Screening for carbapenemase-producing *E. coli* and *K. pneumoniae* in freshwater, bathing water and hospital continuums, and determination of the carbapenemases (bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}) by antibiogram, real-time PCR, immunochromatographic tests and whole genome sequencing

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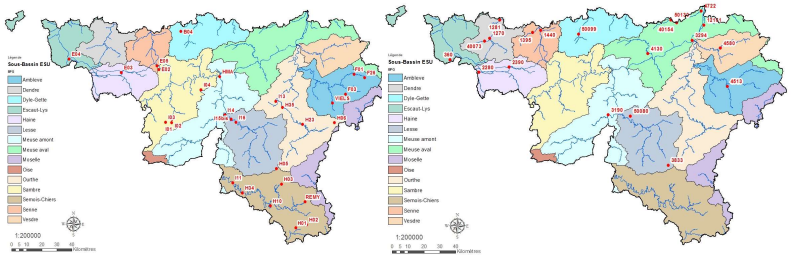
BACKGROUND AND OBJECTIVES

Escherichia coli (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) belong to Enterobacteriales and are present in the intestinal tract of warm-blooded animals. They could be released in the environment through their faeces.

The presence of enteric bacteria in aquatic environments represents a problem of public health, especially due to the fact that such bacteria can carry resistances to antibiotics. Aquatic systems can then become a vector of spreading of antimicrobial resistance (AR) through the environment. This study focused on carbapenemase (CPE)-producing *E. coli* and *K. pneumoniae* in the aquatic environment. Carbapenem resistance is a major concern, as this class of antibiotics, belonging to the β-lactam family, is used as a last resort molecule in hospitals in human medicine.

LOCALISATION & SAMPLING

Screening :



29 bathing waters — 20 surface waters

3 hospital continuums:

- ❑ Hospital A (635 beds) and hospital B (226 beds) – WWTP – Ourthe river
- ❑ Hospital C (438 beds) – WWTP – Meuse river
- ❑ Hospital D (539 beds) – WWTP – Sambre river

WWTP = wastewater treatment plant

MATERIALS & METHODS

➢ Enumeration, isolation and confirmation

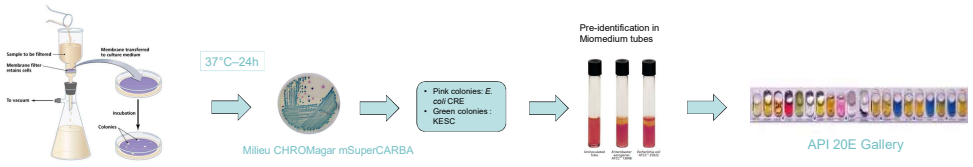


Figure 1. Isolation, pre-identification and confirmation of the CRE *E. coli* and *K. pneumoniae* strains

➢ Determination of CPE genes (bla_{VIM}, bla_{IMP}, bla_{OXA-48}, bla_{KPC} et bla_{NDM})

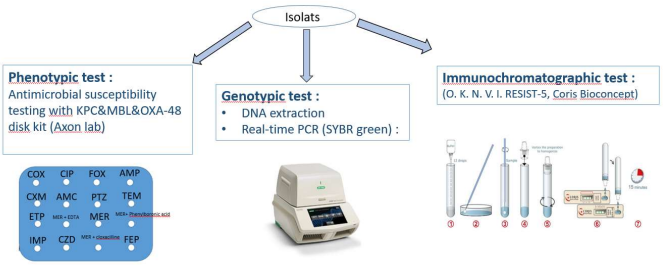


Figure 2. 3 techniques were used for the determination of CPE genes : phenotypic, genotypic and immunochromatographic tests

➢ Sequencing

A subset of isolates (11 *E. coli* et 15 *K. pneumoniae* CPE) were selected for Whole Genome Sequencing (WGS)

RESULTS

➢ Isolation and confirmation

Screening	Number of *E. coli* CRE isolated	Number of *K. pneumoniae* CRE isolated
29 bathing waters	0	0
20 surface waters	4	7
Hospital A et B - WWTP - Ourthe river	19	7
Hospital C - WWTP - Meuse river	0	2
Hospital D - WWTP - Sambre river	1	11
Total	**24**	**27**

Figure 3. Results of the screening and number of *E. coli* and *K. pneumoniae* CPE isolated

➢ Distribution of CPE genes

- **24 *E. coli* CPE**
- **27 *K. pneumoniae* CPE**

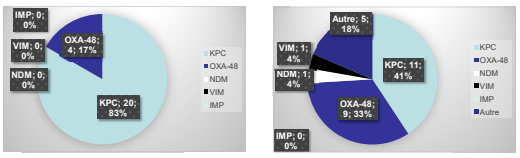


Figure 4. Distribution of CPE genes

➢ Whole Genome Sequencing

	Code	Localisation	Species	Sérotype	MLST	CPE Genes: KPC-3	OXA-48	OXA-244	VIM-1	NDM-5
1	CHUt/-2/7r	Hospital A	*E. coli*	H25	635	x				
2	CHUt/-2/2r			O18:H7	1463	x				
3	CHUt/-2/5r			O18:H8	1463	x				
4	CHUt/-3/6r			O21:H25	401	x				
5	CHUt/-3/2b		*K. pneumoniae*	K51/O3b	16					x
6	INt/7b	Input WWTP Embourg	*K. pneumoniae*	O2afg	512	x				
7	INm-c/-1/2b	Output WWTP Montignies		K38/O3b	11	x				
8	12161/1r	ESU - Ruisseau de Sainte-Julienne à Visé	*E. coli*	O86:H18	38			x		
9	1440/1r	ESU - Hain à Tubize		O105:H10	607		x			
10	3294/1r	ESU - Meuse à Liège		O18:H7	1463	x				
11	4722/1r	ESU - Geer à Bassenge		H21	10		x			
12	1440/1b	ESU - Hain à Tubize	*K. pneumoniae*	K17/O1	101		x			
13	2280/9b	ESU - Haine canalisée à Hensies		K46/O1	461				x	
14	3294/3b	ESU - Meuse à Liège		O5	3318	x				
15	4722/1b	ESU - Geer à Bassenge		K24/O1	15		x			
16	HMCf/-2/2b	Hospital D aile F		K38/O3b	1486	x				
17	HMCf/-2/5b	Hospital D aile F		K10/O3/O3a	147		x			
18	Ag,b,/3b	Ourthe upstream WWTP Grosses Battes		/	1770	x				
19	Bt/1r	Ourthe downstream WWTP Embourg	*E. coli*	O25:H12	607	x				
20	Am-c/1r	Sambre upstream		O86:H30	38		x			
21	Bm-c/7b	Sambre downstream	*K. pneumoniae*	K38/O3b	11	x				
22	1395/3b	ESU - Senne 1395		K27/O4	392		x			
23	OUTt/1ml/4r	Output WWTP Embourg	*E. coli*	O18:H7	1463	x				
24	OUTt/1ml/3b	Output WWTP Embourg	*K. pneumoniae*	O2afg	512	x				
25	OUTm-c/10b	Output WWTP Montignies		K10/O3/O3a	147		x			
26	CHUg/1b	Hospital C		K13/O1	540		x			

Figure 5. Code, localisation, species, serotype, MLST and CPE genes

KEY CONCLUSIONS

24 CPE *E. coli* and 27 CPE *K. pneumoniae* were isolated from surface water and hospital continuums. No strains could be isolated from bathing waters, which indicates their good quality. The work involved in isolating and confirming the bacterial species is fastidious, especially for *K. pneumoniae*, for which there is no selective culture.

Of the 24 CPE *E. coli*, 20 strains, including 16 isolates from the effluent from hospital A, possessed the bla_{KPC} gene. The 4 other CPE *E. coli* had the bla_{OXA-48} gene. For the 27 *K. pneumoniae* isolated, 11 isolates had the bla_{KPC} gene, 9 the bla_{OXA-48} gene, one bla_{NDM}, one bla_{VIM} and 5 had none of the genes tested.

Phenotypic tests were compared with genotypic tests to identify the main genes coding for carbapenemases (bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{IMP} genes) and with rapid immunochromatographic tests. The results obtained with these 3 tests were consistent.

26 strains were sequenced and all possessed CPE genes - bla_{KPC-3} (n=14), bla_{OXA-48} (n=9), $bla_{OXA-244}$ (n=1), bla_{VIM-1} (n=1) *and* bla_{NDM-5} (n=1) - mostly coupled to ESBL-encoding genes.Three *E. coli* CPE serotype O18:H7 ST 1463 were found in the effluent of hospital A, at the output of the Embourg WWTP and in the Meuse river in Liège. The spread of this strain in surface water from the effluent of Hospital A is clearly demonstrated, showing that hospital effluents and WWTP contribute to the dissemination of AR bacteria in the aquatic environment. This study show that *E. coli* and *K. pneumoniae* CPE are present in the Belgian aquatic environment.