

A HOME-MADE 45-PLEX ARRAY FOR THE DETECTION OF ANTIMICROBIAL RESISTANCE GENES IN GRAM-POSITIVE BACTERIA

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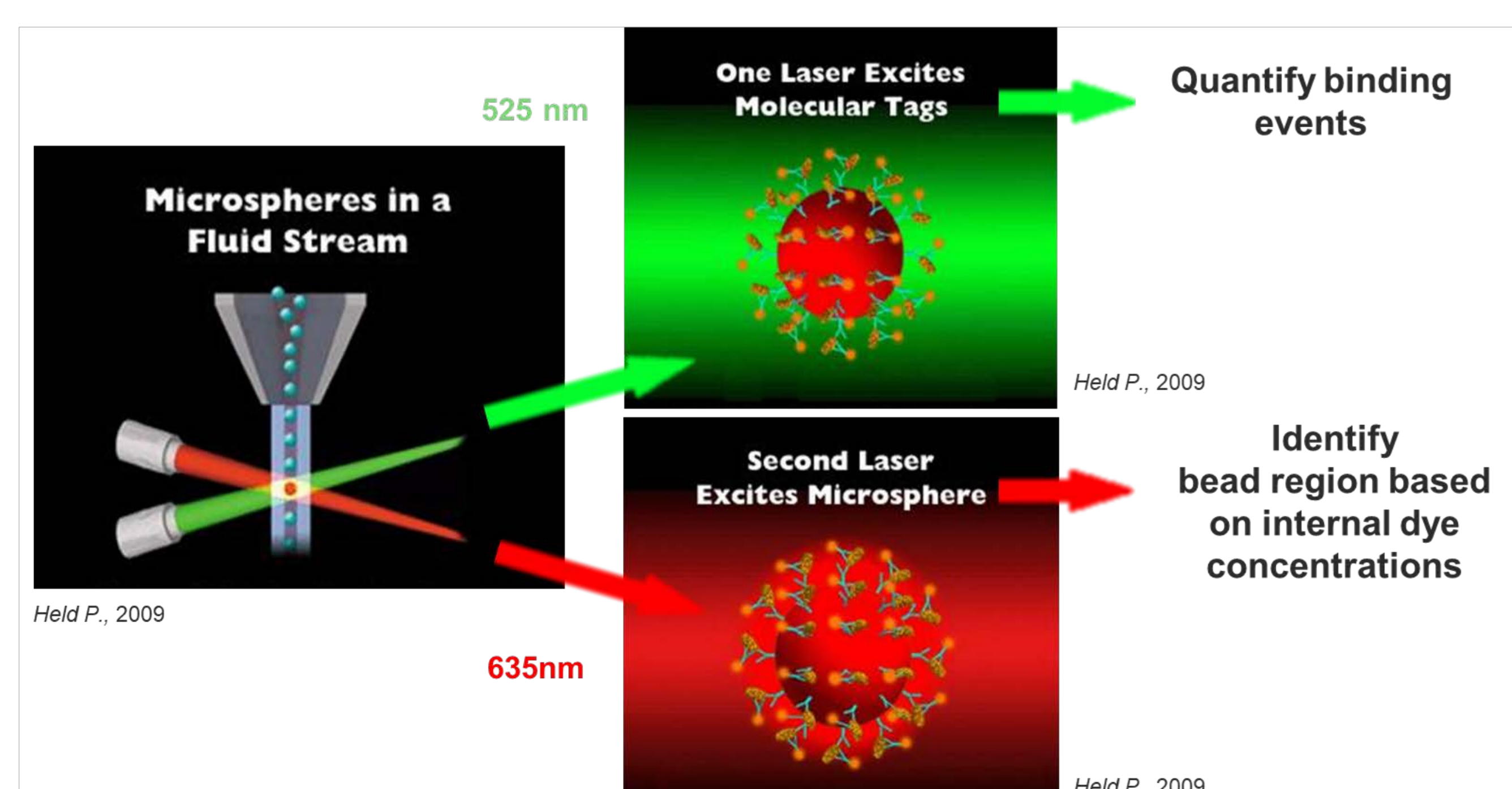
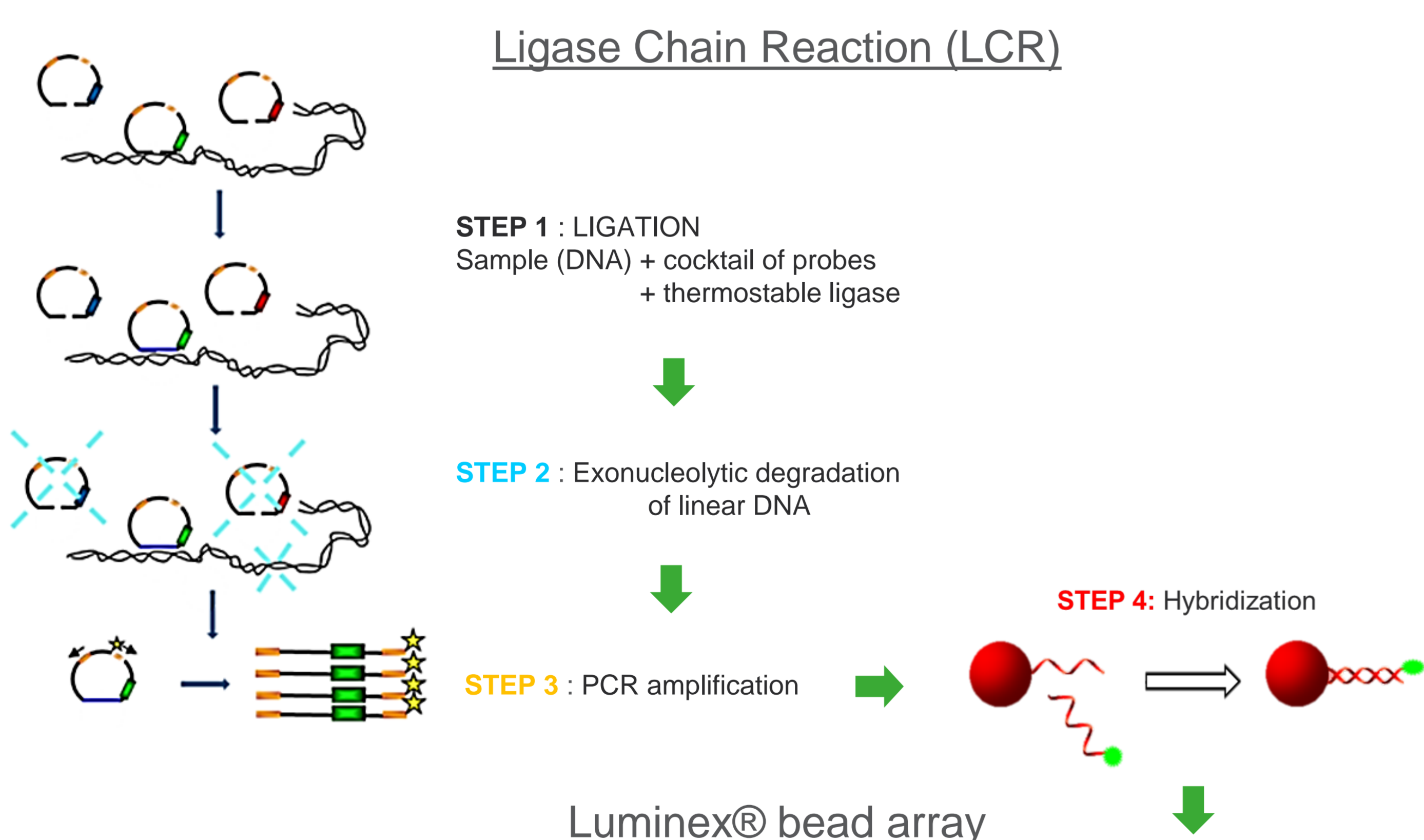
Conclusion

Numerous resistance genes circulating among *Enterococcus* and *Staphylococcus* spp. were detected by this array allowing to screen a large collection of strains in a limited time. Accumulation of genes conferring a same phenotypic resistance in a single isolate was observed repeatedly. Genes conferring resistance to critical antibiotics (linezolid and vancomycin) were detected by this array. The complexity of AMR, particularly the cross-resistance phenomenon, encourages to monitor all putative main AMR sources at the genetic level and consider them as a “One-Health” AMR pool.

Identifying antimicrobial resistance (AMR) genes and determining their occurrence in Gram-positive bacteria provide useful data to understand how resistance can be acquired and maintained in these bacteria, and thereby assist in the establishment of tailor-made guidelines for the appropriate use of antimicrobials. Here we describe a home-made flexible bead array targeting AMR genes of Gram-positive bacteria and allowing their rapid detection all at once at reduced costs.

Methods

- A home-made multiplex Ligase Chain Reaction assay analyzed on a bead-array hybridization platform (Luminex®) was developed to detect the most frequent genes commonly found and particularly shared among *Enterococcus* and *Staphylococcus* spp. conferring resistance to tetracycline, macrolides, lincosamides, pleuromutilins, streptogramins, phenicols, glycopeptides, aminoglycosides, diaminopyrimidines and oxazolidinones.
- This array was used to study a collection of 124 enterococci and 62 staphylococci isolated from healthy livestock animals through the official Belgian AMR monitoring (2018-2020). Results were compared with antibiotic susceptibility test results obtained during official monitoring.



Results

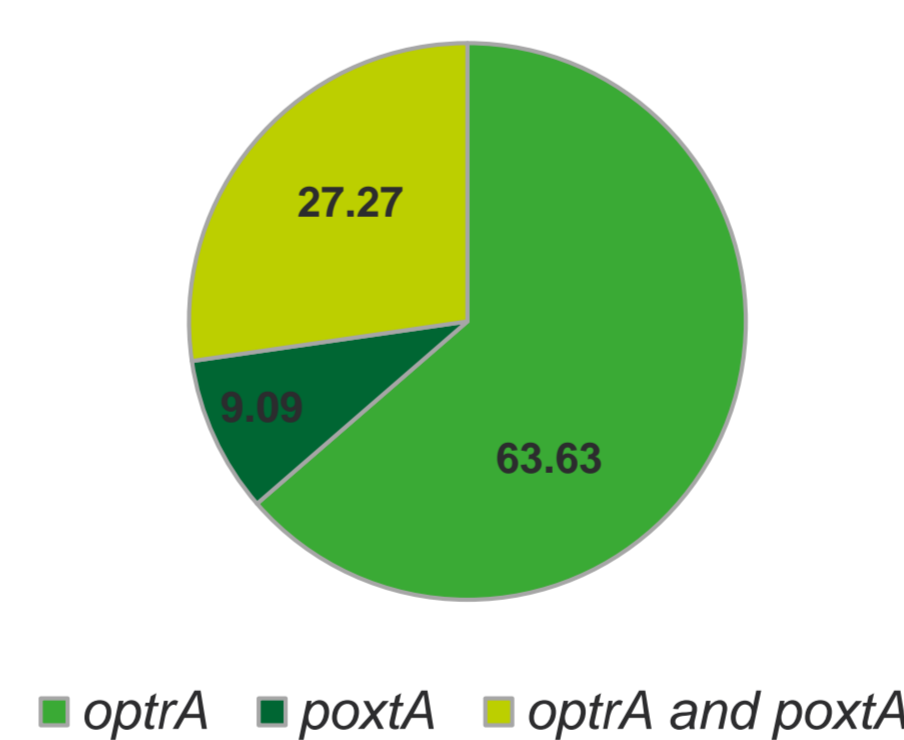
In this collection, 27 out of the 41 targeted AMR genes were detected by this array (in green) and associated with the resistance phenotypes in 93.0% and 89.2% of phenotypes in enterococci and staphylococci, respectively.

Antibiotics classes	Macrolides	Lincosamides	Pleuromutilins	Streptogramins	Diamino pyrimidines	Phenicols
AMR phenotypes	Erythromycin (ERY)	Clindamycin (CLN)	Tiamulin (TIA)	Quinupristin/dalfopristin (Synercid, SYN)	Trimethoprim (TMP)	Chloramphenicol (CHL)
Targeted genes associated with the resistance phenotype	<i>ermA</i>	<i>IsaA</i>	<i>IsaA</i>	<i>IsaA</i>	<i>vatA</i>	<i>catpC194</i>
	<i>ermB</i>	<i>IsaE</i>	<i>IsaE</i>	<i>IsaE</i>	<i>vatB</i>	<i>catpC221/C223</i>
	<i>ermC</i>	<i>ermA</i>	<i>vgaA</i>	<i>ermA</i>	<i>vatC</i>	<i>fexA</i>
	<i>mefA/E</i>	<i>ermB</i>	<i>vgaB</i>	<i>ermB</i>	<i>vatD</i>	<i>cfr</i>
	<i>mphC</i>	<i>ermC</i>	<i>vgaD</i>	<i>ermC</i>	<i>vatE</i>	<i>optrA</i>
		<i>InuA</i>	<i>cfr</i>	<i>vgaA</i>	<i>mefA/E</i>	<i>poxtA</i>
	<i>InuB</i>		<i>vgaB</i>	<i>vgaB</i>		
			<i>vgaD</i>			
Antibiotics classes	Glycopeptides	Oxazolidinones	Tetracyclines	Aminoglycosides		
AMR phenotypes	Vancomycin (VAN)	Linezolid (LZD)	Tetracycline (TET)	Kanamycin (KAN)	Gentamycin (GEN)	Streptomycin (STR)
Targeted genes associated with the resistance phenotype	<i>vanA</i>	<i>cfr</i>	<i>tetO</i>	<i>aadD</i>	<i>aacA-aphD</i>	<i>aadE</i>
	<i>vanB</i>	<i>optrA</i>	<i>tetK</i>	<i>aacA-aphD</i>	<i>aph2-Id/le</i>	<i>aph2-Id/le</i>
	<i>vanC1</i>	<i>poxtA</i>	<i>tetL</i>	<i>aphA3</i>		
	<i>vanC2/3</i>		<i>tetM</i>	<i>aph2-Id/le</i>		
			<i>poxtA</i>			

The studied isolates frequently carried two or more resistance genes conferring the same resistance phenotype, and sometimes genes from the same family.

Linezolid resistance

LZD-resistant *Enterococcus*



- LZD is not used in veterinary medicine.
- 22 LZD-resistant isolates collected through the monitoring of enterococci in food-producing animals in 2019-2020 in Belgium.
- All harbored at least one of the targeted genes: *optrA* and/or *poxtA*.
- The spread of these genes could occur through the use of other AB than LZD since *optrA* and *poxtA* were described to confer resistance to other antibiotics (phenicols and phenicols/TET respectively).

Vancomycin resistance

Rarely observed through the Belgian AMR random monitoring, vancomycin resistance was however another point of interest of this study. *vanA* was detected in one out of the two vancomycin-resistant *Enterococcus faecalis* tested isolates.

Results obtained with the bead array for the two vancomycin-resistant strains. The two first lines are the cut-offs: in red is the negative cut-off and in green is the positive cut-off. The grey cells contain the probe names, with *sodA-fs* probe as positive control of *E. faecalis* species. The results are median fluorescence intensities normalized with the signal of the Gram-positive internal positive control probe.

Strain ID	Animal origin	Genotype	Fluorescence Intensity (FI)				
			<i>vanA</i>	<i>vanB</i>	<i>vanC1</i>	<i>vanC2-3</i>	<i>sodA-fs</i>
20-3786	veal	VAN-R	71.4	4.17	4.17	4.17	60.4
19-4903	pig	No VAN gene	1.2	1.6	1.6	1.9	68.7
<i>E. faecalis</i> ATCC 29212	-	-	1.1	1.5	1.5	1.5	70.2

This work has been published in the following paper: “Kowalewicz, C., Timmermans, M., Fretin, D., Wattiau, P., & Boland, C. (2022). An in-house 45-plex array for the detection of antimicrobial resistance genes in Gram-positive bacteria. *MicrobiologyOpen*, e1341. <https://doi.org/10.1002/mbo3.1341>”.

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