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Introduction & Purpose of the study

Pseudomonas aeruginosa (Pa) is an ubiquitous pathogen capable of surviving the adverse conditions on contaminated sites and can be a source of colonization in the environment. It's an important nosocomial pathogenic agent characterized by an intrinsic resistance to multiple antimicrobial agents which can develop high-level multidrug resistance.

Although, existing studies in Morocco have predominantly addressed changes in clinical Pa strains, there is limited information available regarding genomic findings for environmental Pa. Therefore, in an effort to find an effective strategy to eliminate infections, it is essential to focus on expanding Pa strain collections and origins in Morocco.

Methods

The collection studied concerns environmental Pa strains (n=57), isolated from 200 environment water samples collected during the period between January to December 2021 in Casablanca (Morocco).

To check the purity and to verify the bright green color characteristic of Pa species, a bacterial suspension obtained after incubation in the Brain Heart Infusion medium (BHI) (Bio-rad) at 37°C for 20 to 24 hours was subcultured in cetrimide agar medium (Bio-rad).

Furthermore, they were presumably identified as belonging to Pa species by the standard microbiological and biochemical tests (colony morphology, Gram staining, oxidase test, blue-green pigmentation, and fluorescence under UV light). The identification confirmation of these environmental Pa isolates was carried out using MALDI-TOF.

The Antimicrobial susceptibility was conducted using disk diffusion method on Mueller-Hinton (Bio-Rad) agar according to EUCAST 2021 guidelines and was further confirmed accurately by VITEK® system (BIOMERIEUX).

Carbapenemase production was examined phenotypically using the Biochemical Test Carba NP® rapid diagnostic technique (Rapidec®) and the immunological Carba NG® test (NG.BIOTECH®).

DNA extraction was conducted using the Qiagen (QIAamp® DNA Mini Kit). Short-read next-generation sequencing (NGS) was performed using Illumina DNA Prep kit and the NextSeq S500 technology for sequencing.

Results & Discussion

The antibiotic susceptible testing reveals varying degrees of resistance to different antibiotics. Pa displays widespread resistance across most antibiotics, with notable percentages of resistance observed in figure 1. These results underscore the impact of human activities neither anthropogenic or industrial.

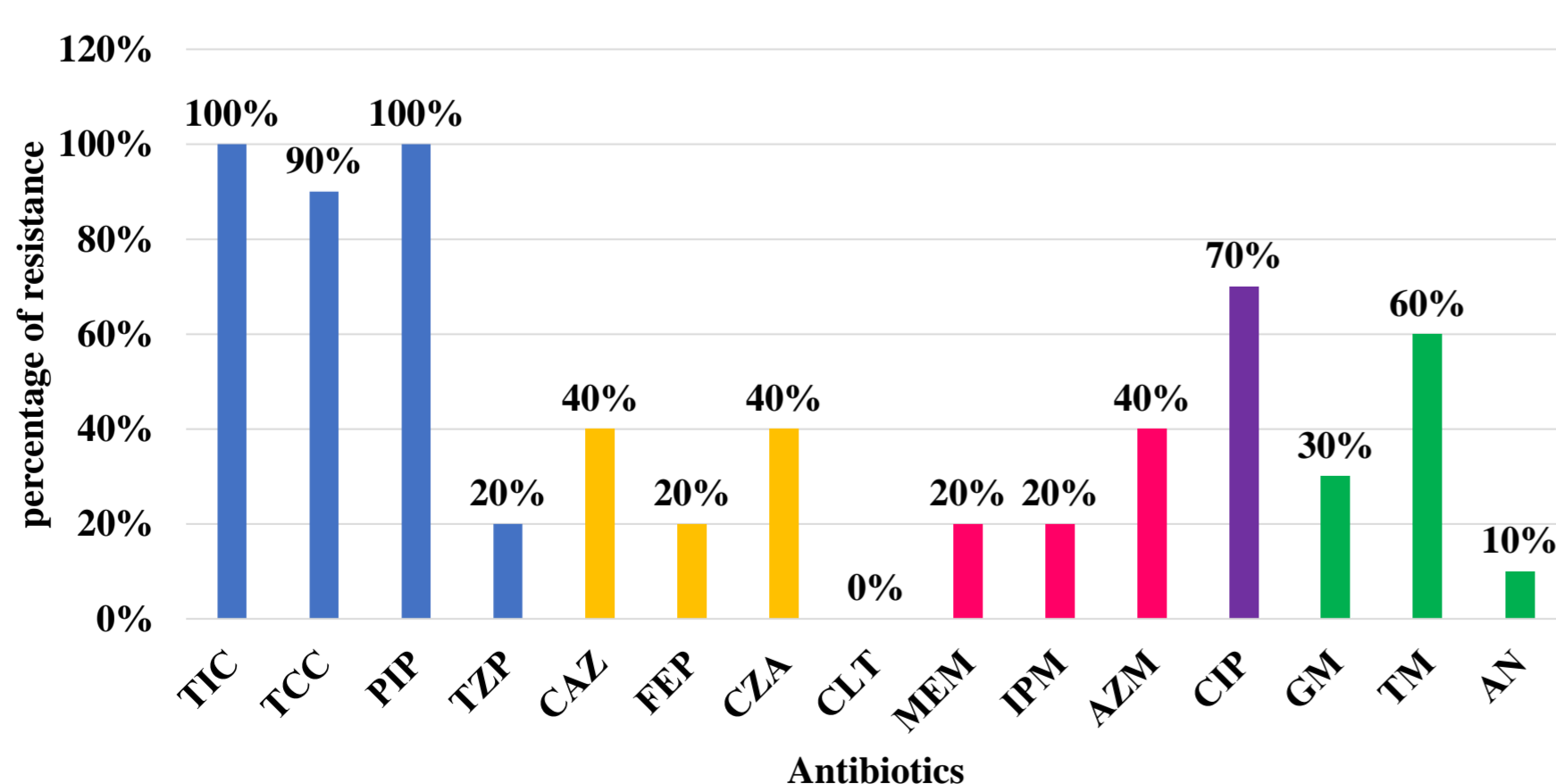


Figure 1: Antibiotic resistance profile of environmental Pa.

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The phenotypic detection of two CRPa strains of environmental origin was negative. The in-depth examination of our genetic results unveils a complex web of resistance and virulence in environmental strains of Pa. The absence of carbapenemases genes in the two environmental strains of Pa, suggests that resistance to carbapenems in these isolates does not stem from the presence of these specific enzymes, this resistance may due to non-enzymatic resistance such as overexpression of oprD. Our results revealed a distribution of various β-lactamase genes among the tested samples in the environmental context, with OXA-846 being the most prevalent at 30%, followed by OXA-50 at 20% (figure 2). The presence of these genes indicates a potential mechanism for intrinsic and acquired resistance to β-lactam antibiotics.

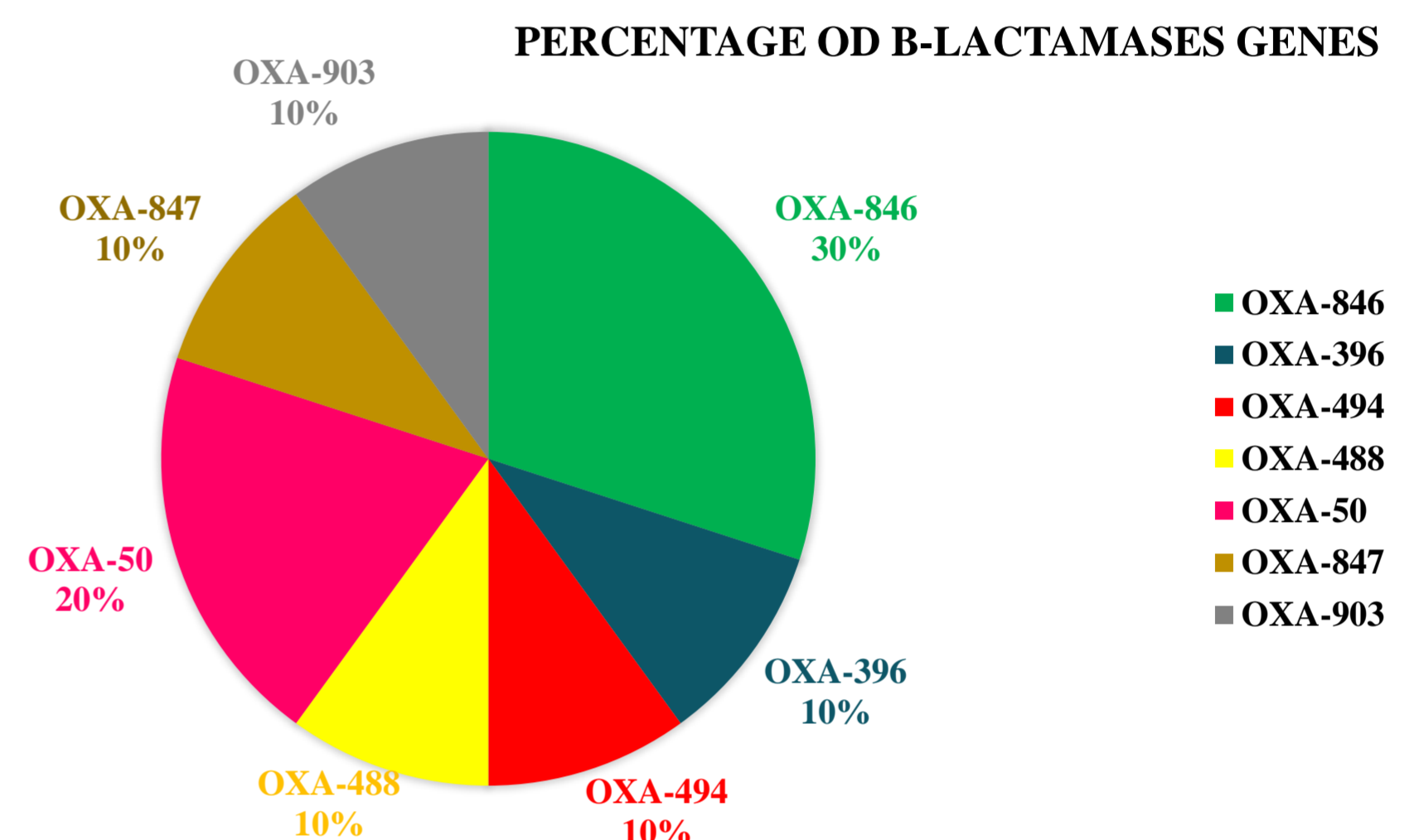


Figure 2 : β-lactamases Oxa-types detected in Pa by NGS

Concerning virulence genes, the identification of crucial factors, remains pivotal. The presented results (figure 3) unveil the prevalence and distribution of key virulence-associated genes in Pa. Notably, genes encoding fimbriae, essential for adhesion and biofilm formation, exhibited moderate prevalence. Regarding flagella-related genes, indicating a substantial yet varied distribution. Moreover, genes contributing to phenazine production, vital for antimicrobial activity, were identified at moderate to high frequencies. The pili system, crucial for motility and attachment, demonstrated a widespread presence, indicating their significant role in pathogenicity. However, genes associated with pyoverdinin synthesis, essential for iron acquisition, and LPS synthesis were less prevalent. Overall, these findings shed light on the genetic landscape of virulence factors in the environmental Pa, providing valuable insights into their pathogenic potential and informing future research directions aimed at effectively combating environmental bacterial infections.

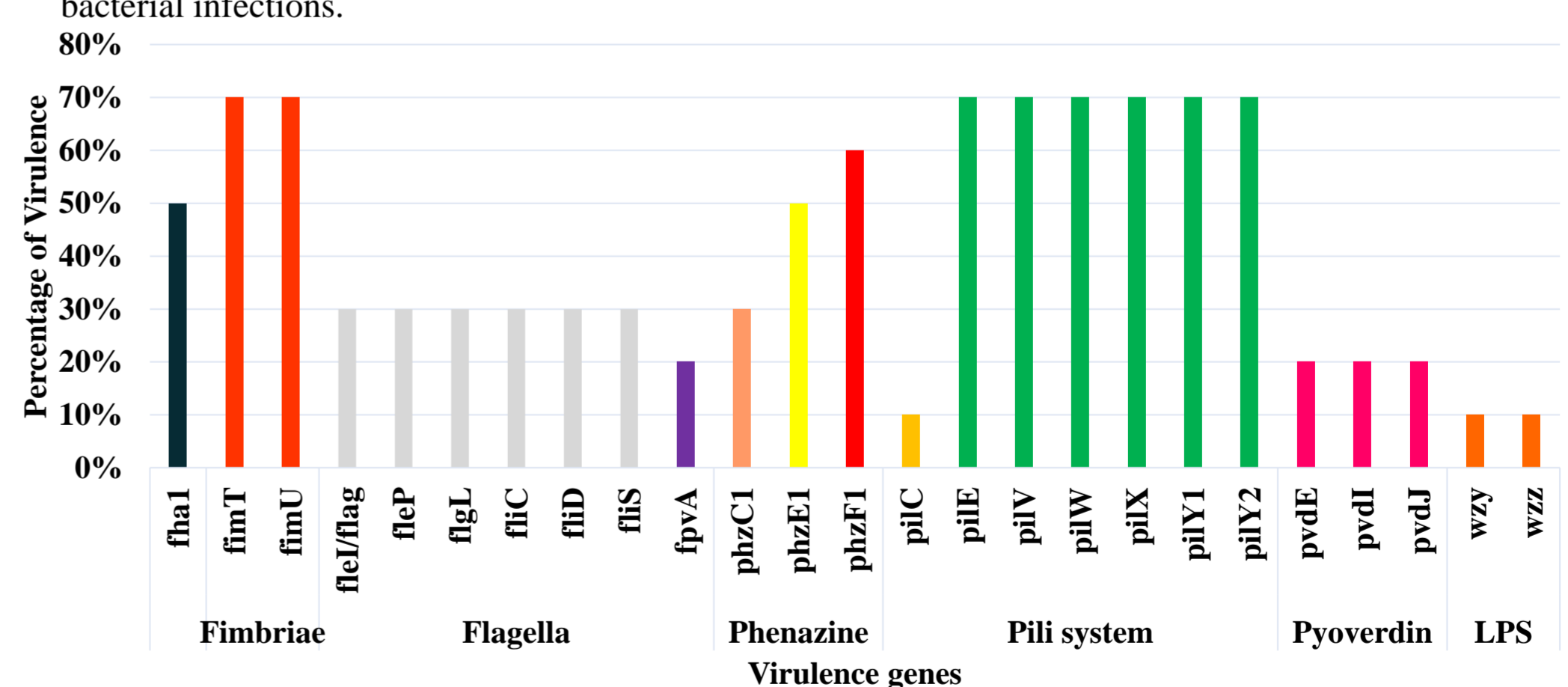


Figure 3 : Virulence genes detected in environmental Pa by NGS

Conclusion

To the best of our knowledge, this is the first molecular characterization of environmental Pa isolates in Morocco. Our analysis provides a comprehensive view of both resistance and virulence profiles in environmental Pa. These findings underscore the complexity of bacterial populations in environmental settings and highlight the need for holistic approaches in managing public health risks. Moving forward, integrating resistance and virulence data will be crucial for developing effective strategies to mitigate the impact of environmental pathogens on human health.