Presence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in the fecal flora of patients from general practice

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Abstract

Objectives: The aim of this study was to determine the extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) carriage in community patients' fecal flora and to characterize the detected ESBLs.

Methods: This study was performed at the University Hospital of Liège (Belgium). From March 2007 to June 2007, a total of 284 fecal specimens were collected from 284 patients who consulted their general practitioner. Each sample was homogenized in 1 ml of sterile saline and aliquots were inoculated on three different selective culture media: ChromID ESBL agar (bioMérieux) and MacConkey agar + ceftazidime (2 mg/L) and Drigalski agar + cefotaxime (1.5 mg/L). All the *Enterobacteriaceae* growing on these media were identified and tested for susceptibility by the Vitek2 (bioMerieux). The detection of ESBL production was performed by combined double disks (ceftazidime, cefotaxime and cefepime disks alone and a disk containing clavulanic acid). Characterisation of these ESBLs was performed by PCR assays targetting *bla*TEM, *bla*SHV, *bla*CTX-M, the most frequent ESBL genes, followed by amplicon sequencing.

Results: Overall, 53 *Enterobacteriaceae* were recovered on the selective media from 284 samples (18.7%). Among these, 25 were identified as ESBL producers: 20 *Escherichia coli*, 3 *Proteus mirabilis*, 1 *Serratia fonticola* and 1 *Enterobacter aerogenes*. These 25 ESBLE originated from 20 patients (7.04%). Among the ESBL-*E.coli*, the following ESBLs were found: TEM 19 (n=1), TEM 52 (n=4), CTX-M 1 (n=5) and CTX-M 15/28 (n=2). The ESBL from the *E. aerogenes* was TEM 52 and those from the 3 *P. mirabilis* were TEM 24. For 9 isolates, 8 *E.coli* and 1 *S.fonticola*, PCR did not demonstrate any ESBL gene of type TEM, SHV or CTX-M.

Conclusion: 1) Out of all screened community patients, 7% were found to be colonized in their fecal flora with ESBL-E strains. 2) *E.coli* accounted for the majority of ESBL-E isolates, while *P.mirabilis* and *E.aerogenes* were in a minor proportion. No ESBL-*Klebsiella sp.* was recovered. 3) Various ESBL genes were identified. 4) TEM- and CTX-M-derived enzymes were the most frequently encountered ESBLs. No SHV-derived enzyme was found. 5) The study shows that antimicrobial resistance among *Enterobacteriaceae* in the community becomes a reality which should probably be taken into account in treatment recommendations.

Background

Infections due to extended-spectrum beta-lactamase (ESBL) producing microorganisms are a well known problem in acute-care hospitals. Unfortunately, after their spread in other healthcare facilities, such as in nursing home, cases of infections by ESBL-producing Enterobacteriaceae (ESBL-E) are now related in the community.

In the context of a FNRS research project in the French-speaking Community of Belgium, three Universities (Louvain, Brussels and Liège) were involved to determine the impact of the large antibiotics use on the acquired resistance of bacteria in the community.

In collaboration with the Department of General Medicine, the Department of Microbiology of the University of Liège has conducted a study on the prevalence of ESBL-E colonizing the digestive tract, considered as a good indicator of resistance.

The aim of this study was to determine the ESBL-E carriage in community patients' fecal flora and to characterize the detected ESBLs.

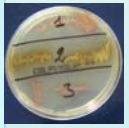
Methods

Clinical data: From March 2007 to July 2007, 284 fecal specimens were collected from different patients who consulted their general practitioner.

For each patient a questionnaire was filled about his age, gender, reason of consultation, taking of antibiotics during the last three months, hospitalization during this period, recent trips or pets at home.

Inoculation: Each fecal specimen was homogenized in 1 ml of sterile saline and 50 µl aliquots were inoculated on three different selective culture media:

ChromID ESBL agar (bioMérieux): Mixture of antibiotics and detection of the production of beta-glucuronidase, beta-glucosidase and desaminase.



Red: *E.Coli*
Green: *Klebsiella spp.*, *Enterobacter spp. …*
Brown: *Proteus spp.*, *Morganella spp. …*

Some *Enterobacteriaceae* and non fermenting gram negative bacilli are uncolored.

MacConkey agar + ceftazidime (2 mg/L)
Drigalski agar + cefotaxime (1.5 mg/L).

Detection of the fermentation of lactose:
Pink on MacConkey
Yellow on Drigalski

Identification and detection of ESBL-E:

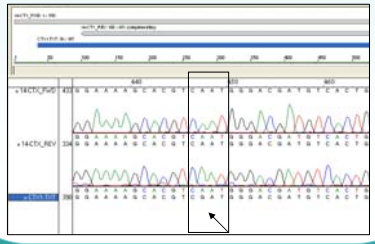
All the *Enterobacteriaceae* growing on these media were identified by the Vitek2 (bioMerieux).

They were screened for ESBL production by combined double disks method (ceftazidime 30 µg, cefotaxime 30 µg and cefepime 30 µg disks and a disk containing clavulanic acid 10 µg).

Genotypic characterization: A Qiagen extraction was performed on each isolated ESBL-E and was followed by 3 different PCR assays targetting *bla*TEM, *bla*SHV and *bla*CTX-M.

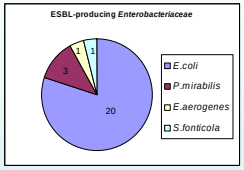
TEM fwd: ATAAAATTCTTGAAGACGAA
TEM rev: ATATGAGTAAGCTTGGTCTGACAG
SHV fwd: GCCTTCACTCAAGGATGTATTGTG
SHV rev: CCCCAAGCTTTTAGCGTTGCCAGTGCTCGATC
CTX-M fwd: GTTACAATGTGTGAGAAGCAG
CTX-M rev: CCGTTTCCGCTATTACAAAC

When the PCR was positive, the amplified DNA was sequenced and mutations were analyzed to determine the identification of the beta-lactamase.



Results

Phenotypic results: 53 *Enterobacteriaceae* were recovered on at least one of the selective media from 284 samples (18.7%). Among these, 25 were identified as ESBL producers:



These 25 ESBL-E originated from 20 patients (7.07%).

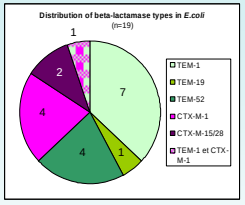
Genotypic results:

•Results of the PCR for the 25 ESBL-E:

One *E.coli* and the *S.fonticola* did not possess any beta-lactamase of type TEM, SHV or CTX-M.

Species (number of isolates)	beta-lactamase			
	TEM	CTX-M	TEM and CTX-M	SHV
E. coli (19)	12	6	1	0
E. aerogenes(1)	1	0	0	0
P. mirabilis(3)	3	0	0	0

• Distribution of beta-lactamase types by species:



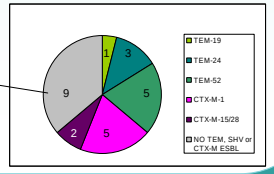
Beta-lactamase in *E.aerogenes* (n=1): TEM-52

Beta-lactamases in *P.mirabilis* (n=3): TEM-24

• Characterization of the ESBLs from the 25 ESBL-E:

23 strains harbored a TEM or CTX-M gene. Among these, no ESBL TEM gene was demonstrated for 7 *E. coli*.

1 *S.fonticola*
8 *E.coli*



Expected risk factors for ESBL-E carriage:

Among this limited population, no association between the following factors and the ESBL-E carriage was demonstrated.

	ESBL-E carriers (Total=20)	ESBL-E negative carriers (Total=264)	
Recent antibiotics taking	4	74	p > 0.05
Pets at home	11	135	p > 0.05
Recent trips	1	43	p > 0.05
Recent hospitalization	2	15	p > 0.05

Conclusion

1) Out of all screened community patients, 7% were found to be colonized in their fecal flora with ESBL-E strains phenotypically characterized. This prevalence is higher than reported in other studies in the community (5.5 % found by Valverde et al. in Spain in 2003)

2) *E.coli* accounted for the majority of ESBL-E isolates, while *P.mirabilis* and *E.aerogenes* were in a minor proportion. No ESBL-*Klebsiella sp.* was recovered.

3) Various ESBL genes were identified.

4) TEM- and CTX-M-derived enzymes were the most frequently encountered ESBLs. No SHV-derived enzyme was found.

5) 36 % of the phenotypically characterized ESBL-E did not possess any ESBL of type TEM, SHV or CTX-M. Other ESBL types should be searched for these isolates.

6) The study shows that antimicrobial resistance among *Enterobacteriaceae* in the community becomes a reality which should probably be taken into account in treatment recommendations.

References

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Acknowledgements

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