Antimicrobial resistance and relatedness of bacterial organisms obtained from feed farm and human contact

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

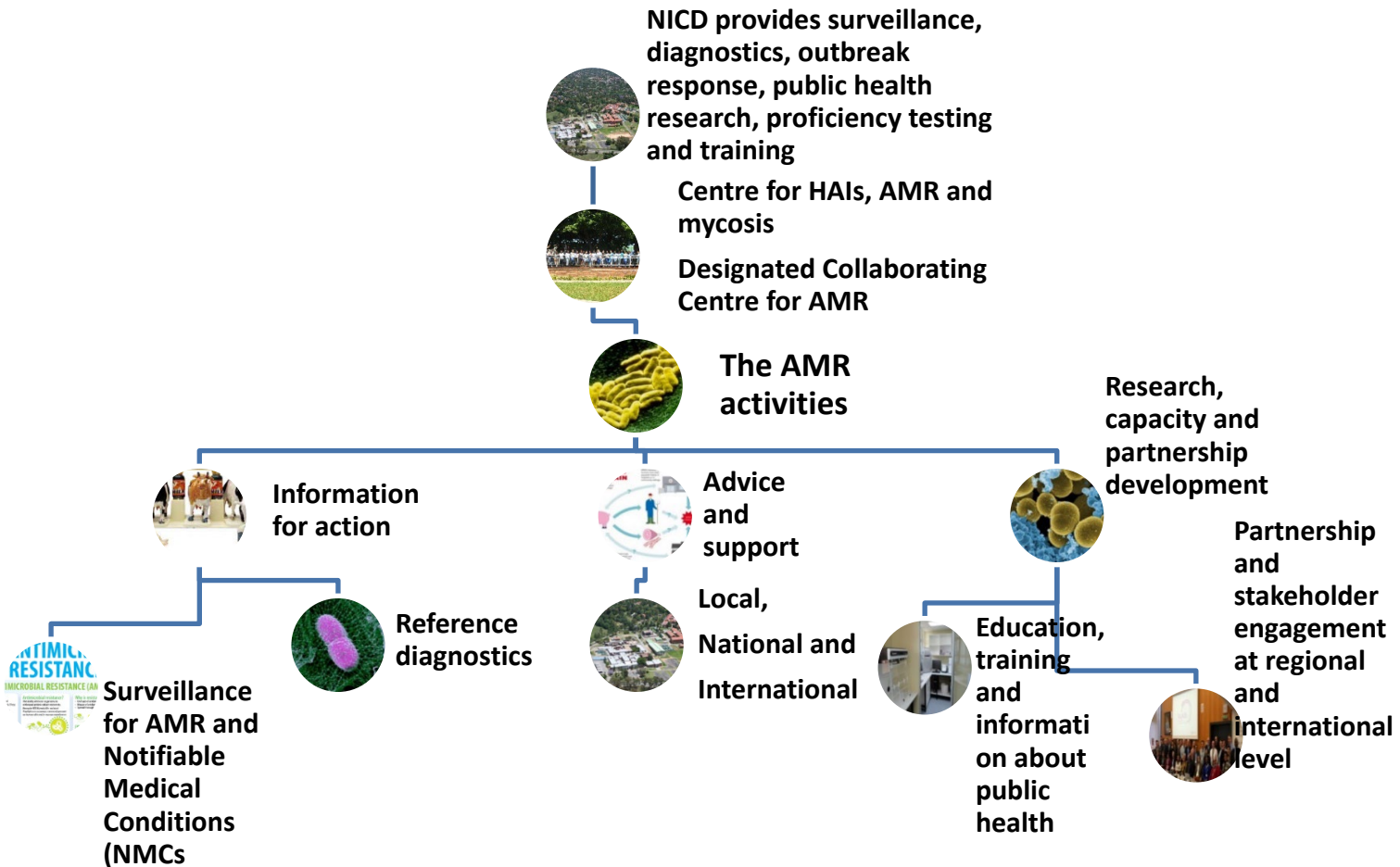**Division of the National Health Laboratory Service**

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Head of WHO Collaborating Centre for AMR,
University of Witwatersrand

Presentation outline

1. One Health surveillance for AMR
2. Whole genome sequencing for data analysis
3. Conclusions

Responsibilities for AMR surveillance at Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses (CHARM)

NICD provides surveillance, diagnostics, outbreak response, public health research, proficiency testing and training

Centre for HAIs, AMR and mycosis

Designated Collaborating Centre for AMR

The AMR activities

Information for action

Advice and support

Research, capacity and partnership development

Partnership and stakeholder engagement at regional and international level

Surveillance for AMR and Notifiable Medical Conditions (NMCs

Reference diagnostics

Local, National and International

Education, training and information about public health

Two tiers surveillance for AMR

**Laboratory based antimicrobial surveillance (LARS) since 2010 at GERMS**

- At sentinel sites as population-based surveillance was not feasible
- National reference laboratory for confirmation of isolates identification and AST, detection of unusual resistance and outbreak, implement national laboratory standards
- Laboratories networking

Electronic surveillance since 2013

- Collection of national data from LIS according case definition exclusion and inclusion criteria and defining denominator data-SDW
- Notifiable Medical Condition (NMC) system throughout surveillance data warehouse (SDW) on monthly bases for MDROs.

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Figure 1.2 Satellite approaches to enhance integrated surveillance on AMR around GLASS and Tricycle[iii]
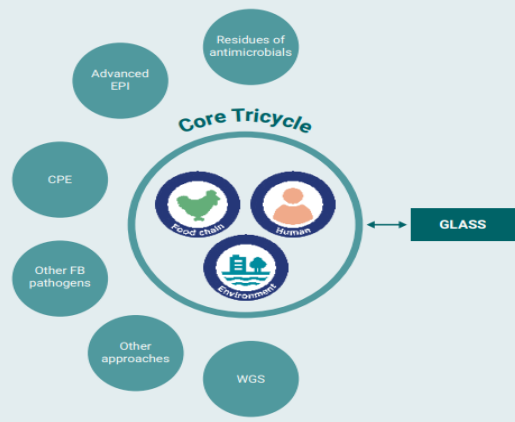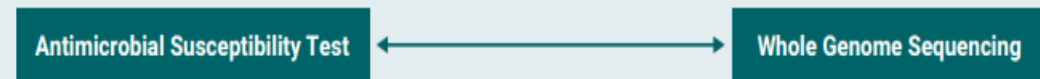

iii CPE: carbapenem-producing Enterobacteriaceae; FB: foodborne; WGS: whole genome sequencing; EPI: epidemiology.

Figure 4.4 Added value of sequencing analysis of the isolates from the three work packages

Integrated Surveillance of AMR: ESBL-Ec Tricycle surveillance

Sequencing analysis of isolates

| Species ID | Sub-type | AMR | Plasmids | Virulence | Phylogeny |

Antimicrobial Susceptibility Test ⟷ Whole Genome Sequencing

Phenotype — Genotype

Conceptual added value in WGS:
- More discriminatory methodology
- Better understanding of AMR
- Possibility for rapid re-analysis
- Easy data sharing
- Electronic data storage
- Direct comparison of data between sectors

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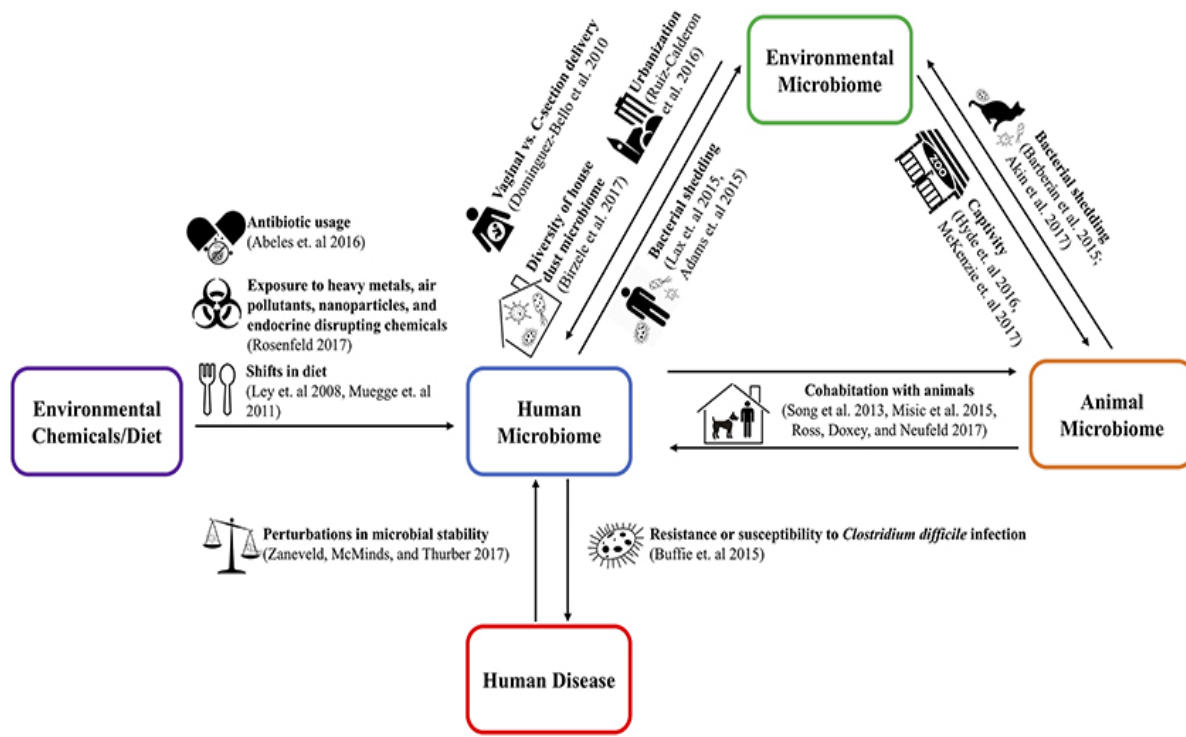
Antimicrobial resistance prevalence and transmission between animal feed and humans

Investigators	Role
Investigators from NICD	
Olga Perovic	Principal Investigator
Ashika Singh-Moodley	Senior Medical Scientist, CHARM, NICD
Wilhelmina Strasheim	Medical Scientist and PhD candidate, CHARM, NICD
Michelle Lowe	Medical Scientist, CHARM, NICD
Marshagne Smith	Laboratory manager, CHARM, NICD
Investigators from other organizations	
Peter Evans	Consulting veterinarian, CSV veterinary service and South African Pork Producer's Organisation (SAPPO)
Annelize Jonker	Consulting veterinarian (Veterinary laboratory)
Japhta Mokoele	Consulting veterinarian (Pig herd health)
Eric Etter	Consulting veterinarian (Veterinary epidemiologist)

BILL & MELINDA GATES foundation

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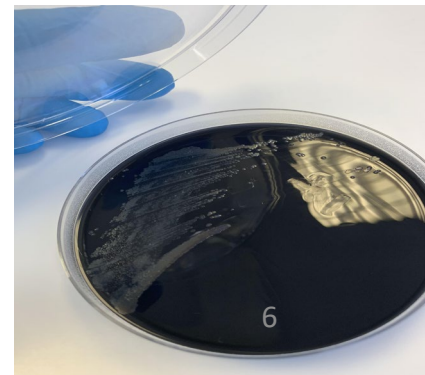YUNIBESITHI YA PRETORIA

Study design

Aim

The aim of the study will be to determine the prevalence of AMR, measure the association between antimicrobial usage and the emergence of AMR, as well as determine the risk of transmission of AMR genes between the farm environment, farm employees and animals in commercial pig farms in South Africa.
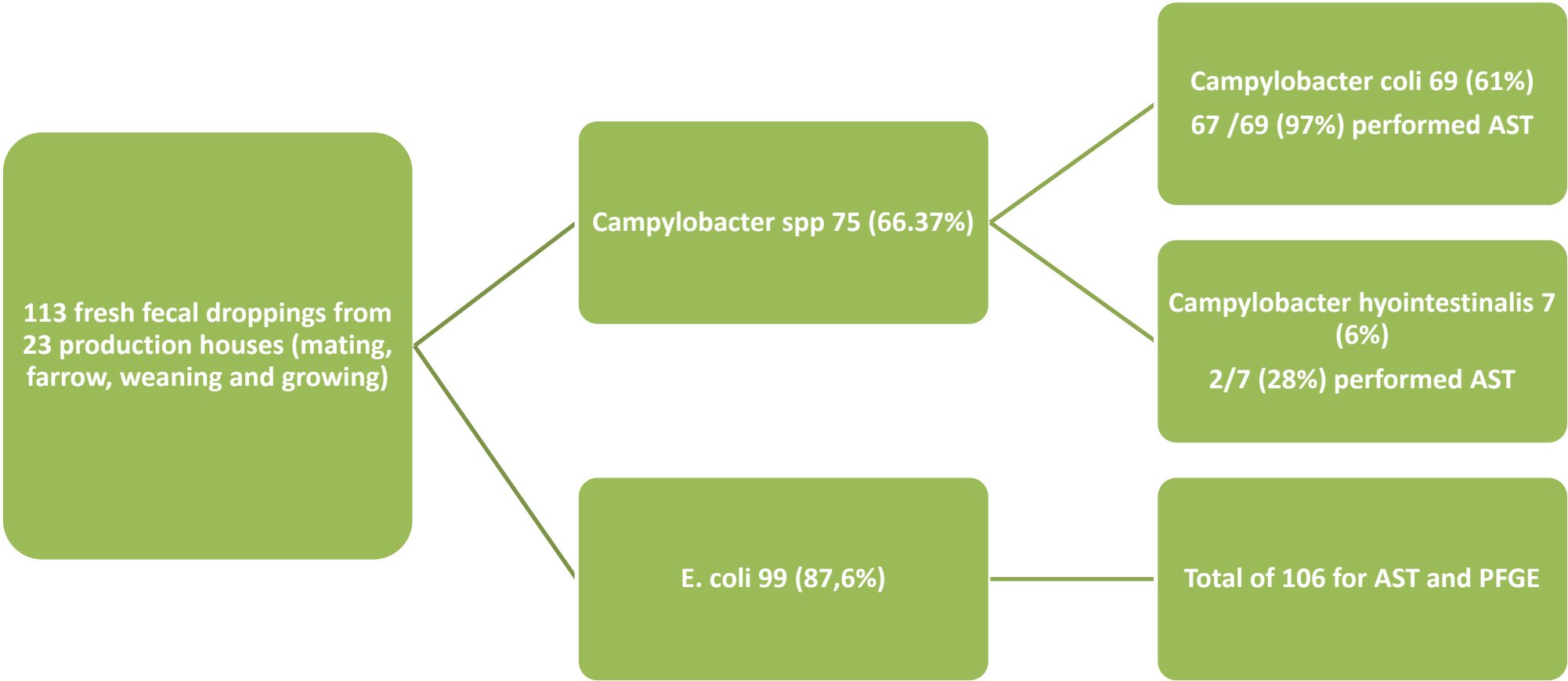
Farm recruitment

- Three commercial farms expressed interest in the study, but only a single farm was successfully enrolled after the completion of informed consent.
- 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.
- A total of five fresh, undisturbed pig faecal droppings were collected aseptically (Minimum weight = 10 g) from randomly selected pens per production house.

Laboratory methods

- Standard laboratory procedure were used except for isolation of Campylobacter species
    - Two isolation methodologies were done in parallel:
    - Direct plating of the swab on commercial selective media (CHROMagar) -red brick colonies (ISO 10272 standard)
    - Bolton broth enrichment (Oxoid, UK) - subculture onto mCCDA agar - smooth grey colonies (Bruker Daltonics, USA) (Fleming Fund protocol)
    - Both characteristic colonies were confirmed on MALDI-TOF MS (Bruker Daltonics, USA)

Results from pig faecal droppings

113 fresh fecal droppings from 23 production houses (mating, farrow, weaning and growing)

Campylobacter spp 75 (66.37%)

Campylobacter coli 69 (61%)
67 /69 (97%) performed AST

Campylobacter hyointestinalis 7 (6%)
2/7 (28%) performed AST

E. coli 99 (87,6%)

Total of 106 for AST and PFGE

Results from human specimens

The 64 human volunteers were recruited

E. coli was isolated from 78% (50/64) of the self-collected rectal swabs

Some morphologically distinct were included total of 63 isolates.

Most participants were male (85.9% 49/57) and in average 39 years old (±11 years)
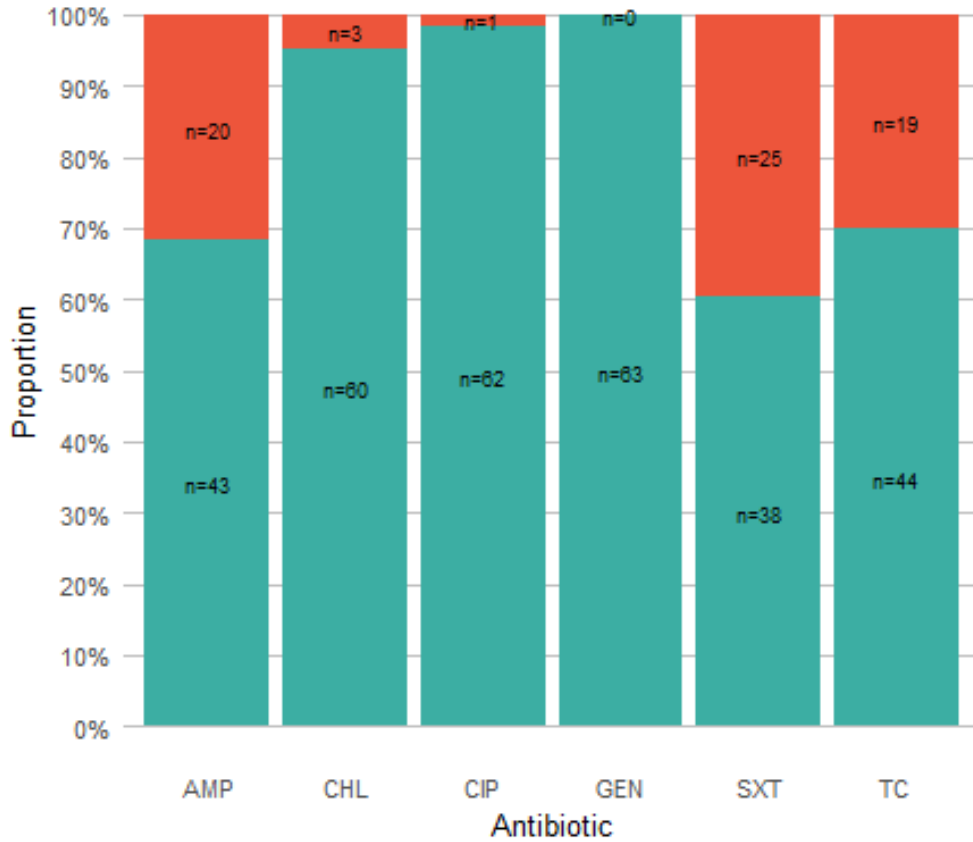
The remaining staff were involved in maintenance and housekeeping of the farm (29%; 16/55) or responsible for the transportation of live pigs to the abattoir (11%; 6/64) (Assigned duties unknown for 9 participants
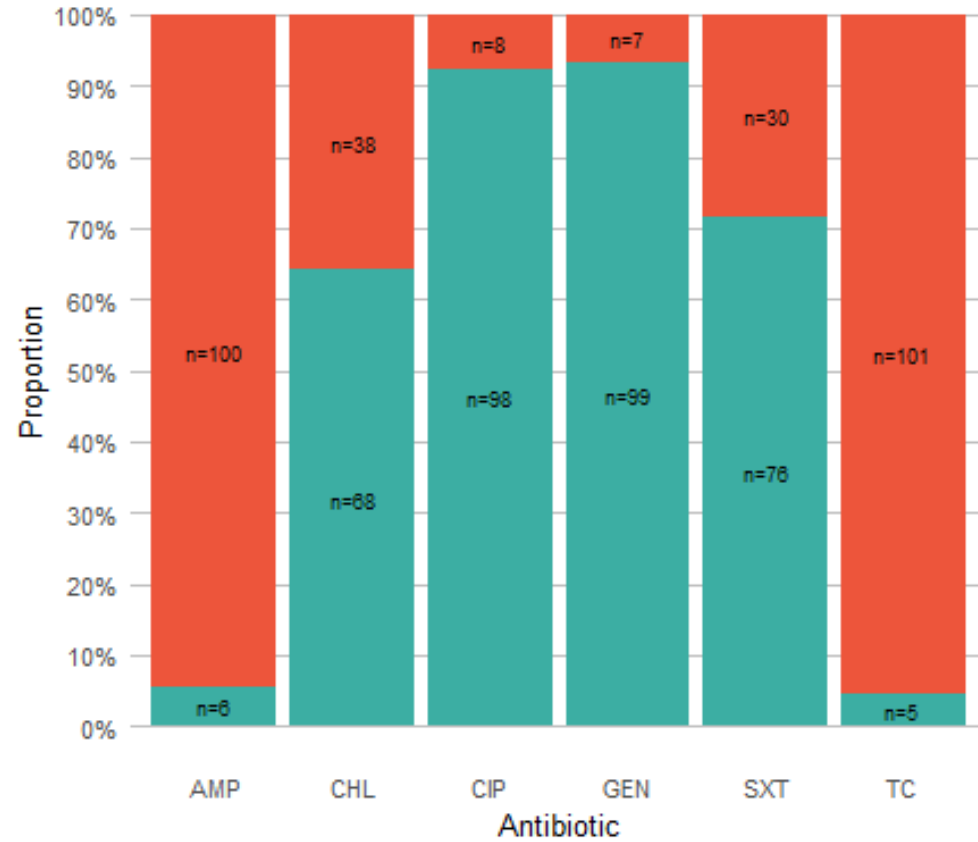
RESULTS

Phenotypic antibiotic susceptibility profiles

E. coli isolated from humans (n =63)

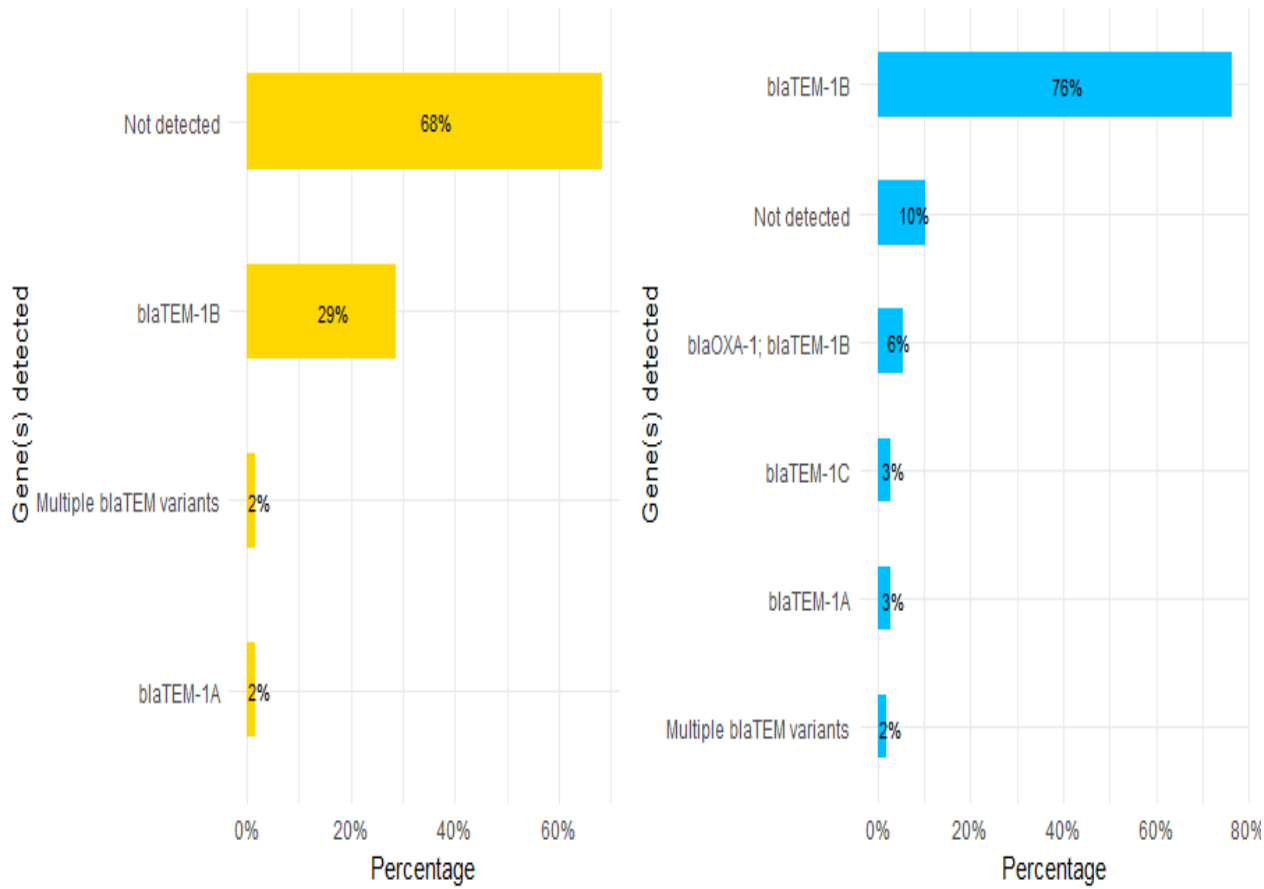**E. coli** isolated from pigs (n =106)

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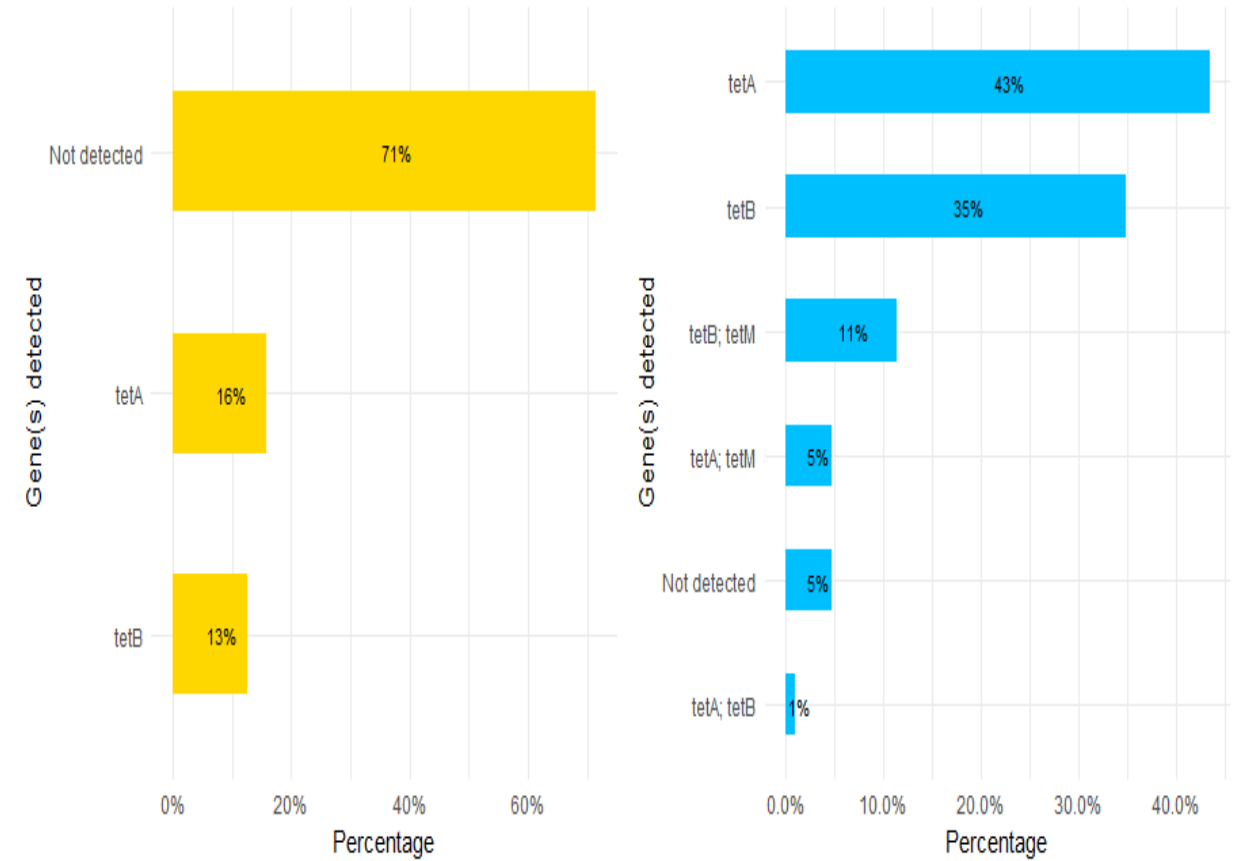Sulfamethoxazole
TC = Tetracycline

interpretation ■ S ■ R

RESULTS

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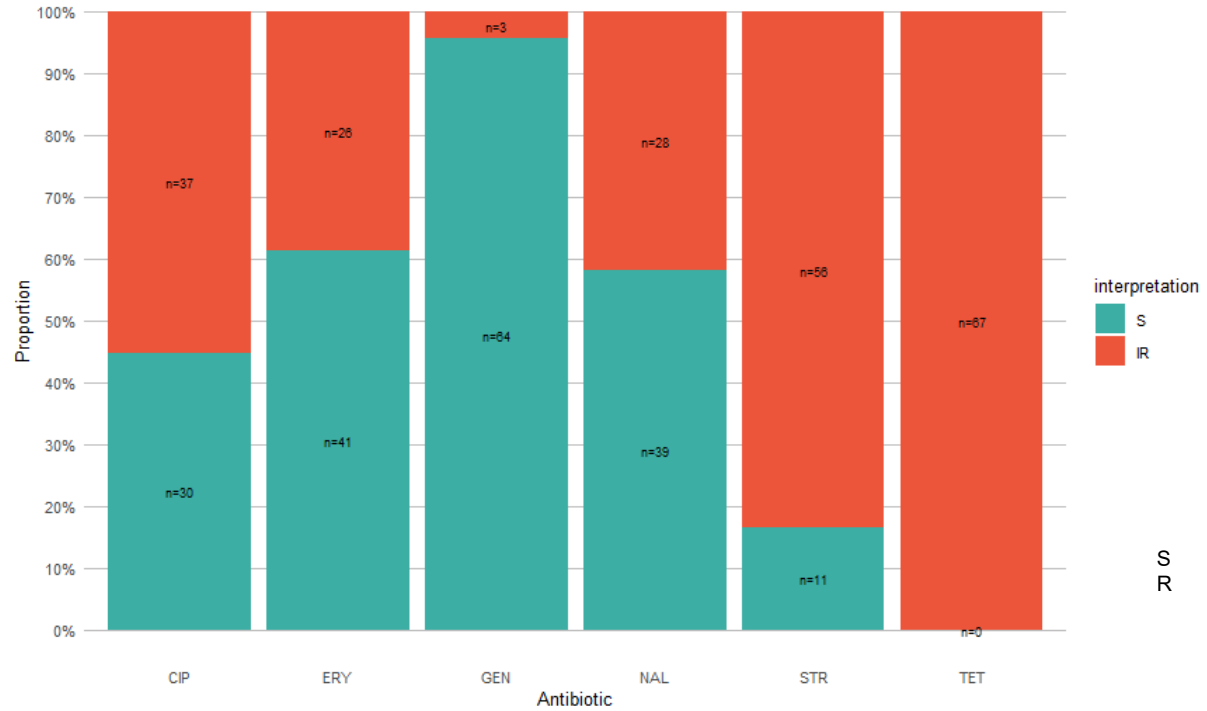
Distribution of tetracycline resistance genes

RESULTS

Antibiotic susceptibility profiles for *Campylobacter* species

Campylobacter coli isolated from pigs (n = 67)

CIP = Ciprofloxacin
ERY = Erythromycin
GEN = Gentamicin
NAL = Nalidixic acid
STR = Streptomycin
TET = Tetracycline
S = susceptible (wild-type)
R = resistant (non-wild type, acquired resistance

Antibiotic	MIC for non-WT
CIP	> 0.5
ERY	> 8
GEN	> 2
NAL	> 16
STR	> 4
TET	> 2

Bioinformatics analysis was done using the JEKESA pipeline – it is an automated bash pipeline build by Dr Stanford Kwenda for bacterial WG assembly and typing from Illumina sequencing data.

NICD approach is to perform tests of the public health importance
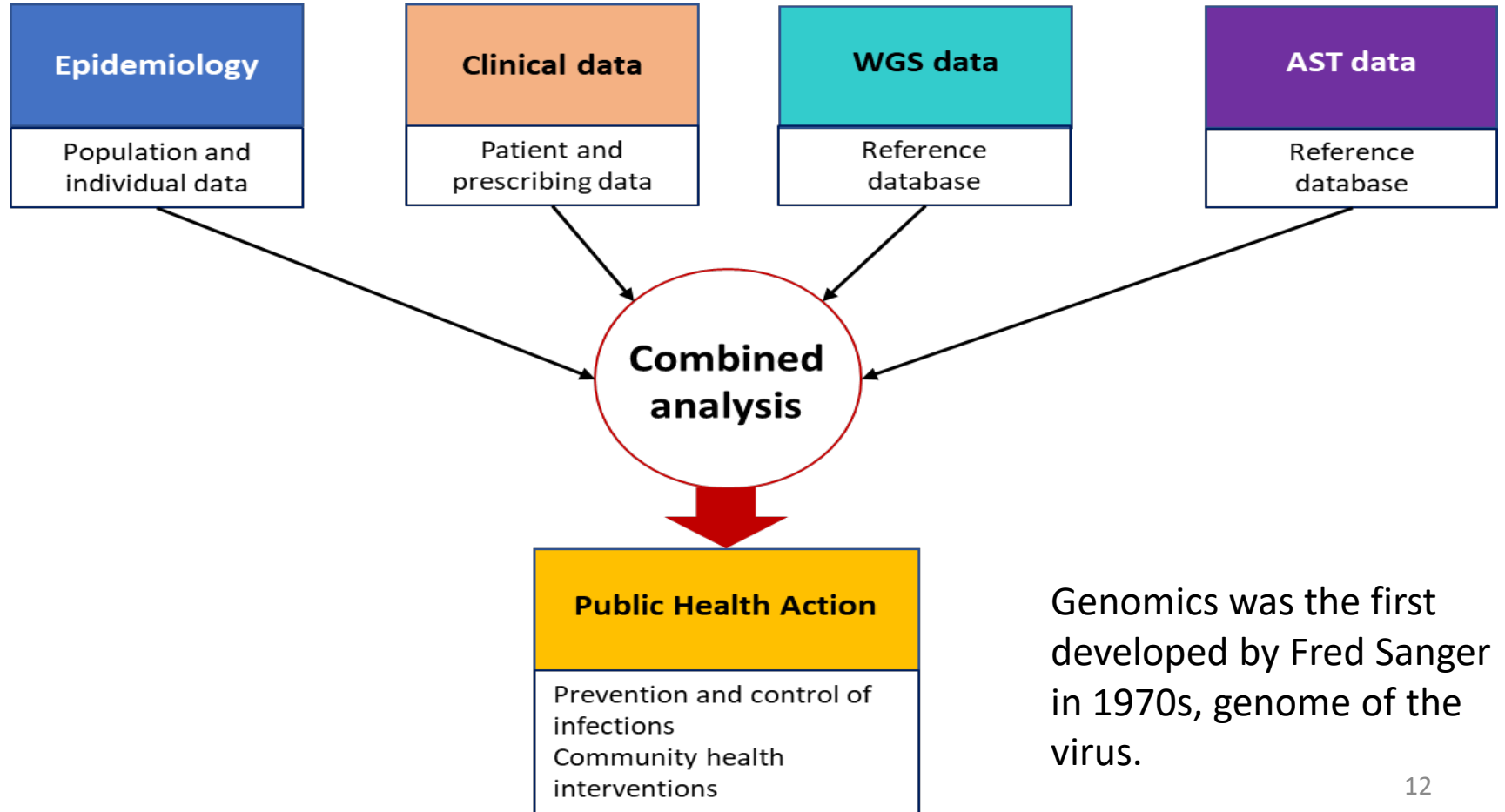
Use of NGS at NICD

Surveillance

Outbreak investigations

Research

Overview of interlinking of different data to produce public health results

| Epidemiology | Clinical data | WGS data | AST data |
| Population and individual data | Patient and prescribing data | Reference database | Reference database |

Combined analysis

Public Health Action

Prevention and control of infections
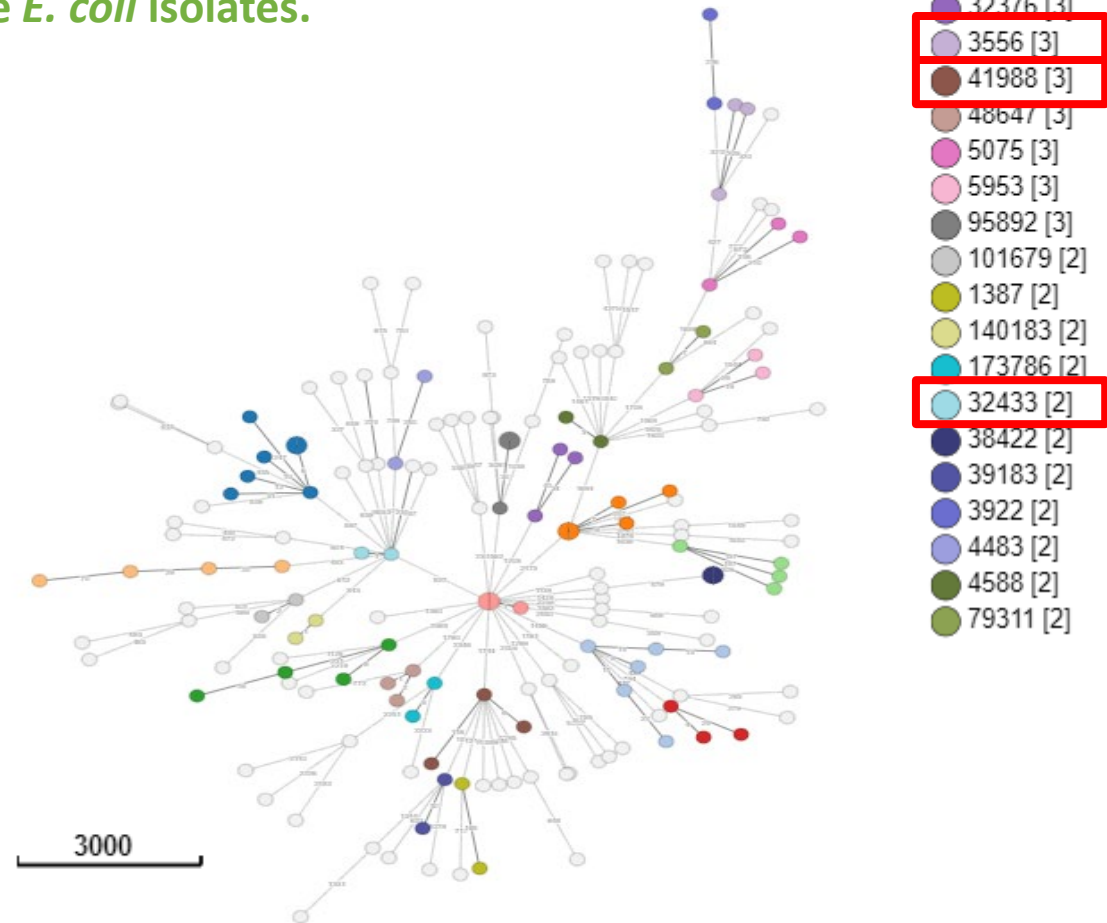Community health interventions

Genomics was the first developed by Fred Sanger in 1970s, genome of the virus.

RESULTS

Core genome multilocus sequence type (cgMLST) and hierarchical clustering (HierCC) on EnteroBase
Level: HC200
phylogeny of minimum spanning tree according to source niche.
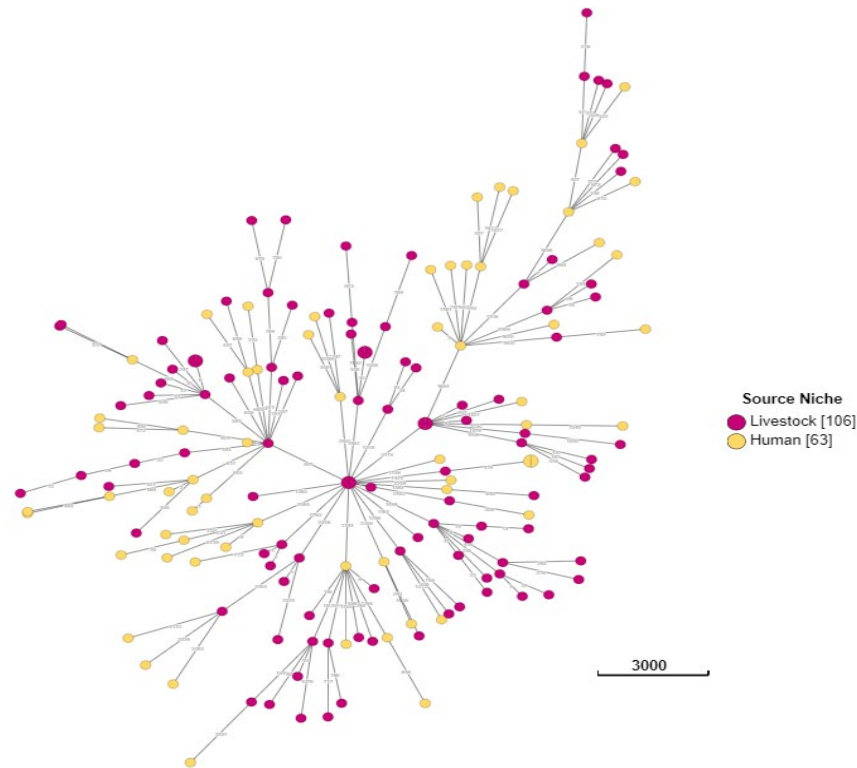
27 clusters were detected, of which three clusters (HC200_3556, HC200_32433, HC_41988) consisted of human and porcine *E. coli* isolates
Other clusters at HC200 consisted of only human or porcine *E. coli* isolates.

Source Niche
- Livestock [106]
- Human [63]

3000

3000

Legend:
- 5628 [7]
- 63117 [6]
- 176 [5]
- 173778 [4]
- 32 [4]
- 4848 [4]
- 173773 [3]
- 173811 [3]
- 32376 [3]
- 3556 [3]
- 41988 [3]
- 4647 [3]
- 5075 [3]
- 5953 [3]
- 95892 [3]
- 101679 [2]
- 1387 [2]
- 140183 [2]
- 173786 [2]
- 32433 [2]
- 38422 [2]
- 39183 [2]
- 3922 [2]
- 4483 [2]
- 4588 [2]
- 79311 [2]

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Phylogeny of *Campylobacter coli* (n = 27)

Colours by Stage
- Breeding
- Farrowing
- Growing
- Weaning

Colours by ST
- 1096
- 1125
- 1186
- 1556
- 2704
- 3336
- 830
- 854
- Unassigned

Colours by resistance gene
- Not detected
- Detected

ST = Sequence Type
AAD = Adenylyl transferases
ANT = nucleotidyltransferases
APH = phosphotransferases
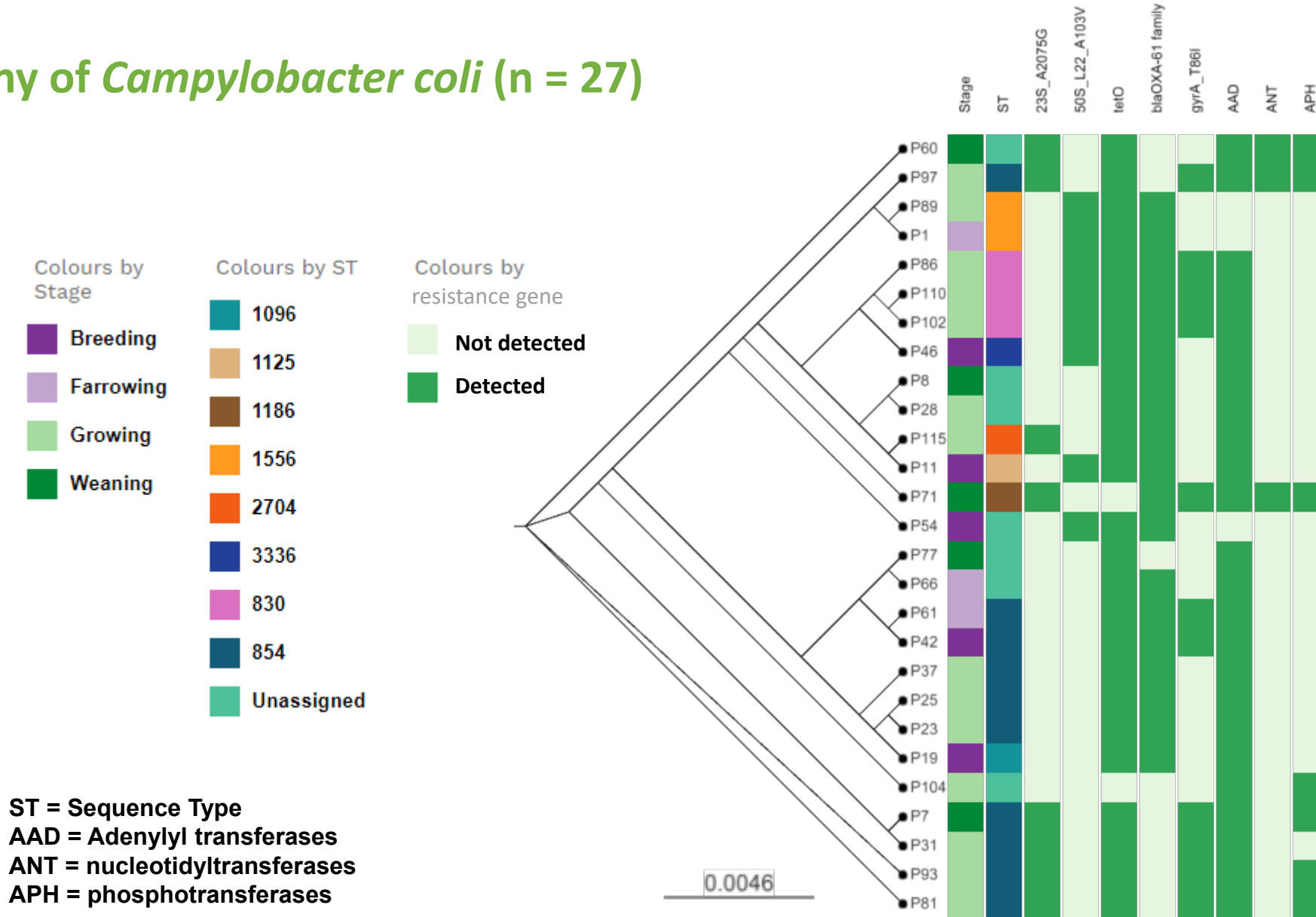
RESULTS
Antibiotic resistance genes detected of *Campylobacter coli*

Macrolide resistance:
- Two mutations were observed
  - 50S L22 ribosomal unit A103V (8/27; 29.63%)
  - 23S ribosomal unit A2075G (8/27; 29.63%)

Tetracycline resistance:
  - Mediated by *tet*O (25/27; 92.59%)

Aminoglycoside resistance:

Multiple aminoglycoside modifying enzymes (AMEs):
- Adenylyl transferases (ADD)
  - *aad*E-Cc (23/27; 85.19%)
  - *aad*9 (7/27; 25.93%)
  - *aad*E (6/27; 22.22%)
  - *aad*D1 (3/27; 11.11%)
- Phophotransferases (APH) (*aph*(3')-IIIa: (7/27; 25.93%)
- Nucleotidyltransferases (ANT) (*ant*(6)-Ia) (3/27; 11.11%)
  Other genes associated with resistance: *sat*4 (3/27; 11.11%) and arsenite efflux transporter Acr3 (11/27; 40.74%) were detected.

Beta-lactam resistance were not determined phenotypically
- OXA-61 family class D beta-lactamases detected (19/27; 70.37%):
  - OXA-193 (13/19; 68.42%)
  - OXA-578 (4/19; 21.05%)
  - OXA-489 (2/19; 10.53%)

Fluoroquinolone resistance:

- Point mutations in *gyr*A gene:
  - T86I (11/27; 40.74%)

Study conclusions

- Porcine *E. coli* isolates were more resistant to antibiotics tested, apart from more resistance in human *E. coli* isolates for trimethoprim-sulfamethoxazole.
- Overall, porcine *E. coli* isolates harboured a higher diversity of antibiotic resistance genes, and potentially indicates the selection pressure by antibiotics used in the farm setting.
- Human and porcine *E. coli* isolates were genetically diverse, with evidence of hierarchical clustering

- Two species were isolated, *Campylobacter coli* and *C. hyointestinalis*
- High levels of resistance towards streptomycin and tetracycline were detected in *Campylobacter coli* isolates, as well as resistance towards fluoroquinolones and macrolides.

THANK YOU!

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