Prevalence and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* in freshwaters, hospital effluents and wastewaters in Belgium

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BACKGROUND AND OBJECTIVES

Escherichia coli (or *E. coli*), a gram-negative bacillus that belongs to the coliform group, is present in the intestinal tract of warm-blooded animals. It is released in the environment through their faeces. *E. coli* is thus usually used as an indicator of faecal contamination and water quality.

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.

The purpose of this study was to evaluate the prevalence of extended-spectrum β-lactamase-producing *E. coli* (ESBL-EC) in freshwaters, hospital effluents and wastewaters during two sampling campaigns (winter/summer) in 2021 and to characterize them. A total of 24 stations were sampled including 17 freshwaters, 3 hospital effluents and the input/output of 2 wastewater treatment plants (WWTPs) in the Ourthe watershed.

LOCALISATION & SAMPLING

Sampling locations : Ourthe river

- ❑ 181 km long and located in Northeastern Wallonia
- ❑ Flows into the Meuse river in Liège, in a heavily urbanized environment
- ❑ Two sampling campaigns : the 1st campaign in winter 2021 and the 2nd in summer 2021
- ❑ **24 sampling locations** along the river

Code	Locations	Origin
A	Ourthe at Lavacherie	freshwater
B	Ourthe at Ortho	freshwater
C	Ourthe at Mabompré	freshwater
D	Ourthe at Maboge	freshwater/bathing location
E	Ourthe at Hampteau	freshwater
F	Ourthe at Hotton	freshwater/bathing location
G	Ourthe at Noiseux	freshwater/bathing location
H	Petite Somme	freshwater
I	Ourthe at Bomal	freshwater
J	Ourthe at Comblain-Fairon	freshwater
K	Ourthe at Aval MR Esneux	freshwater
L	Upstream of WWTP Tiff-Embourg in the Ourthe	freshwater
M	Downstream of WWTP Tiff-Embourg in the Ourthe	freshwater
N	Input WWTP Tiff-Embourg	Hospital/community effluent
O	Output WWTP Tiff-Embourg	Treated effluent
P	Upstream of WWTP Angleur in the Ourthe	freshwater
Q	Downstream of WWTP Angleur in the Ourthe	freshwater
R	Input WWTP Angleur	Hospital/community effluent
S	Output WWTP Angleur	Treated effluent
T	Ourthe at Angleur-Chênée	freshwater
U	Meuse	freshwater
V	Hospital A	Hospital effluent
W	Veterinary faculty	Hospital/community effluent
X	Hospital B	Hospital effluent

MATERIALS & METHODS

Enumeration, isolation and confirmation

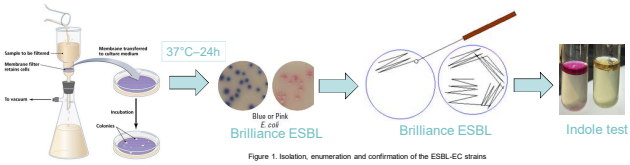


Figure 1. Isolation, enumeration and confirmation of the ESBL-EC strains

Phenotypic test : antimicrobial susceptibility testing

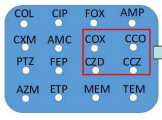
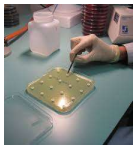


Figure 2. Antibiotics tested and disks disposition on the Mueller-Hinton agar

BLSE confirmation (EUCAST)

Antibiotics tested			
COL	Colistin	PTZ	Piperacillin + Tazobactam
CIP	Ciprofloxacin	FEP	Cefepime
FOX	Cefoxitin	CZD	Ceftazidime
AMP	Ampicillin	CCZ	Ceftazidime + clavulanic acid
CXM	Cefuroxime	AZM	Azithromycin
AMC	Amoxicillin + clavulanic acid	ETP	Ertapenem
COX	Cefotaxime	MEM	Meropenem
CCO	Cefotaxime + clavulanic acid	TEM	Temocillin



Genotypic test : PCR triplex of blaCTX-M 1, 2 and 9 gene's group

DNA extraction

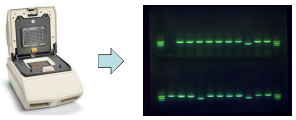


Figure 3. DNA extraction, PCR and gel electrophoresis

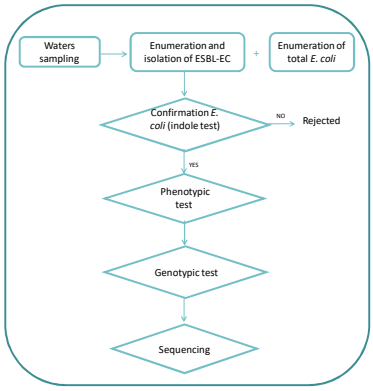


Figure 4. Flow chart of the analyses performed on the isolates

Sequencing

A subset of isolates (n=40) were selected for whole genome sequencing

RESULTS

- ❑ 372 ESBL-EC isolated in the 1st campaign
- ❑ 272 ESBL-EC isolated in the 2nd campaign

Enumeration of total *E. coli* and ESBL-EC

Code	Locations	1st campaign *E. coli*/100 ml TBX	1st campaign ESBL-EC/100 ml	1st campaign % ESBL-EC	2nd campaign *E. coli*/100 ml TBX	2nd campaign ESBL-EC/100 ml	2nd campaign % ESBL-EC
A	Ourthe at Lavacherie	2.0×10^3	94	4.7	3.2×10^3	15	0.5
B	Ourthe at Ortho	1.1×10^3	25	2.3	6.1×10^3	5	0.8
C	Ourthe at Mabompré	9.7×10^2	11	1.1	8.3×10^2	5	0.6
D	Ourthe at Maboge	6.1×10^2	8	1.4	3.2×10^2	4	1.3
E	Ourthe at Hampteau	6.5×10^2	6	0.9	2.8×10^2	2	0.6
F	Ourthe at Hotton	4.7×10^2	5	1.1	4.3×10^2	2	0.5
G	Ourthe at Noiseux	6.5×10^2	8	1.3	6.2×10^2	11	1.7
H	Petite Somme	1.5×10^4	52	0.3	2.1×10^4	12	0.6
I	Ourthe at Bomal	1.3×10^4	86	0.6	9.8×10^3	5	0.5
J	Ourthe at Comblain-Fairon	9.9×10^3	1.5×10^2	1.5	1.5×10^3	17	1.2
K	Ourthe at Aval MR Esneux	1.2×10^4	1.3×10^2	1.1	1.5×10^3	20	1.3
L	Upstream of WWTP Tiff-Embourg in the Ourthe	2.6×10^3	33	1.3	1.0×10^4	95	0.9
M	Downstream of WWTP Tiff-Embourg in the Ourthe	1.3×10^3	60	4.7	7.2×10^3	1.4×10^2	1.9
N	Input WWTP Tiff-Embourg	2.5×10^4	2.3×10^3	9.2	5.4×10^4	1.1×10^4	20.5
O	Output WWTP Tiff-Embourg	1.2×10^3	3.1×10^2	2.6	3.2×10^3	1.5×10^2	4.6
P	Upstream of WWTP Angleur in the Ourthe	2.8×10^3	42	1.5	1.2×10^4	1.4×10^2	1.1
Q	Downstream of WWTP Angleur in the Ourthe	3.6×10^3	43	1.2	1.5×10^4	1.9×10^2	1.3
R	Input WWTP Angleur	7.3×10^3	1.6×10^2	0.02	1.0×10^4	2.0×10^3	2.0
S	Output WWTP Angleur	1.2×10^4	7.6×10^2	0.6	1.9×10^4	2.4×10^2	1.2
T	Ourthe at Angleur-Chênée	8.6×10^3	2.1×10^2	2.5	8.5×10^3	1.1×10^2	1.3
U	Meuse	1.3×10^4	2.4×10^2	1.9	9.2×10^3	1.7×10^2	1.9
V	Hospital A	7.3×10^3	1.1×10^3	15.6	4.3×10^3	1.1×10^3	25.7
W	Veterinary faculty	1.1×10^5	6.7×10^3	6.3	3.2×10^4	4.1×10^3	0.1
X	Hospital B	4.0×10^3	6.3×10^3	0.2	1.0×10^4	2.0×10^3	2.0

Resistances by antibiotic

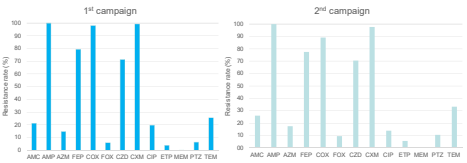


Figure 5. Resistance rates by antibiotic measured for strains isolated during the 1st and 2nd campaign

Distribution of CTX-M 1, 2 and 9 gene's group

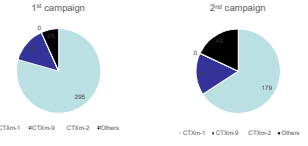
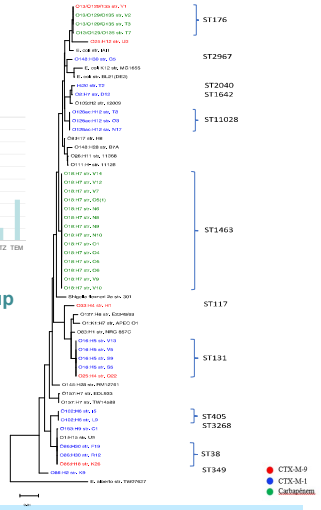


Figure 6. Distribution of the CTX-M 1, 2 and 9 gene's group for strains isolated during the 1st and 2nd campaign

Sequencing - Phylogenetic tree



KEY CONCLUSIONS

For all sampling location, ESBL-EC were enumerated and 644 ESBL-EC strains were isolated on Brilliance ESBL agar medium. The proportion of ESBL-EC to total *E. coli* (% ESBL-EC) ranged from 0.3 to 4.7% in freshwater and was highest in hospital effluent, up to 25%. Strains tested by the disk-diffusion assays showed the highest resistance for AMP (100%-100%), COX (98.1%-89.3%) and CXM (98.7%-97.8%) for the 1st and 2nd campaign respectively. A small number of ESBL-EC were resistant to the carbapenems tested (15 ETP-resistant strains and 1 MEM-resistant strain for each campaign).

Genes belonging to the blaCTX-M-1 and CTX-M-9 groups were detected in (79.3%-65.8%) and (14-16.2%) of the isolated strains, respectively for the 1st and 2nd campaign. No genes of blaCTX-M-2 group were found. The proportion of genes other than those belonging to these 3 groups (blaCTX-M-1, 2 and 9) was higher during the summer (2nd campaign).

A subset of isolates (n=40), selected for their high number of antibiotic resistance, were subjected to whole genome sequencing. *E. coli* O18:H7 serotype with ST1463 sequence types was predominant (n=14). The β-lactamase genes identified were blaCTX-M (n=21), with blaCTX-M-15 the most represented (n=15), as well as blaTEM (n=6), blaOXA (n=9) and blaSHV (n=9). One of the most observed concerns was the large number of strains containing carbapenemase genes- blaKPC-3 (n=19), blaNDM-1 (n=1) and blaVIM-1 (n=2) -even in freshwaters. This study shows that hospital effluents and WWTPs contribute to the dissemination of AR into the environment.