Molecular epidemiology from the national survey of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in Belgium

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Revised abstract

Background
Carbapenemase-producing *Enterobacteriaceae* (CPE) isolates represent a serious threat for global public health. We conducted a nationwide survey to monitor the evolution of the epidemiology of carbapenemase-producing *K. pneumoniae* (CPKP) and *E. coli* (CPEC) in Belgium.

Methods
The survey includes 75 laboratories requested to refer non-duplicate consecutive *K. pneumoniae* (KP) and *E. coli* (EC) isolates collected from clinical specimens (fecal samples excluded) between May and October 2019 to the National Reference Center for decreased carbapenem susceptibility. Isolates were tested for susceptibility phenotype by disk diffusion and for carbapenemase production by colorimetric hydrolysis test, immunochromatographic lateral flow assay (LFA) and PCRs. Whole-genome sequencing using Illumina technology was performed on CPE isolates to characterize their resistance determinants and clonal distribution.

Results
Of the 232 referred isolates from 54 laboratories, 144 KP and 31 EC collected from 46 laboratories were confirmed as CPE. Among KP, the distribution of the carbapenemase enzymes was: OXA-48 (74%), KPC-3 (13%), NDM-1 (7%), while OXA-181, OXA-232, VIM-1, NDM-5 and IMP-8 were present in ≤3 isolates each. Among EC, OXA-48 (52%), OXA-244 (32%) were predominant, while NDM-5 (10%), NDM-1 (3%) and VIM-1 (3%) were also found. Compared to a previous survey including the same laboratories in 2015, there was an increase in the proportion of laboratories reporting CPKP/CPEC (47% to 61%; p=0.07), the number of CPKP/CPEC grouped cases defined as >1 case of the same CPE type in the same hospital (24 to 37; p=0.027) and the number of laboratories reporting ≥2 different carbapenemase types (8 to 13; p=0.21). The genomic population analysis among KP revealed ST15, ST307, ST512, ST405, ST17 as the top 5 clones (54% of the total 45 MLST-types) with regional clusters, while other high-risk clones (ST147 and ST392) were also detected. For EC, 20 different MLST-types were identified with no predominant clone.

Conclusions
OXA-48-like remained the predominant carbapenemase among CPKP/CPEC in Belgium in 2019 but increasing laboratories reporting more than one carbapenemase type from clinical specimens strongly suggests their spread and diversification. Our data also demonstrated the emergence of carbapenemase variants (OXA-244, NDM-5) and high-risk clones (KP CC147).

Introduction

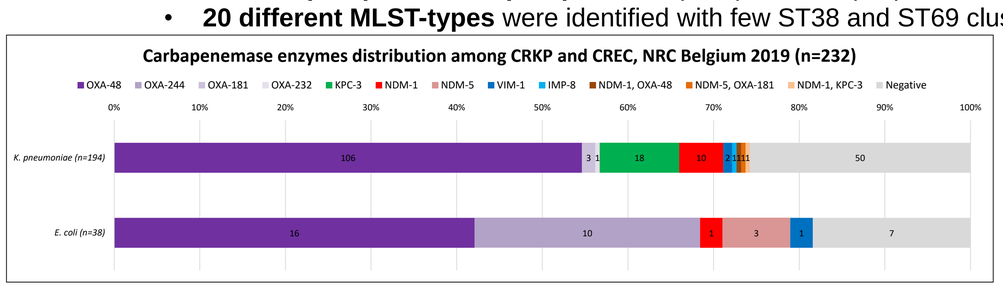
- The rapid evolution and spread of carbapenemase-producing *Enterobacterales* (CPE) causing outbreaks with limited treatment options have become a global public health concern.[1]
- In Belgium, two previous multicentric surveys demonstrated a significantly increased proportion of CPE among clinical *Enterobacterales* isolates in hospitals from 2012 to 2015.[2] *Klebsiella pneumoniae* and OXA-48 were the most frequent CPE species and carbapenemase type encountered in Belgium.
- We conducted a nationwide survey to monitor the evolution of the epidemiology of carbapenemase-producing *K. pneumoniae* (CPKP) and *E. coli* (CPEC) in Belgium during year 2019.

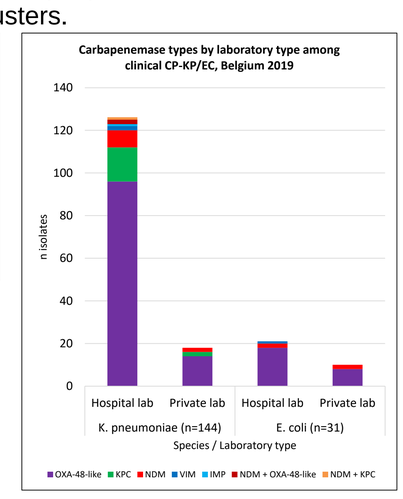
Methods

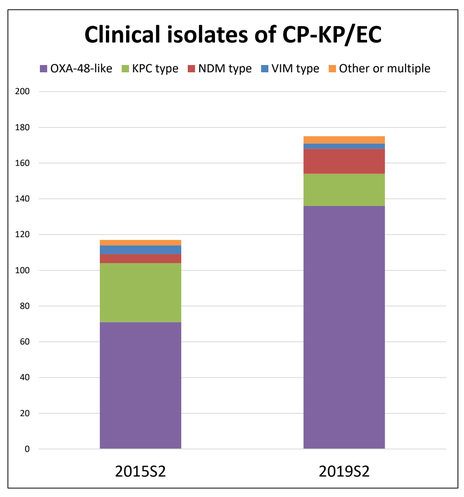
- Active national survey: 75 (63 hospital-based and 12 private community-serving) laboratories requested to refer all non-duplicate consecutive *K. pneumoniae* (KP) and *E. coli* (EC) isolates for decreased carbapenem susceptibility collected from clinical specimens (fecal samples excluded) between May and October 2019 to the National Reference Center (CHU UCL Namur, Yvoir, Belgium).
- All isolates were tested for susceptibility phenotype by disk diffusion and for carbapenemase production by hydrolysis-based tests (βCARBA test, BioRad), immunochromatographic lateral flow assays (LFA) targeting KPC, OXA-48-like, VIM, NDM, IMP enzymes (RESIST K-Set, Coris Bioconcept) and/or PCRs targeting their encoding genes. Other less prevalent carbapenemases were eventually sought by molecular methods in case of suspicious CPE phenotype (multidrug-resistance) with negative hydrolysis-based tests result and by multiplex PCR for the 5 major carbapenemase encoding genes).
- Whole-genome sequencing using Illumina technology was performed on confirmed CPKP and CPEC isolates to characterize their resistance determinants and clonal structure.
- Epidemiological indicators were compared to those of the active surveillance performance during the same 6-month period in 2015.

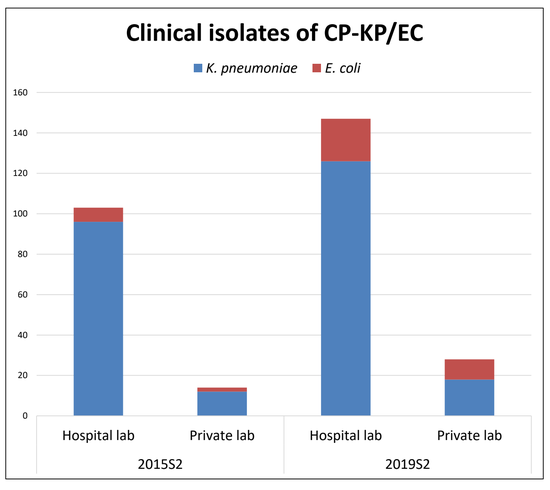
Results

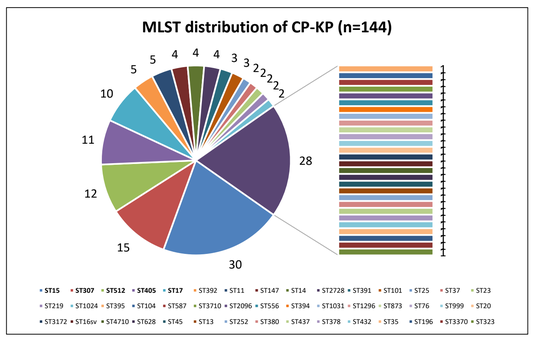
- 175 isolates from 46 laboratories (74% of the 232 referred by 54 labs) confirmed as CPE:
  - **CPKP (n=144):**
    - **OXA-48 (74%), KPC-3 (13%), NDM-1 (7%),** OXA-181 (2%), OXA-232 (<1%), VIM-1 (<1%), NDM-5 (<1%) and IMP-8 (<1%)
    - **ST15, ST307, ST512, ST405, ST17** as the top 5 clones (54% of the total **45 MLST-types**)
  - **CP-EC (n=31):**
    - **OXA-48 (52%), OXA-244 (32%),** NDM-5 (10%), NDM-1 (3%) and VIM-1 (3%)
    - **20 different MLST-types** were identified with few ST38 and ST69 clusters.
- 2019 compared to 2015 (same 6-month period, same 75 labs):
  - ↑ **number (proportion) of laboratories reporting CPKP/CPEC** from 35 (47%) in 2015 to 46 (61%) in 2019 (p=0.07)
  - ↑ **CPKP/CPEC grouped cases** (>1 case of the same CPE type) from 24 in 2015 to 37 in 2019 (p=0.027)
  - ↑ **≥2 different carbapenemase types** (n=8 in 2015 and n=13 in 2019; p=0.21)

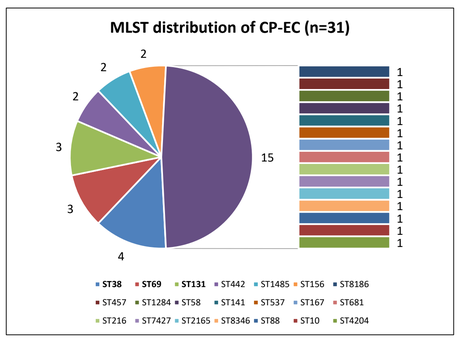


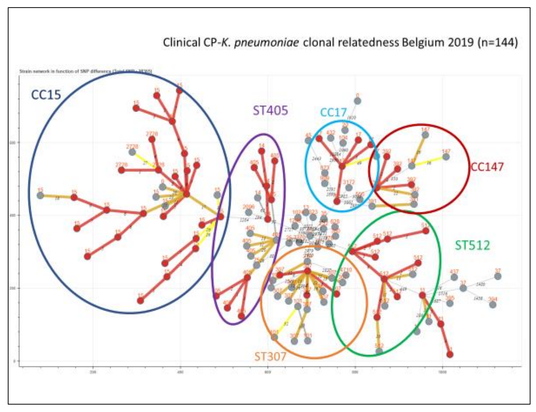


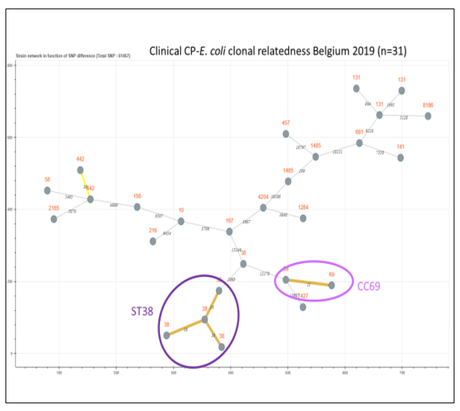


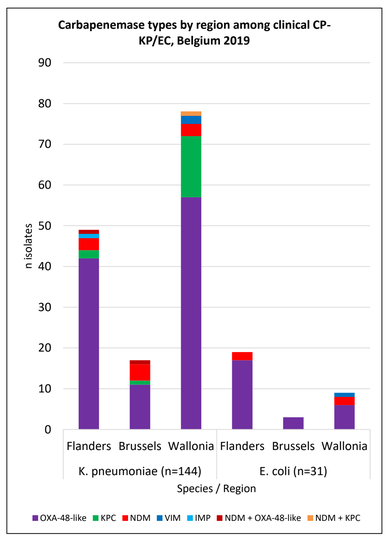


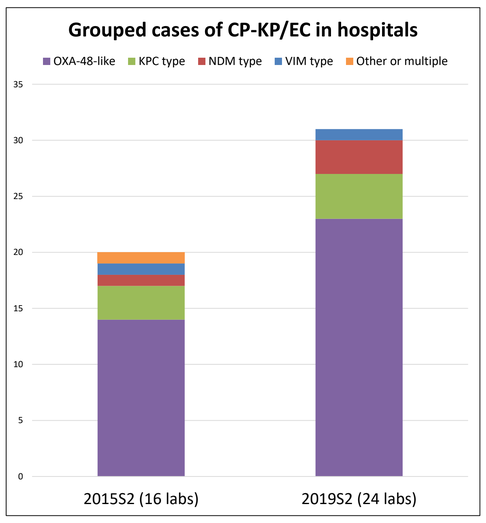


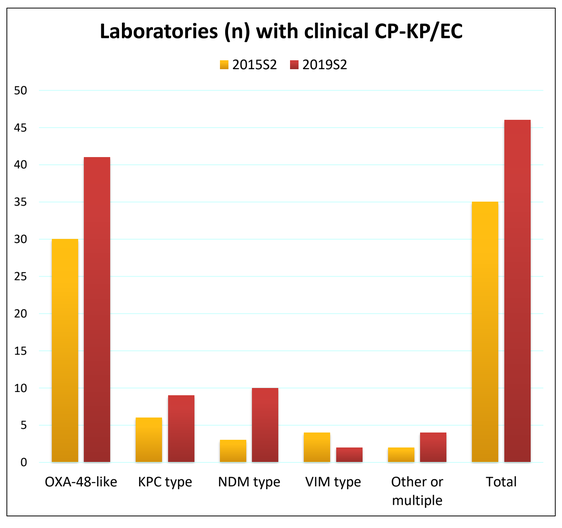












Conclusions

- ✓ **OXA-48-like carbapenemases** remained the predominant enzymes among CPKP/CPEC from clinical samples in Belgium in 2019
- ✓ There was a **significant increase in laboratories reporting clinical CPKP/CPEC and grouped cases from 2015 to 2019**. The increasing laboratories reporting more than one carbapenemase type from clinical specimens strongly suggested their **spread and diversification**.
- ✓ Genomic analysis of **CP-KP** highlighted **5 major clonal complexes (CC15, CC307, CC512, CC405, CC17)** among 45 MLST-types, while a very high clonal diversity was observed among **CP-EC**.
- ✓ Our data demonstrated the emergence of carbapenemase variants (**OXA-244, NDM-5**) and high-risk clones (**KP CC147, KP CC307 and EC CC38**).

References

1. **Brolund A** et al, European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) capacity survey group. Worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, assessment by national experts from 37 countries, July 2018. Euro Surveill. 2019;24(9):pii=1900123.
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