Prevalence of Extended Spectrum β-lactamase-producing *Enterobacteriaceae* and vancomycin-resistant enterococci in hospitals and faecal carriage in the community in the Northern Netherlands.

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Objectives

Extended spectrum β-lactamase (ESBL), plasmid mediated AmpC β-lactamase (pAmpC) and carbapenemase (CP)-producing enterobacteria have emerged globally. The increase of these bacteria in the hospital setting is a major problem, but their emergence in the community is also a matter of concern. In addition, vancomycin resistant enterococci (VRE) emerged as an important nosocomial pathogen worldwide. The increase of *E. faecium* infections is mainly due to the so-called specific hospital associated (HA) *E. faecium*, which are amoxicillin resistant (AREfm). The aim of this study was to determine the prevalence of ESBL/pAmpC/CP- *Enterobacteriaceae* and HA *E. faecium* (AREfm and VREfm) in Dutch hospitals and the community

Material and Methods

In total 445 rectal swabs were prospectively collected from patients in 4 hospitals in the Northern Netherlands between 2012-2013. The following high-risk wards for antibiotic- resistant microorganisms were selected: intensive care units (ICU), vascular surgery, internal medicine haematology/oncology and dialysis wards (both for in- and out-patients). Wards for which a low prevalence was expected were also included for comparison: gynaecology and neurology. Additionally 400 stool samples were collected from healthy people in the community between 2010-2012. Detection of ESBL/pAmpC-*Enterobacteriaceae* and VRE was done on selective medium after pre-enrichment. Species identification was done by MaldiTof-MS and susceptibility testing by the VITEK®2 system according to EUCAST breakpoints. DNA array assays were used to determine ESBL genes. Typing of ESBL/pAmpC positive *E. coli* was performed using multiple locus sequence typing (MLST) (Wirth *et al*.). *E. coli* phylogenetic groups were determined by a multiplex PCR assay as described by Clermont *et al*. Enterococci intermediate or resistant to amoxicillin and/or resistant to vancomycin were screened by PCR for the presence of IS16, *vanA* and *van*B genes.

Ward	**ESBL/pAmpC producing *Enterobacteriaceae***	**Amoxicillin resistant *E. faecium***	**Vancomycin resistant *E. faecium***
Intensive care unit (n=102)	6 (5.9%)	31 (30.4%)	1 (1%)
Vascular surgery (n=54)	6 (11.1%)	15 (27.8%)	1(1%)
Internal medicine hematology/oncology (n=81)	1 (1.2%)	36 (44.4%)	2 (2.5%)
Dialysis (n=91)	6 (6.6%)	17 (18.7%)	2 (2.2%)
Subtotal high risk (n=328)	19 (5.8 %)	99 (30.2%)	6 (1.8%)
Gynaecology (n=55)	3 (5.5%)	1 (1.8%)	0 (0%)
Neurology (n=62)	5 (8.1%)	5 (8.1%)	0 (0%)
Subtotal low risk (n=117)	8 (6.8%)	6 (5.1%)	0 (0%)
Total (n= 445)	27 (6.1%)	105 (23.6%)	6 (1.3%)

Table 1. Distribution of ESBL/pAmpC producing *Enterobacteriaceae*, amoxicillin and vancomycin resistant *E. faecium* among the different wards studied in Dutch hospitals.

Results

A total of 34 hospital isolates from 27 patients (6.1%) were confirmed to be ESBL and/or pAmpC positive. Thirty-two isolates were *E. coli,* of which 29 were ESBL positive (CTX-M-1-like (n=17), CTX-M-9 group (n=9), TEM_{ESBL} (n=2), SHV_{ESBL} (n=1)) and 3 were pAmpC producers (CMY-II (n=2) and DHA (n=1)). ST131-phylogroup B2 was the most prevalent among *E. coli* isolates (15.6%). The other two positive isolates were an *E. cloacae* (containing a CTX-M-1-like gene) and a pAmpC CMY-II producing *P. mirabilis*. At high risk wards 19 patients (5.8%) were found with ESBL/pAmpC positive isolates compared to 8 patients (6.8%) at low risk wards (Table 1) (NS).

In the community samples, 10 *E. coli* (2.5%) were confirmed to be ESBL positive (CTX-M-1 like (n=5), CTX-M-15-like (n=3), CTX-M-9 group (n=1) and SHV_{ESBL} (n=1)), and 1 (0.3%) was pAmpC CMY-II positive. Among the 11 *E. coli* community isolates ST10-Cplx was the most prevalent (27.3%) whereas only one isolate belonged to ST131 (phylogroup F).

A total of 105 hospitalized patients (23.6%) were colonized with ampicillin resistant *E. faecium* (AREfm), six (1.3%) of them were also colonized with vancomycin resistant *E. faecium* (VREfm). All AREfm were positive for the IS16. All VREfm had the *vanB* gene. Colonization of AREfm (and VREfm) was associated with high risk wards (p<0.001), prolonged hospitalization (p<0.001) and use of antibiotics (p=0.05), especially fluoroquinolones (p=0.009) (Table 2). Six AREfm (1.5%) were found in the community samples, three (0.75%) of them were confirmed *IS16* positive and one was a *vanA*-VREfm (0.25%).

Variables	**AREfm n=105**	**No AREfm n=340**	***p*-value**
Hospitalization days median (range)	12 (1-127)	3 (1-107)	*P*<0.001
Ward			*P*<0.001
- High risk (n=328)	99 (94.3%)	229 (67.4%)	
- Low risk (n=117)	6 (5.7%)	111 (32.6%)	
Antibiotic use (n=145)	62 (59%)	83 (24.4%)	*P*<0.001
- Broad spectrum penicillins	15 (14.3%)	23 (6.8%)	*P*=0.016
- Fluoroquinolones	28 (26.7%)	15 (4.4%)	*P*<0.001
- 3rd gen cephalosporins	11 (10.5%)	19 (5.6%)	*P*=0.081

Table 2. Variables associated with carriage of amoxicillin-resistant *E. faecium* (AREfm)

Conclusions

A higher prevalence of ESBL/pAmpC-*Enterobacteriaceae* was observed in hospitals compared to the community in the Northern Netherlands. The most prevalent ESBL gene appeared to be CTX-M-1-like in both settings. The results suggest ESBL/pAmpC-*E.coli* circulate in the hospital and the community. AREfm is a more prevalent nosocomial pathogen whereas VREfm prevalence was low both in the hospital environment and the community.