Regional dissemination of an IS*911*-mediated *bla*$_{AmpC}$-hyperexpressing *Escherichia coli* ST131 clone

C. Ramberg[1], D.H. Skutlaberg[2], C.G. Giske[3], A. Sundsfjord[1,4] and Ø. Samuelsen[1*]

[1]Reference Centre for Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway. [2]Haukeland University Hospital, Department of Microbiology, Bergen, Norway.
[3]Karolinska Institutet – MTC, Clinical Microbiology, Karolinska University Hospital, Solna, Stockholm, Sweden. [4]Research Group for Host-Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway.

Objective

To investigate a possible regional dissemination of clonally related *E. coli* isolates hyperexpressing the chromosomal AmpC β-lactamase.

Materials and methods

Strain collections
Two strain collections of *E. coli* isolates submitted to the National Reference Centre for Detection of Antimicrobial Resistance from 2006-2010 were analysed in this study:

- ***HUH collection***: All 111 *E. coli* isolates submitted from Haukeland University Hospital (HUH) in Western Norway
- ***Nationwide collection***: 100 *E. coli* isolates from other laboratories.

The selection criteria for the isolates included an AmpC-phenotypic profile, negative PCR for plasmid-mediated AmpC genes[1] and phenotypically ESBL-negative. Clinical data were collected retrospectively.

Identification and antimicrobial susceptibility testing
Bacterial identification was performed using Vitek2 ID-GNB systems (BioMérieux). Antimicrobial susceptibility testing was performed by Etest (BioMérieux) and the results were interpreted according to the clinical breakpoints set by EUCAST.

Screening for insertions in the *bla*$_{AmpC}$ promoter region
All 211 bacterial isolates were screened for an insertion in the *bla*$_{AmpC}$ promoter region by PCR using primers located upstream of the *bla*$_{AmpC}$ promoter region and in the *bla*$_{AmpC}$ gene. Linkage between *bla*$_{AmpC}$ and IS*911* was confirmed by an IS*911*-*bla*$_{AmpC}$ linkage PCR.

Sequencing of insertions in the *bla*$_{AmpC}$ promoter region
Sequencing of the PCR product in 13 insertion positive isolates were performed using BigDye 3.1 technology (Applied Biosystems). Sequence alignment was performed using DNAStar Lasergene (DNAStar).

Molecular typing of isolates
Multilocus sequence typing (MLST) were performed on selected isolates according to the Achtman scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli). The presence of sequence type (ST)131 in both strain collections was investigated using a real-time PCR as described by Dhanji H. *et al.*[2]

References

1. Brolund A. *et al*. 2010 *J. Microbiol. Methods* **82**:229-233
2. Dhanji H. *et al*. 2010 *Int. J. Antimicrob. Agents* **36**:355-358
3. Mulvey MA. *et al.* 2005 *Antimicrob. Agents Chemother.* **49**:358-365

Results

Screening for insertions in the *bla*$_{AmpC}$ promoter region
Screening for insertions in the *bla*$_{AmpC}$ promoter region showed that in total 42% of the HUH isolates had an insertion while only 3% in the nationwide collection was positive for an insertion (Table 1).

Table 1: Distribution of insertions in the *bla*$_{AmpC}$ promoter region.

	HUH collection		Nationwide collection	
Year	Insertion-positive	Insertion-negative	Insertion-positive	Insertion-negative
2006	9	16	0	20
2007	3	40	0	20
2008	17	5	1	19
2009	14	1	2	18
2010	4	2	0	20
Total	47	64	3	97

Sequencing of insertions in the *bla*$_{AmpC}$ promoter region
Sequencing of the insertion in 10 isolates from HUH revealed that in all isolates IS*911* was inserted in the *bla*$_{AmpC}$ promoter region between the -35 box and -10 box (Figure 1). The IS911 insertion site was as described previously in a Canadian study on cefoxitin resistant *E. coli*.[3]

Linkage PCR of IS*911* and *bla*$_{AmpC}$ confirmed the insertion of IS*911* in all insertion-PCR positive HUH isolates.

In the 3 insertion positive isolates from the nationwide collection IS*10* was inserted in the *bla*$_{AmpC}$ promoter region.

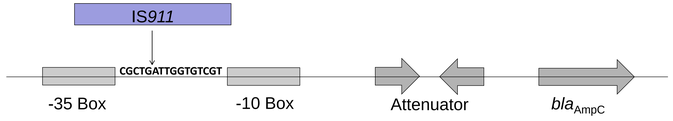

Figure 1: Schematic diagram showing the insertion of IS*911* in the *bla*$_{AmpC}$ promoter region of HUH isolates.

Molecular typing of isolates
Initial MLST analysis of ten IS*911*-positive isolates from the HUH collection showed that all isolates were of sequence type (ST)131. Subsequent screening using a ST131 real-time PCR on all isolates from both collections showed that 98% of the IS*911*-*bla*$_{AmpC}$ positive isolates from HUH were ST131 (Table 2). The non-ST131 isolate were typed to ST978.

The three IS*10*-*bla*$_{AmpC}$ positive isolates from the nationwide collection were typed to ST23 and ST405 (*n*=2).

Among the insertion-negative isolates 11/64 (17%) and 9/100 (9.0%) were ST131 in the HUH collection and in the nationwide collection, respectively (Table 2).

Table 2: Distribution of ST131 in the HUH collection and nationwide collection.

	HUH collection		Nationwide collection	
	IS*911*-positive	IS*911*-negative	IS*10*-positive	Insertion-negative
ST131	46	11	0	9
Non-ST131	1	53	3	88
Total	47	64	3	97

Antimicrobial susceptibility profile of IS*911*-*bla*$_{AmpC}$ ST131 HUH isolates
The antimicrobial susceptibility profile of the 46 IS*911*-*bla*$_{AmpC}$ ST131 isolates are shown in Table 3.

Clinical data of IS*911*-*bla*$_{AmpC}$ HUH isolates
The 47 IS*911*-*bla*$_{AmpC}$ HUH isolates were isolated from 39 patients. In 7 patients ≥ 2 isolates were obtained with the longest time period between isolations of ~11 months indicating long term colonization.

Based on the first isolation of an IS*911*-*bla*$_{AmpC}$ isolate, the majority of samples originated from patients admitted to nursing homes (56%) or hospitals (26%). 18% were from general practitioners.

Forty five isolates were from urine and 2 isolates were from blood culture. The patient age ranged from 58-97 years (mean 84 years) and 79% of the patients were female.

Table 3: Antimicrobial susceptiblity of IS*911*-*bla*$_{AmpC}$ ST131 HUH isolates (*n*=46)

	Proportion of isolates (%)		
Agent[1]	Susceptible	Intermediate	Resistant
AMC	0	-	100
TZP	74.0	21.7	4.3
CPD	0	-	100
CTX	0	30.4	69.6
CAZ	0	47.8	52.2
FOX	0	-	100
MEM	100	0	0
CIP	0	0	100
GEN	10.9	0	89.1
TOB	10.9	0	89.1
AMK	100	0	0
SXT	32.6	0	67.4
FOF	87.0	-	13.0
NIT	100	-	0
MEC	95.7	-	4.3

[1]AMC: amoxicillin-clavulanate; TZP: piperacillin-tazobactam;
CPD: cefpodoxime; CTX: cefotaxime; CAZ: ceftazidime;
FOX: cefoxitin; MEM: meropenem; CIP: ciprofloxacin; GEN: gentamicin;
TOB: tobramycin; AMK: amikacin; SXT: trimethoprim-sulfamethoxazole;
FOF: fosfomycin; NIT: nitrofurantioin; MEC: mecillinam.

Conclusion

We have shown a regional clonal dissemination of an ESBL-negative ciprofloxacin resistant *E. coli* ST131 clone hyperexpressing the chromosomal AmpC induced by an IS*911* insertion in the promoter region.

Acknowledgement
Part of this study was supported by a grant from the Northern Norway Regional Health Authority.
Bjørg Haldorsen and Bettina Aasnæs is acknowledged for excellent technical assistance.

***Contact information:** E-mail: orjan.samuelsen@unn.no