**Additional Evaluation on Escherichia coli**

To further evaluate the generalization capacity of our model, we conducted additional experiments using microscopic images of Escherichia coli. In most cases, the segmentation produced by the YOLOv8-based architecture was not sufficiently precise, highlighting the challenges of applying a model trained exclusively on Staphylococcus aureus to bacteria with different morphological and growth characteristics. These findings emphasize that the network cannot be directly applied to other bacterial species without retraining. Morphological and growth-pattern differences between bacteria significantly affect segmentation quality and, consequently, the accuracy of inhibition zone measurement. Therefore, for each new bacterial species to be analyzed, it is necessary either to retrain the model with a sufficiently large dataset specific to that bacterium or to develop a dedicated model per species. This requirement is fundamental to ensuring reliable performance across diverse microorganisms.

For transparency and reproducibility, all segmentation outputs are available in the folder Ecoli\_Segmentation, while the corresponding original microscopic images are provided in the folder Original\_Images.

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| **Table 1.** Performance of the model when applied to microscopic images of E. coli. Results show limited segmentation precision, reinforcing the need to retrain the network with species-specific datasets or develop dedicated models for each bacterium. | |
| Original image | Segmented image |
|  |  |
|  |  |
|  | The distance is: 0.062 mm |
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