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# POST-PANDEMIC EFFECTS ON FAECAL POLLUTION OF AQUATIC SYSTEMS

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#### Introduction

Hospitals are hotspots for antimicrobial resistant bacteria and play a major role in both their emergence and spread, respectively. Large numbers of these microorganisms will be released form hospitals into natural emisaries via wastewater systems. Effluents of wastewater treatment plants (WWTP) could represent an important source of pathogens of the aquatic environments. Generally, wastewater treatment plants collect various amounts of municipal watewater including from hospital discharges depending on the size and type of communities connected to a single seweage system. Hospital wasterwater is quite different from the domestic wastewater discharges due to its hazardous and infectious potential. In hospitals, up to one third of patients receive antibiotic therapy on any given day and consequently hospitals may be important hubs for the emergence and spread of antibiotic resistance genes, especialy Gram-negative pathogens. It is known that the period of 2019-present was afected by the SarsCov-2 pandemic when many antiseptics and disinfectants were used, especially in the medical field. The continued exposure to antibiotics and biocides in different settings may result an increasing trend of resitance or co-resistance bacterial presence in wastewater. The latter fact may result in maintaining the density of bacteria with insignifiant changes based on the selection of microorganisms under pressure factors. Bacterial indicators of fecal contamination are present in wastewater and their variation in density and resistance profile could provide information about the that antibiotics and biocides impact on the environment.

### Materials and methods

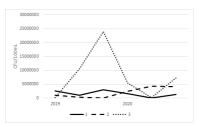
In order to highlight the impact of the pandemic phenomenon, especially on the wide use of antibiotics and biocides on bacterial communities, 3 Romanian interest regions were studied during 2019-2020: SE, NV and NE. Every year, three wastewater sampling campaigns were organized from Covid-19 hospitals and WWTPs. The effluents were subjected to quantitative analysis for the determination of fecal coliform bacteria by the MPN (Most Probable Number) method using Colilert-18 medium (Idexx). The samples were collected in sterile containers with a volume of 1L and transported in maximum 24 hours to the laboratory in isothermal boxes at a temperature of 4-5°C. After sowing, the samples were incubated at 37°C for 20 hours, then the result was interpreted according to the Idexx table.

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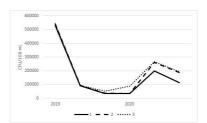
The positive control (*Escherichia coli ATCC25922*, *Citrobacter freundii* ATCC 8090 and *Enterobacter aerogenes ATCC 13048*) and the negative one (*Enterococcus faecalis ATCC 29212*) were tested. In the same time, a blank control with sterile distilled water was analyzed.

## Results and conclusions

The beginning of the pandemic period did not have a major impact on hospitals, the results indicating low values of fecal bacteria density in all three points analysed. The evolution of the pandemic phenomenon triggered an increase of CFU / 100 mL in hospitals, having the highest value at  $24 \times 10^6$  CFU/100 mL in the mid-2019 in point 3 (Figure 1). This fact is also backed-up by a large number of cases positives to Covid-19 reported at that time in the North-Eastern part of Romania.



**Fig.1.** Density (CFU/100 mL) of faecal coliform bacteria in 3 Romanian hospitals effluents in 2019-2021. Legend: 1- SE Romanian site; 2-NV Romanian site; 3-NV Romanian site.



**Fig.2.** Density (CFU/100 mL) of faecal coliform bacteria in 3 effluents Wastewater Treatment plants from Romania in 2019-2021.

Legend: 1- SE Romanian site; 2-NV

Legend: 1- SE Romanian site; 2-NV Romanian site; 3-NV Romanian site.

Hospital effluents did not show high densities of coliform bacteria at the beginning of the pandemic, but the values of these indicators were high (an average of over  $5 \times 10^5$  CFU/100mL) in the WWTP. With the evolution of the pandemic, the treatment plants have streamlined their treatment processes, so that by the end of 2019 the densities of coliform bacteria remained around  $5 \times 10^4$  CFU/100mL. While in mid-2020 the density of coliform bacteria decreased in hospital effluents, an increase can be observed in WWTP (Figure 2). These results could be explained by the contribution of massive municipal discharges during the quarantine period.

In conclusion, the inversely proportional distribution of the densities of coliform bacteria in the hospital compared to WWTP, could be explained by the action of biocides and antibiotics on the microorganisms in the hospital environment that determined their decrease in wastewater.

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