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Posted Date: 15 May 2024

doi: 10.20944/preprints202405.0998.v1

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Whole Genome Sequencing of Human and Porcine *Escherichia coli* Isolates on a Commercial Pig Farm in South Africa

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Abstract: *Escherichia coli* is an indicator microorganism in One Health antibiotic resistance surveillance programmes. The purpose of the study was to describe and compare *E. coli* isolates obtained from pigs and human contacts from a commercial farm in South Africa using conventional methods and whole genome sequencing (WGS). Porcine *E. coli* isolates were proportionally more resistant phenotypically and harboured a richer diversity of antibiotic resistance genes, as compared to human *E. coli* isolates. Different pathovars, namely ExPEC (12.43%, 21/169), ETEC (4.14%; 7/169), EPEC (2.96%, 5/169), EAEC (2.96%, 5/169) and STEC (1.18%, 2/169) were detected at low frequencies. Sequence type complex (STc) 10 were the most prevalent (85.51%, 59/169) among human and porcine isolates. Six STcs (STc10, STc86, STc168, STc206, STc278 and STc469) were shared at the human-livestock interface according to multilocus sequence typing (MLST). Core genome MLST and hierarchical clustering (HC) showed that human and porcine isolates were overall genetically diverse, but some clustering at HC2 to HC200 were observed. In conclusion, even though the isolates shared a spatio-temporal relationship, there were still differences in the virulence potential, antibiotic resistance profiles, and cgMLST and HC according to the source of isolation

Keywords: *Escherichia coli*; pigs; close human contacts; One Health; antibiotic resistance; whole genome sequencing; virulence factors; core genome MLST and hierarchical clustering; sequence type complex 10; South Africa

1. Introduction

Wild-type *Escherichia coli* is intrinsically susceptible to most antibiotics, but this bacterium has the ability to acquire a vast range of mobile genetic elements (i.e., virulence and antibiotic resistance genes) through horizontal gene transfer, which allows it to become pathogenic and/or resistant [1,2]. It is for this reason that *Escherichia coli* is used as an indicator microorganism in One Health antibiotic

 doi:10.20944/preprints202405.0998.v1

resistance surveillance programmes, as the antibiotic susceptibility profile of this bacterium demonstrates the existing antibiotic pressure inflicted on an environment [3,4].

Certain *E. coli* strains can cause a wide spectrum of diseases in both humans and animals [1]. The diseases in humans caused by *E. coli* can be broadly be classified as: i) diarrhoeagenic or ii) extraintestinal diseases (i.e., urinary tract infections (UTIs), peritonitis, bacteriaemia and meningitis) [5,6]. Diarrhoeagenic *E. coli* can further be classified into seven different pathovars, namely i) enteropathogenic *E. coli* (EPEC), ii) shiga toxin-producing *E. coli* (STEC), iii) enterotoxigenic *E. coli* (ETEC), iv) enteroaggregative *E. coli* (EAEC), v) enteroinvasive *E. coli* (EIEC), vi) diffusely adherent *E. coli* (DAEC) and adherent invasive *E. coli* (AIEC) [7].

Previously, the classification of *E. coli* pathovars relied on the presence or absence of group-specific virulence genes detected by a polymerase chain reaction (PCR) assay [8]. However, this method can only detect a limited number of virulence genes simultaneously [8]. This has changed in the era of whole genome sequencing (WGS) as it is now possible to detect multiple virulence genes simultaneously without having any prior knowledge of the potential pathovar under investigation [6,8,9]. Whole genome sequencing had shown that specific virulence factors, previously thought to be pathovar-specific, can be shared among different pathovars [6,9]. It is therefore important to define the epidemiological and clinical significance of different *E. coli* pathovars using WGS [6].

The primary ecological niche of *E. coli* is the gastro-intestinal tract of its vertebra host, where it normally exists as a commensal [6,10]. *Escherichia coli* may also be found in secondary habitats, such as water and soil sediments and is used as an indicator of environmental faecal contamination [10,11]. The overlapping ecological niches of *E. coli* between humans, animals and the environment present the opportunity for the transmission of virulence and antibiotic-resistant genes, either i) directly between humans and animals due to close proximity or ii) indirectly through an intermediary vehicle, such as through the food chain or insect vectors [1,12]. Furthermore, virulence genes and antibiotic resistance genes are often located on mobile genetic elements, which can be transferred between groups of unrelated bacteria [2].

It is important to understand the transmission of antibiotic resistance and virulence factors, within a bacterium's phylogenetic background, to develop interventions to reduce the burden of disease and antibiotic resistance [8,13]. However, the spread of antibiotic resistance at the human-livestock interface are somewhat controversial [13,14]. Earlier studies, using multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis, found the same sequence types (STs) and pulsotypes circulating in pigs and farmer workers [15]. However, MLST is based on seven different housekeeping genes and has a lower resolution than WGS [16]. Nowadays, WGS is considered as the gold standard, but there is still conflicting evidence of transmission at the human-livestock interface using a higher resolution technique [13,16]. The use of WGS combined with the appropriate study design can overcome these limitations and increase our understanding of complex transmission pathways [13]. Multiple studies on antibiotic resistance have been conducted in South Africa [17]. However, these studies focused either exclusively on only animals or only human clinical isolates. Additionally, isolates did not share a spatio-temporal relationship and only a limited number of studies used WGS as a typing technique [17]. The purpose of the study was to describe and compare human and porcine *E. coli* isolates, which shared a temporal and spatial relationship on a commercial pig farm in South Africa, using WGS to ultimately investigate antibiotic resistance, virulence potential and phylogeny.

2. Materials and Methods

2.1. Study setting

Farm recruitment have been described previously [18]. The farm is situated in the North West province of South Africa and have a population of more than 1 000 sows. The farm produces approximately 3 000 metric tons of pork meat per year and has 25 production houses. Pigs are housed in four operational stages, namely: i) breeding, ii) farrowing, iii) weaning and iv) growing.

 doi:10.20944/preprints202405.0998.v1

2.2. Participant Recruitment, Sample Collection and Transportation

Sample collection was done in December 2019. All farm employees (age ≥ 18 years) were invited to participate in the study, after the completion of informed consent. Participants self-collected a dual-tip rectal swab (BD BBL CultureSwab EZ Swab, USA) and completed a study questionnaire to collect the following variables: i) age, ii) sex and iii) routine farm duties (i.e., animal handler, market transportation, routine maintenance and housekeeping or other). Four to five, fresh, undistributed pig faecal droppings, weighing approximately 10 grams (g), were collected aseptically from randomly selected pens per production house for all production stages. All samples were transported by road and processed within 48 h of collection at the Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses (CHARM), National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service (NHLS).

2.3. Isolation, Identification and Antibiotic Susceptibility Testing (AST) of E. coli

A primary inoculum was made with the self-collected rectal swab on a MacConkey agar plate (Diagnostic Media Products, Johannesburg, South Africa). The inoculum was streaked for single colonies and incubated aerobically at 35°C (±2°C) for 18 h to 24 h. Ten grams (10 g) of the pig faecal droppings were added to 90 mL of buffered peptone water (BPW) (Oxoid, Thermo Fisher Microbiology, Basingstoke, Hampshire, United Kingdom) and vortexed to homogenize the sample. The inoculated BPW was incubated at 35°C (±2°C) for 18 h to 24 h. The next day, 10 µL of the BPW was plated onto MacConkey agar (Diagnostic Media Products, Johannesburg, South Africa) and incubated under the same conditions.

The identity of all (both human rectal swabs and pig faecal droppings) presumptive *E. coli* colonies were confirmed with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Billerica, Massachusetts, USA). The MicroScan WalkAway plus (Beckman Coulter, Brea, California, USA) system was used for antibiotic susceptibility testing using the NM44 panel. The minimum inhibitory concentration (MIC) of colistin was determined with the Sensititre system using custom made plates (FRCOL) (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The MIC for ciprofloxacin was determined using a gradient diffusion method (Etest®, BioMerieux SA, Marcy l'Etoile, France) to detect low level ciprofloxacin resistance (0.06 µg/mL to 0.5 µg/mL). The gradient diffusion method (Etest®, BioMerieux SA, Marcy l'Etoile, France) was also used to detect the MIC of streptomycin, as this antibiotic was not included on the commercial NM44 panel. The Clinical and Laboratory Standards Institute (CLSI) (M100, 2020) guidelines were used for the interpretation of MIC for all antibiotics, except for streptomycin, colistin and tigecycline. The epidemiological cut-off (ECOFF) value of ≥32 µg/mL, as established by the US National Antimicrobial Resistance Monitoring System (NARMS) were used for streptomycin, whereas the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (v.13.0, 2023) were used for colistin and tigecycline [19,20].

2.4. Total Genomic DNA Extractions and Whole Genome Sequencing (WGS)

A purity plate prepared during AST was used to inoculate Brain Heart Infusion (BHI) broth (Diagnostic Media Products, Johannesburg, South Africa) for each *E. coli* isolate. The BHI broth was incubated at 35°C (±2°C) for 18 h to serve as starting material for DNA extractions. Total genomic DNA extractions was done using the QIAamp DNA Minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA extracts were submitted to the Sequencing Core Facility (SCF), NICD for WGS. The Nextera DNA Flex library prep kit (Illumina, San Diego, California, USA) was used for library preparation with the inclusion of a gBlock Gene Fragment (Integrated DNA technologies, Coralville, Iowa, USA) as a quality control measure. The Illumina NextSeq 550 instrument (Illumina, USA) was used for sequencing at 100× coverage, using 2 × 150 base pairs (bp) paired-end sequencing for each flow cell with the addition of a PhiX v.3 (Illumina, San Diego, California, USA) to serve as a cluster generation and sequencing control.

 doi:10.20944/preprints202405.0998.v1

2.5. Bioinformatics Analysis

The JEKESA pipeline (v 1.0) was used in part for the processing of sequencing reads [21]. In short, quality control was done using FastQC (v.0.11.9) (Available online: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/, accessed on 23 October 2021) and paired-end reads were trimmed with Trim Galore! (v.0.6.7) (Available online: https://github.com/FelixKrueger/TrimGalore, accessed on 5 August 2021) with a Q-score and read length parameters set at > 30 bp and > 50 bp, respectively. The presence of contaminating sequences other than *E. coli* were assessed with Kraken [22]. Assembly was done using SPAdes (v.3.14.1) and polished in shovill (v.1.1.0) (Available online: https://github.com/tseemann/shovill, accessed on 5 August 2021) [23]. Final read assembly was assessed using QUAST [24].

Annotation of the assembled genomes was performed using VirulenceFinder 2.0 and ResFinder 4.1 hosted on the Centre for Genomic Epidemiology server, with the pre-set parameters [25–29]. Enteroaggregative *E. coli* was defined based on the definition proposed by Boison and colleagues (2020), which states that an *E. coli* strain should contain AggR (*agg*R together with its Agg-R-activator regulator protein encoded by aar gene) and a complete aggregative adherence fimbriae (AAF) (I-V) or CS22 colonization factor gene cluster [30]. Enteropathogenic *E. coli* was defined based on the presence of the locus of enterocyte effacement (LEE) pathogenicity island, as well as the presence of non-LEE effectors [31]. Enterotoxigenic *E. coli* isolates were defined based on virulence factors listed by Duan *et al*., 2012 and Pakbin *et al*., 2021 [32,33]. Extraintestinal pathogenic *E. coli* isolates were defined based on the presence of Group II or Group III capsules, which protect ExPEC from phagocytosis and complement-mediated killing by the host's immune system [34,35]. An *E. coli* isolate had to harbour at least two of the above-mentioned virulence factors simultaneously to be classified as ExPEC. Shiga-toxin producing *E. coli* was defined as any isolate that harboured the *stx* genes.

Raw sequencing data (FastQ files for paired-end reads) were uploaded to the EnteroBase platform (Available online: https://enterobase.warwick.ac.uk/species/index/ecoli, accessed on 23 October 2021), where various tools were used to investigate phylogeny, namely: i) MLST, ii) Clermont phylogroups and iii) core-genome MLST (cgMLST) and hierarchical clustering (HC) among human and porcine *E. coli* isolates [36–39]. The genomic relationships were visualized using GrapeTree with the MSTree V2 algorithm based on the cgMLST V1 + HierCC V1 scheme [40]. All sequencing reads have been deposited in the National Centre for Biotechnology Information (NCBI)'s GenBank under BioProject number: PRJNA994298.

2.6. Statistical Analysis

Data were captured in Microsoft Excel. Data cleaning and analysis were done in R (v4.0.2) using the janitor, dplyr and rstatix packages [41–43]. Categorical variables were summarized as numbers and percentages, The Pearson's chi-squared test with Yates' continuity correction was used to compare differences between human and porcine *E. coli* isolates. A *p*-value of 0.05 was considered as significant.

3. Results

3.1. Participant Demographics and the Isolation Rate of E. coli

Sixty-four (64) farm workers were recruited, of which the majority were male (76.56%, 49/64). The participants were on average 40 years old (range: 22 years to 67 years). The routine farm duties were unknown for six participants (9.38%, 6/64). The remaining participants were involved in various duties, which included animal-handling (56,90%, 33/58), maintenance and housekeeping (31.03%, 18/58), transportation of pigs to the abattoir (8.62%, 5/58) and working in the feeding mill (3.45%, 2/58). Each participant (n =64) self-collected a rectal swab and *E. coli* was isolated from 78.13% (50/64) of the swabs. Different colony morphologies of *E. coli* isolates were observed from the same rectal

 doi:10.20944/preprints202405.0998.v1

swab in 13 instances and two isolates were therefore processed. Thus, a total of 63 human *E. coli* isolates obtained from 50 rectal swabs underwent further testing.

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 was isolated from every production house (100%, 23/23) across all production phases (100%, 4/4). *Escherichia coli* isolates with different colony 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.

3.2. Phenotypic Antibiotic Resistance Testing

The phenotypic resistance rates for human and porcine *E. coli* isolates are shown in Table 1.

Table 1. Phenotypic antibiotic resistance rates of human and porcine *E. coli* isolates.

Antibiotic class		R MIC breakpoint (µg/mL)	Human % (n=63)	Pigs % (n =106)	Total % (n = 169)	*p*-value
Penicillins						
	Ampicillin	≥ 32	33.33 (21)	94.34 (100)	71.60 (121)	**<0.001**
	Piperacillin	≥ 128	31.75 (20)	84.91 (90)	65.09 (110)	**<0.001**
ß-lactam combination agents						
	Amoxicillin-clavulanate	≥ 32/16	0.0 (0)	0.0 (0)	0.0 (0)	**-**
	Ampicillin-sulbactam	≥ 32/16	9.52 (6)	40.57 (43)	28.99 (49)	**<0.001**
	Piperacillin-tazobactam	≥ 128/4	0.0 (0)	0.00 (0)	0.0 (0)	-
Cephems						
	Cefepime	≥ 16	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
	Cefotaxime	≥ 4	0.0 (0)	0.0 (0)	0.0 (0)	-
	Cefotaxime-clavulante	> 0.5	0.0 (0)	0.0 (0)	0.0 (0)	-
	Cefoxitin	≥ 32	0.0 (0)	0.0 (0)	0.0 (0)	-
	Ceftazidime	≥ 16	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
	Ceftazidime-clavulante	> 0.25	0.0 (0)	0.0 (0)	0.0 (0)	-
	Cefuroxime	≥ 32	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
Monobactams						
	Aztreonam	≥ 16	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
Carbapenems						
	Doripenem	≥ 4	0.0 (0)	0.0 (0)	0.0 (0)	-
	Ertapenem	≥ 2	0.0 (0)	0.0 (0)	0.0 (0)	-
	Imipenem	≥ 4	0.0 (0)	0.0 (0)	0.0 (0)	-
	Meropenem	≥ 4	0.0 (0)	0.0 (0)	0.0 (0)	-
Aminoglycosides						
	Amikacin	≥ 64	0.0 (0)	0.0 (0)	0.0 (0)	-
	Gentamicin	≥ 16	0.0 (0)	6.6 (7)	4.14 (7)	0.0922
	Streptomycin#	≥ 32	22.22 (14)	28.3 (30)	26.04 (44)	0.4904
	Tobramycin	≥ 16	0.0 (0)	3.77 (4)	2.37 (4)	0.2996
Tetracyclines						
	Tetracycline	≥ 16	30.16 (19)	95.28 (101)	71.01 (120)	**<0.001**
Quinolones						
	Ciprofloxacin*	≥ 1	1.59 (1)	16.04 (17)	10.65 (18)	0.0072
	Levofloxacin	≥ 2	0.0 (0)	4.72 (5)	2.96 (5)	0.2004
Other						
	Chloramphenicol	≥ 32	4.76 (3)	35.85 (38)	24.26 (41)	**<0.001**
	Colistin#$	> 2	0.0 (0)	0.0 (0)	0.0 (0)	-

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Fosfomycin	≥ 256	0.0 (0)	1.89 (2)	1.18 (2)	0.7179
Tigecycline#	0.5	0.0 (0)	0.94 (1)	0.59 (1)	1
Trimethoprim-sulfamethoxazole	≥ 4/76	39.68 (25)	28.3 (30)	32.54 (55)	0.1747

MIC = Minimum inhibitory concentration; R = resistant; # Epidemiological cut-off values (ECOFF) reported and interpreted according to EUCAST guidelines (2023) NARMS; *As determined by the commercial broth microdilution method; $ MIC for colistin was determined using Sensititre. *P*-values indicated in bold were considered statistically significant.

Overall, high levels of resistance were observed for ampicillin (71.60%, 121/169) and tetracycline (71.01%, 120/170), whereas moderate levels of resistance were observed for trimethoprim-sulfamethoxazole (32.54%, 55/169), streptomycin (26.04%, 44/169), chloramphenicol (24.26%, 41/169) and ciprofloxacin (10.65%, 18/169). Low levels of resistance towards cephems (0.59%, 1/169), monobactams (0.59%, 1/169), fosfomycin (1.18%, 2/169) and gentamicin (4.14%, 7/169) were observed. Colistin and carbapenem resistance were not detected. A single porcine *E. coli* isolate (P74) had a MIC breakpoint of >2 µg/mL for tigecycline. Porcine *E. coli* isolates were more resistant than human isolates for ampicillin, piperacillin, ampicillin-sulbactam, tetracycline, ciprofloxacin and chloramphenicol ($p < 0.05$).

3.3. Genotypic Antibiotic Resistance Profiles

The genotypic resistance rates as determined by ResFinder (v4.1) for human and porcine *E. coli* isolates are shown in Table 2.

Table 2. Antibiotic resistance genes detected in human and porcine *E. coli* isolates using ResFinder (v.4.1).

Antibiotic resistance gene class	Human % (n=63)	Pigs % (n =106)	Total % (n = 169)	*p*-value
Aminoglycoside modifying enzymes				
Acetyltransferases				
ACC(3)-IId	0.0 (0)	0.94 (1)	0.59 (1)	1
ACC(3)-IId; ACC-Ib-cr	0.0 (0)	5.66 (6)	3.55 (6)	0.1354
Not detected	100.00 (63)	61.11 (99)	95.86 (162)	0.0922
Nucleotidyltransferases				
ANT(3'')-Ia	19.05 (12)	50.0 (53)	38.46 (65)	**<0.001**
Not detected	80.95 (51)	50.0 (53)	61.54 (104)	**<0.001**
Phosphotransferases				
APH(3')-Ia	0.0 (0)	12.26 (13)	7.69 (13)	**0.0095**
APH(3'')-Ib; APH(6)-Id	30.16 (19)	22.64 (24)	25.44 (43)	0.3669
APH(3')-Ia; APH(3'')-Ib; APH(6)-Id	0.0 (0)	11.32 (12)	7.10 (12)	**0.0139**
Not detected	69.84 (44)	53.77 (57)	59.76 (101)	0.0578
ß-lactam resistance genes				
bla$_{TEM-1A}$	1.59 (1)	2.83 (3)	2.37 (4)	1
bla$_{TEM-1B}$	28.57 (18)	76.42 (81)	58.58 (99)	**<0.001**
bla$_{TEM-1C}$	0.0 (0)	2.83 (3)	1.78 (3)	0.4563
ß-lactam resistance genes				
bla$_{TEM-1B}$ and *bla*$_{OXA-1}$	0.0 (0)	5.66 (6)	3.55 (6)	0.1354
Multiple *bla*$_{TEM}$ variants	1.59 (1)	1.89 (2)	1.78 (3)	1
Not detected	68.25 (43)	10.38 (11)	31.95 (54)	**<0.001**
Colistin				
*pmr*B(V161G)	0.0 (0)	0.94 (1)	0.59 (1)	1
Macrolides				
*mdf*A	95.24 (60)	97.17 (103)	96.45 (163)	0.8209

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*mdf*A, *mph*A	4.76 (3)	2.83 (6)	3.55 (6)	1
Phenicols				
*cat*A1	0.00 (0)	1.89 (2)	1.18 (2)	1
*cml*A1	1.59 (1)	4.72 (5)	3.55 (6)	0.5265
*cml*A1, *cat*B3	0.0 (0)	0.94 (1)	0.59 (1)	1
*cml*A1, *cat*B4, *flo*R	0.0 (0)	4.72 (5)	2.96 (5)	0.2004
*flo*R	0.0 (0)	1.89 (2)	1.18 (2)	1
Not detected	98.41 (62)	85.85 (91)	90.53 (153)	**0.0152**
Quinolones				
Chromosomal mutations				
*gyr*A (S83A)	4.76 (3)	5.66 (6)	5.33 (9)	1
*gyr*A (S83L)	6.35 (4)	23.58 (25)	17.16 (29)	0.0078
*par*C (A56T)	1.59 (1)	2.83 (3)	2.37 (4)	1
*par*C (S80I)	1.59 (1)	0.94 (1)	1.18 (2)	1
*par*E (I355T)	1.59 (1)	0.94 (1)	1.18 (2)	1
Plasmid mediated				
OqxAB	3.17 (2)	32.08 (34)	21.30 (36)	**<0.001**
OqxAB, acc-(6′)-Ib-cr, *qnr*S2	0.0 (0)	4.72 (5)	2.96 (5)	0.2004
OqxAB, *qnr*S1	0.0 (0)	6.60 (7)	4.14 (7)	0.0922
OqxAB, *qnr*S2	0.0 (0)	0.94 (1)	0.59 (1)	1
*qnr*S1	7.94 (5)	6.60 (7)	7.10 (12)	0.9868
Not detected	88.89 (56)	49.06 (52)	63.91 (108)	**<0.001**
Rifampicin				
arr-3	0.0 (0)	5.66 (6)	3.55 (6)	0.1354
Not detected	100.00 (63)	94.34 (100)	96.45 (163)	0.1354
Sulphonamides				
*sul*1	4.76 (3)	12.26 (13)	9.47 (16)	0.1805
*sul*1, *sul*2	11.11 (7)	3.77 (4)	6.51 (11)	0.1218
*sul*1, *sul*3	0.0 (0)	0.94 (1)	0.59 (1)	1
*sul*2	22.22 (14)	10.38 (11)	14.79 (25)	0.0610
*sul*2, *sul*3	0.0 (0)	1.89 (2)	1.18 (2)	1
*sul*3	1.59 (1)	10.38 (11)	7.10 (12)	0.0655
Not detected	60.32 (38)	60.38 (64)	60.36 (102)	1
Tetracyclines				
*tet*A	15.87 (10)	43.40 (46)	33.14 (56)	**<0.001**
*tet*A, *tet*M	0.0 (0)	4.72 (5)	2.96 (5)	0.2004
*tet*B	12.70 (8)	34.91 (37)	26.63 (45)	**0.0029**
*tet*B, *tet*M	0.0 (0)	11.32 (12)	7.10 (12)	**0.0139**
*tet*A, *tet*B	0.0 (0)	0.94 (1)	0.59 (1)	1
Not detected	71.43 (45)	4.72 (5)	29.59 (50)	**<0.001**
Trimethoprim				
*drf*A1	9.52 (6)	2.83 (3)	5.33 (9)	0.1286
*drf*A1, *drf*A14	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
*drf*A12	3.17 (2)	25.47 (27)	17.16 (29)	**<0.001**
*drf*A12, *drf*A21	0.0 (0)	3.77 (4)	2.37 (4)	0.2996
*drf*A14	12.70 (8)	4.72 (5)	7.69 (13)	0.1131
*drf*A17	4.76 (3)	2.83 (3)	3.55 (6)	0.8209
*drf*A21	0.0 (0)	0.94 (1)	0.59 (1)	1
*drf*A5	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
*drf*A7	4.76 (3)	0.0 (0)	1.78 (3)	0.0960
*drf*A7, *drf*A14	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
Not detected	60.32 (38)	59.43 (63)	59.76 (101)	1

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P-values indicated in bold were considered statistically significant.

No acquired antibiotic resistance genes (i.e., no hits found) were detected for fosfomycin, fusidic acid, nitroimidazoles, oxazolidinones, and glycopeptides. A single porcine isolate (P42) harboured a point mutation (V161G) in the *pmr*B gene, but was not phenotypically resistant to colistin. The antibiotic resistance genes for aminoglycosides, ß-lactams, quinolones and tetracyclines are further summarized below:

3.3.1. Aminoglycoside-Modifying Enzymes (AMEs)

All three classes of aminoglycoside modifying enzymes (AMEs), namely acetyltransferases (ACC), nucleotidyltransferases (ANT) and phosphotransferases (APH) were detected in human and porcine *E. coli* isolates. In addition, multiple combinations of APHs, that encodes resistance towards streptomycin and kanamycin, were detected. A richer diversity of APHs and ANTs were detected in porcine *E. coli* isolates when compared to human *E. coli* isolates ($p < 0.05$). Acetyltransferases (ACCs), that encodes resistance towards gentamicin, were only detected in porcine *E. coli* isolates. The *acc*-(6')-Ib-cr gene, that also mediate resistance towards fluoroquinolones, was detected in combination with ACC(3)-IId in six porcine *E. coli* isolates (5.66%, 6/106).

3.3.2. ß-Lactam Resistance Genes

Beta-lactam resistance was predominately mediated by different variants of the *bla*$_{\text{TEM}}$ gene in both human and porcine *E. coli* isolates (64.50%, 109/169), with the exception of the detection of *bla*$_{\text{OXA-1}}$ in combination with *bla*$_{\text{TEM-1B}}$ in 5.66% (6/106) of the porcine *E. coli* isolates. Sixty-eight percent (68.25%, 43/63) of human *E. coli* isolates did not harbour a ß-lactam antibiotic resistance gene, whereas the majority of the porcine *E. coli* isolates harboured a resistance gene associated with ampicillin resistance (89.62%, 95/106) ($p < 0.001$).

3.3.3. Quinolone Resistance Genes

Quinolone resistance was mediated by chromosomal point mutations, as well as antibiotic resistance determinants residing on plasmids. The S83L point mutation in DNA gyrase (*gyr*A) (17.16%, 29/169) was the most common chromosomal point mutation, whereas the multidrug OqxAB efflux pump (21.30%, 36/169) was the most common plasmid-mediated quinolone resistant determinant. It was more likely to detect both the S83L point mutation in *gyr*A (and OqxAB pump in porcine than in human *E. coli* isolates ($p < 0.05$). Human *E. coli* isolates were more likely not to harbour any plasmid-mediated antibiotic resistant determinants for fluoroquinolones than porcine *E. coli* isolates ($p < 0.001$).

3.3.4. Tetracycline Resistance Genes

A total of 71.43% (45/63) human *E. coli* isolates did not harbour a tetracycline resistance gene, in contrast to the 4.72% (5/106) of porcine *E. coli* isolates without a tetracycline resistance determinant ($p < 0.001$). Overall, tetracycline resistance was mediated by three genes, namely *tet*A, *tet*B and *tet*M, of which *tet*A occurred in the highest frequency (36.69%, 62/169), followed by *tet*B (34.32%, 58/169). The *tet*M gene was not detected in human isolates and was always detected in combination with either *tet*A (4.72%, 5/106) or *tet*B (11.32%, 12/106) in porcine isolates. A single porcine *E. coli* isolate harboured the *tet*A and *tet*B gene simultaneously.

3.4. Virulence Potential

Overall, 103 different types of virulence genes were detected. Thirteen virulence genes were only detected in porcine *E. coli* isolates (12.62%, 13/103), whereas 47 different virulence genes (45.63%, 47/103) were only detected in human *E. coli* isolates ($p < 0.001$). All isolates (100%, 169/169) harboured the tellurite resistance (terC) gene. The occurrence of virulence genes was further interpreted based

 doi:10.20944/preprints202405.0998.v1

on the virulence gene combinations that grouped into gene clusters, pathogenicity islands and pathotypes (Table 3).

Table 3. Virulence factors grouped according to pathotype in human and porcine *E. coli* isolates.

Virulence gene combinations	Human % (n=63)	Pigs % (n =106)	Total % (n = 169)	*p*-value
Enteroaggregative *E. coli* (EAEC)				
Dispersin (*aap*)	12.70 (8)	0.0 (0)	4.73 (8)	<0.001
Dispersin transporter protein (*aat*A)	9.52 (6)	0.0 (0)	3.55 (6)	0.0050
*aai*C, ORF4 and ORF4	4.76 (3)	0.0 (0)	1.78 (3)	0.0960
*aai*C	0.0 (0)	0.94 (1)	0.59 (1)	1
Biogenesis of AFA-III				
*afa*ABCDE	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
*afa*ABCDE8	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
*afa*D	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
Biogenesis of AAF-I				
*agg*ACD	3.17 (2)	0.0 (0)	1.18 (2)	0.2671
*agg*ABCD	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
AggR transcriptional activation				
*agg*R and *aar*	4.76 (3)	0.0 (0)	1.78 (3)	0.0960
Other genes associated with EAEC				
air	7.94 (5)	0.94 (1)	3.55 (6)	0.0517
pet	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
pic	4.76 (3)	0.0 (0)	1.78 (3)	0.0960
sat	15.87 (10)	0.0 (0)	5.92 (10)	<0.001
*sig*A	14.29 (9)	0.0 (0)	5.33 (9)	<0.001
Enteropathogenic *E. coli* (EPEC)				
Genes harboured on LEE pathogenicity island				
eae	1.59 (1)	3.77 (4)	2.96 (5)	0.7326
*esp*B	0.0 (0)	1.89 (2)	1.18 (2)	0.7179
*esp*A	1.59 (1)	3.77 (4)	2.96 (5)	0.7326
*esp*F	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
*tcc*P	0.00 (0)	0.94 (1)	0.59 (1)	1
tir	1.59 (1)	3.77 (4)	2.96 (5)	0.7326
Non-LEE effectors				
cif	1.59 (1)	3.77 (4)	2.96 (5)	0.7326
*esp*J	1.59 (1)	3.77 (4)	2.96 (5)	0.7326
*nle*A	1.59 (1)	3.77 (4)	2.96 (5)	0.7326
*nle*B	1.59 (1)	3.77 (4)	2.96 (5)	0.7326
Enterotoxigenic *E. coli* (ETEC)				
*ast*A	11.11 (7)	17.92 (19)	15.38 (26)	0.3337
F18 fimbriae (*fed*AF)	0.0 (0)	0.94 (1)	0.59 (1)	1
*ltc*A	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
*mcb*A	1.59 (1)	0.94 (1)	1.18 (2)	1
STb toxin (*stb*)	0.00 (0)	3.77 (4)	2.37 (4)	0.2996
Extraintestinal *E. coli* (ExPEC)				
Group 2 capsule				
Only *kps*E	1.59 (1)	1.89 (2)	1.78 (3)	1
*kps*M	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
*kps*MII	1.59 (1)	3.77 (4)	2.96 (5)	0.7326
K1	7.94 (5)	0.0 (0)	2.96 (5)	0.0133

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	K5	7.94 (5)	0.0 (0)	2.96 (5)	0.0133
	K23	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
	K52	4.76 (3)	0.0 (0)	1.78 (3)	0.0960
	*neu*C	15.87 (10)	0.0 (0)	5.92 (10)	<0.001
Group 3 capsule					
	K96	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
	K98	1.59 (1)	0.0 (0)	0.59 (1)	0.7919
Shiga toxin-producing *E. coli* (STEC)					
	Stx-2 (*stx*2AB)	0.00 (0)	1.89 (2)	1.18 (2)	0.7179

AFA = afimbrial adhesion; AAF = aggregative adherence fimbria; LEE = locus of enterocyte effacement pathogenicity island.

The following pathovars were detected: i) extraintestinal *E. coli* (ExPEC) (12.43%, 21/169), ii) ETEC (4.14%, 7/169), iii) EAEC (2.96%, 5/169), iv) EPEC (2.96%, 5/169) and v) STEC (1.18%, 2/169) in low frequencies as described in more detail below. Overlap between the ETEC and ExPEC pathovars in three porcine isolates, as well as in EAEC and ExPEC pathovars in two human isolates were observed. The majority of ETEC (85.71%, 6/7), EPEC (80.00%, 4/5) and all STEC (100.00%, 2/2) pathovars were isolated from pigs, whereas all EAEC (100.00%, 5/5) pathovars and the majority of ExPEC (80.95%, 17/21) pathovars were isolated from healthy human volunteers.

3.4.1. Enteroaggregative E. coli (EAEC)

The complete molecular gene signatures associated with EAEC were only detected in three human *E. coli* isolates (H24-2, H31 and H59-2) and not in any of the porcine isolates. These three human isolates also harboured the *aai*C gene cluster with the putative proteins ORF3 and ORF4, as well as the dispersin (*aap*) and the dispersin transporter protein (*aat*A) genes simultaneously. Two human *E. coli* isolates (H21 and H54) harboured a complete AFA-III gene cluster (H21: *afa*ABCDE; and H54-1: *afa*ABCDE8), but the AggR transcriptional activator were not detected. Various other genes (*sat* and *sig*A) previously reported to be associated with EAEC were also mostly detected in the human *E. coli* isolates ($p < 0.05$).

3.4.2. Enteropathogenic E. coli (EPEC)

A total of five *E. coli* isolates were classified as EPEC, of which a single isolate originated from a human (H27-2) and four isolates (P6-2, P75, P105 and P107) originated from pigs. All porcine EPEC isolates were detected in different production houses, but originated from two production stages, namely weaning phase (P6-2 and P75) and growing phase (P105 and P107).

3.4.3. Enterotoxigenic E. coli (ETEC)

Virulence factors associated with ETEC detected in this study included *ast*A, *ltc*A, *mbc*A, F18 fimbriae (*fed*AF) and STb toxin (*stb*). An *E. coli* isolate was defined as ETEC in this study, if it harboured at least two of the above mentioned virulence factors simultaneously. A total of six porcine *E. coli* isolates (P10-2, P91, P102, P97, P98-1 and P35) and a single human *E. coli* isolate (H29) harboured the following gene combinations: i) *ast*A-*stb* (P10-2; P102; P97 and P98-1); ii) *ast*A-*mcb*A (P91), iii) *ast*A-*fed*AF (P35) and iv) *ast*A-*ltc*A (H29).

3.4.4. Extraintestinal Pathogenic E. coli (ExPEC)

Human ExPEC harboured different Group II capsules, namely K1, K5, K23 and K52. Four pig isolates (P60, P97, P98-1 and P102) harboured the *kps*MII capsule, whereas two porcine isolates (P68 and P113) only harboured the *kps*E transporter protein. Three porcine isolates (P97, P98-1 and P102) also simultaneously harboured genes associated with ETEC, whereas two human isolates (H21 and H54-1) also simultaneously harboured genes associated with EAEC.

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3.4.5. Shiga Toxin-Producing E. coli (STEC)

Two porcine isolates [P92 (ST162, phylogroup B1, O8:H28) and P109 (ST23, phylogroup B1, O8:H9)] from the growing production phase were classified as STEC. These isolates harboured the Shiga toxin-type 2 (Stx2) (*stx*2AB) and were detected in different production houses (House 19 and House 22).

3.4.6. Other Virulence Genes Detected that Are Not Associated with a Specific Pathovars

Multiple other virulence genes that overlap between the different pathovars or that are not associated with a specific pathovars were also detected. These virulence factors were further grouped according to function and are available in the supplementary material (Table S1). Multiple virulence factors encoding bacteriocins were detected. In addition, genes associated with iron acquisition, colonization and toxins (*hly*A and *tox*B) were also detected.

3.5. Phylogeny

3.5.1. Clermont Phylogroups

Overall, most *E. coli* isolates typed to Clermont phylogroup A (63.31%, 107/169), followed by phylogroup B1 (23.08%, 39/169), whereas phylogroups B2, C, D, E and F were detected at low frequencies (Table 4).

Table 4. Phylogroups in human and porcine *E. coli* isolates according to Clermont typing by EnteroBase.

Phylogroups	Human % (n=63)	Pigs % (n = 106)	Total % (n =169)	*p*-value
A	46.03 (29)	73.58 (78)	63.31 (107)	**< 0.001**
B1	31.75 (20)	17.92 (19)	23.08 (39)	0.0610
B2	4.76 (3)	0.0 (0)	1.78 (3)	0.0960
C	0.0 (0)	0.94 (1)	0.59 (1)	1
Cryptic	1.59 (1)	0.94 (1)	1.18 (2)	1
D	11.11 (7)	1.89 (2)	5.33 (9)	**0.0259**
E	1.59 (1)	1.89 (2)	1.78 (3)	1
F	1.59 (1)	0.94 (1)	1.18 (2)	1
U/cryptic	1.59 (1)	1.89 (2)	1.78 (3)	1

P-values indicated in bold were considered statistically significant.

Clermont phylogroup A were more frequently detected in porcine *E. coli* isolates than in human isolates ($p < 0.001$), whereas Clermont phylogroup D were more frequently detected in human isolates than in porcine isolates ($p = 0.0259$).

3.5.2. Multilocus Sequence Typing – Sequence Type Complexes

The sequence type complex (STc) could not be assigned for 36.09% (61/169) of *E. coli* isolates. Among the remaining isolates, 18 different STcs were detected overall, of which four STcs (STc23, STc32, STc165 and STc467) were only detected in porcine isolates and eight STcs (STc69, STc95, STc101, STc155, STc156, STc394, STc399 and STc522) were only detected in human isolates (Table 5).

Table 5. Sequence type complexes in human and porcine *E. coli* isolates according to MLST.

STc*	**Humans % (n=63)**	**Pigs % (n=106)**	**Total % (n=169)**	***p*-value**
10*	30.16 (19)	37.74 (40)	34.91 (59)	0.4052
23 (ST23)	0.0 (0)	0.94 (1)	0.59 (1)	1

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32 (ST137)	0.0 (0)	1.89 (2)	1.18 (2)	0.7179
69 (ST69)	6.35 (4)	0.0 (0)	2.37 (4)	**0.0355**
86 (ST453; ST641; ST877)*	6.35 (4)	6.60 (7)	6.51 (11)	1
95 (ST95; ST12411)*	3.17 (2)	0.0 (0)	1.18 (2)	0.2671
101 (ST101)	1.59 (1)	0.0 (0)	0.59 (1)	0.7179
155 (ST155)	3.17 (2)	0.0 (0)	1.18 (2)	0.2671
156 (ST12350)	1.59 (1)	0.0 (0)	0.59 (1)	0.7179
165 (ST165; ST1114; ST1178; ST5455)	0.0 (0)	6.60 (7)	4.14 (7)	0.0922
168 (ST93; ST484)	1.59 (1)	2.83 (3)	2.37 (4)	1
206 (ST793; ST4995)*	1.59 (1)	0.94 (1)	1.18 (2)	1
278 (ST336; ST795)*	1.59 (1)	1.89 (2)	1.78 (3)	1
394 (ST394)	1.59 (1)	0.0 (0)	0.59 (1)	0.7179
399 (ST399)	3.17 (2)	0.0 (0)	1.18 (2)	0.2671
467 (ST480; ST2325)*	0.0 (0)	2.83 (3)	1.78 (3)	0.4563
469 (ST162)	1.59 (1)	0.94 (1)	1.18 (2)	1
522 (ST3075)	1.59 (1)	0.0 (0)	0.59 (1)	0.7179
Complex not assigned*	34.92 (22)	36.79 (39)	36.09 (61)	0.9367

*STc = Sequence type complex. ST = Sequence Type. The individual STs of which the STc constitute are indicated in brackets if not more than four STs. The distribution of the individual STs within each STc is shown in the supplementary, if more than four different STs were detected in a complex (Supplementary Table 2). *P*-values indicated in bold were considered statistically significant.

Six different STcs (STc10, STc86, STc168, STc206, STc278 and STc469) were shared between human and porcine isolates. There was no statistical significant difference in the distribution between STs among human and porcine *E. coli* isolates, except for STc 69, which were more frequently detected in human *E. coli* isolates ($p = 0.0355$).

The composition of each STc with the different STs are listed in Supplementary Table 2. Sequence type 10 (22.49%, 38/169), followed by ST542 (5.92%, 10/169) were detected in the highest frequency. Different STs were detected for human and porcine isolates within the same STc (i.e., STc168, STc206 and STc278). For example, within STc168, ST93 were only detected in porcine *E. coli* isolates, whereas ST484 were detected in a single human *E. coli* isolate.

3.5.3. Core Genome Multilocus Sequence Typing (cgMLST) and Hierarchical Clustering (HC)

The overall phylogeny of *E. coli* isolates is shown in the minimum spanning tree (Figure 1). Five clusters of genetically indistinguishable isolates were detected at HC level 0 (HC0), namely HC0:ST171955, HC0:ST173766, HC0:ST173767, HC0:ST173811 and HC0:ST173815. Each cluster consisted out of two isolates. HC0:171955 was only detected in humans, whereas HC0:ST173767 originated from the same pig, but were morphologically distinct. The three other STs (i.e., HC0:ST173766, HC0:ST173811 and HC0:ST173815) originated from the same production stage (i.e., weaning), but each cluster of weaner *E. coli* isolates originated from a different production house (House 2, House 12 and House 16).

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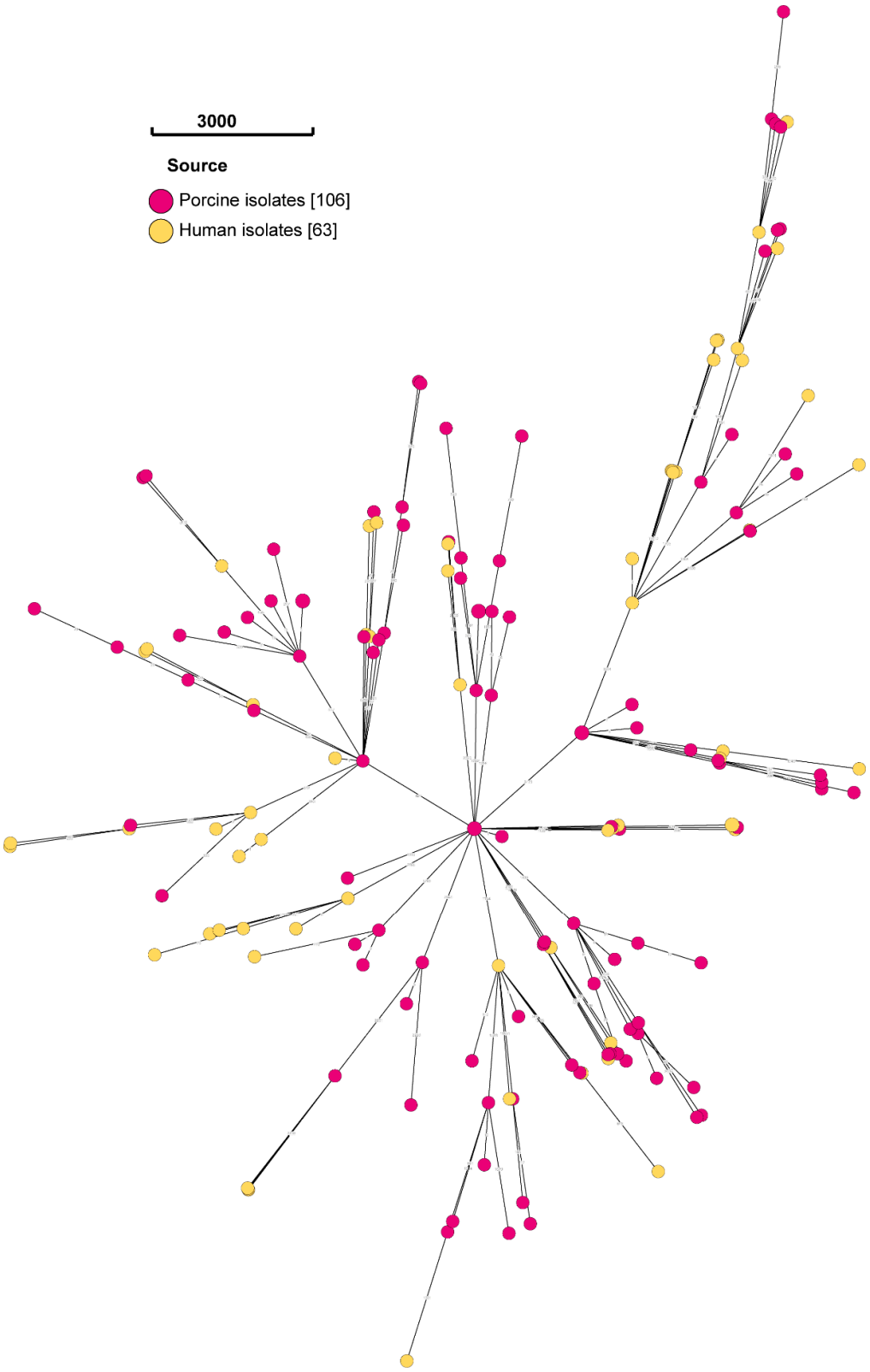

Figure 1. Minimum spanning tree based on the core genome MLST and hierarchical clustering (HC) of human (yellow dots) and porcine (pink dots) *E. coli* isolates on EnteroBase, visualized using GrapeTree.

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A total of nine clusters were detected at HC level 2 (HC2), namely HC2:173881, HC2:171955, HC2:173701, HC2:173719, HC2: 173721, HC2:173766, HC2:173767, HC2:173815 and HC2:174541 (Figure 2).

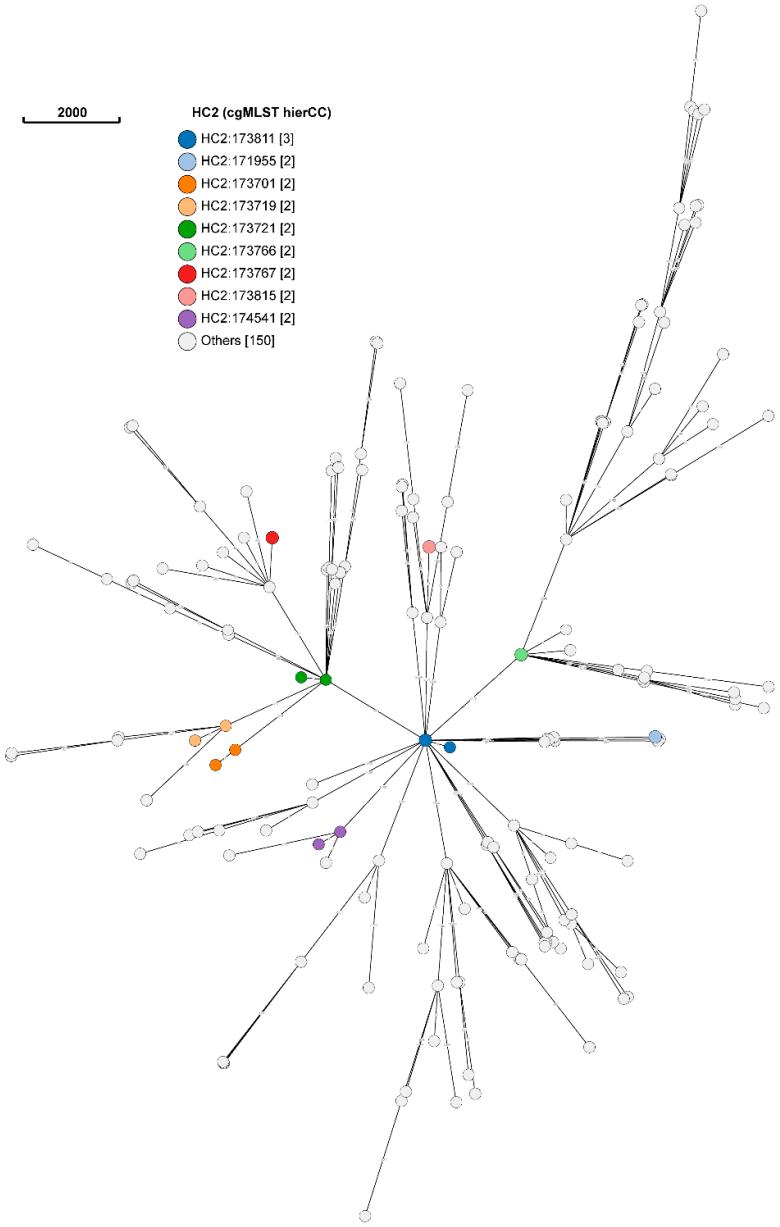

Figure 2. Minimum spanning tree of human and porcine *E. coli* isolates at hierarchical level 2 on EnteroBase, visualized using GrapeTree.

Each cluster consisted out of two isolates, with the exception of HC2:173811 that consisted out of three isolates. Three clusters (i.e., HC2:171955, HC2:173701 and HC2:173719) consisted out of only human isolates, whereas five clusters (i.e., HC2:173766, HC2:17367, HC2:173811, HC2:173815 and HC2:174541) consisted out of only porcine isolates. Hierarchical cluster 2:173721 (HC2:173721) consisted of a human (H47) and porcine (P101) E. coli isolate, which provides some evidence for the transmission and subsequent spread of antibiotic resistance genes between humans and animals.

Additional evidence of clustering of human and porcine isolates were evident at HC10 with the detection of HC10:173841. This HC consisted of a single human E. coli isolate (H15-1) isolated from a female animal handler working in the weaning production stage and a porcine E. coli isolate (P52)

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isolated from a dry sow at the same site (i.e., Site B) on the farm. A total of three clusters of human and porcine isolates were detected at HC level 200 [i.e., HC200:3556 consisting of human E. coli (H27-1) and porcine E. coli (P4) isolate; HC200:41988 previously HC10:173841, but with an additional porcine E. coli P53 isolate and HC200:32433 previously HC2:173721], whereas a total seven clusters of human and porcine E. coli isolates were detected at HC level 400 (i.e., HC400:13, HC400:37, HC400:82, HC400:4483, HC400:4993, HC400:5951 and HC400:31574).

4. Discussion

Escherichia coli serves as an indicator microorganism in antibiotic resistance surveillance programmes [4]. Whole genome sequencing can concurrently provide information on an isolate's antibiotic resistance profile, it's virulence potential and genetic relationship to other *E. coli* isolates [44]. This can provide insights into the spread of antibiotic resistance genes among the One Health continuum [44]. The study found that porcine *E. coli* isolates were more resistant and harboured a richer diversity of antibiotic resistance genes, than *E. coli* isolated from close human contacts on the same farm, specifically for penicillins (i.e., *bla*$_{\text{TEM-1}}$), tetracyclines (i.e., *tet*A and *tet*B), ciprofloxacin (i.e., S83L point mutation in *gyr*A and OqxAB efflux pump) and phenicols ($p < 0.05$). On the other hand, a richer diversity of virulence genes associated with the specific pathotypes were detected in human *E. coli* isolates ($p < 0.001$). Although, the same STc were circulating in both pigs and close human contacts, only a single set of human and porcine *E. coli* isolates showed clonality at HC2, which is an indication of a recent transmission event.

Antibiotic resistance rates in this study were similar to a study done in an intensive pig production system in KwaZulu Natal, South Africa, where the highest rate of resistance detected was towards tetracyclines and the lowest rate of resistance rate towards carbapenems [45]. It is well established that tetracycline resistance in the South African agricultural setting is high, which may be a reflection on the antibiotic practices in South Africa [46–48]. Tetracycline can be purchased over the counter and used at the farmers own's discretion [49]. A previous study by the authors (2022) showed that tetracyclines were the antibiotic class used in the highest quantity (i.e., 453.65 ± 35.49 kg or 135.16 mg/kg ± 3.31 mg/kg) on the same farm, which will explain why tetracycline resistance was high (95.28%. 101/113) in porcine isolates and were predominately mediated by the *tet*A and *tet*B genes [18].

Tetracycline and ampicillin resistance determinants are often harboured on the same plasmid, which may explain why ampicillin and tetracycline resistance were equivalent in the porcine *E. coli* isolates [50]. Ampicillin resistance was predominately mediated by the *bla*$_{\text{TEM-1}}$ gene, which has previously been reported in non-O157 *E. coli* isolates in cattle farming from the same province [48]. The level of ampicillin resistance in this study was also higher when compared to a study done on swine farms in southern Brazil [12]. Brisola and colleagues (2019) found that the level of ampicillin resistance was 11.76% (8/103) compared to 94.34% (100/113) found in porcine isolates in this study [12]. The high levels of ampicillin resistance are again potentially a reflection of antibiotic practices on the farm, where ampicillin is used as metaphylaxis for post-weaning diarrhoea [18].

Multiple porcine *E. coli* isolates harboured the multidrug efflux pump, OqxAB, that confers resistance to a veterinary growth promotor, olaquindox, but also to chloramphenicol, tigecycline, nitrofurantoin, fluoroquinolones, detergents and disinfectants [51,52]. This efflux-pump is located on a plasmid and flanked by two insertion sequence (*IS*26) elements, which means it can easily be acquired by other bacteria, such as *Klebsiella pneumoniae*, through horizontal gene transfer [52,53]. Strasheim and colleagues (2022) previously reported that olaquindox was the antibiotic class used in the second highest quantity (i.e., 258.33 ± 8.04 kg or 77.07 mg/kg ± 3.93 mg/kg) on the farm [18]. Antibiotic growth promotors are not banned in South Africa, but their use have well documented consequences of co-selection of resistance towards other antibiotics, which may lead to co-transmission of multiple antibiotic resistance genes [49,52]. The use of olaquindox as a growth promotor should be reconsidered in the South African context, taking food security, economic productivity, animal welfare and emergence of antibiotic resistance into account. Plasmid-mediated resistance towards colistin was not detected in human or porcine *E. coli* isolates collected from this

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farm. This may be linked to the ban on colistin use in livestock by the South African Veterinary Council, after the detection of the *mcr*-1 gene in South Africa in 2015 by Perreten and colleagues (2016) in animals and by Coetzee and colleagues (2016) in humans [54–56].

A study by Founou and colleagues (2022) investigated five extended-spectrum β-lactamse producing (ESBL) porcine *E. coli* isolates obtained from two abattoirs in South Africa [57] . Isolates from the same abattoir were closely-related and harboured the *bla*$_{\text{CTX-M-1 and 15}}$ genes together with multiple other resistance determinants to fluoroquinolones, aminoglycosides, tetracyclines and trimethoprim-sulfametaoxazole [57]. In this study, ESBL *E. coli* isolates were not detected, potentially because the isolates were grown on non-selective media. It is well known that media supplemented with antibiotics leads to different isolation rates than non-selective media [4]. Some authors state that the use of non-selective media may underestimate the true prevalence of ESBL producers [58,59], whereas others state that it is important to monitor both dominant (non-selective media) and subdominant (selective media) microflora to accurately define the true extent of antibiotic resistance rates [60]. In future, media used for the isolation of bacteria in antibiotic resistance monitoring should be standardized to enhance the comparability of antibiotic resistance rates.

The majority of human and porcine *E. coli* isolates were non-pathogenic in this study, but different pathovars, namely EAEC, EPEC, ETEC, ExPEC and STEC were detected at low frequencies. Enteroaggregative *E. coli* was only detected in humans, where EPEC, ETEC and STEC were predominantly isolated from pigs. Overlap of pathovar-specific genes were also observed as evident with the detection of the *ast*A gene, which encodes the enteroaggregative *E. coli* heat-stable enterotoxin (EAST1). This gene has previously been associated with EAEC in humans, but it is also reported as an important virulence factor of ETEC in piglets [30,61]. In this study, the *ast*A gene was also detected in the porcine EPEC isolates as well as human EAEC isolates. In addition, the *ast*A gene were more frequently detected in porcine *E. coli* isolates than in human *E. coli* isolates. This suggest that the *ast*A gene may play a different role in disease based on the host species.

Sequence type complex 10 was the most dominant STc in both human and porcine *E. coli* isolates, based on Achtman's 7-gene MLST scheme. A study by Peng and colleagues (2022) sequenced 1 871 *E. coli* isolates obtained from pigs and their immediate environment from 31 provinces in China and compared these genomes with publicly available human *E. coli* genomes [62]. The authors also found STc10 as the most predominant ST, as in this study.

Core genome MLST and HC showed that human and porcine *E. coli* isolates were overall genetically diverse in this study, but there was some evidence (albeit very little) of HC of isolates at levels HC:2 to HC:200. This indicates: i) transmission between pigs from different production houses, phases and sites, ii) transmission between pigs and humans, potentially due to proximity and iii) transmission between humans, potentially due to shared facilities [14]. These findings are in line with a previous study by Muloi and colleagues (2022) in Nairobi, Kenya, which showed transmission of *E. coli* between humans and animals, can occur, but it remains an infrequent event [63]. The findings are also in agreement a study done by Leekitcharoephon and colleagues (2021), who investigated the genetic relatedness among 627 poultry, porcine and veal *E. coli* isolates from different farms in six European countries during 2014 to 2015, using WGS [64]. *Escherichia coli* isolated from the same farm, as well as different farms, within the same country, showed some level of clonality but were genetically diverse between different animal species and different countries [64]. In addition, a study by Ludden and colleagues (2019) found limited evidence of clonality within a large collection of *E. coli* isolated from livestock and the food chain when compared to isolates implicated in bloodstream infections in eastern England using WGS [65]. However, the isolates from the different sources were not isolated during the same time and the majority of clinical isolates were healthcare-associated, which could be potential reasons for the distinct lineages detected and limited evidence of transmission at the human-livestock interface in this setting [14,65].

The study has some limitations as only a single pig farm was included, which has implications on the generalizability of the findings. African Swine Fever was in circulation in South Africa at the time of sample collection and multiple farms could therefore not be visited due to the fear of spreading disease. In addition, shortly after the first farm visit, the COVID-19 pandemic emerged.

 doi:10.20944/preprints202405.0998.v1

Although, only a single recent transmission event between a human and porcine *E. coli* isolate was detected on this farm, it remains important to strengthen antibiotic stewardship practices, reduce antibiotic use for growth promotion and metaphylaxis to mitigate the risk of transmission and spread of resistance linked to antibiotic usage. In future, this study can serve as a blue-print to implement One Health antibiotic resistance surveillance programmes in South Africa on a broader scale.

Supplementary Materials: The following supporting information are available at the website of this paper posted on Preprints.org, T**able S1:** Other virulence genes detected in human and porcine *E. coli* isolates; **Table S2:** Sequence type complex composition with the different STs detected in human and porcine *E. coli* isolates

Author Contributions: Conceptualization, WS, OP and ECM; methodology, WS, ML, OP and AMS; validation, WS and ML; formal analysis, WS; investigation, WS and ML; resources, OP and AMS; data curation, WS and ML; writing—original draft preparation, WS; writing—review and editing, WS, ML, EMC, AMS and OP; visualization, WS; supervision, OP, AMS and EMC; project administration, WS and ML; funding acquisition, OP and AMS. All authors have read and agreed to the published version of the manuscript.

Funding: The South African Medical Research Council (SAMRC) funded this research as a sub-grant received from the Bill and Melinda Gates Foundation (Grant number: 96086). The contents and findings reported/ illustrated are the sole deduction, view and responsibility of the researcher and do not reflect the official position and sentiments of the SAMRC or the Bill and Melinda Gates Foundation. Whole genome sequencing was made possible by support from the SEQAFRICA project which is funded by the Department of Health and Social Care's Fleming Fund using UK aid. The views expressed in this publication are those of the authors and not necessarily those of the UK Department of Health and Social Care or its Management Agent, Mott MacDonald. WS was also supported by a Fogarty International Center Global Infectious Disease research-training grant, National Institutes of Health, to the University of Pittsburgh and the National Institute for Communicable Diseases (D43TW011255).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by different human and animal health research ethics committees (HREC/AREC) from three statutory bodies, namely: i) The University of the Witwatersrand (Medical HREC M190244; 10/05/2019), ii) the University of Pretoria (AREC of Faculty of Veterinary Science REC0055-20; 6/07/2020; REC of Faculty of Humanities HUM027/0620; 25/06/2020 and HREC of Faculty of Health Sciences 406/2020; 12/08/2020) and iii) the National Institute for Communicable Diseases (NICD) (AEC003-19; 23/06/2019). In addition, a Section 20 clearance certificate (project reference number: 12/11/1/1/13; 23/04/2019) was issued by the Department of Agriculture, Land Reform and Rural Development of the Republic of South Africa. Informed consent was obtained from all subjects involved in the study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Sequencing data is available at the NCBI's GenBank under Bioproject number PRJNA994298 (https://www.ncbi.nlm.nih.gov/sra/PRJNA994298). Additional data generated during this study has been placed in an online repository (https://doi.org/10.6084/m9.figshare.23677266.v2). Accession numbers are listed in the supplementary materials together with the isolate's unique identifier.

Acknowledgments: The authors would hereby like to acknowledge the farmer and his veterinarian in charge, for providing access to the farm. The authors will also like to thank Ashika Singh-Moodley, Marshagne Smith, Naseema Bulbulia, Rosah Mabokachaba, Nompumelelo Shezi and Nokuthula Linda for assistance in processing isolates in the laboratory. Else Marais is hereby acknowledged for initiating the overall project idea. The Sequencing Core Facility at the National Institute for Communicable Diseases is also acknowledged for providing whole genome sequencing services.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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