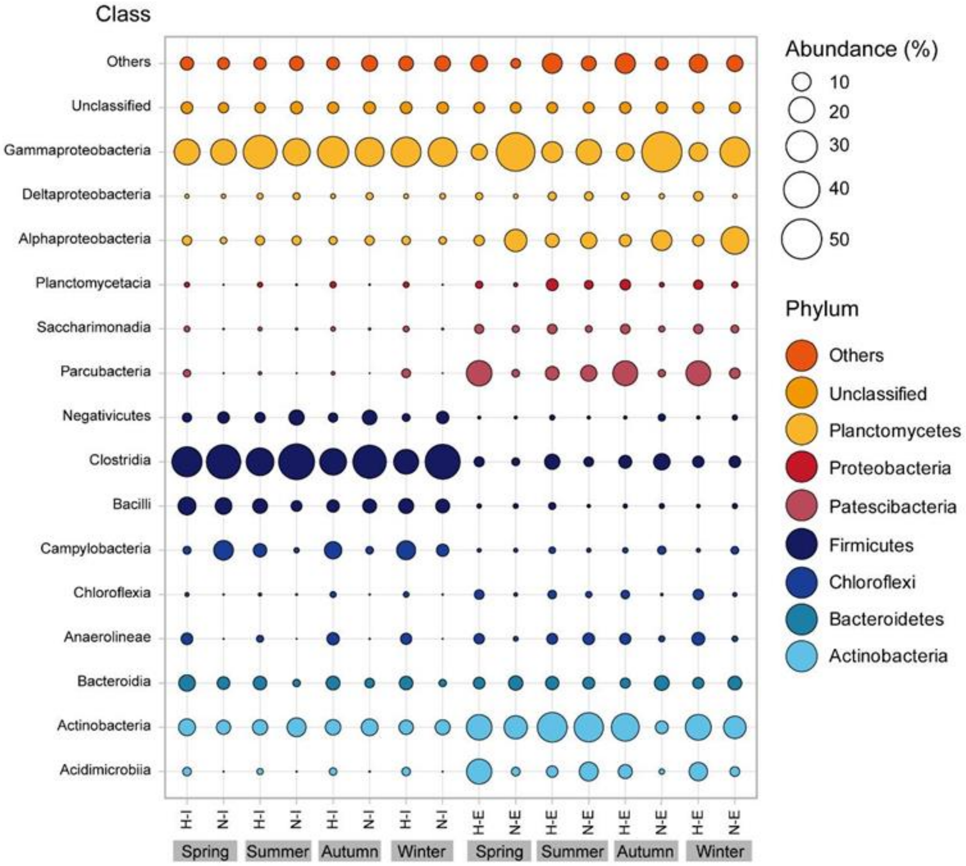

Fig. 4. Class level seasonal breakdown of the relative abundances of bacterial taxonomic groups. Classes belonging to the same phyla are represented by the same color. Bubble size corresponds to the relative abundance, and only major taxonomic groups (.1% total abundance) were included in the graphic. H-I, hospital influent; H-E, hospital effluent; NI, non-hospital influent; N-E, non-hospital effluent. Phyla and classes with abundances ,1% are designated as "Others (Teban-Man et. al., 2022).

Among the predominant bacterial families, some important pathogenic taxa were identified. For example, *Clostridium perfringens*, *Escherichia-Shigella*, *Enterococcus* spp., and *Streptococcus* spp. were observed. These are commonly found in human-impacted water habitats and are considered pollution indicators (Grøndahl et. al., 2014; Hussain, 2019). *Aeromonas* and *Pseudomonas* included in the WHO AMR priority pathogens list (Fouz et. al., 2020) are considered environmental bacteria susceptible to developing antibiotic resistance (Numberger et. al., 2019). The presence of *Legionella*, *Leptospira*, and *Mycobacterium* genera in the wastewaters could represent a potential health risk once they enter the receiving rivers, as they are considered dangerous waterborne pathogens. Although less abundant, *Serratia marcescens* and *Bacillus* spp. may be used as indicator taxa for cadmium (Cd), lead (Pb), pesticides, and detergent contamination (Hussain, 2019).

Regarding the comparative analyses of the relative and absolute abundance of CRGs, it was observed that abundance of the 16S rRNA gene decreased significantly after the wastewater treatment for both types of water (H and N). With a similar effect on the relative abundance, the *bla*KPC gene decreased after water treatment, but it shows a significant difference between groups being more abundant in H-type waters. The relative abundance of the *bla*VIM, *bla*NDM, and *bla*OXA-48 genes remains constant throughout the wastewater treatment. Except for the *bla*NDM gene, which was found predominantly in H-type samples, there are no significant differences between the tested groups. The *bla*IMP gene shows a significantly higher relative abundance in treated than untreated waters for both H and N samples (Fig. 5) (Teban-Man et al., 2022).

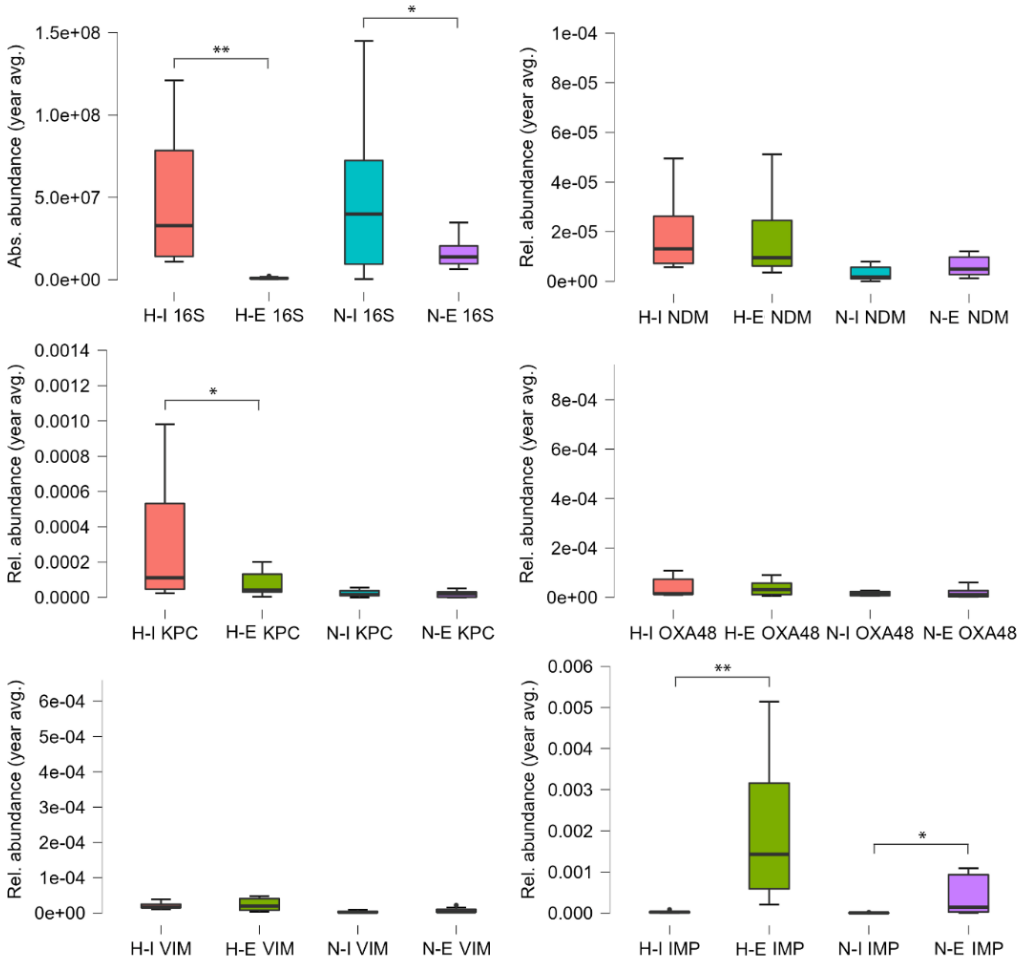

Fig. 5. Comparative analysis (yearly average) of absolute (16S rRNA gene) and relative (target CRG copies/16S rRNA gene copies) gene abundances in the influent and effluent of hospital and non-hospital type WWTPs. H-I, hospital influent; H-E, hospital effluent; N-I, non-hospital influent; N-E, non-hospital effluent. NDM, *bla*NDM; KPC, *bla*KPC; OXA48, *bla*OXA-48; VIM, *bla*VIM; IMP, *bla*IMP. *, P, 0.05; **, P, 0.01 (Teban-Man et. al., 2022).

The positive correlations between different OTUs and CRGs underlined that *bla*IMP is the most frequent gene associated with several bacterial groups within *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Patescibacteria*, *Planctomycetes*, and *Verrucomicrobia* in the investigated wastewaters. The strong association with representatives of the *Actinobacteria* phylum, especially the *Mycobacterium* genus, which had a significant presence in the effluent, might be responsible for the increased number of *bla*IMP gene copies in the treated wastewaters. The second most frequent CRG family was *bla*NDM, which was positively correlated with taxa belonging to *Acinetobacter*, *Flavobacterium*, *Moraxella*, and *Streptococcus* genera. *Bla*KPC gene was correlated with *Aeromonas* and the *bla*OXA-48 gene with *Arcobacter* and the *Acinetobacter* genus. Also, some CRGs were positively associated with some physico-chemical parameters of wastewater such as: the 16S rRNA gene with TSS, *bla*OXA-48 with CBO and HEM, *bla*NDM with TSS, etc. (Teban-Man et al., 2022).

Finally, it was attempted to monitor the presence of CRG in the human population using WBE methods. Thus, for *bla*NDM, *bla*KPC, and *bla*VIM genes, both types of wastewaters (H and N) followed a similar temporal pattern throughout the year. The *bla*KPC and *bla*NDM genes are more prevalent during the cold months (early fall to late winter), while the *bla*VIM gene is more prevalent during the warm months (spring and summer). In warm months, the *bla*OXA-48 gene had a low number of copies/1PE, with their abundance increasing from mid-autumn to winter, especially in N-type samples. The *bla*IMP group appeared in elevated values during spring, late autumn, and winter months. The highest copies/1PE were identified for *bla*KPC and *bla*OXA-48, with the difference that H-I was dominated by, while *bla*OXA-48 was predominant in N-I.

4. Conclusions

In conclusion, the result of the research activity of this Ph.D. thesis highlighted the importance and potential of using urban wastewater to monitor carbapenem resistance in a general population associated with the tested treatment plants. Valuable scientific data on carbapenemase-producing bacteria, such as multi-resistant *Klebsiella pneumoniae*, were also obtained. The structure and composition of bacterial communities were characterized, and specific carbapenem resistance genes were quantified and analyzed for possible positive correlations with wastewater physico-chemical parameters. As a result, the collected data is a valuable source of information that can serve as a foundation for future research aimed at reducing carbapenem resistance in the population.

Disemination

Articles published in international ISI journals, as first author (from the subject of the Ph.D. thesis)

Teban-Man, A., Szekeres, E., Fang, P., Klümper, U., Hegedus, A., Baricz, A., Berendonk, T.U., Pârvu, M., Coman, C., Municipal wastewaters carry important carbapenemase genes independent of hospital input and can mirror clinical resistance patterns, Microbiology Spectrum, 2022, 2, 10, https://doi.org/10.1128/spectrum.02711-21
IF 7.171 (2020)

Teban-Man, A., Farkas, A., Baricz, A., Hegedus, A., Szekeres, E., Pârvu, M., Coman, C, (2021), Wastewaters, with or without hospital contribution, harbour MDR, carbapenemase-producing, but not hypervirulent *Klebsiella pneumoniae*, Antibiotics, 2021, 10, 361, https://doi.org/10.3390/antibiotics10040361
IF 3.893 (2019)

Articles published in international ISI journals, as author with equal contribution with the first author

Baricz, A., **Teban, A.,** Chiriac, C.M., Szekeres, E., Farkas, A., Nica, M., Dascălu, A., Oprișan, C., Lavin, P., Coman, C., (2018). Investigating the potential use of an Antarctic variant of *Janthinobacterium lividum* for tackling antimicrobial resistance in a One Health approach, Scientific Reports, 8, 15272, https://doi.org/10.1038/s41598-018-33691-6, **IF** 4.011 (2018)

Articles published in international ISI journals, as co-author

Farkas, A., Coman, C., Szekeres, E., **Teban-Man, A.,** Carpa, R., Butiuc-Keul, A, Molecular typing reveals environmental dispersion of antibiotic-resistant Enterococci under anthropogenic pressure, Antibiotics, 2022, 11, 1213, doi: 10.3390/antibiotics11091213,

IF 4.639 (2022)

Research project member:

Biodiversity as an ecological barrier for the spread of clinically relevant antibiotic resistance in the environment (ANTIVERSA) contract 117/2020, 01/02/2020 - 31/12/2022, ERANET-BIODIVHEALT-ANTIVERSA PNCDI III, Institutul de Cercetări Biologice Cluj-Napoca, director de proiect Dr. Cristian Coman.
https://tu-dresden.de/bu/umwelt/hydro/hydrobiologie/limnologie/forschung/projekte/antiversa

Scientific communications:

Teban-Man, A., Farkas, A., Baricz, A., Hegedus, A., Szekeres, E., Pârvu, M., Coman, C., Molecular epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in wastewaters, 7th Edition of BIOTA Symposium: Biodiversity, Traditions and Current Affairs, 2021, Romania

Bibliografie

Alexander, J., Hembach, N., Schwartz, T., Evaluation of antibiotic resistance dissemination by wastewater treatment plant effluents with different catchment areas in Germany, Scientific Reports, 2020, 10, 8952, doi: 10.1038/s41598-020-65635-4

Codjoe, F. S., Donkor, E.S., Carbapenem resistance: a review, Journal of Medical Sciences, 2017, 6, 1, doi: 10.3390/medsci6010001

Fouz, N., Pangesti, K.N.A., Yasir, M., Al-Malki, A.L., Azhar, E.I., Hill-Cawthorne, G.A., Abd El Ghany, M., The contribution of wastewater to the transmission of antimicrobial resistance in the environment: implications of mass gathering settings, Tropical Medicine and Infectious Disease, 2020, 5, 33, doi: 10.3390/tropicalmed5010033

Grøndahl-Rosado, R.C., Tryland, I., Myrmel, M., Aanes, K.J., Lucy, J., Robertson detection of microbial pathogens and indicators in sewage effluent and river water during the temporary interruption of a wastewater treatment plant, Water Qual Expo Health, 2014, 6, 155–159, doi.org/10.1007/s12403-014-0121-y

Hussain, Q.A., Bacteria: the natural indicator of environmental pollution, Freshwater Microbiology, 2019, 393-420, doi: org/10.1016/B978-0-12-817495-1.00010-4

Laurens, C., Jean-Pierre, H., Licznar-Fajardo, P., Hantova, S., Godreuil, S., Martinez, O., Jumas-Bilak, E., Transmission of IMI-2 carbapenemase-producing *Enterobacteriaceae* from river water to human, J Glob Antimicrob Resist, 2018, 15, 88-92, doi: 10.1016/j.jgar.2018.06.022

Manaia, C.M., Rocha, J., Scaccia, N., Marano, R., Radu, E., Biancullo, F., Cerqueira, F., Fortunato, G., Iakovides, I.C., Zammit, I., Kampouris, I., Vaz-Moreira, I., Nunes, O.C., Antibiotic resistance in wastewater treatment plants: Tackling the black box, Environmental Internation, 2018, 115, 312-324, doi: 10.1016/j.envint.2018.03.044

Mills, M.C., Lee, J., The threat of carbapenem-resistant bacteria in the environment: Evidence of widespread contamination of reservoirs at a global scale, Environmental Pollution, 2019, 255, 113143, doi: 10.1016/j.envpol.2019.113143

Müller, H., Sib, E., Gajdiss, M., Klanke, U., Lenz-Plet, F., Barabasch, V., Albert, C., Schallenberg A., Timm, C., Zacharias, N., Schmithausen, R.M., Engelhart, S., Exner, M., Parcina, M., Schreiber, C., Bierbaum, G., Dissemination of multi-resistant Gram-negative bacteria into German wastewater and surface waters, FEMS Microbiology Ecology, 2018, 94, doi: 10.1093/femsec/fiy057

Numberger, D., Ganzert, L., Zoccarato, L., Mühldorfer, K., Sauer, S., Grossart, H.P., Greenwood, A.D., Characterization of bacterial communities in wastewater with enhanced taxonomic resolution by full-length 16S rRNA sequencing, Scientific Reports, 2019, 9, 9673, doi: 10.1038/s41598-019-46015-z

Rozen, Y., Belkin, S., Survival of enteric bacteria in seawater: molecular aspects. In:Oceans and Health: Pathogens in the Marine Environment, 2015, 93–107, doi: 10.1007/0-387-23709-7_4.

Sorgen, A., Johnson, J., Lambirth, K., Clinton, S.M., Redmond, M., Fodor, A., Gibas, C., Characterization of environmental and cultivable antibiotic-resistant microbial communities associated with wastewater treatment, Antibiotics, 2021, 10, 352, doi: 10.3390/antibiotics10040352

Teban-Man, A., Farkas, A., Baricz, A., Hegedus, A., Szekeres, E., Pârvu, M., Coman, C., Wastewaters, with or without hospital contribution, harbour MDR, carbapenemase-producing, but not hypervirulent *Klebsiella pneumoniae*, Antibiotics, 2021, 10, 361, doi:10.3390/antibiotics10040361

Teban-Man, A., Szekeres, E., Fang, P., Klümper, U., Hegedus, A., Baricz, A., Berendonk, T.U., Pârvu, M., Coman, C., Municipal wastewaters carry important carbapenemase genes independent of hospital input and can mirror clinical resistance patterns, Microbiology Spectrum, 2022, 2, 10, doi: 10.1128/spectrum.02711-21

Vaz-Moreira, I., Nunes, O.C., Manaia, C.M., Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome, FEMS Microbiol. Rev., 2014 38, 761–778, doi: 10.1111/1574-6976.12062.