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MICROBIAL CHARACTERIZATION OF A RECREATIONAL POND

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Introduction

Swimming in natural water can expose swimmers to health risks, if the water quality is microbiologically poor. Water quality could be affected by anthropic activities but also by the microorganisms associated with the natural aquatic flora and animals. Although there are regulations regarding the bacterial load from natural waters, there are other important parameters such as antibiotic microbiological resistance and virulence which could generate environmental and humans health issues. It is important to analyze an overall picture composed from the microbiological load to their antibiotic resistance and virulence.

Materials and methods

Water samples were taken during August-September 2024, in Bucharest, Romania from two locations, a pond (used for recreational swimming), and a well that replenishes it. All samples were microbiologically analysed in order to quantify the bacterial load and to characterize antibiotic resistance and virulence factors of the isolated strains. *Quantification and isolation*: the bacterial load [coliforms, *Escherichia coli (E.coli)* and enterococci] was quantified according to ISO 9308-2:2014 and 7899-2:2022 by using a membrane filtration technique. Beside the media requested by the standards, samples were seeded on manitol salt agar, pseudomonas agar and acinetobacter agar in order to detect and isolate *Staphylococcus, Pseudomonas* and *Acinetobacter*. *Identification*: bacterial species isolated from culture media were identified using MALDI-TOF technique. *Antibiotic resistance: E. coli* and *Pseudomonas aeruginosa (P. aeruginosa)* strains were tested for their antimicrobial resistance to antibiotics through the Kirby-Bauer technique [tested antibiotics were ampicillin (AMP), amoxicillin-clavulanate (AMC), cefazolin (KZ), cefepime (FEP), ceftriaxone (CRO), cefoxitin (FOX), aztreonam (ATM), imipenem (IPM), tetracycline (TE), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT) for *E. coli* and piperacillin (PIP), ceftazidime (CAZ), FEP, ATM, IPM, meropenem (MEM), tobramycin (TOB), amikacin (AK), CIP for *P. aeruginosa*]. *Virulence characterization*: The pathogenic potential and the virulence of the isolated strains was evaluated using agars with different substrata: lipids metabolism was determined analysing the expression of amylases

(agar with starch), esterase (agar with Tween 80) and lecithinase (agar with egg york); proteases presence was observed using agar with milk for caseinases and gelatin for gelatinases, bacterial DN-ases were detected using DNA agar while siderophores were evaluated through esculin hydrolyses. Biofilm production was assessed by crystal violet staining assay.

Results and conclusions

Microbiological analyses of the water samples collected classified the water as suitable for swimming, the bacterial load being in accordance with the legal criteria (Romanian HG546/2008). Our MALDI-TOF study identified 57 species, most of them belonging to genus *Acinetobacter, Enterobacter, Enterococcus, Escherichia and Pseudomonas*. There were no significat differences among the samplig points regarding the detected species, some of the bacteria identified in the well were not recovered in the pond, for instance *Acinetobacter baumannii, A. calcoaceticus, Aeromonas eucrenophila, A. media, Enterobacter asburiae, E. cloacae, E. ludwigii, Glutamicibacter mysorens,* while others were isolated from all the sampling points (*Acinetobacter junii, A. pittii, Citrobacter braakii, C. freundii, E. coli* and *P. aeruginosa)*. The *E. coli* strains were 100% susceptible to cephepim and imipenem. We noticed some differences between the sampling points, the presence of amoxicillin, aztreonam, ciprofloxacin and trimethroprim-sulfamethoxazole resistance only in the pond isolated strains. Regarding the virulence factors for *E. coli,* all strains presented enzymes for starch hydrolasys and proteases. The rest of the virulence factors were detected to a lesser extent. The *P. aeruginosa* strains were sensitive to all the tested antibiotics and provided positive reactions to the phenotypical testing of the virulence factors with no differences between the sampling points. Beside the already mentioned species, 104 strains (most of them Gram negative bacilli belonging to *Enterobacteriaceea)* have been tested for the phenotypic expression of enzymes that can act as virulence factors and was observed that most of the strains can hydrolase proteins and esculin (Figure 1). The strains selected in the study proved to be weak biofilm producers, which can be explained by the fact that sampled bacteria were free floating in the natural water body.

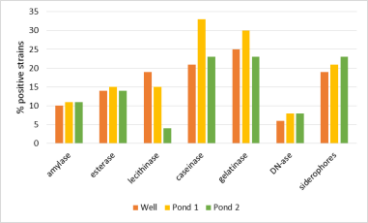

Figure 1. Percentage of strains that express phenotypically enzymes associated with virulence factors

The analyzed pond was characterized by a diverse microbiome, meeting the requirements for fecal indicators, with strains susceptible to antibiotics that do not endanger swimmers' health.