SURFACE WATERS VS HOSPITAL WASTEWATERS: ESBL PRODUCING *KLEBSIELLA SPP.*, ANTIMICROBIAL RESISTANCE, AND ANTIBIOTIC RESISTANCE GENES

S. Araújo [1*], V. Silva [1-4], J. Eduardo [1,5,6], L. Maltez [1,5,6], G. Igrejas [2-4], P. Poeta [1,5,6]

[1] Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

[2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

[3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

[4] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Portugal

[5] Veterinary and Animal Research Centre, Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

[6] Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

***saravanessaaraujo07@gmail.com**

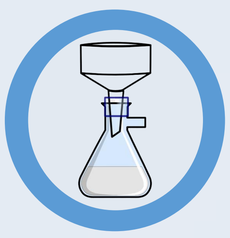
1 Background

Klebsiella spp. is recognized as an emerging threat to public health worldwide mainly due to its increasing prevalence in healthcare-associated infections caused by multidrug-resistant strains producing extended-spectrum β-lactamases (ESBL). However, comparable data on its occurrence in surface waters and hospital wastewaters are scarce. Therefore, we aimed to investigate the prevalence of ESBL-producing *Klebsiella spp.*, in surface waters and hospital wastewaters, and characterize the isolates phenotypically and genotypically.

2 Methodology

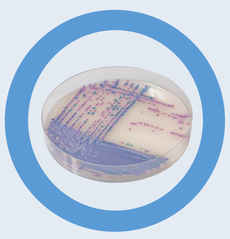
Water samples collection

77 samples were collected from surface waters; on the other hand, 44 samples were collected from Hospital wastewaters.



Water filtration

Water filtration through a 0.45 µm cellulose nitrate pore membrane filter.

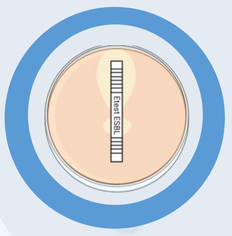


Klebsiella spp. isolation

The filtration membrane was immersed in BHI broth, and 24h after, streaked on selective media for the isolation of *Klebsiella spp.*.

Antimicrobial susceptibility testing

Antibiograms were performed using the Kirby-Bauer disk diffusion method against 11 antibiotics according to EUCAST guidelines.



Screening of phenotypic ESBL

Screening for ESBL phenotypic production was performed by double-disk synergy.

3 Results

From the 77 samples collected from different surface waters in the North of Portugal, 35 (45.5%) were positive for *Klebsiella spp.*. Conversely, 41 (93,2%) *Klebsiella spp*. were isolated from 44 samples collected in hospital wastewaters in northern Portugal. Regarding antibiotic resistance, a high prevalence of resistance to cefotaxime was found, both in surface waters (n=16; 45,7%) and hospital wastewaters (n=31; 75,6%) **[Fig. 1].** ESBL production was detected in 11 (31,4%) of 35 *Klebsiella spp.* isolated in surface waters, and 20 (48,8%) of 41 *Klebsiella spp*., in hospital wastewaters **[Fig. 2].** The presence of different carbapenem genes (blaOXA, blaIMP, and blaKPC) was analyzed by PCR in *Klebsiella spp.* isolates, as well as the presence of other resistance genes (blaCTX, tetA, tetB, aac(3)-II, aac(3)-IV, and blaSHV). The one with the highest prevalence, in the surface waters, was blaCTX (25,7%); however, in hospital wastewater, it was blaSHV (54,7%). Nevertheless, in carbapenem genes, amplification was not verified, in surface waters, although in hospital wastewater, it was verified in the blaKPC (9,8%).

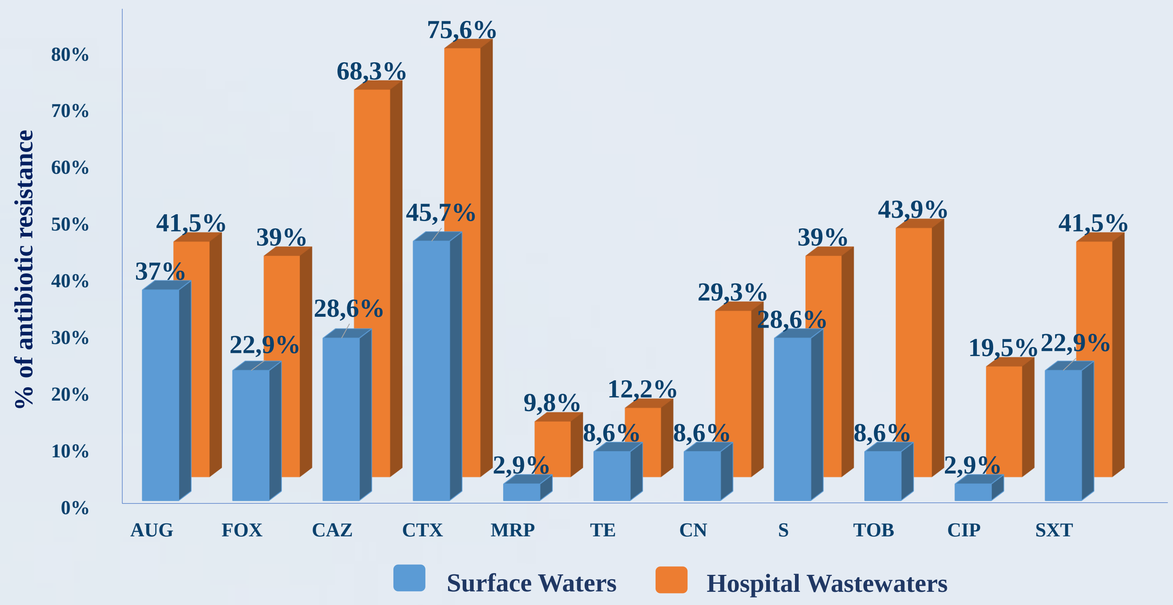


Fig. 1- Percentage of resistance in *Klebsiella spp.* strains

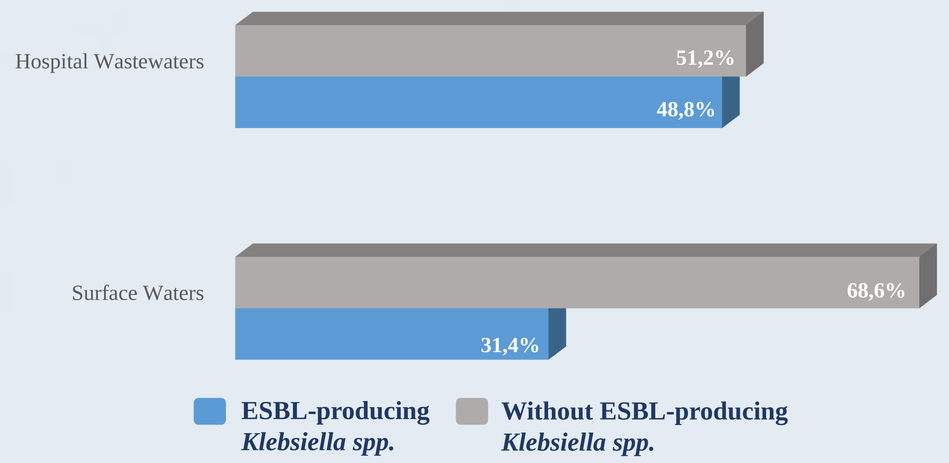


Fig. 2- Prevalence of ESBL-producing *Klebsiella spp.*

4 Conclusion

This study revealed a high incidence of resistance to antibiotics commonly used to treat *Klebsiella spp.* infections in animals and humans, such as cefotaxime and ceftazidime. Furthermore, there was a high prevalence of ESBL-producing *Klebsiella spp.*, which is concerning, as they cause the reduction or even ineffectiveness of existing therapies. As this is one of the main concerns, it is important to monitor the evolution of the ESBL situation, and applying a One Health approach is essential to keep this problem under control, to prevent its spread to humans and animals. However, it is still important to note that the development of new antibiotics is necessary for the successful treatment of these infections.

Acknowledgments

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