

# *enilab*AMR project

(**e**xpansion of **n**etwork and **i**mprovement of  
**l**ab capacity for better **AMR** surveillance)

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# Background, aims

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- ▶ **Increasing prevalence of resistant Gram-negative bacteria**
  - ▶ Emerging spread of carbapenemases (KPC, NDM, OXA-48, OXA-23, VIM etc)
- ▶ **Incomplete data about ESBL- CARBA epidemiology in Baltic Sea region and neighbouring countries**
  - ▶ Prevalence/incidence
  - ▶ Molecular classes
  - ▶ MLST (Multilocus sequence typing) types
  - ▶ etc
- ▶ **Need for standardized methodology, algorithms**
- ▶ **Application of new techniques**



# Some project details

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- ▶ 9 participant countries: Estonia, Latvia, Lithuania, Russia, Belarus, Ukraine, Georgia, Poland and Moldova
  - ▶ 5 new participants to BEEp team
- ▶ Focus of interest – carbapenem screening positive/R/I Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter* sp.
- ▶ Collection period of 3 months – April till June 2015
- ▶ Clinically relevant strains (infection site, no colonisation/environmental strains)
- ▶ Sensitive strains from each participation country (sequencing)



# Management team

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- ▶ Sweden – Petra Edquist and Karin Sjöström
  - ▶ Financial support and management
- ▶ Estonia – Paul Naaber, Marina Ivanova, Anastasia Pavelkovich, Tiiu Rööp
  - ▶ General management
    - ▶ Data collection
    - ▶ Consumables planning and delivery
    - ▶ Workshop arrangements
    - ▶ Strains collection, storage and maintenance



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▶ Achievements and first results



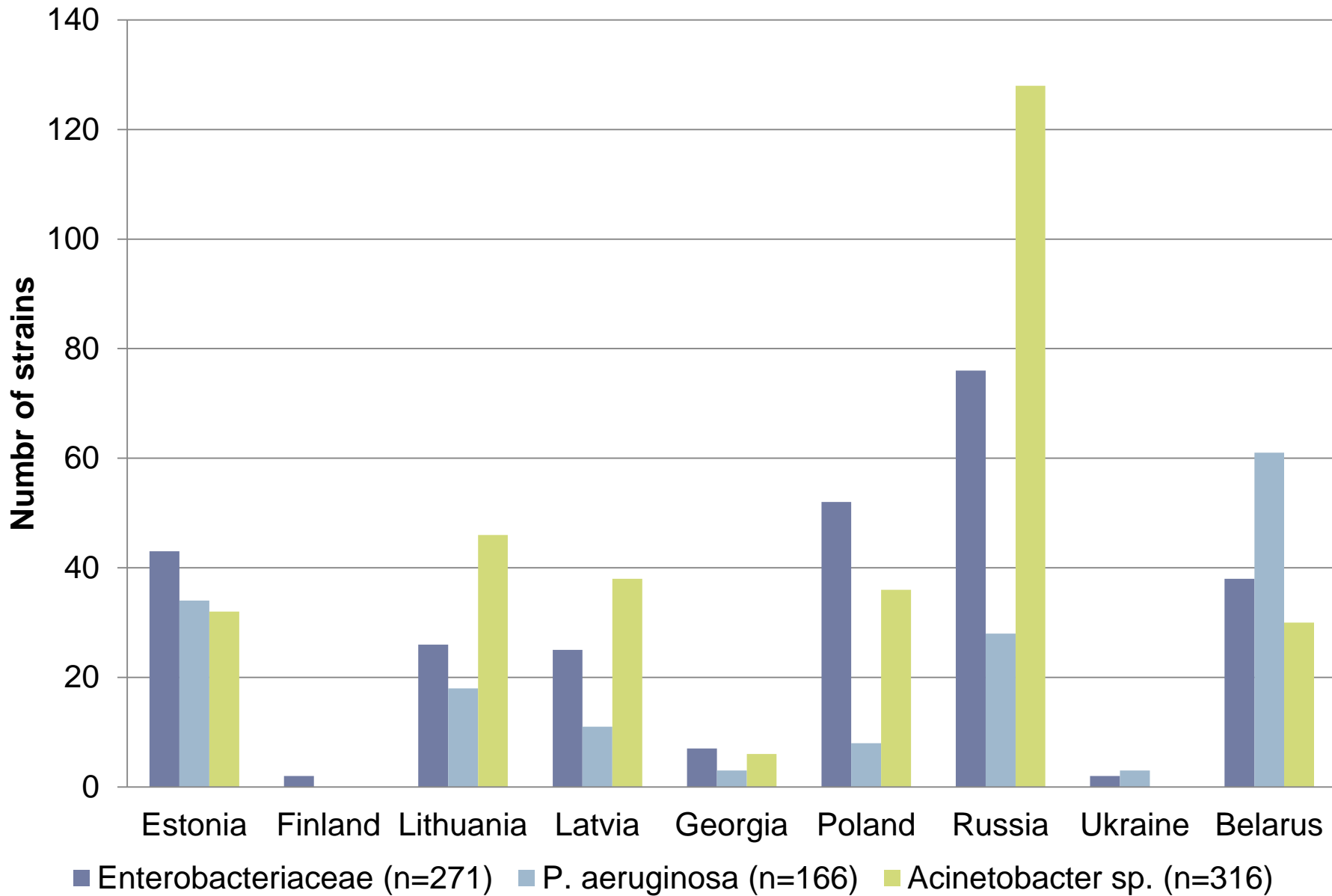
# Final participants' number

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- ▶ Georgia workshop – March 2015
  - ▶ 9 countries
  - ▶ 38 participant labs
- ▶ Estonia workshop – October 2015
  - ▶ 9 countries ( - Moldova, + Finland)
  - ▶ 38 participant labs ( - Moldova, - 1 Estonian lab, + 1 Finnish lab, + 1 Ukrainian lab):
    - ▶ 10 labs provide service only for hospitals
    - ▶ 3 labs provide service only for outpatients
    - ▶ 23 labs: „mixed“ service, reference service, public health

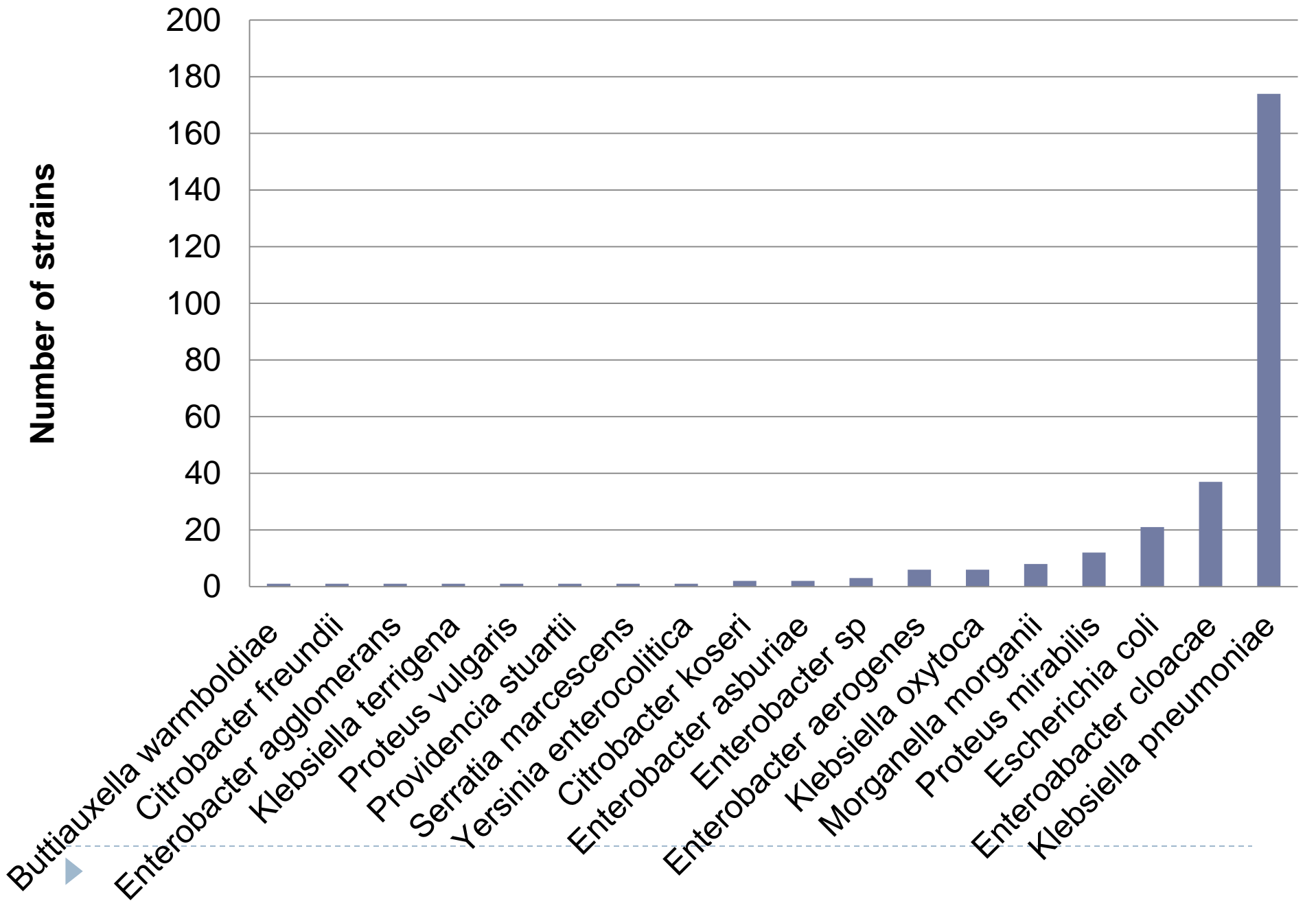


## Collected screening positive strains



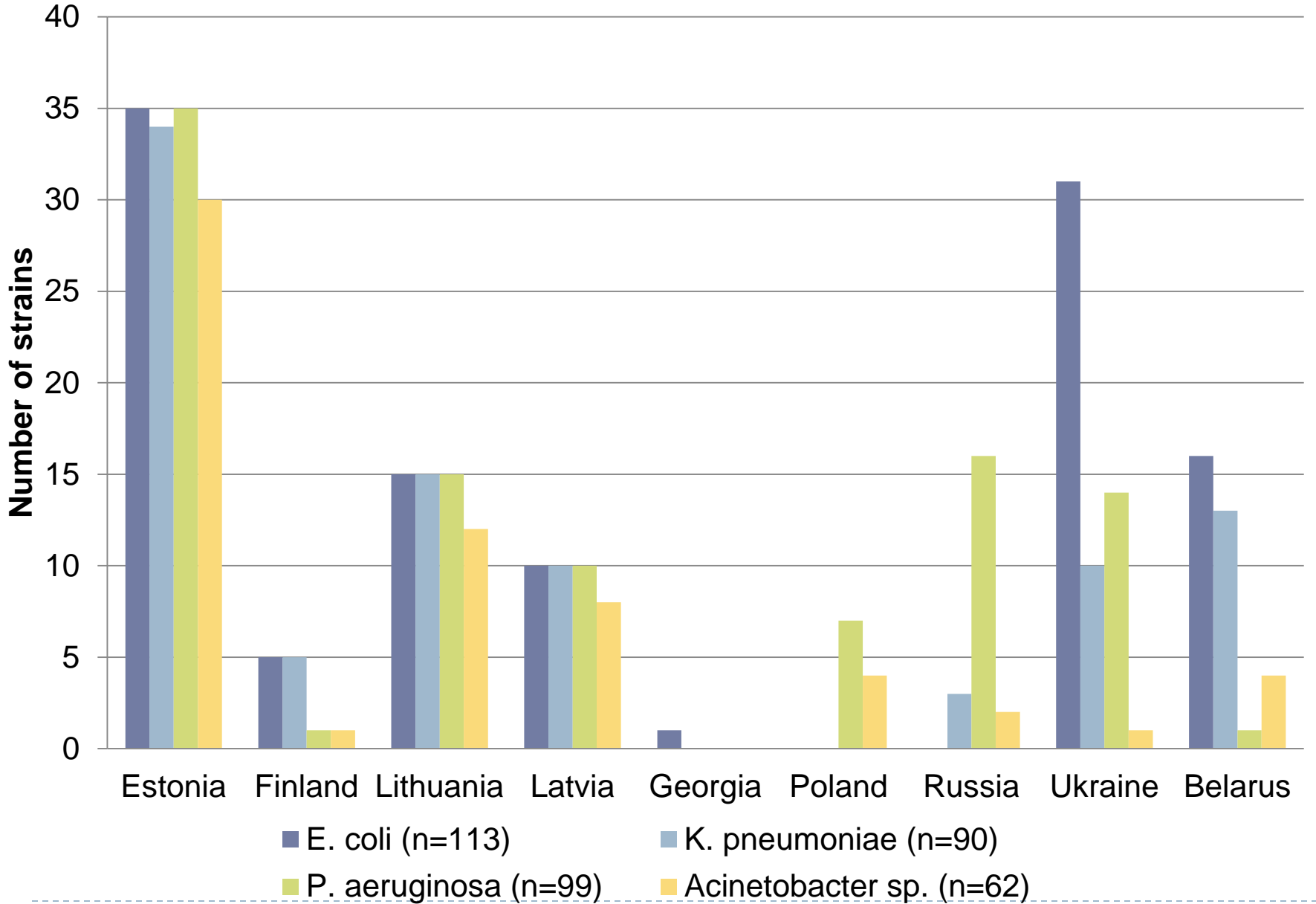
Updated 29.11.15

# Screening positive Enterobacteriaceae



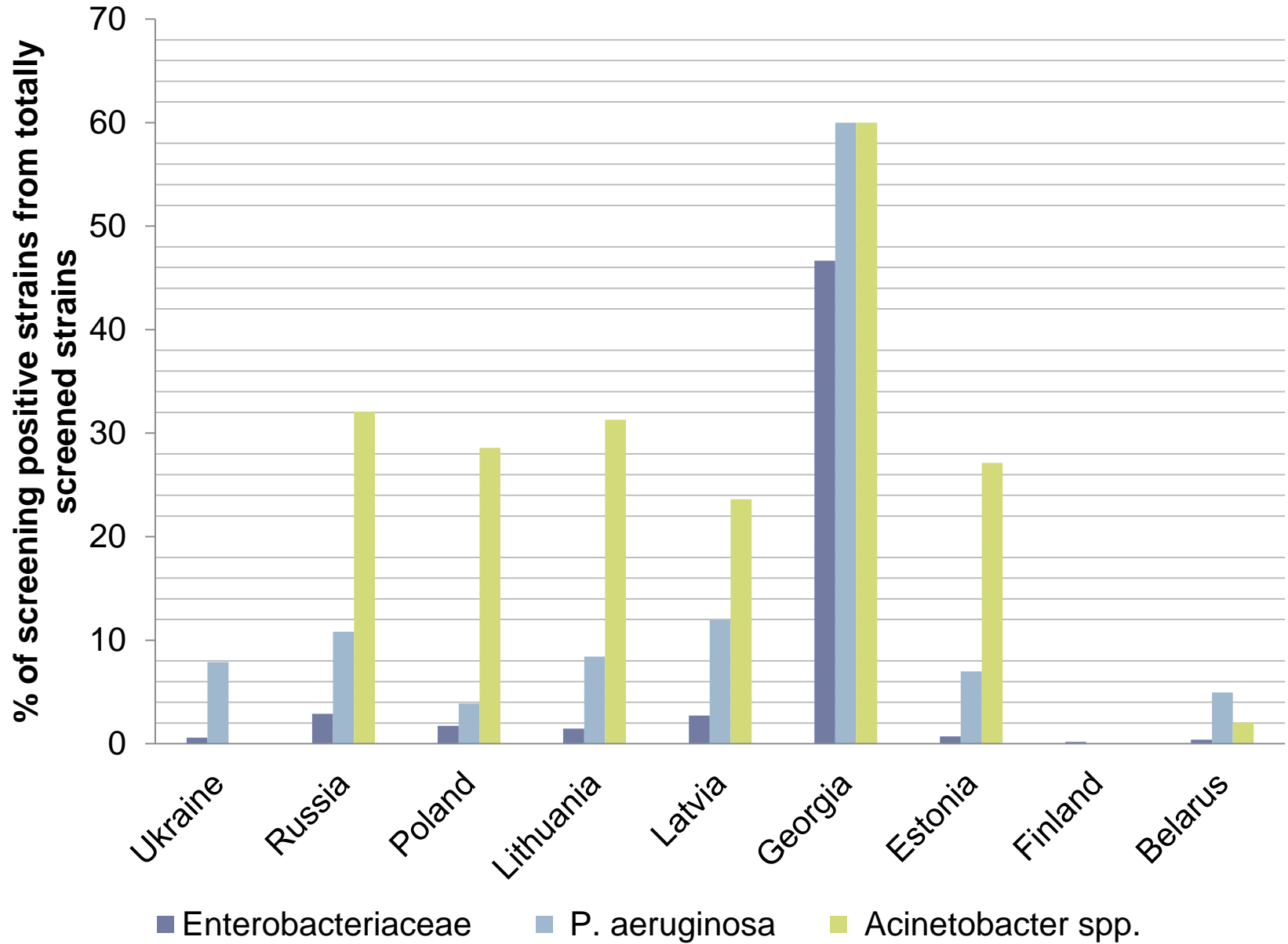


# Collected sensitive strains



Updated 29.11.15

## Screening positive strains from all screened strains



# First stage – Enterobacteriaceae

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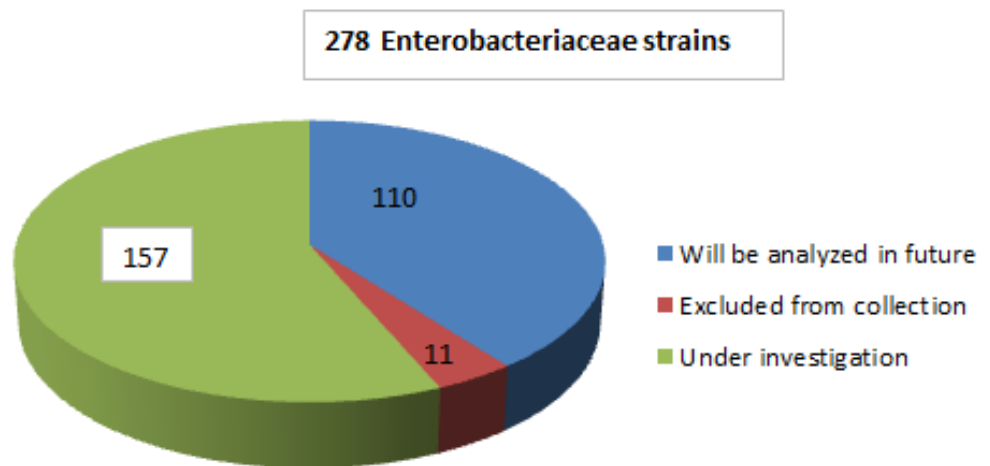


# Enterobacteriaceae investigations

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- ▶ Maldi identification
- ▶ DNA extraction
- ▶ CTX-M genes detection by Luminex in-house multiplex panels
- ▶ Carbapenemases genes (IMP, VIM, KPC, GIM, OXA48, NDM) detection by Luminex
- ▶ Whole Genome

Sequencing by Illumina

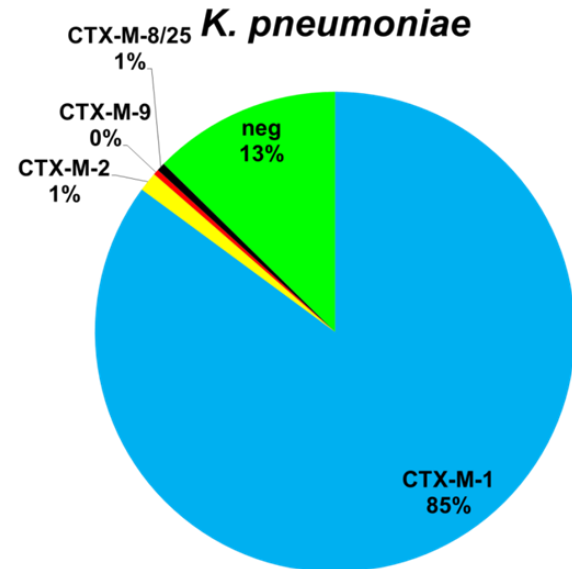


# Results – CTX-M genes

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- ▶ 156 *Klebsiella pneumoniae* confirmed isolates
  - ▶ 140 have **CTX-M1** group gene
  - ▶ 1 strain has both CTX-M1 and CTX-M9 group genes
  - ▶ 2 strains have CTX-M2 group gene
  - ▶ in 13 strains no CTX-M genes were found

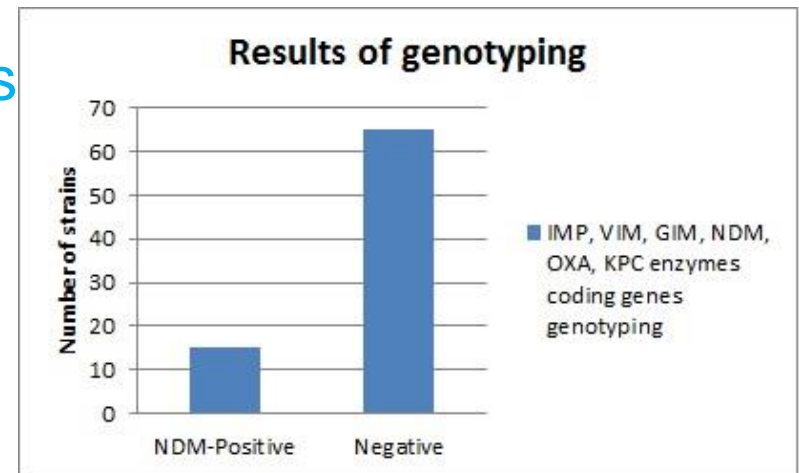
BEEp results



# Results – carbapenemase genes

- ▶ Carbapenemase genes were found in 75 *K. pneumoniae* strains
  - ▶ The most dominant was NDM gene – 48 strains
  - ▶ OXA-48 – 23 strains
  - ▶ VIM (Latvia) – 1 strain
  - ▶ KPC (Russia) – 1 strain
  - ▶ GIM (Poland) – 1 strain

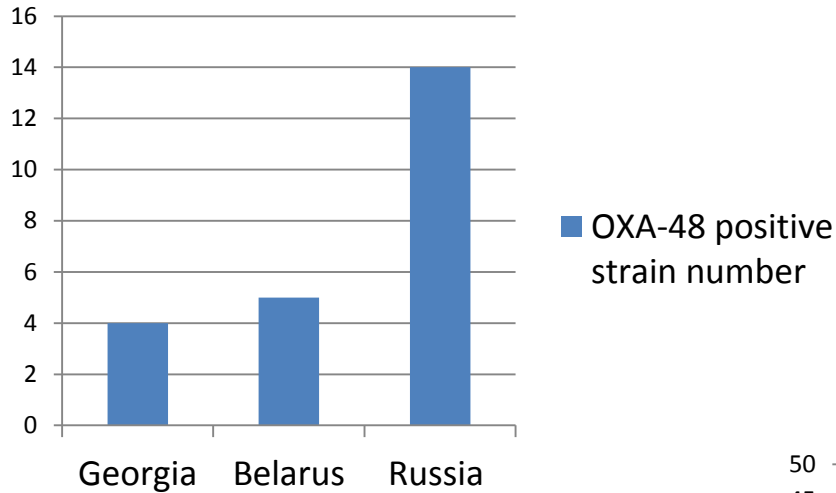
BEEp results



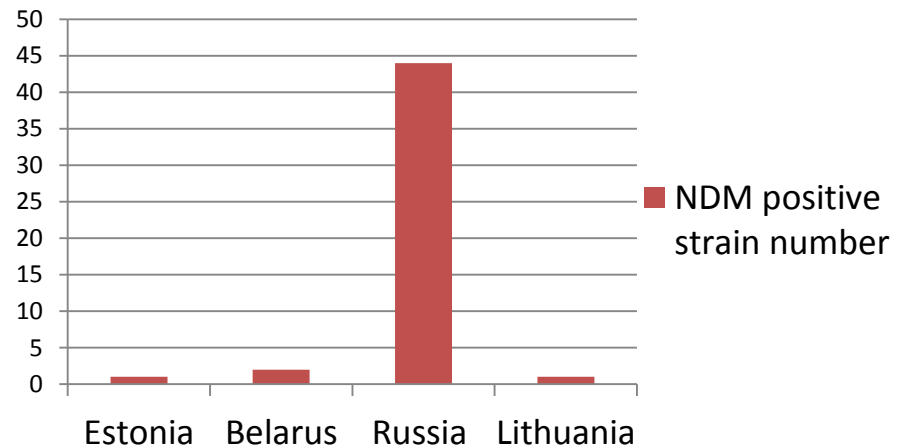
# Distribution by country

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## OXA-48 positive



## NDM positive



# Future plans

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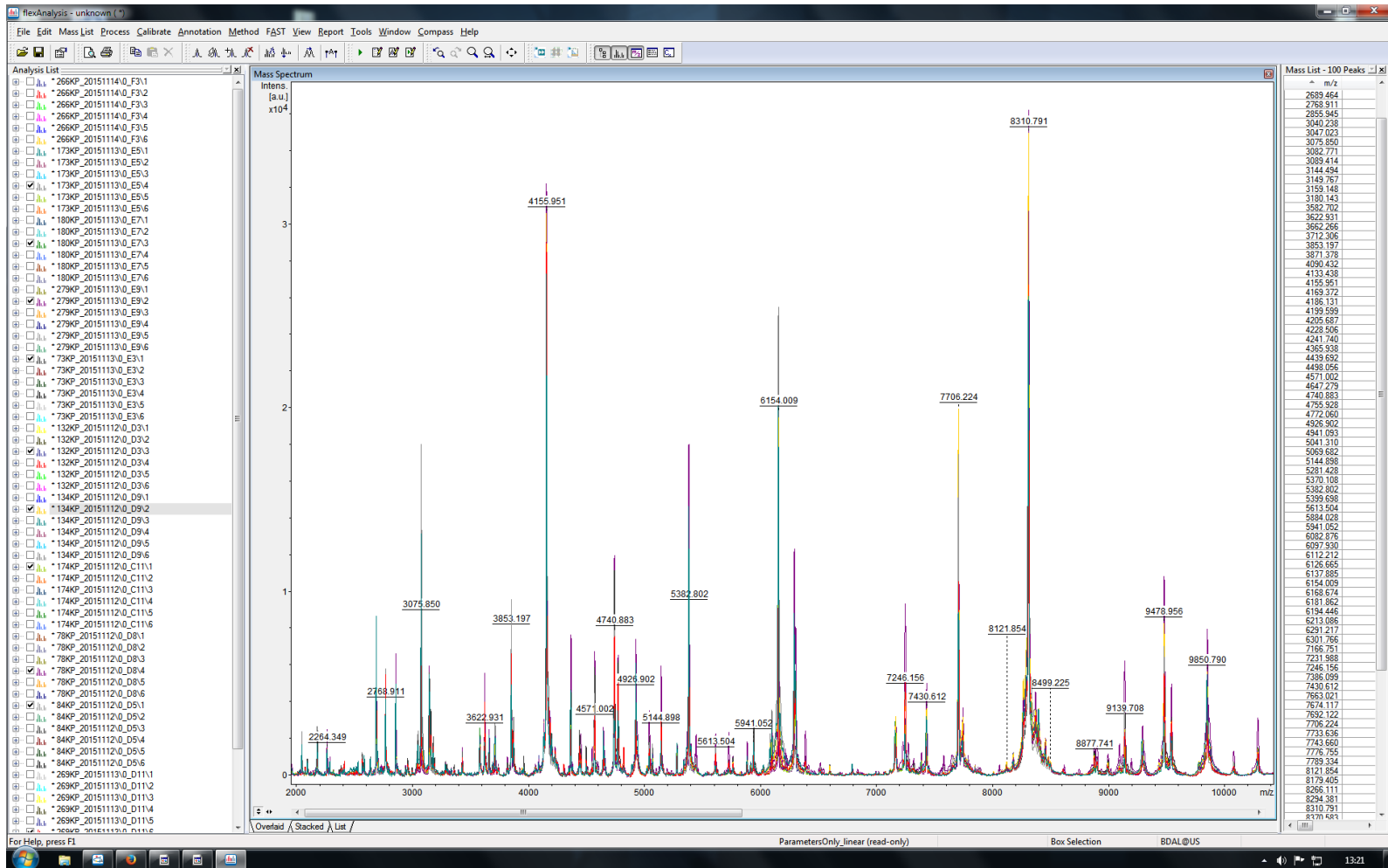
- ▶ Molecular tests
  - ▶ WGS to all Enterobacteriaceae strains
  - ▶ MALDI subtyping
  - ▶ Data analysis
  - ▶ Publications
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- ▶ Investigations in PA and AC strains





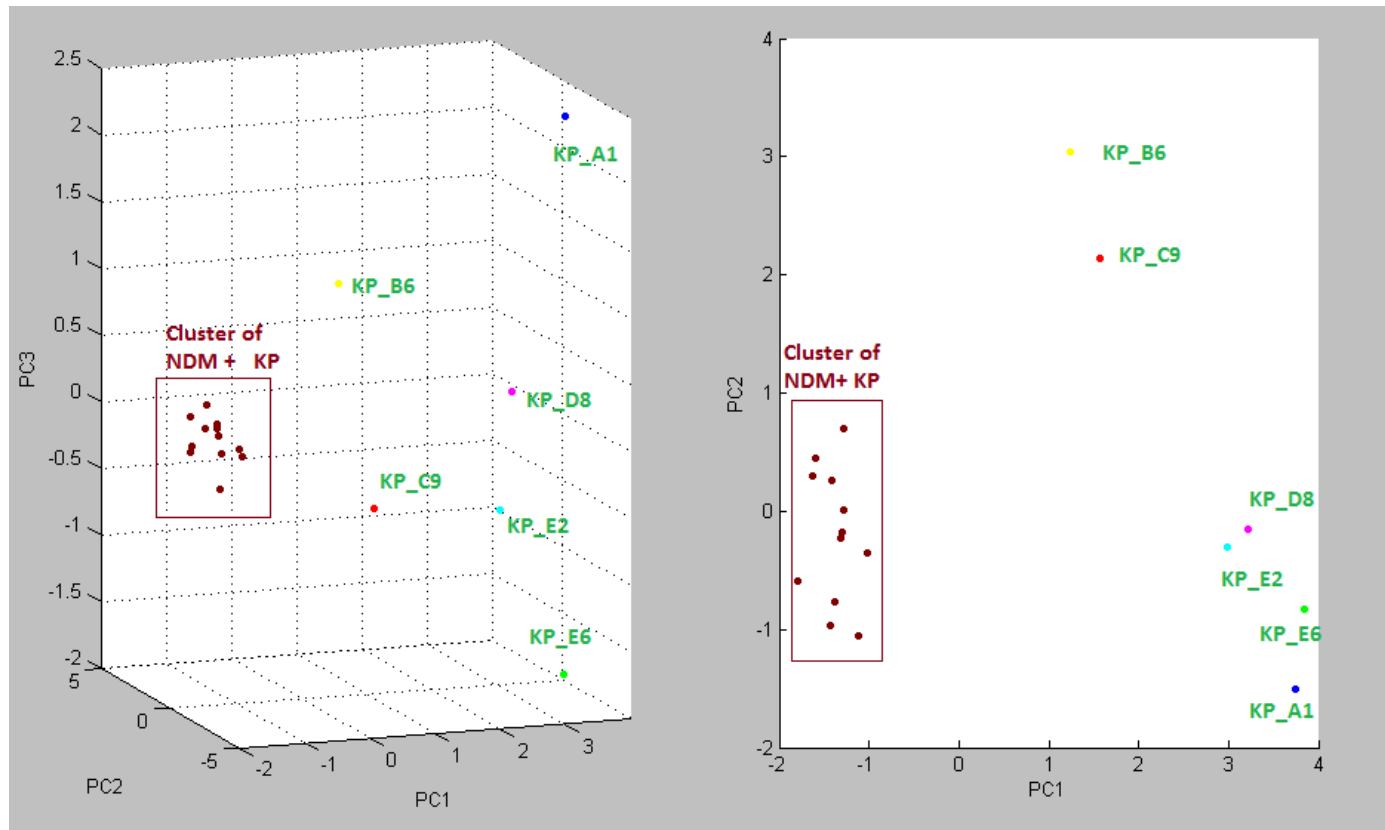
# Visual evaluation of spectra.

## Peak list creation



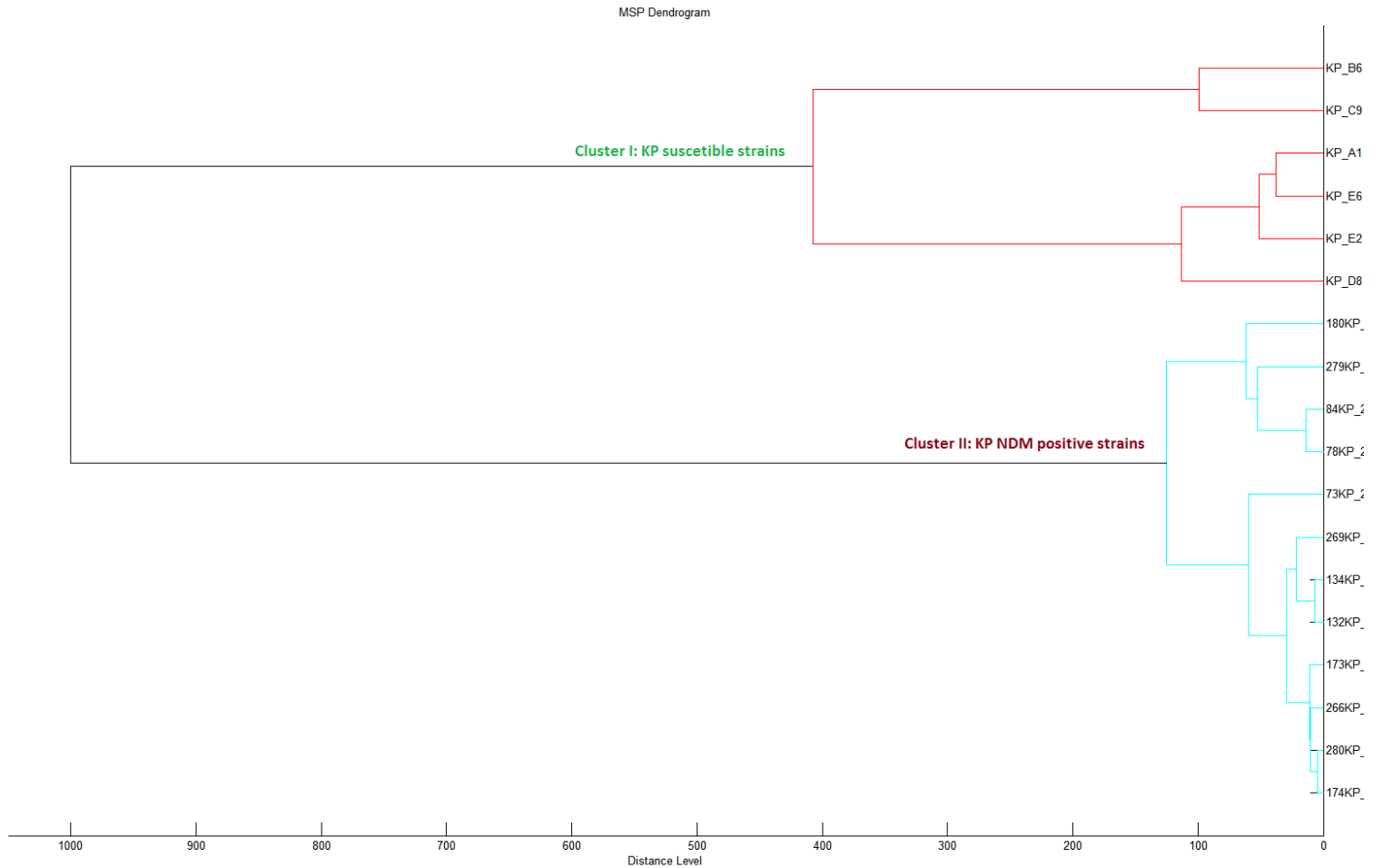
Totally list of 37 peaks were created

# Application of Principal Component Analysis (PCA) in 2D/3D



PCA analysis indicates 2 clusters of *Klebsiella pneumoniae* isolates corresponding to **NDM producers** and **non-producers**

# Application of Main Spectrum (MSP) dendrogram



► What is right distance level score to securely distinguish cluster difference?