UNIVERSITY OF HELSINKI
FACULTY OF VETERINARY MEDICINE

NATIONAL INSTITUTE FOR HEALTH AND WELFARE

Epidemiological comparison of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* from equine patients at the Finnish Veterinary Teaching Hospital in 2011-2014

Eklund Marjut[1], Thomson Katariina[1], Jalava Jari[2], Niinistö Kati[1], Grönthal Thomas[1], Piiparinen Heli[1], Rantala Merja[1]

[1]University of Helsinki, Faculty of Veterinary Medicine, Finland, [2]National Institute for Health and Welfare, Turku, Finland

Objectives:

At the end of 2011, the first ESBL producing *Enterobacteriaceae* isolates were observed from equine specimens at the Equine Veterinary Teaching Hospital of the University of Helsinki. Due to the increasing number of infections in fall 2012 and spring 2013, enhanced surveillance was initiated. This included culturing of infection sites, and – if ESBL was detected – screening of contact patients. In this work we characterize equine ESBL isolates collected from 83 horses between 10/2011 – 05/2014.

Materials and methods:

- **Bacterial isolates:** 117 ESBL-isolates, of which 92 and 25 were from screening and infection specimens, respectively
- **Identification and susceptibility testing:** API20E (Biomerieux, France), CLSI disk diffusion method.
- **ESBL production:** cefotaxime/ceftatzidime with or without clavulanic acid and ESBL/AmpC detection disks (MAST, UK)
- **Genotyping:** To detect clusters, PFGE with *Xba*I digestion was performed for each species (PulseNetO157, CDC). MLST was done for *Klebsiella pneumoniae* (bigsdb.web.pasteur.fr/klebsiella) and *Escherichia coli* (mlst.warwick.ac.uk). The genes coding for CTX-M, TEM, SHV were detected by multiplex PCR; sequencing was performed to identify ESBL-variant

Results:

- 117 isolates represented 6 bacterial species: *K. pneumoniae* (44*), Enterobacter cloacae* (32), *E. coli* (29), *Klebsiella oxytoca* (6), *Citrobacter* spp. (4), *Enterobacter aerogenes* (2)
- 28 /83 (34%) horses had more than one *Enterobacteriaceae* species
- One major *K. pneumoniae* clone with 40 members (Figure 2) was observed; were of ST307 and carried CTX-M-1 ESBL enzyme (Table 1). These were isolated mainly during April – August 2013 (Figure 1)
- *E. cloacae* distributed into 12 PFGE types with 6 clusters of 2 to 9 isolates. All carried SHV and TEM; phenotypically they showed SHV activity
- *E. coli* scattered into 20 PFGE types with 4 clusters of 2 to 7 isolates (Table 1). Both CTX and SHV pheno- and genotypes were observed. 10 isolates were of ST167 with 2 pulsotypes
- Among the 6 *K. oxytoca,* 4 were of same PFGE-type. The isolates were CTX-M, TEM and SHV positive, but phenotypes indicated the presence of SHV
- *Enterobacter* and *Citrobacter* spp. commonly showed both ESBL and AmpC phenotype. All carried TEM and SHV, but phenotypes suggested presence of SHV
- Of all ESBL isolates, 74/117 (63%) were multi-drug resistant. No resistance to amikacin was observed

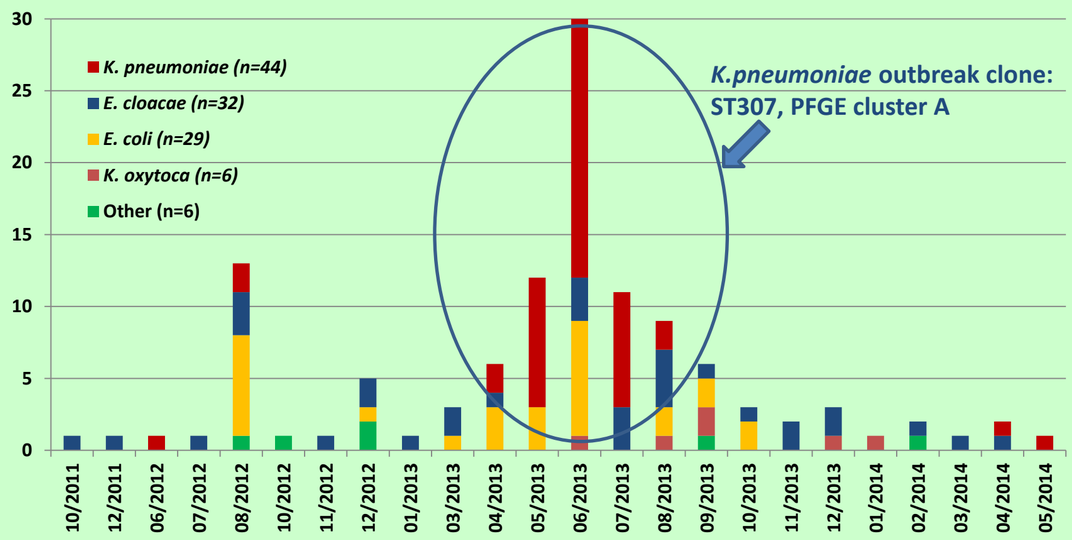

Figure 1. Temporal occurrence of ESBL isolates from equine specimens

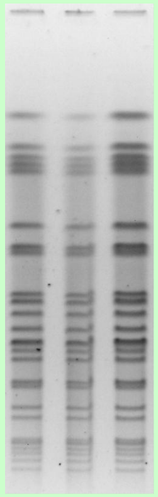

Figure 2. *K. pneumoniae* ST307 clone

Table 1. Characteristics of *K. pneumoniae* and *E. coli* clusters

Species	PFGE - cluster	No. of isolates	MLST type	ESBL		Antibiogram***		
				gene*	phenotype**	SXT	ENR	CN
K. pneumoniae	A	40	ST307	CTX-M-1*	CTX, SHV	R	R	R
	B	4	ST107	CTX-M, SHV	CTX, SHV	R	S	R
E. coli	C	7	ST167	CTX-M, TEM	CTX	R	R	R
E. coli	D	3	ST167	CTX-M, TEM	CTX	R	R	R
E. coli	E	2	ST141	SHV, TEM	SHV	R	S	R
E. coli	F	2	New type	CTX-M, TEM	CTX	R	I	R

**ESBL gene for ST307 was confirmed by sequencing; the rest were screened by multiplex-PCR; ST307 had also SHV and TEM, but these were not ESBL variants*

**phenotype as indicated by the results of cefotaxime /ceftatzidime (+/- clavulanate) double disk test

***SXT=trimethoprim-sulfamethoxazole, ENR=enrofloxacin, CN=gentamicin

Conclusions: The results indicate nosocomial spread of a *K. pneumoniae* ST307 clone with spatial and temporal association. Concurrent temporal clustering of heterogeneous ESBL species, as well as simultaneous presence of multiple species in the same horse may suggest plasmid transmission between bacterial genera.