High prevalence of Beta-lactamase producing Enterobacteriaceae: also in captivity animals from zoological gardens in Belgium

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Introduction and Purpose

Over the last decade, there has been a worldwide increase in the occurrence of extended-spectrum ß-lactamases (**ESBLs**) and of plamidic-AmpC ß-lactamases (**pAmpCs**) which account as major causes of antibiotic resistance. There are only scarce data on the prevalence of the distribution of these resistance mechanisms in **zoo animals** (1, 2, 3). Here we analyzed 389 **fecal samples** from 86 different animal zoo species for the presence of ESBLs and pAmpCs.

Methods

Bacterial strains identification

From September 2014 to July 2015, 389 faecal samples were collected from various animals at Antwerp Zoo and at Wild Animal Park Planckendael (mammals [n=184], birds [n=167] and reptiles [n=38]). In search for possible reservoirs, 120 animal source food items were also examined (meat, rats, mice, rabbits, 1 day-old chicks, herring, sprat, roach, crickets, grasshoppers, meal- and buffalo worms).

Antimicrobial susceptibility, phenotypic detection of resistance

The samples were cultured on MacConkey agar plus cefotaxime disc after enrichment in broth containing 1µg/ml cefotaxime. Cefotaxime-resistant colonies were identified and subjected to antimicrobial susceptibility testing by disc diffusion according to CLSI standards.

Molecular testing

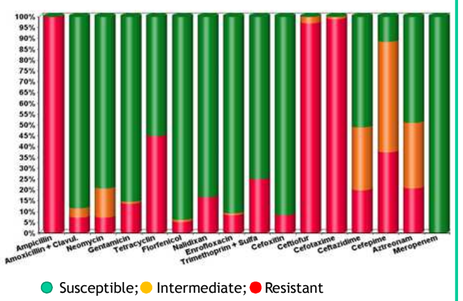
Multiplex PCRs targeting ESBLs and pAmpCs genes were performed by in house ISO15189 multiplex PCR. Analysis of β-lactamase genes were performed by CT103XL array (Check-Points, Wageningen, The Netherlands) and by PCR-sequencing targeting carbapenemases, ESBLs and minor ESBLs (GES, VEB, PER, BEL).
A subset of strains was typed by MLST and assigned by PCR to phylogenetic groups A, B1, B2, C, D, E or F along with sequencing of the ESBL/pAmpC genes (4).
Plasmids were extracted by the method of Kieser and analyzed on 1% agarose gel in presence of ethidium bromide.

Results

110 ESBL/AmpC producing-isolates were recovered from 46 mammals, 47 birds, 6 reptiles and 9 food samples (frozen chicken)

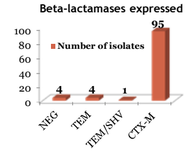
Species identification	Number of isolates
E. coli	104
C. freundii	2
E. cloacae	2
E. aerogenes	1
K. pneumoniae	1
Total	110

Antibiotic susceptibility profiles

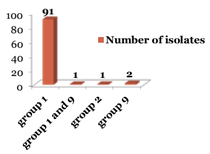


22.3 % of the mammals, 27.5% of the birds and 7.9 % of the reptiles were colonized by ESBL/AmpC-producing *Enterobacteriaceae*

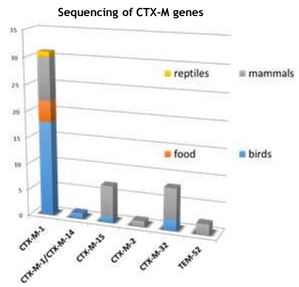
Beta-lactamases expressed



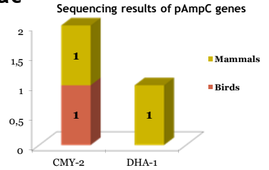
Repartition of CTX-M group



Sequencing of CTX-M genes

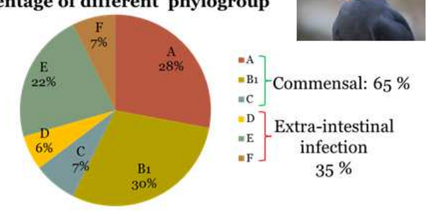


Sequencing results of pAmpC genes

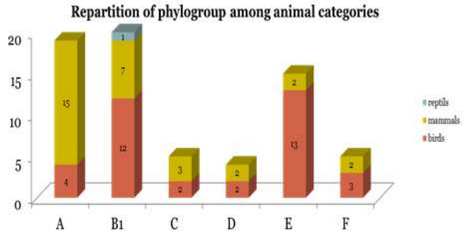


65 % and 35 % of the *E. coli* belongs to commensal and hypervirulent extra-intestinal phylogroups respectively

Percentage of different phylogroup

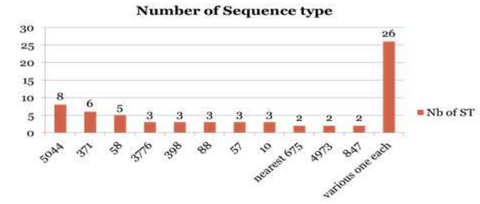


Repartition of phylogroup among animal categories

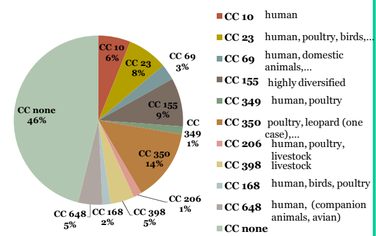


E. coli belong to 37 different STs and present multiple plasmid profiles

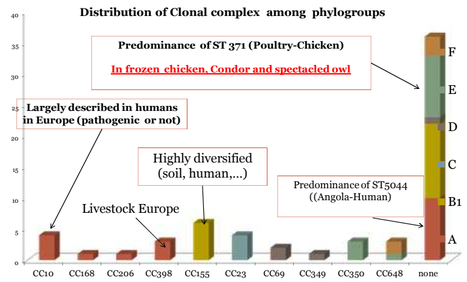
Number of Sequence type



Repartition of Clonal Complex among isolates

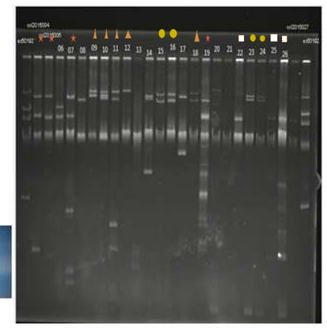


Phylogroup VS Clonal complex (58 strains)



CC350: ST371, ST57
CC155: ST58, 155
CC23: ST23, 88
CC10: ST10, 48
CC648: ST 648, 1485, 2112
CC none: 18 STs

Plasmid profiles of representative *E. coli*



Food/water transmission to be further investigated

- **6 birds with *E. coli* ST371 (CC350) Phylogroup E**
- **6 CTX-M-1**
- **4 in frozen chickens**
- **2 in prey birds (Condor, Spectacled owl)**
- **All TET ans SSS co-resistant**

Conclusions

- More than 20 % of ESBL carriage in animal zoo
- CTX-M-1 is the major ESBL
- 35 % of *E. coli* Hypervirulent phylogroups are detected
- Routes of transmission have to be investigated including by WGS

References

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2. Wang, Y. et al.(2012): Prevalence of ESBLs and PMQR genes in fecal Escherichia coli isolated from the non-human primates in six zoos in China. Vet Microbiol. 159(1-2), 53-9
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