INAUGURAL ONE HEALTH CONFERENCE

1 - 3 November 2021

Whole genome sequencing of human and porcine *Escherichia coli* isolates from a commercial pig farm in South Africa

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INTRODUCTION

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Background

Escherichia coli **is used as a indicator microorganism in One Health antibiotic resistance (ABR) surveillance programmes:**

i) **Wild-type *E. coli* is susceptible to all antibiotics and this bacterium could acquire vast array of antibiotic resistance genes through horizontal gene transfer**

ii) **Occurs in all One Health ecological niches (humans, animals and environment) and allows comparison**

iii) **Easy to grow in a laboratory and allows standardization of isolation methodologies across different laboratories**

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Background

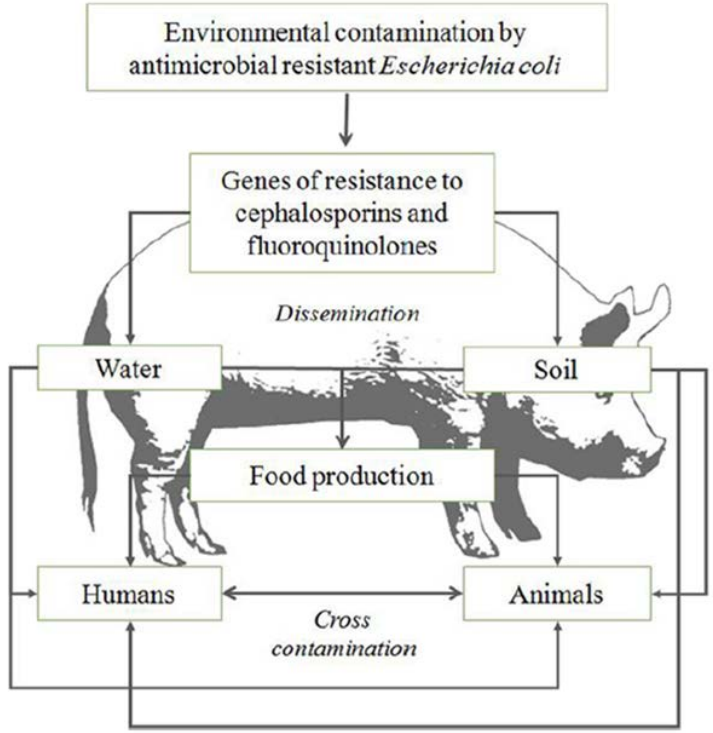
The primary ecological niche for *E. coli* is the gastro-intestinal tract of its vertebra host, where it normally exists as a commensal

***E. coli* may also exist in secondary habitats, such as water and sediments**

The overlapping ecological niches of *E. coli* present opportunities for the transmission and spread of ABR genes, which can either be through:

i) **direct transmission between human and animals due to proximity**
ii) **indirect transmission between the two populations through an intermediatory vehicle**

Important to understand transmission dynamics in the development of evidence-based One Health interventions to reduce the burden of ABR



Brisola *et al*., 2019 *Science of the Total Environment* **647:** 362-368

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INTRODUCTION

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Aim

To describe the antibiotic susceptibility profiles and the genetic relatedness among human and porcine *E. coli* isolates on a commercial farm using whole genome sequencing

METHODS

Farm recruitment

- The study protocol was presented to the Pig Veterinary Society (PVS) of the South African Veterinary Association (SAVA) and to the South African Pork Producers Organisation (SAPPO)
- Veterinarians were requested to provide more information to their clients (i.e. farmers) and to invite them to participate by completing an online form
- Three commercial farms expressed interest in the study, but only a single farm was successfully enrolled after the completion of informed consent

METHODS

Farm setting

- Located in North-West province
- In-operation since 1954 and employees 75 workers full-time
- Consist of two production sites, with 25 production houses, managed by the same farmer
- Sow population = 1 415
- All-in, all-out, farrow-to-finish, closed production system
- Sows are impregnated through artificial insemination
- No new breeding pigs have been purchased since 2011

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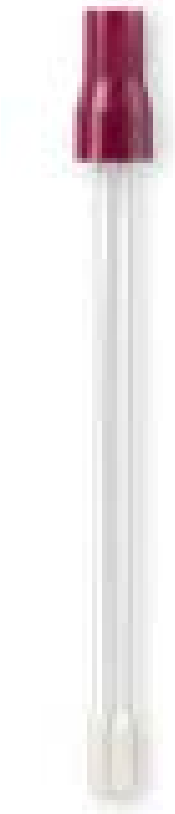
METHODS

Participant recruitment and self-collection of a rectal swab

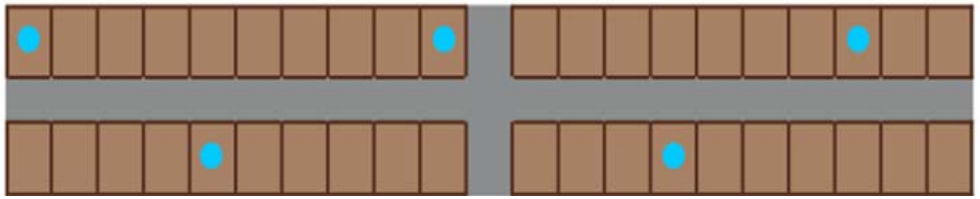


- **Employees (> 18 years old) were invited to participate after the completion of informed consent**
- **Participation involved:**
  - **Completion of a structured questionnaire in either Afrikaans, English, Xhosa and Zulu**
  - **Self-collection of a dual tip rectal swab in the privacy of a restroom**

METHODS

Sample collection from pigs

- All production houses were eligible for sampling
- A total of five fresh, undisturbed pig faecal droppings were collected aseptically (Minimum weight = 10 g) from randomly selected pens per production house
- Sample collection preceded from pigs with a high susceptibility to disease (i.e farrow stage) to pigs with a lower susceptibility to disease (i.e. growers)



METHODS

Isolation and identification of *E. coli* from humans and pigs

Rectal swab were placed into 2 mℓ of saline and vortexed

↓

10 µℓ of saline were plated onto MacConkey agar and incubated for 18 h to 24 h

↓

Characteristic colonies underwent MALDI-TOF MS (Bruker Daltonics, USA)

***E. coli* ATCC 25922 included as positive control**

10 g of faeces weighed and added to 90 mℓ of buffered peptone water (BPW). Incubated aerobically at 35°C (±2°C) for 18 h to 24 h

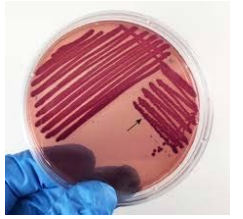
↓

10 µℓ of BPW were plated onto MacConkey agar and incubated for 18 h to 24 h

↓

Characteristic colonies underwent MALDI-TOF MS (Bruker Daltonics, USA)

***E. coli* ATCC 25922 included as positive control**



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METHODS

Antibiotic susceptibility testing of *E. coli*

Minimum inhibitory concentrations (MICs) (µg/mℓ) were determined for 21 antibiotics covering 12 antibiotic classes using two commercial broth microdilution systems:

- **Colistin MICs determined by Sensititre (ThermoFischer Scientific, USA)**
- **The MICs of the remaining antibiotics were determined with the MicroScan WalkAway plus (Beckman Coulter, USA) using the NM 44 panel**
- **Interpreted according to the Clinical Laboratory Standards Institute (M100, 2020) guidelines**

METHODS

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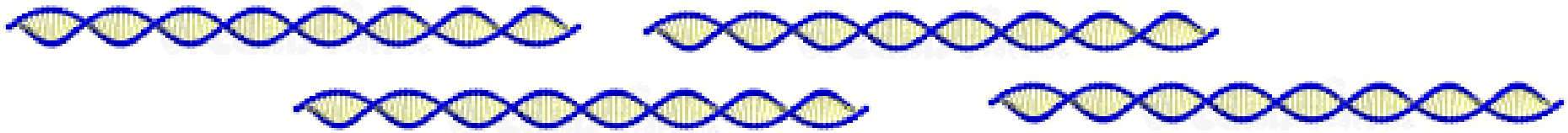
Total genomic DNA extraction and whole genome sequencing

The QIAamp DNA Minikit (QIAGEN, USA) was used for extractions

Whole genome sequencing was performed at the Core Sequencing Facility, NICD:

- **Library preparation was done using the Nextera DNA Flex kit**
- **Sequencing was done on NextSeq500 instrument at 100× coverage with and output of 2 × 150 base pairs (bp) paired-end reads**



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METHODS

Bioinformatic processing and data analysis

RStudio

ggplot2

tidyverse

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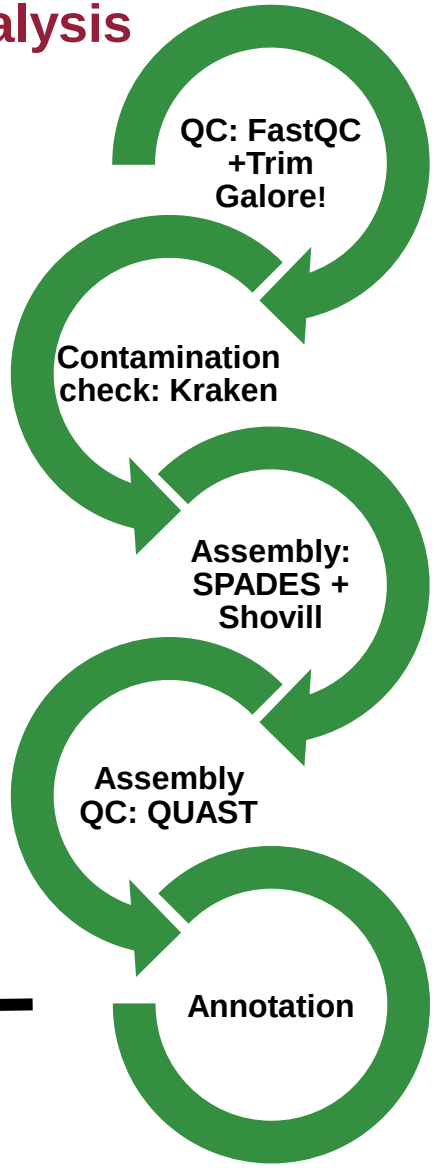
JEKESA pipeline



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stanikae/**jekesa**

Jekesa (Illuminate) is an automated bash pipeline for bacterial whole genome assembly and typing using Illumina paired-end sequencing data.

1 Contributors · 0 Issues · 3 Stars · 0 Forks

https://github.com/stanikae/jekesa

ablab/**spades**

SPAdes Genome Assembler

CAB

48 Contributors · 151 Issues · 311 Stars · 83 Forks

ResFinder 4.1

Center for Genomic Epidemiology

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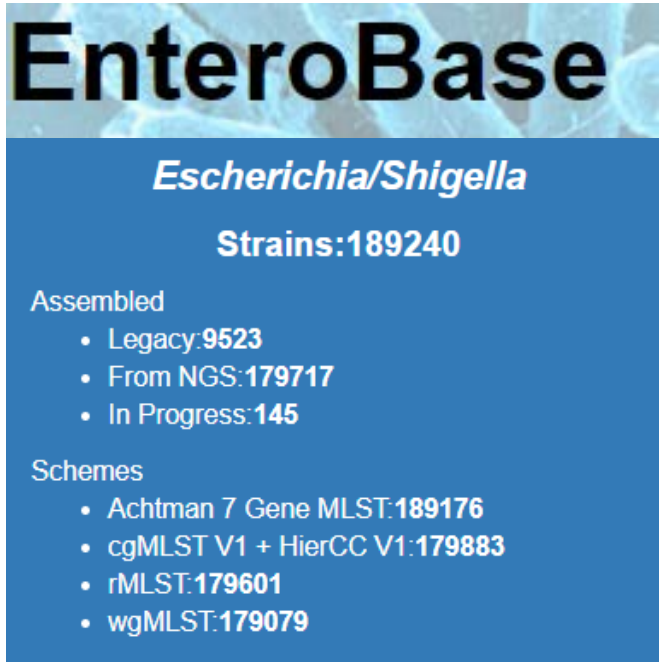
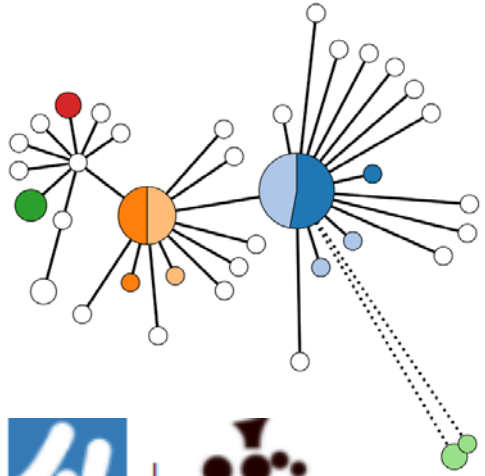
METHODS

Phylogenetic analysis

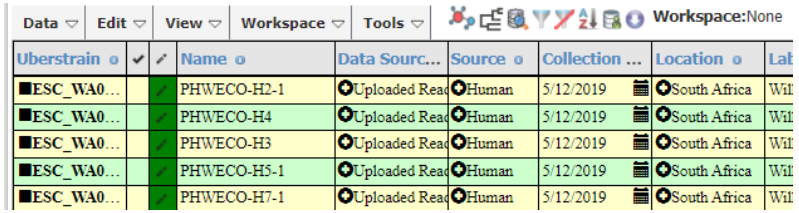
Raw reads





GrapeTree

Curate Strains



.TXT

R Studio

tidyverse

ggplot2

https://enterobase.warwick.ac.uk/

- **Achtman 7-gene MLST sequence type**
- **Clermont phylogroups**
- **Core genome MLST and hierarchical clustering (HC)**

Zhou *et al.*, 2018
Zhou *et al.*, 2019

METHODS

Ethical approvals

Protocol #	Name of ethical or institutional review board
REC0055-20	Research Ethics Committee, Faculty of Veterinary Science, University of Pretoria, Pretoria
REC0055-20	Animal Ethics Committee, Faculty of Veterinary Science, University of Pretoria, Pretoria
HUM027/0620	Research Ethics Committee, Faculty of Humanities, University of Pretoria, Pretoria
406/2020	Faculty of Health Sciences Research Ethics Committee, University of Pretoria, Pretoria
AEC003-19	Animal Research Ethics Committee, National Institute for Communicable Diseases (NICD), Johannesburg
M190244	Human research ethics committee (Medical), University of the Witwatersrand, Johannesburg
12/11/1/1/13	Section 20 clearance, Department of Agriculture, Land Reform and Rural Development (DALRRD) of the Republic of South Africa

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rural development & land reform
Department:
Rural Development and Land Reform
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RESULTS

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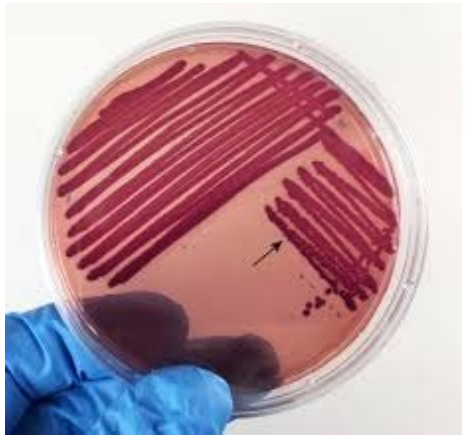
Isolation rate of *E. coli* from humans



- **Sixty-four (64) farm workers were recruited:**
  - **Male (76.56%; 49/64) on average 40 years old**
  - **Performed various duties on the farm:**
    - **Animal-handling (43.75%; 28/64)**
    - **Maintenance and housekeeping (29.69%; 19/64)**
    - **Transportation of pigs to the abattoir (9.38%; 6/64)**
    - **Animal feed preparation (3.13%; 2/64)**
    - **Unknown (14.06%; 9/64)**
  - ***Escherichia coli* was isolated from 77.78% (49/63) of the swabs**
    - **Different morphologies were observed from the same rectal swab in 13 instances and two isolates were therefore processed. T**

~ A total of 63 human *E. coli* isolates
obtained from 49 rectal swabs underwent further testing ~

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Isolation rate of *E. coli* from pigs

- **A total of 113 pig faecal droppings were collected from 23 production houses:**
  - ***Escherichia coli* was not isolated from every pig faecal dropping (detection rate: 88.5%, 100/113), but were isolated from every production house (100%, 23/23)**
  - **Different morphologies were also observed from five pig faecal droppings (two different colony morphologies from four droppings and three different colony morphologies from a single dropping)**

Thus, a total 106 porcine *E. coli* isolates obtained from 100 pig faecal droppings underwent further testing

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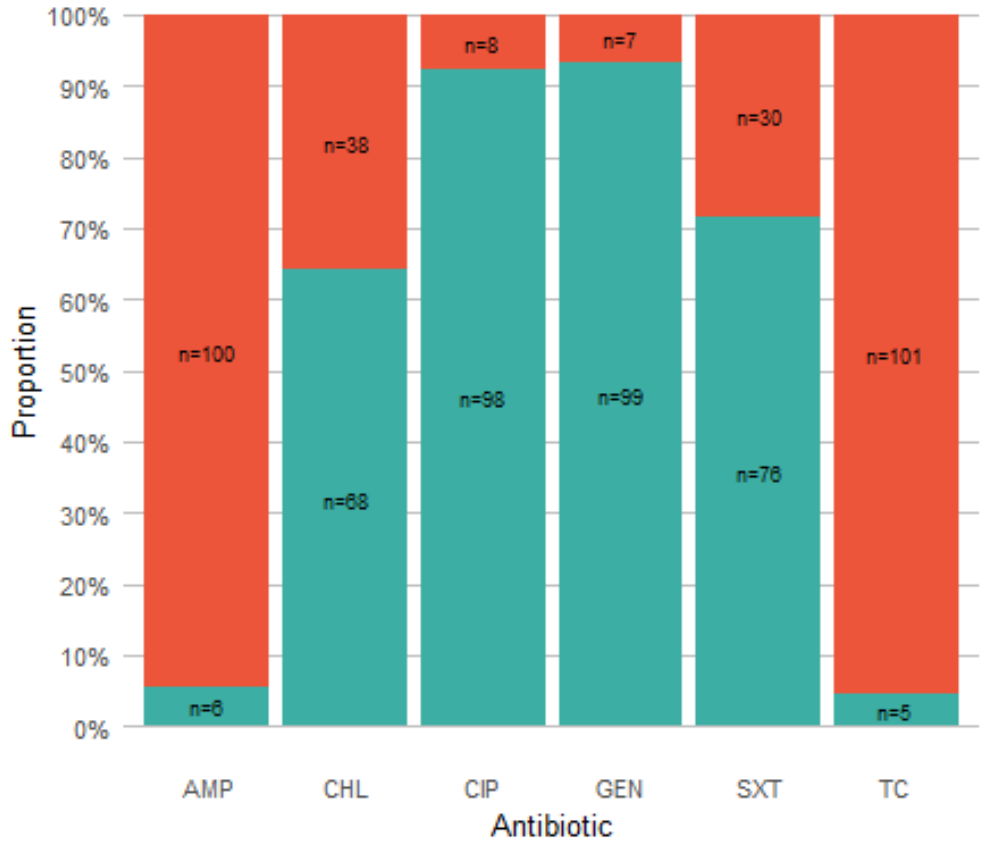
RESULTS

Phenotypic antibiotic susceptibility profiles

***E. coli* isolated from humans (n =63)**

***E. coli* isolated from pigs (n =106)**



Antibiotics:
AMP = Ampicillin
CHL = Chloramphenicol
CIP = Ciprofloxacin
GEN = Gentamicin
SXT = Trimethoprim/ Sulfamethoxazole
TC = Tetracycline

RESULTS

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Phenotypic antibiotic susceptibility profiles

- **Resistance towards other β-lactam antibiotics (i.e. Cephems such as ceftazidime and cefepime) and carbapenems (i.e. ertapenem, meropenem, imipenem and doripenem) were not detected**

- **Distribution of colistin MICs and genotypic resistance:**

MIC (µg/mL)	Human % (n=63)	Pigs % (n=106)
0.5	7.94 (5)	9.43 (10)
1	88.89 (56)	84.91 (90)
2	3.17 (2)	5.66 (6)

***M*obile *c*olistin *r*esistance (*mcr*) genes were not detected and a single porcine isolate harboured the V161G mutation in *pmr*B gene**

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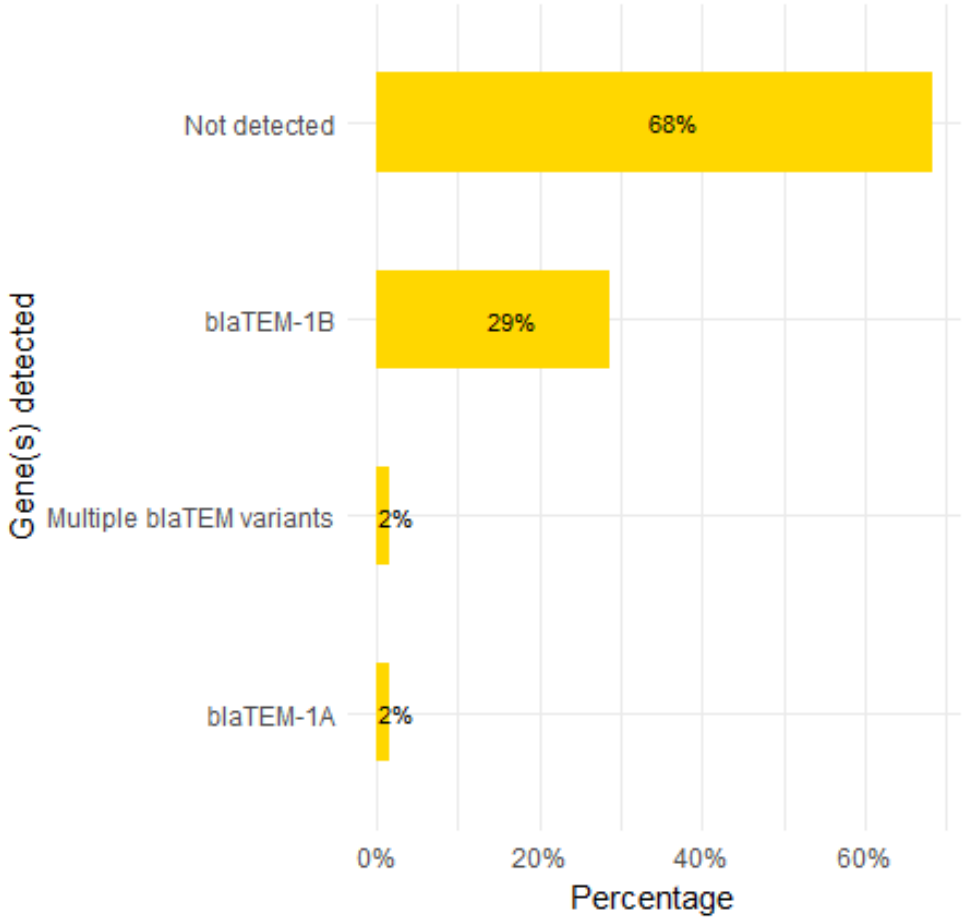
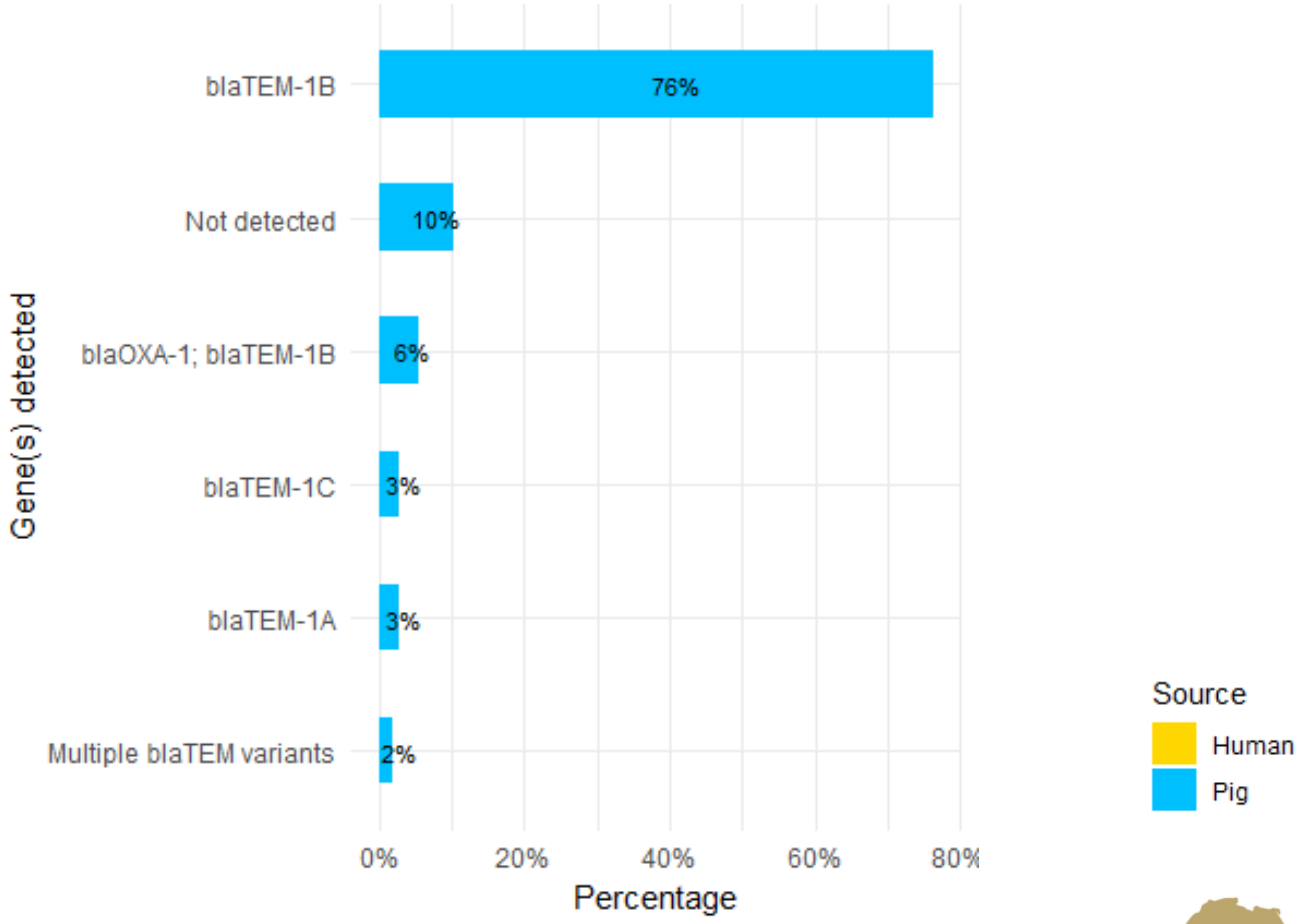
RESULTS

Antibiotic resistance genes: distribution of β-lactam resistance genes in *E. coli*



n=63



n=106

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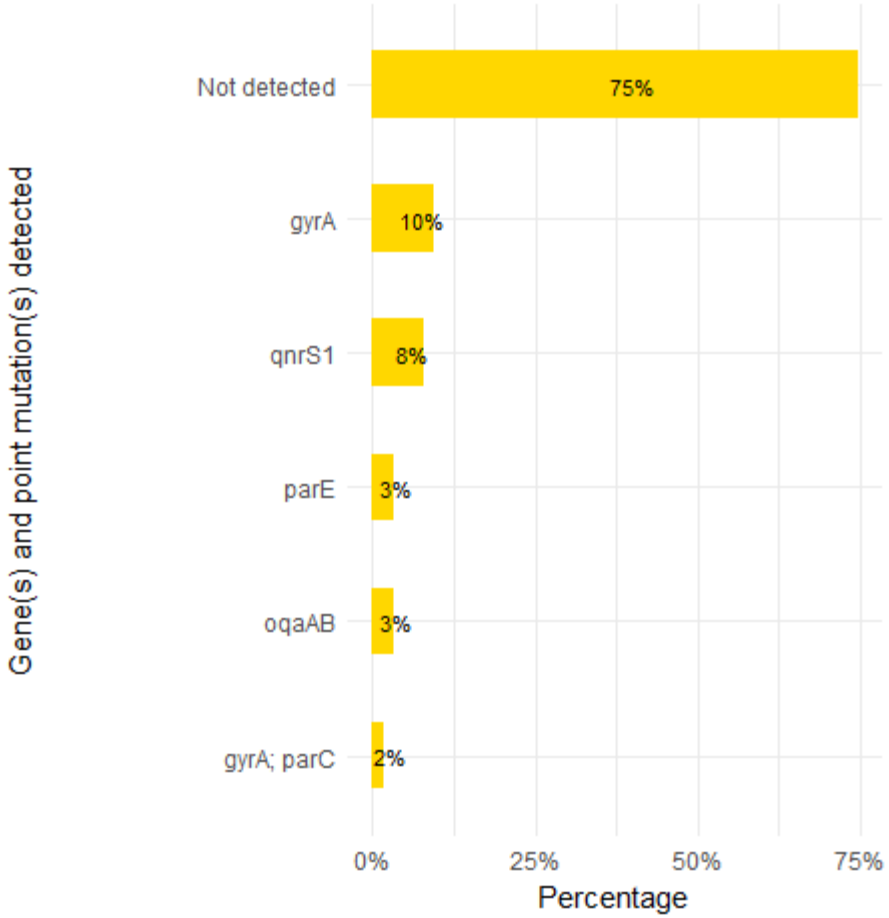
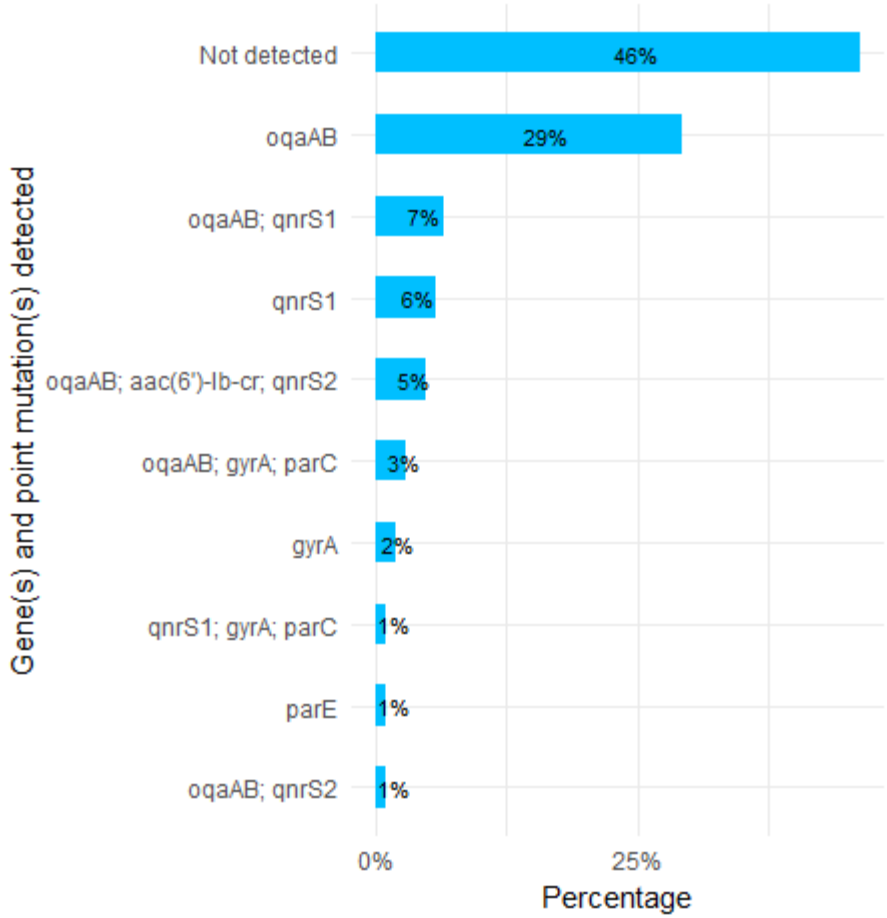
RESULTS

Antibiotic resistance genes: distribution of quinolones resistance determinants in *E. coli*



n=63



n=106

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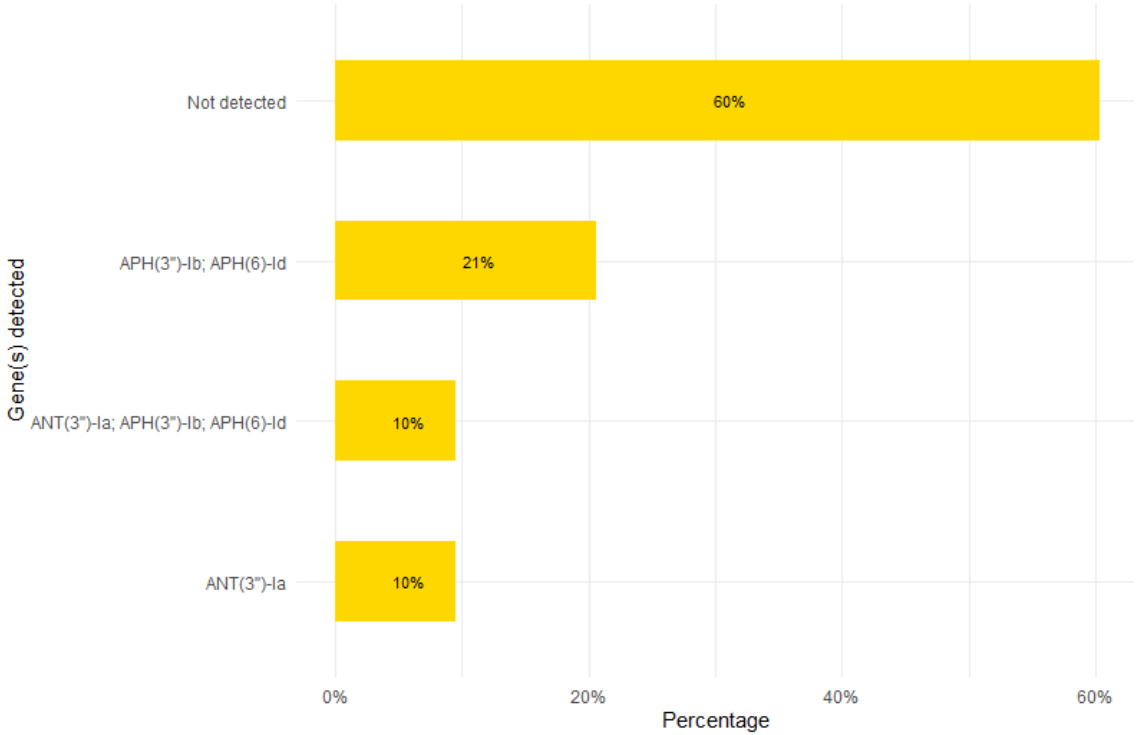
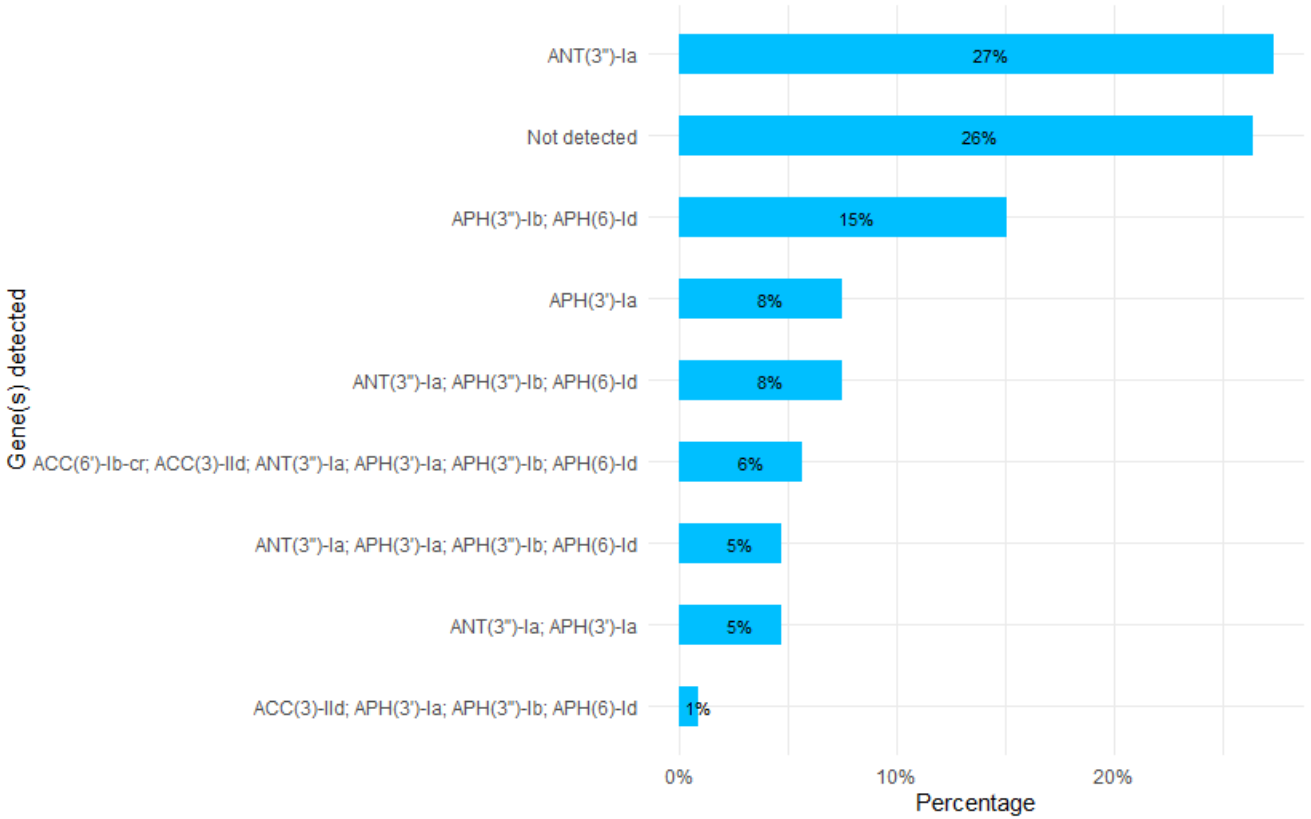
RESULTS

Antibiotic resistance genes:
distribution of aminoglycoside-modifying enzymes (AMEs) in *E. coli*



n=63



n=106

AMEs:

- **ANT = nucleotidyltransferases (resistance towards streptomycin)**
- **APH = phosphotransferases (resistance towards streptomycin and kanamycin)**
- **ACC = Acetyltransferases (resistance towards gentamicin)**

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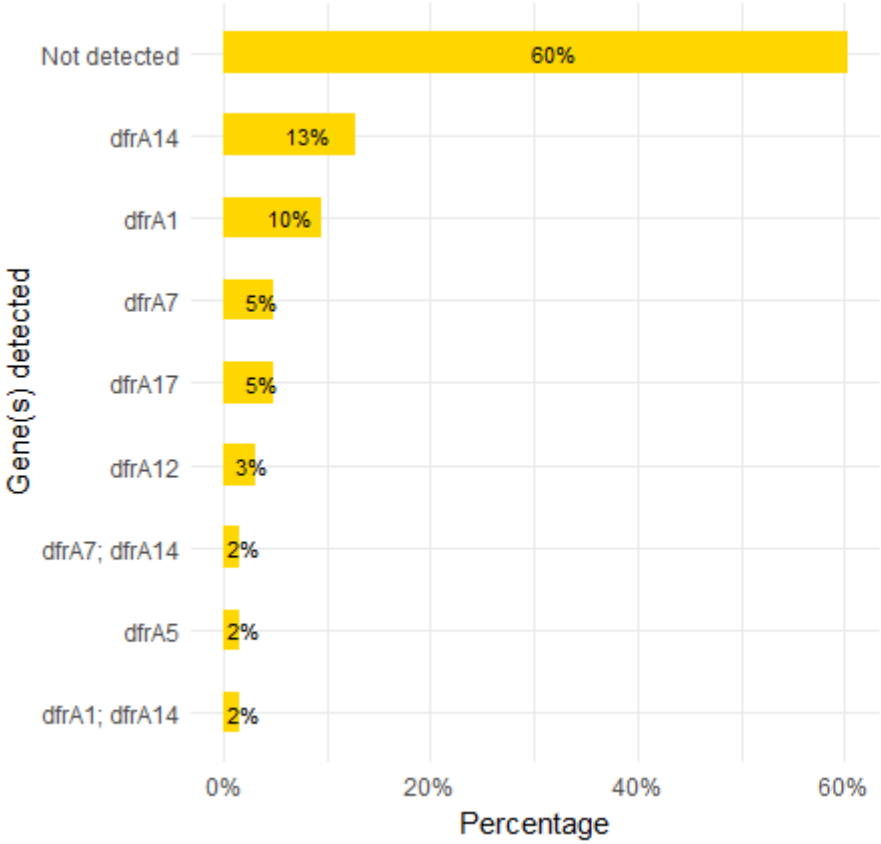
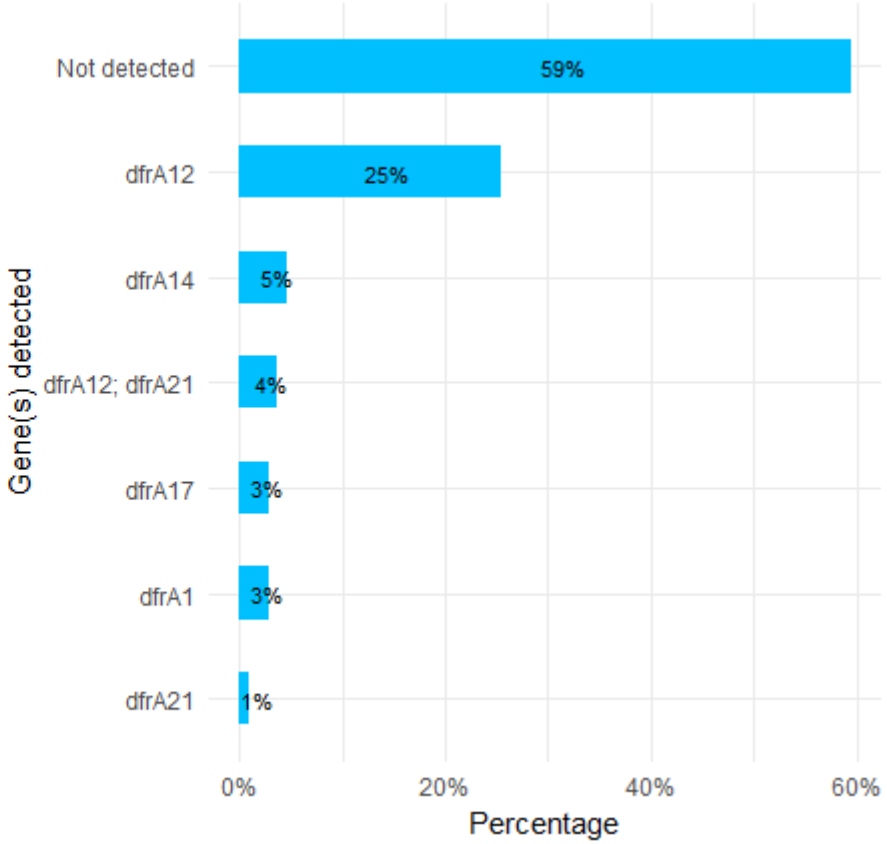
Antibiotic resistance genes: distribution of dihydrofolate reductases (trimethoprim resistance) in *E. coli*



n=63



n=106

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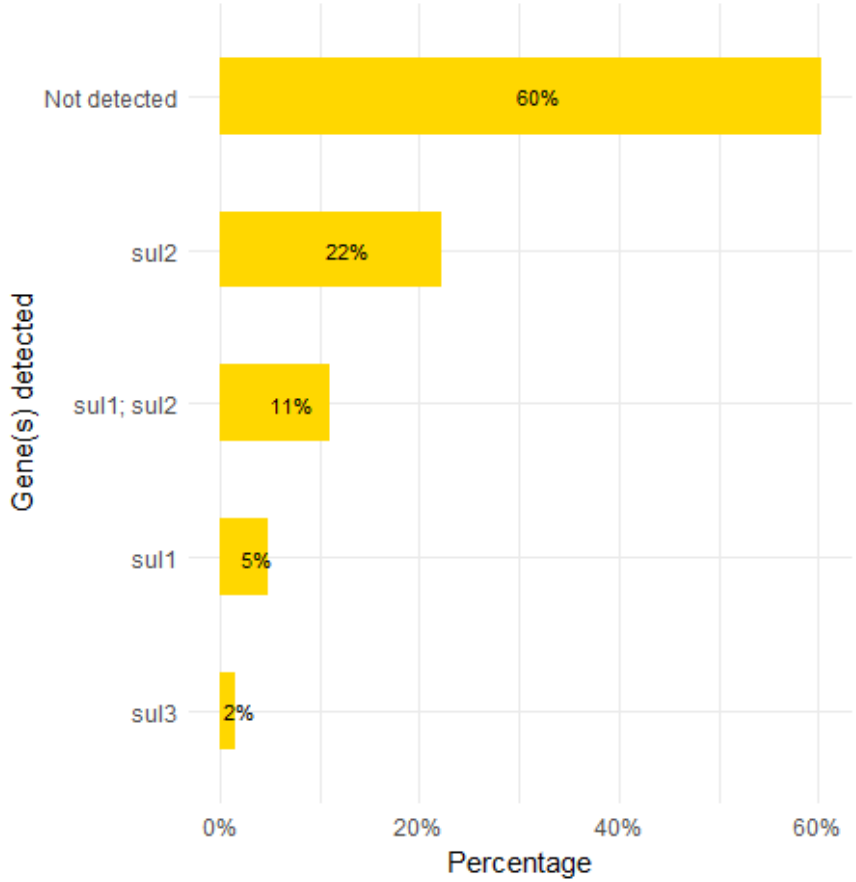
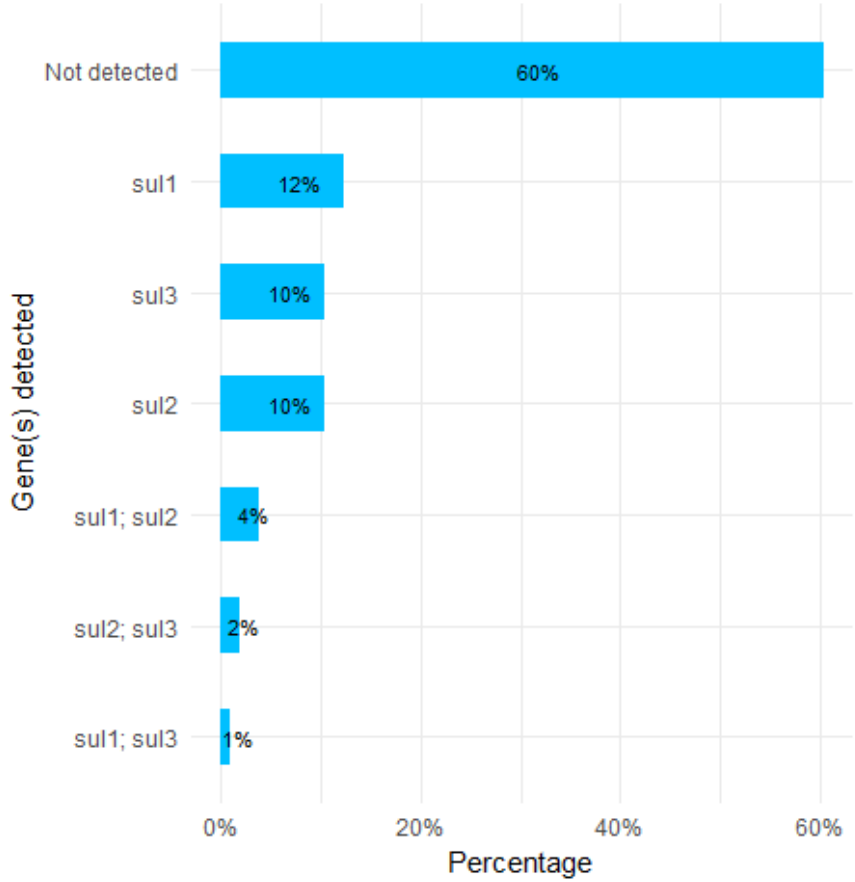
RESULTS

Antibiotic resistance genes: distribution of sulphonamide resistant dihydropteroate synthase (*sul*) in *E. coli*



n=63



n=106

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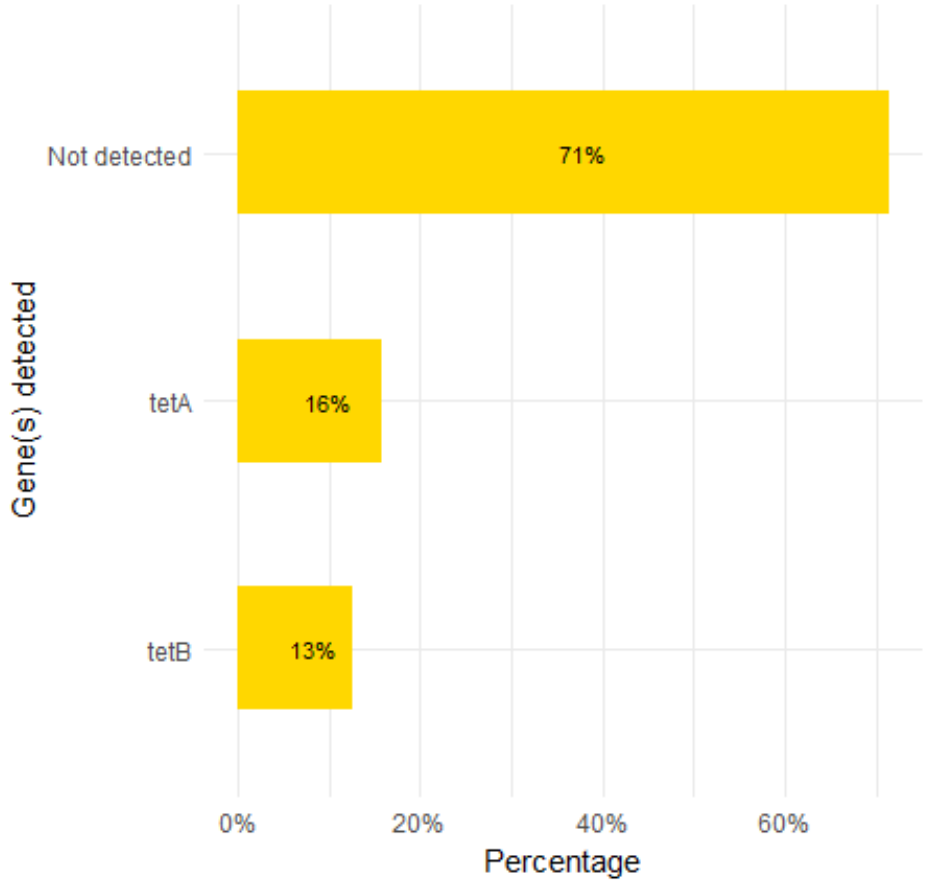
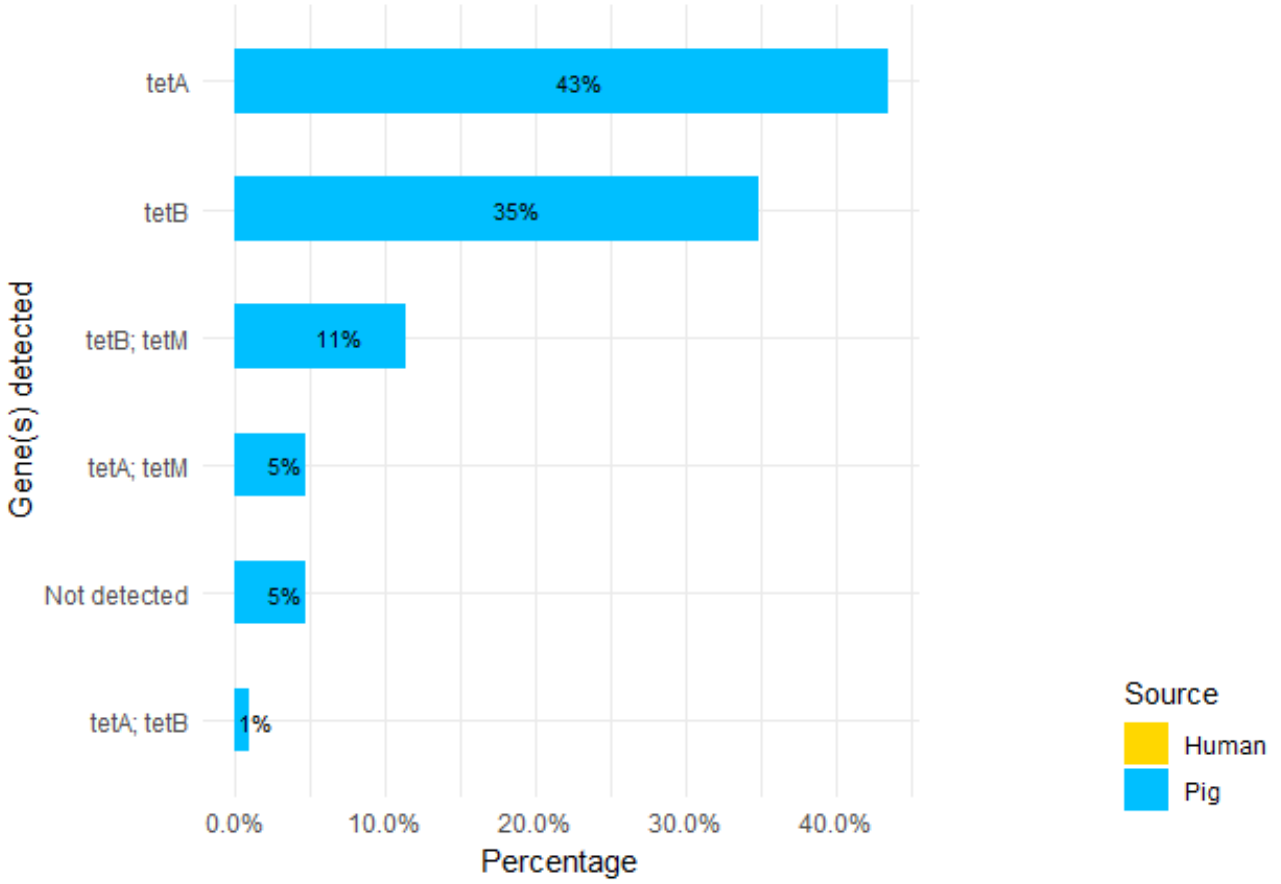
RESULTS

Antibiotic resistance genes: distribution of tetracycline resistance genes in *E. coli*



n=63



n=106

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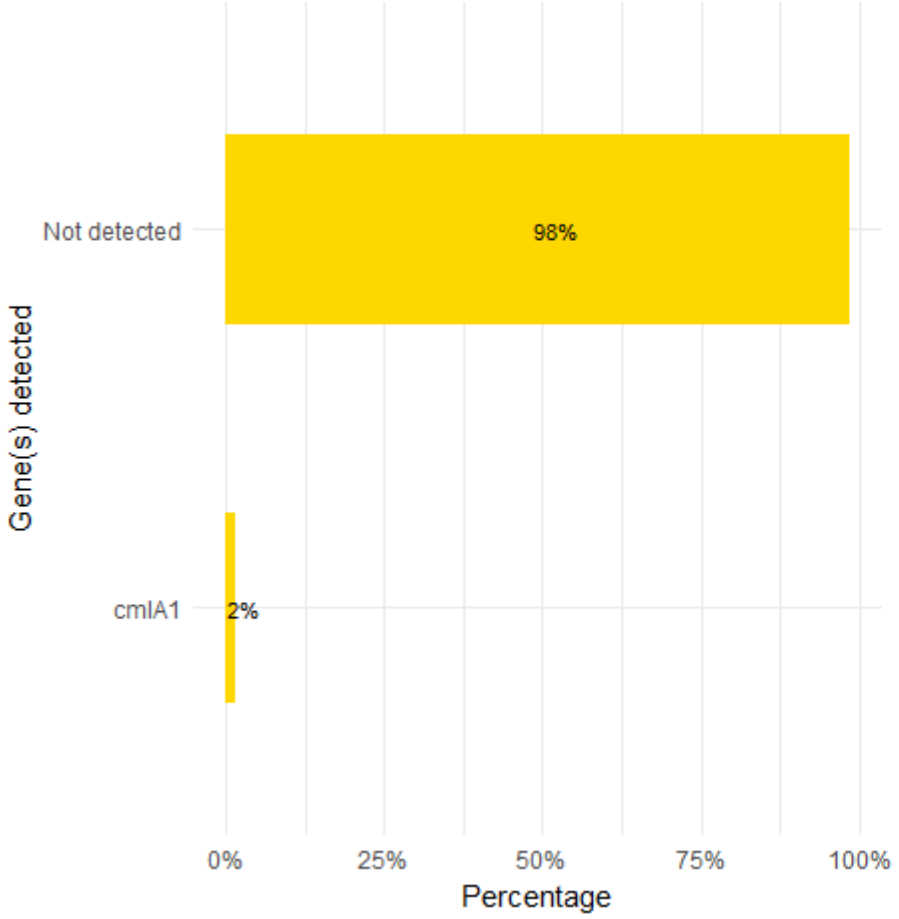
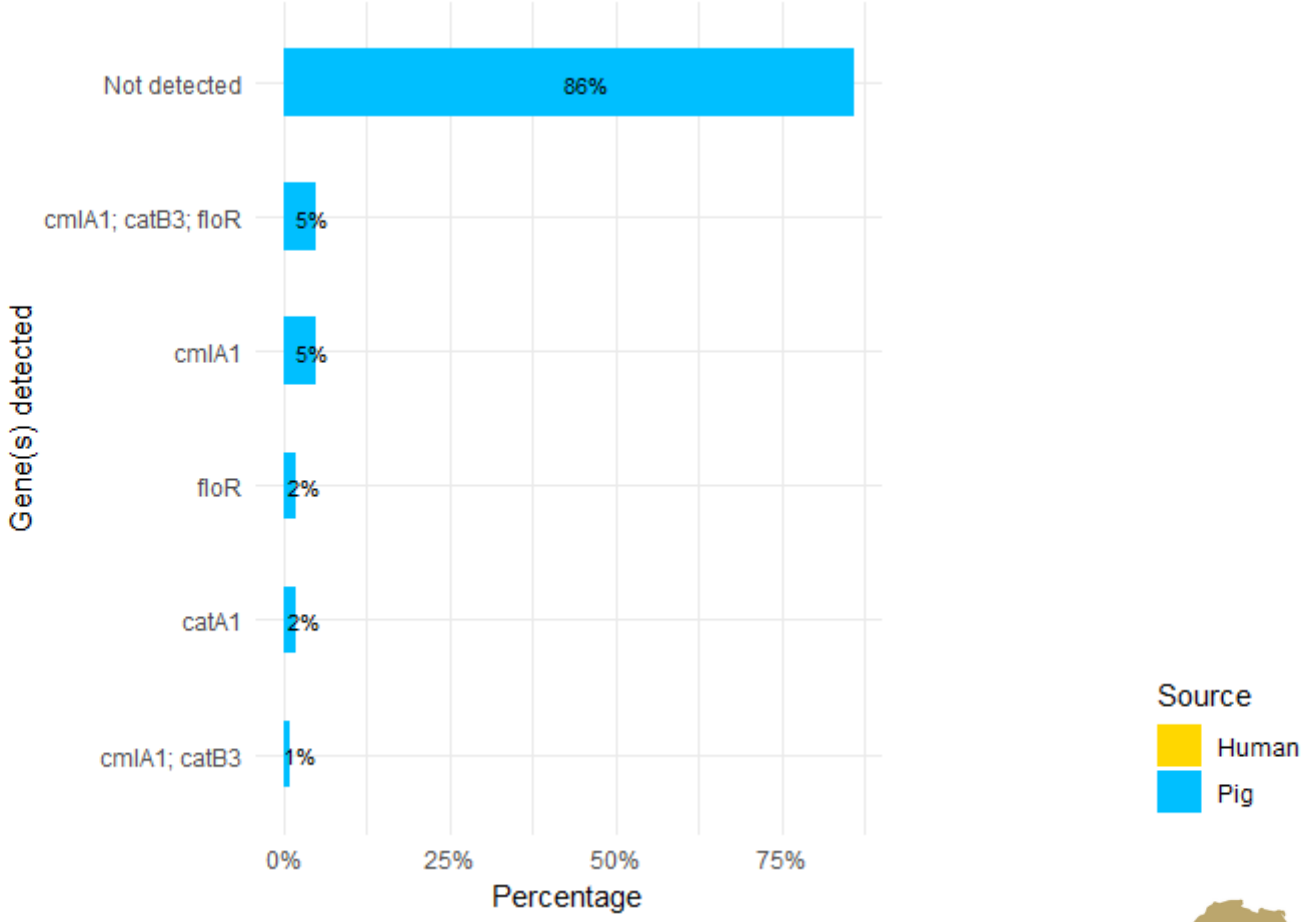
Antibiotic resistance genes: distribution of chloramphenicol resistance genes in *E. coli*



n=63



n=106

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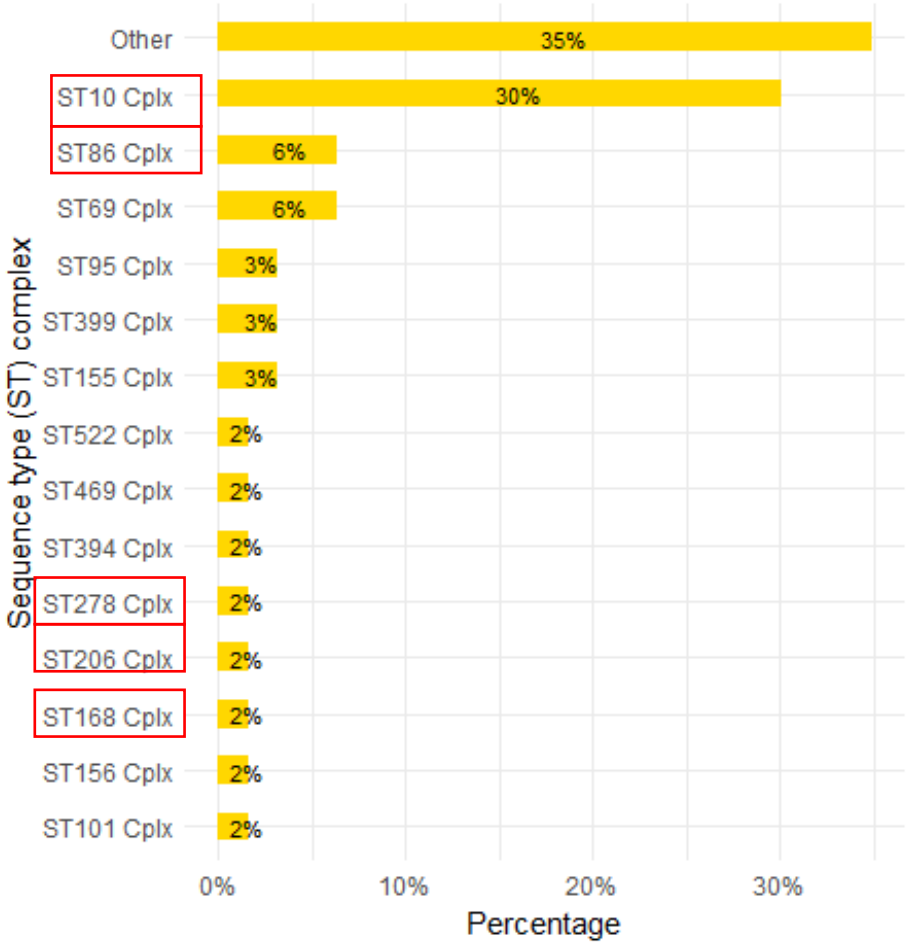
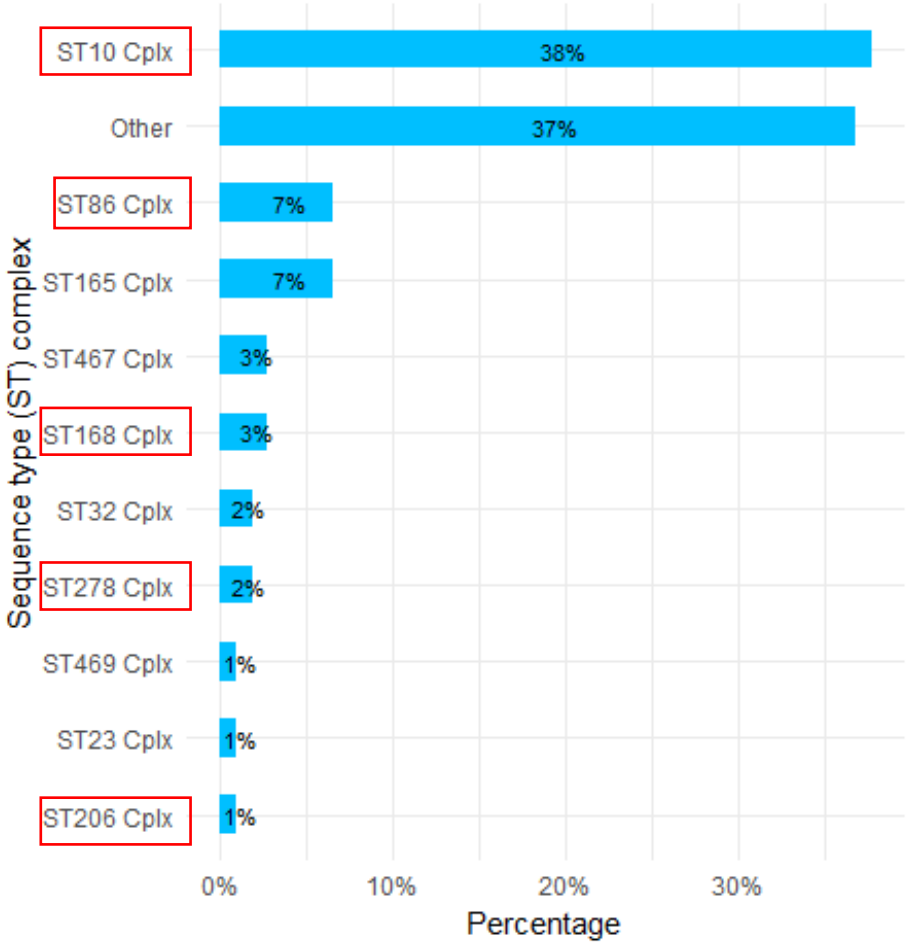
RESULTS

Phylogeny: Achtman 7-gene multilocus sequence type (MLST)



n=63



n=106

Shared STs:
- ST10
- ST86
- ST168
- ST206
- ST278

Source
Human
Pig

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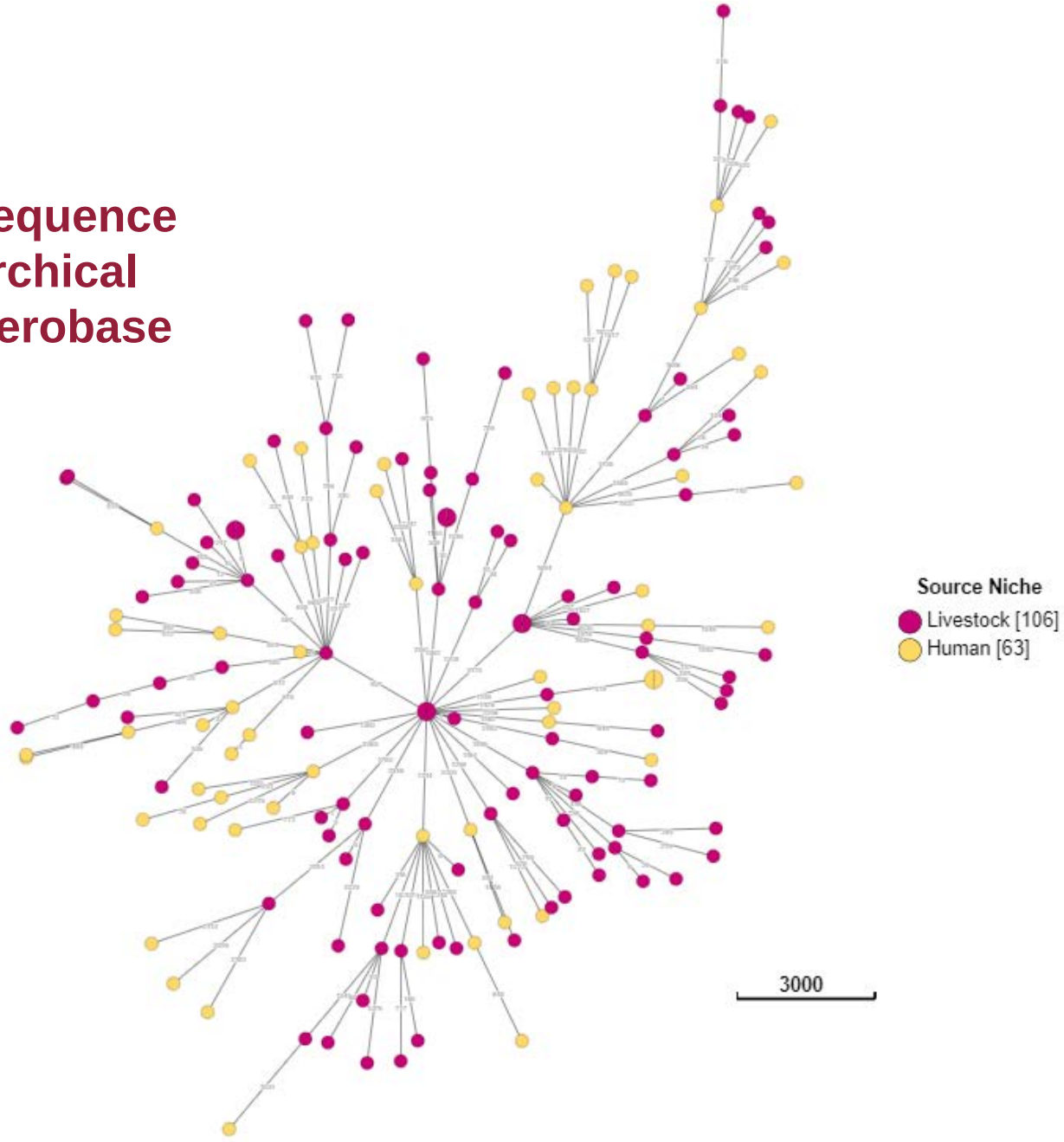
RESULTS

Core genome multilocus sequence type (cgMLST) and hierarchical clustering (HierCC) on Enterobase

Overall phylogeny of minimum spanning tree according to source niche



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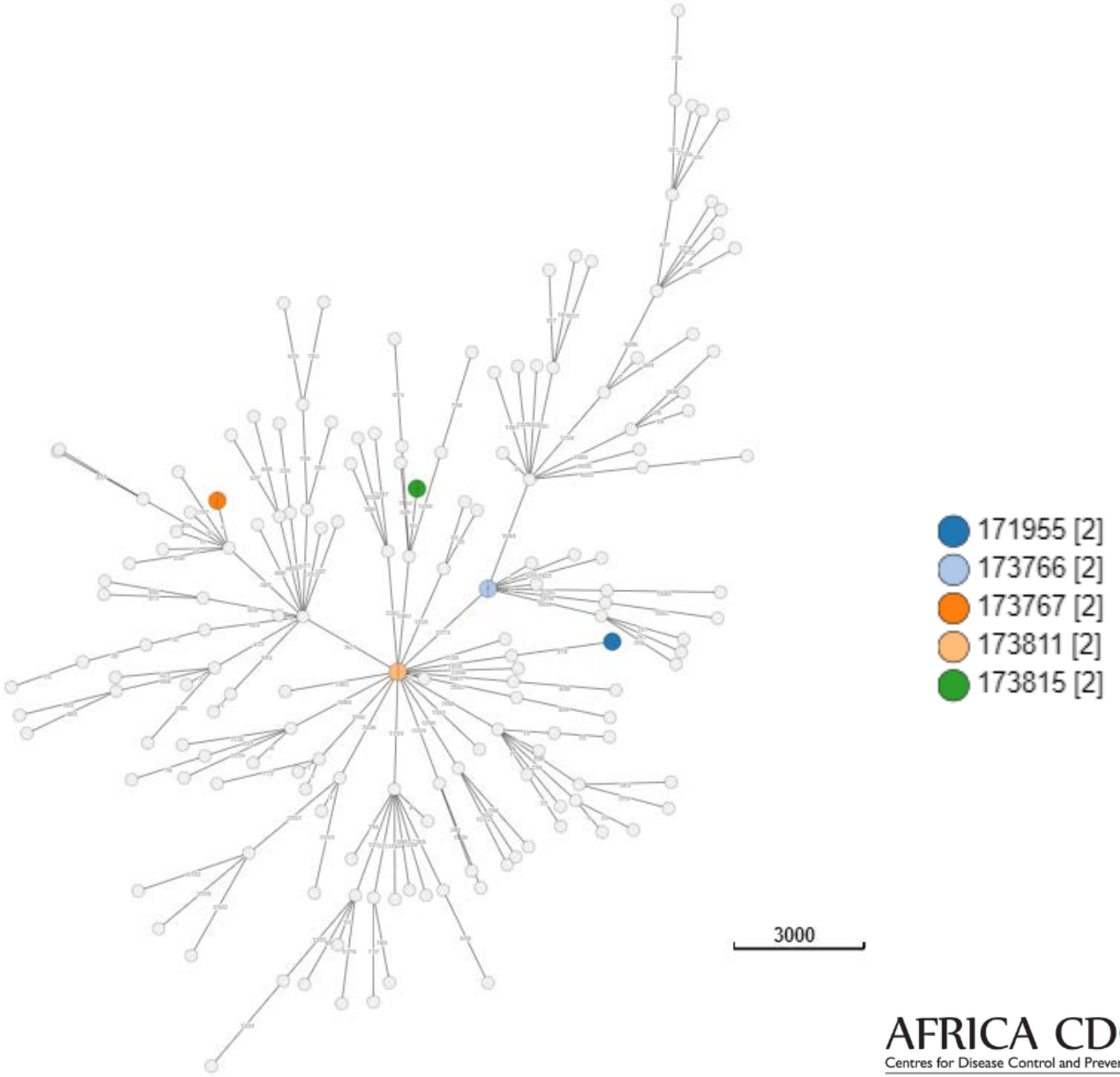
RESULTS

Core genome multilocus sequence type (cgMLST) and hierarchical clustering (HierCC) on Enterobase

Level: HC0

Five clusters, consisting of two isolates each, were genetically indistinguishable:

- **ST171955 (Humans)**
- **ST173766 (Pigs: Site A, House 2, Weaning)**
- **ST173767 (Single pig: Morphologically distinct isolates)**
- **ST173811 (Pigs: Site B, House 12, Weaning)**
- **ST173815 (Pigs: Site B, House 16, Weaning)**



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RESULTS

Core genome multilocus sequence type (cgMLST) and hierarchical clustering (HierCC) on Enterobase

Level: HC0

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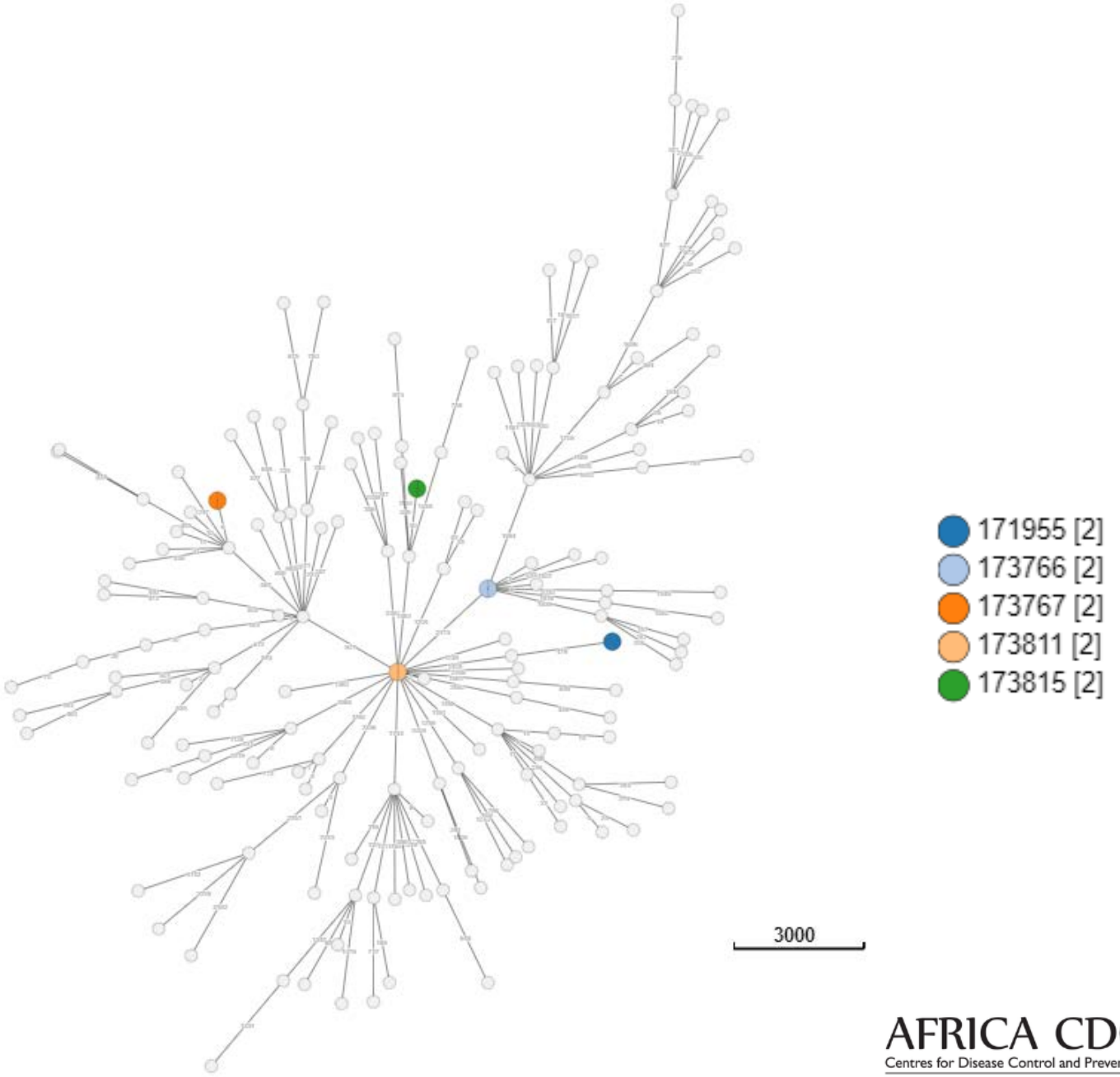

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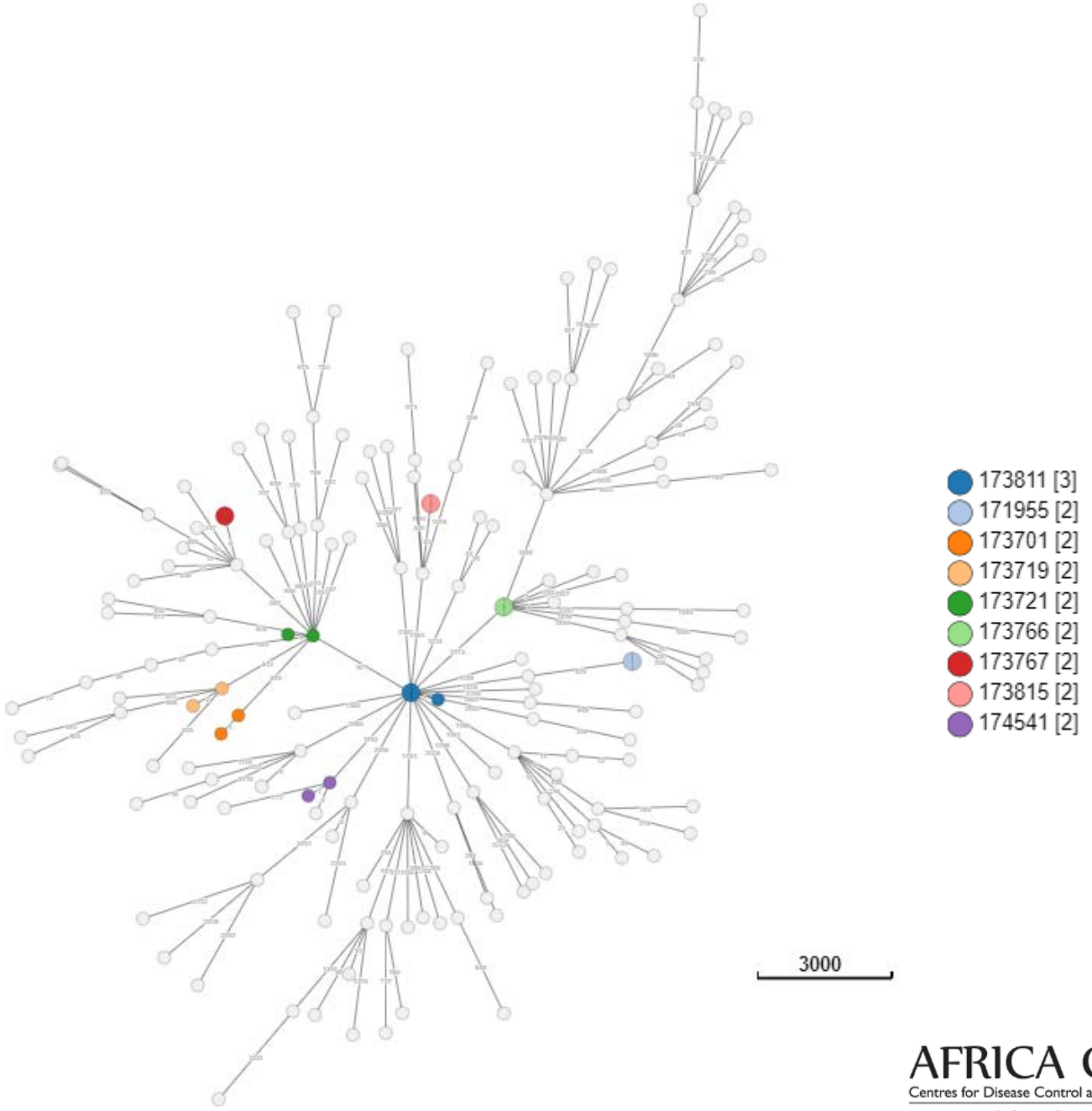
RESULTS

Core genome multilocus sequence type (cgMLST) and hierarchical clustering (HierCC) on Enterobase

Level: HC2

Nine clusters, consisting of two to three isolates each, were detected:

Cluster name	Source	Description		
		Site	House	Phase
171955	Humans			
173701	Humans	B		
173719	Humans	A		
173721	Pig + human	A (human) B (pig)	21	Growing
173766	Pigs	A	2	Weaning
173767	Same pig	A	2	Weaning
173811	Pigs (3)	B	12	Weaning
173815	Pigs	B	16	Weaning
174541	Pigs	B	20+21	Growing



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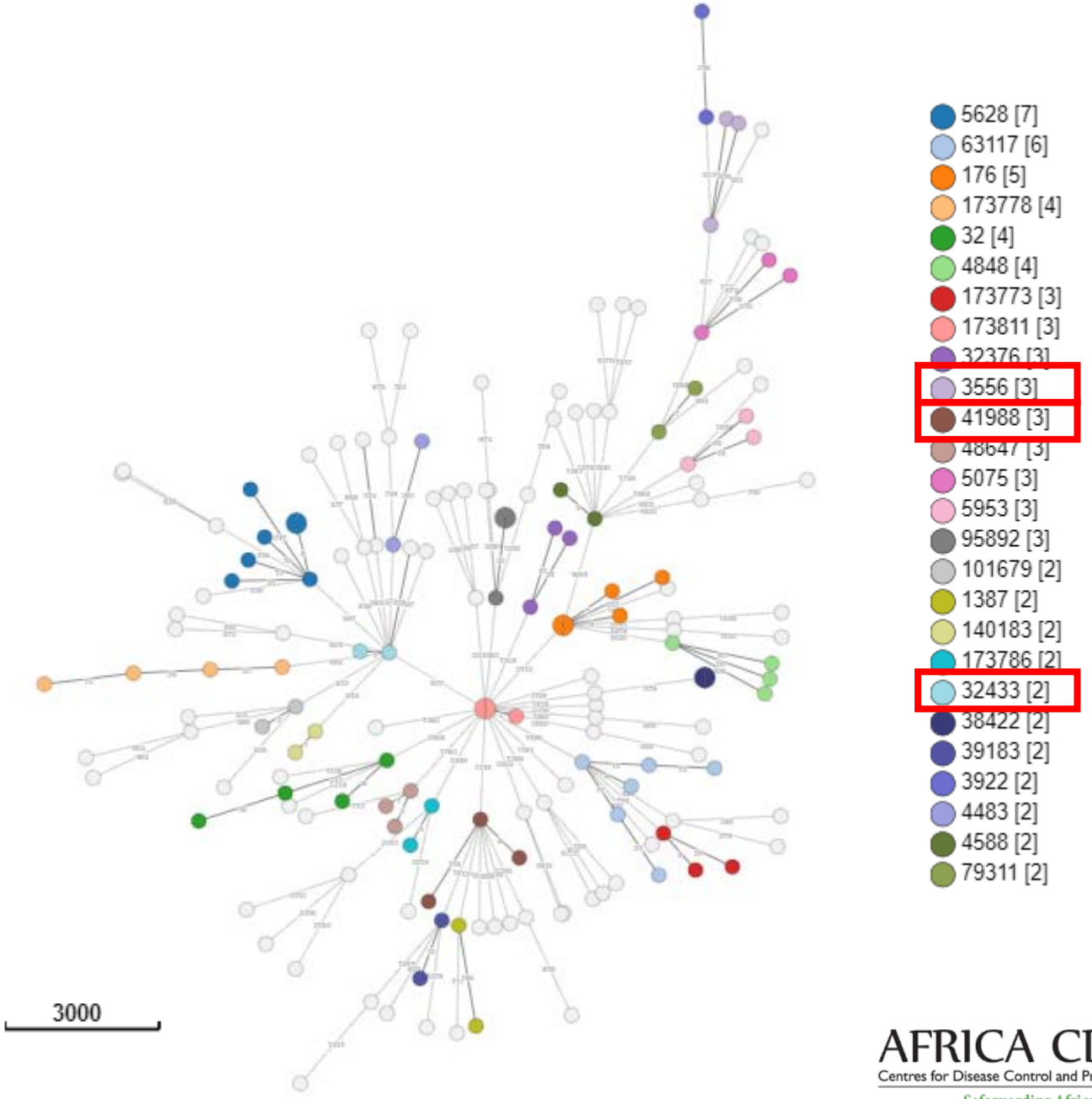
RESULTS

Core genome multilocus sequence type (cgMLST) and hierarchical clustering (HierCC) on Enterobase

Level: HC200

27 clusters were detected, of which three clusters (HC200_3556, HC200_32433, HC_41988) consisted of human and porcine *E. coli* isolates

Other clusters at HC200 consisted of only human or porcine *E. coli* isolates



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CONCLUSION

"Take home messages"

- **Porcine *E. coli* isolates were proportionally more resistant to antibiotics tested, except for trimethoprim-sulfamethoxazole where human *E. coli* isolates were slightly more resistant**

- **Overall, porcine *E. coli* isolates harboured a higher diversity of antibiotic resistance genes, which is an indication of high genetic variability of the porcine *E. coli* accessory genome and potentially indicates the selection pressure excerted by antibiotics used in the farm setting**

- **ST10 was the most predominant ST in both human and porcine *E. coli* isolates, based on Achtman's 7-gene MLST scheme**

- **Human and porcine *E. coli* isolates were genetically diverse, with evidence of hierarchical clustering at level 2 to 200, which potentially indicates:**
  - **i) a transmission risk between animals from different production houses, phases and sites**
  - **ii) a transmission risk between animals and humans, potentially due to proximity**
  - **iii) a transmission risk between humans, potentially due to shared facilities**

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FUTURE WORK

To be continued…

- **Concordance between phenotypic and antibiotic susceptibility profiles for human and porcine *E. coli* isolates**
- **Description and comparison of virulence genes for human and porcine *E. coli* isolates**
- **Description and comparison of mobile genetic elements for human and porcine *E. coli* isolates**

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THANK YOU

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