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ANTIBIOTIC RESISTANCE ANALYSIS OF BACTERIA ISOLATED FROM DRINKING WATER DISTRIBUTION SYSTEMS

AUTHORS

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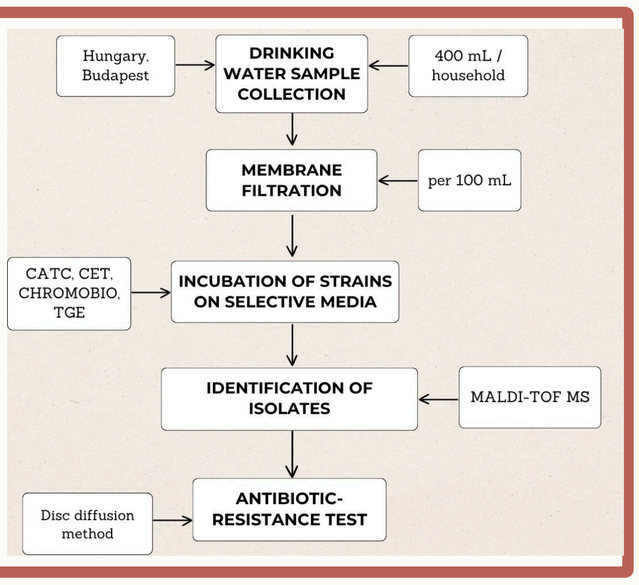
INTRODUCTION

Despite the fundamental importance of safe and regulated drinking water for human health, access to such resources remains insufficient in many regions. Waterborne bacterial infections, notably gastroenteritis, continue to represent a major source of preventable morbidity and mortality, particularly among populations lacking access to clean water. The emergence of pathogenic bacteria within drinking water distribution systems (DWDS) is an increasingly pressing public health concern. In recent years, particular attention has been directed toward the presence of antibiotic-resistant bacteria (ARB) in treated drinking water, raising further questions about microbial safety.

This project set out to examine the bacterial composition of drinking water distribution systems in households across Hungary. A key focus was identifying antibiotic resistance in these bacteria, with special attention given to opportunistic pathogenic bacteria. Furthermore, the project sought to uncover any potential correlations between these findings and other influencing factors.

MATERIAL AND METHODS

Drinking water samples (400 mL) were collected from households in five Hungarian municipalities and eight districts of Budapest. Samples were filtered in 100 mL portions, and filters were incubated for 48 hours on four selective media. Bacterial colonies were identified using MALDI-TOF MS, and 26 isolates were selected for antimicrobial susceptibility testing against nine antibiotics. The Kirby–Bauer disk diffusion method was applied on three agar media, with resistance determined by inhibition zone diameters.



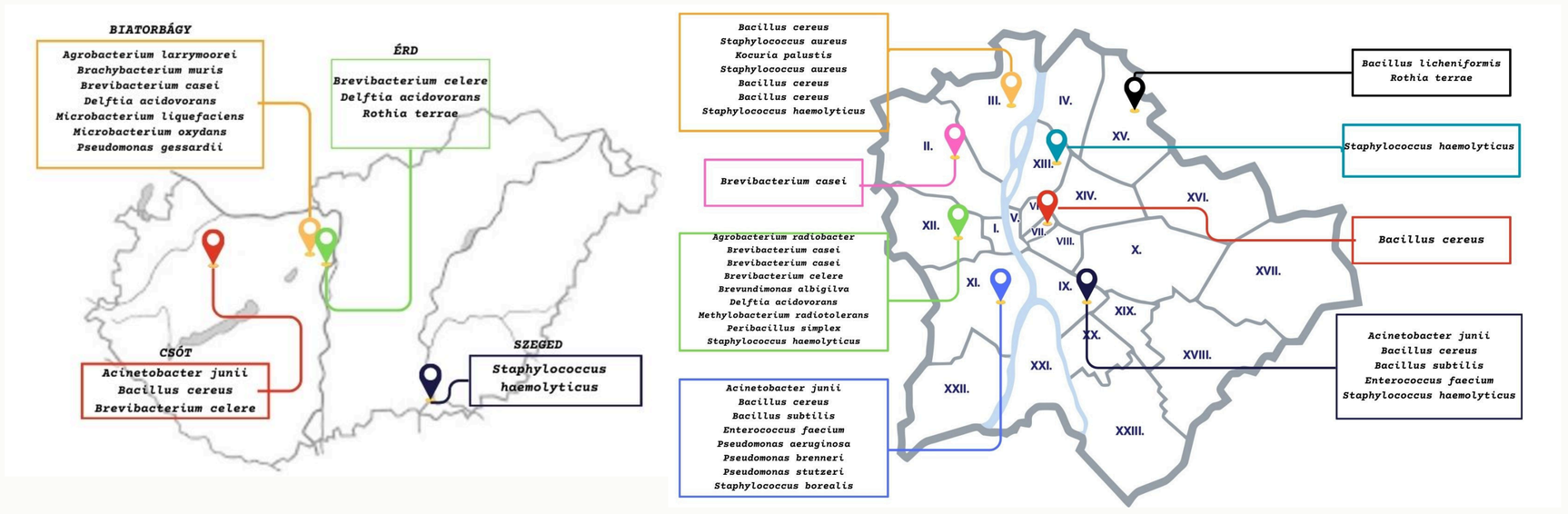


Figure 1 Geographical distribution of bacterial isolates detected in drinking water across Hungary (left), including Budapest (right).

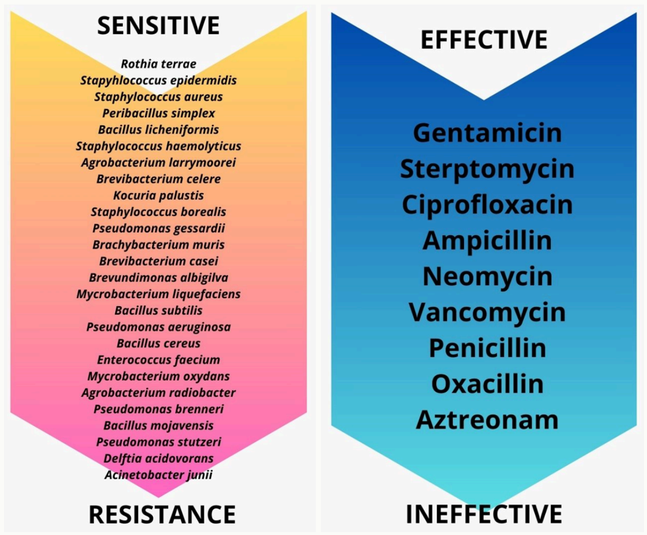


Figure 2 The sequence of bacteria isolated from drinking water is presented in order of sensitivity and resistance (left). The efficacy of the antibiotics tested was ranked in order of effectiveness (right)

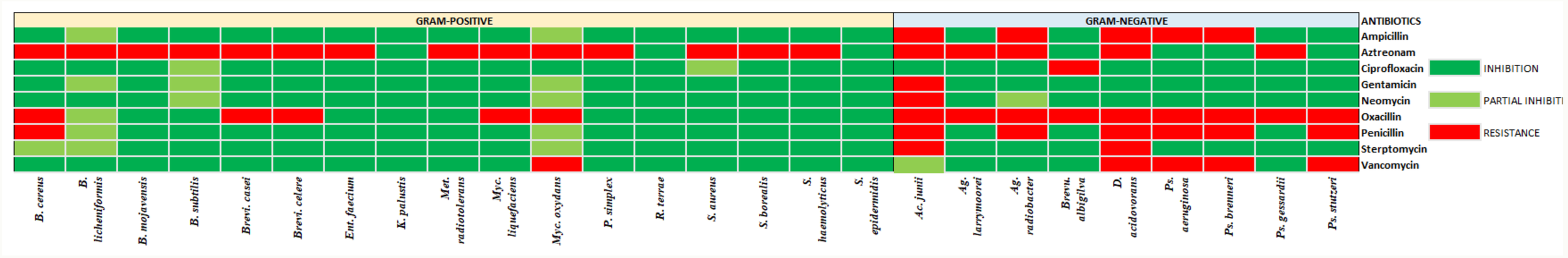


Figure 3 The inhibition, partial inhibition and resistance to antibiotics of all isolated bacteria

RESULTS AND DISCUSSION

A total of 26 bacterial strains were identified from water samples collected across Hungary, including Budapest. Their geographical distribution is presented in **Figures 1**. Of the strains identified, 67% were Gram-positive and 33% were Gram-negative.

Based on the results of antibiotic susceptibility testing—conducted on 3 different culture media, which showed no substantial differences—a consolidated rank order was established for the bacterial strains, ranging from most sensitive to most resistant (**Figure 2**). **Figure 3** illustrates the antibiotic sensitivity and resistance profiles of all isolates. Among the antibiotics tested, gentamicin and streptomycin proved to be the most effective, as nearly all strains showed clear inhibition zones. The most resistant strain was Acinetobacter junii, a Gram-negative opportunistic pathogen commonly associated with DWDS.The 3 most resistant bacteria were ***Acinetobacter junii***, ***Delftia acidovorans***, and ***Pseudomonas stutzeri***—all Gram-negative, opportunistic pathogens previously detected in DWDS (Giao et al., 2011; Douterelo et al., 2014; Zhao et al., 2014; Chen et al., 2017; Liu et al., 2017; Jing et al., 2021).

Gentamicin proved to be the most effective antibiotic, producing inhibition zones in 25 out of 26 bacterial isolates, with an average diameter of 11 mm (**Figure 4**). In contrast, **Aztreonam** showed the highest level of bacterial resistance (**Figure 5**); only 8 isolates exhibited inhibition zones, with an average diameter of 2 mm. This observation aligns with the general trend that Gram-negative bacteria tend to exhibit higher resistance to antibiotics.

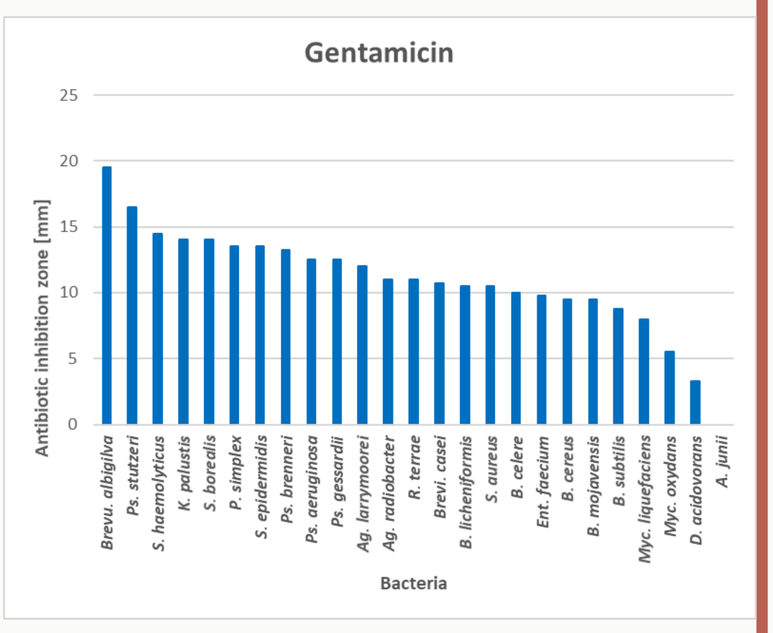


Figure 4 The size of the inhibition zone for **Gentamicin** against 26 distinct bacterial strains

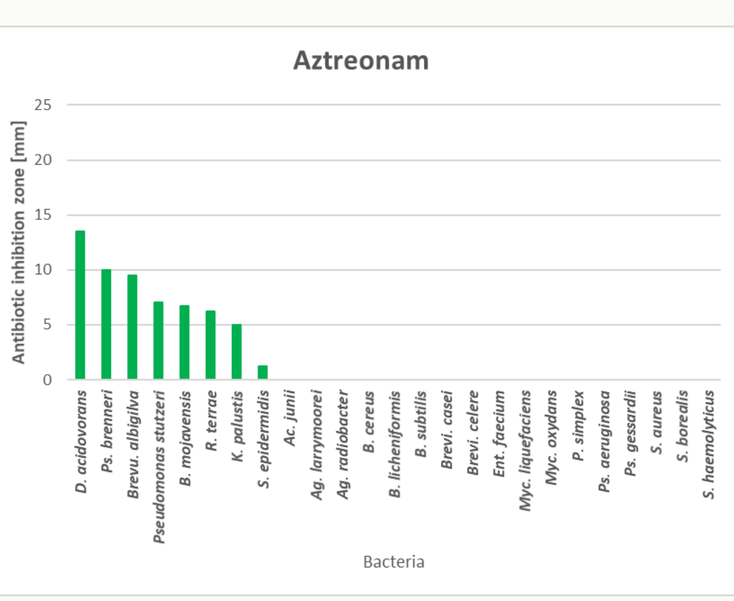


Figure 5 The size of the inhibition zone for **Aztreonam** against 26 distinct bacterial strains

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