

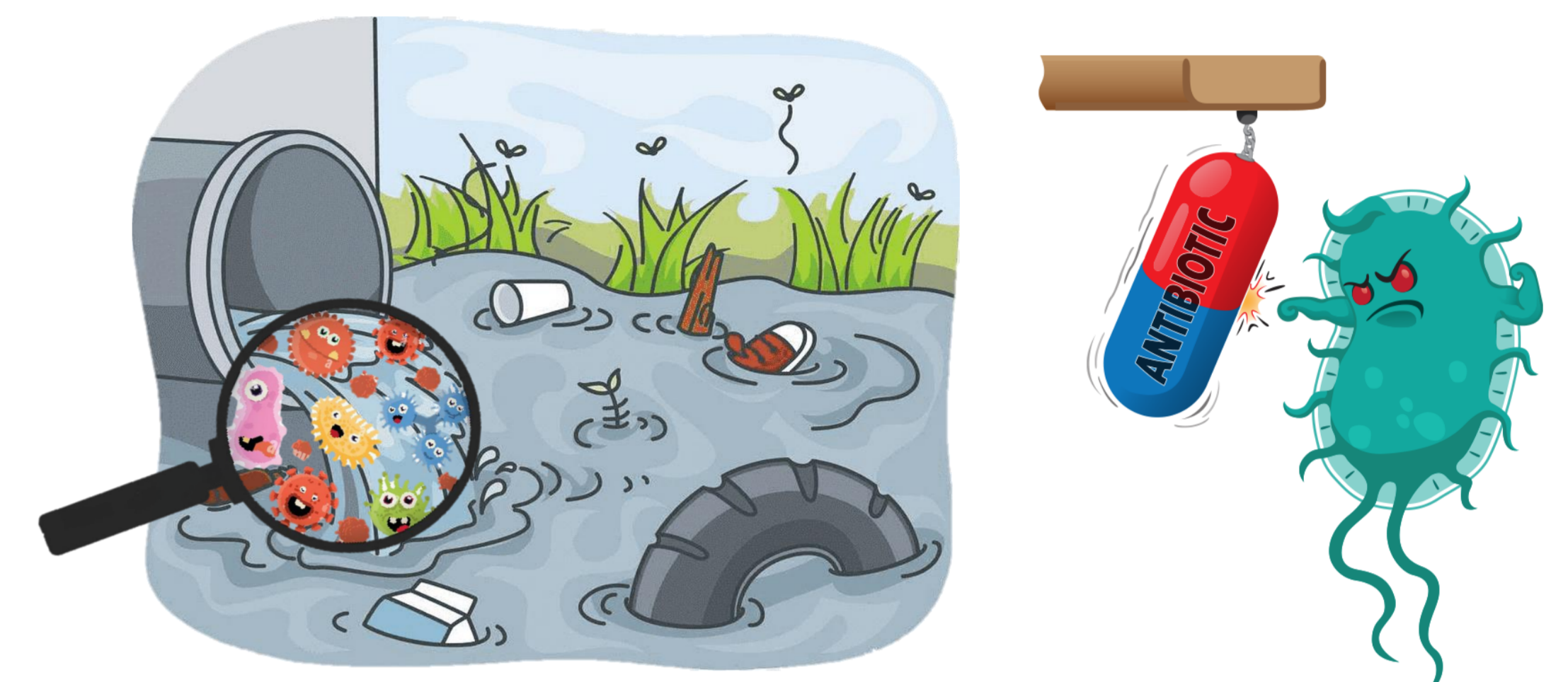
Advancing Wastewater Surveillance: Development and Validation of AMR Detection Methods

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Context

Accurate surveillance is a key component in the fight against antimicrobial resistance (AMR) genes. **Wastewater** is progressively becoming a **new player in AMR gene surveillance**, enabling a cost-effective real-time tracking of AMR and global monitoring of AMR profiles in specific regions. Moreover, the new revision of the **EU Wastewater Treatment Directive (WWTD)** will require member states to **monitor antibiotic resistance** in their wastewater treatment plants (WWTP) in the coming years.



Challenges

Digital droplet PCR (ddPCR) is gaining **more interest for wastewater and AMR surveillance**, as it provides the advantage of simultaneously detecting and quantifying AMR genes in wastewater. However, there are **currently no ddPCR methods** for AMR surveillance that have been **validated** according **international standard** performance criteria (ENGL 2015, 2023).



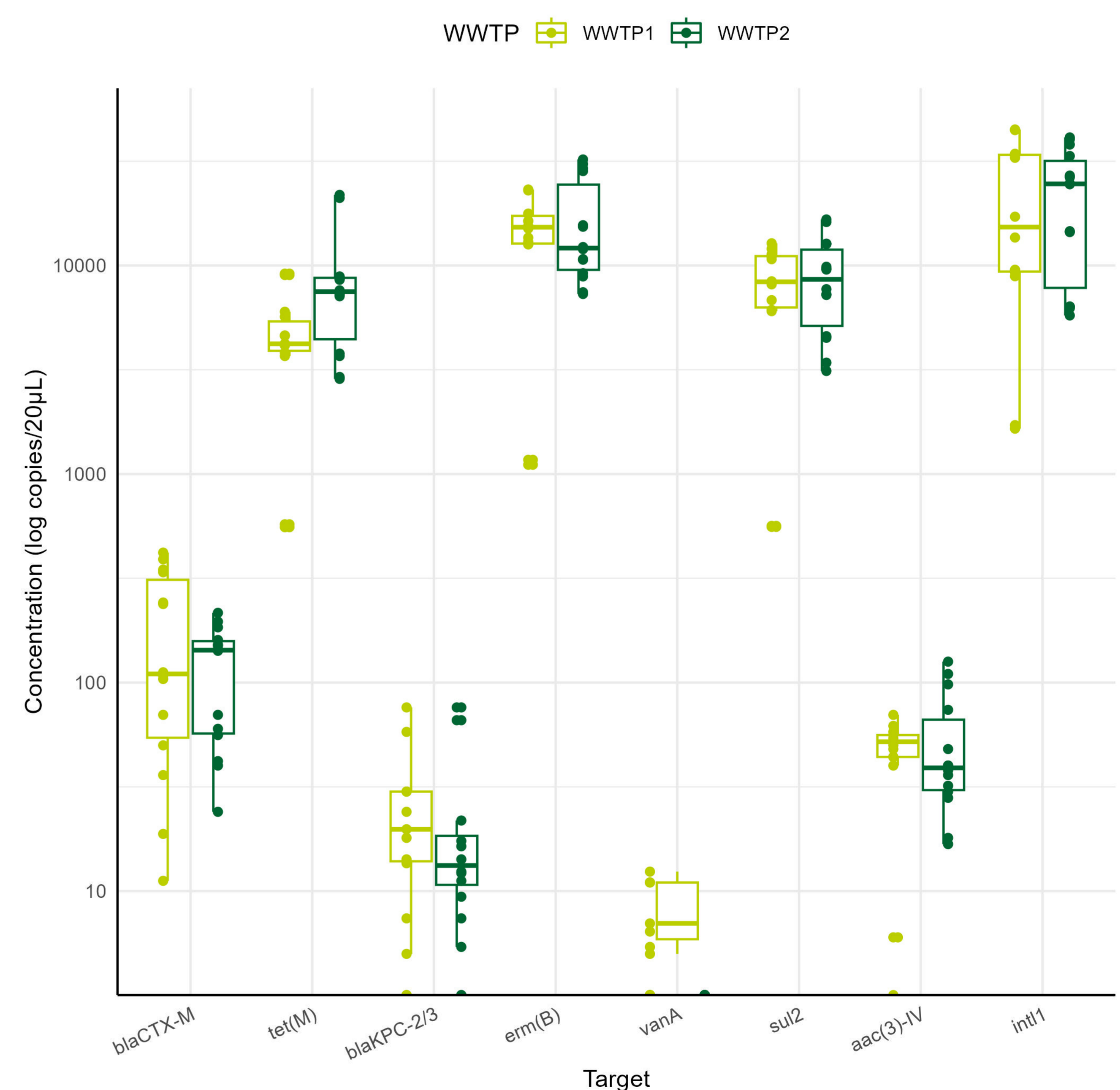
Methods and Results

A **workflow to develop and validate ddPCR methods** responding to these **international standard** performance criteria was proposed, focusing on 4 duplex ddPCR methods including a total of 8 clinical relevant AMR targets:

The ddPCR method **applicability** was also tested on 14 wastewater samples collected by auto-samplers (24-hour composite) at the influent of two Belgian treatment plants. The results align with previous scientific literature observations (AMR target abundance), confirming their reliability.

ddPCR methods	AMR targets	Limit of detection (LOD _{95%} ; target copy number)
Duplex-1	bla _{CTX-M} R-extended spectrum beta-lactam	16
	tet(M) R-tetracycline	9
Duplex-2	bla _{KPC-2/3} R-carbapenem	15
	erm(B) R-erythromycin	13
Duplex-3	vanA R-vancomycin	8
	sul2 R-sulfonamide	16
Duplex-4	aac(3)-IV R-aminoglycoside	7
	intl1 Class I integron	14

These duplex ddPCR methods were successfully assessed as **specific** (no false positives and negatives) **and sensitive** (LOD_{95%} < 25 copies) according international standard performance criteria.



Conclusion

This study proposes a **workflow for the development and validation** of ddPCR methods helping to build a **harmonized and global AMR surveillance** in the framework of the EU WWTD.



ACKNOWLEDGEMENTS

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