Prospective One Health genetic surveillance in Vietnam identifies distinct blaCTX-M-harbouring Escherichia coli in food-chain and human-derived samples

Prospective One Health genetic surveillance in Vietnam identifies distinct *bla*$_{CTX-M}$-harbouring *Escherichia coli* in food-chain and human-derived samples

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ABSTRACT

Objectives: We performed a One Health surveillance in Hanoi – a region with a high-density human population and livestock production, and a recognized hotspot of animal-associated antimicrobial resistance (AMR) – to study the contribution of *bla*$_{CTX-M}$-carrying *E. coli* and plasmids from food-animal sources in causing human community-acquired urinary tract infections (CA-UTIs).

Methods: During 2014-2015, 9,090 samples were collected from CA-UTI patients (urines, n=8,564), pigs/chickens from farms and slaughterhouses (faeces, carcasses, n=448), and from the slaughterhouse environment (surface-swabs, water, n=78). *E. coli* were identified in 2,084 samples. ESBL production was confirmed in 235, and *bla*$_{CTX-M}$ in 198 strains by PCR that underwent short-read plasmid sequencing. Fourteen strains were long-read sequenced to enable plasmid reconstruction.

Results: Majority of the ESBL-producing *E. coli* harboured *bla*$_{CTX-M}$ (n=198/235, 84%). High clonal diversity (48 STs) and distinct, dominant STs in human (ST1193, n=38/137; ST131, n=30/137) and non-human sources (ST155, n=25/61) indicated lack of clonal transmission between habitats. Eight *bla*$_{CTX-M}$ variants were identified; 5 were present in at least two sample sources. Human and food-animal strains did not show similar plasmids carrying shared *bla*$_{CTX-M}$ genes. However, IS*6* elements flanking IS*Ecp1*–*bla*$_{CTX-M}$–*orf477*/IS*903B* structures were common across habitats.

Conclusions: In this study, animal-associated *bla*$_{CTX-M}$-*E. coli* or *bla*$_{CTX-M}$-plasmids were not direct sources of CA-UTIs or ESBL resistance in humans, respectively, suggesting evolutionary bottlenecks to their adaptation to a new host species. Presence of common IS*6* elements flanking *bla*$_{CTX-M}$ variants in different plasmid backbones, however, highlighted the potential of these transposable elements for AMR transmission either within or across habitats.

INTRODUCTION

Of all antimicrobials sold globally, more than 70% are used in raising food-animals, either as therapeutic agents or as growth promoters (1). One Health studies have demonstrated the potential of antimicrobial resistance (AMR) transfer to humans via food, and by exchange of mobile elements harbouring AMR genes between animal, human and environmental habitats (2, 3).

Escherichia coli is, arguably, the most common bacterium traversing all habitats as benign microbe or pathogen and a poster child for One Health studies attempting to understand microbe or plasmid flow between habitats. Similarly, beta-lactams represent the most common antibiotic class used in both humans and animals, and genes encoding beta-lactam resistance – extended-spectrum beta-lactamases (ESBL) of the $bla_{\text{CTX-M}}$ class harboured on a variety of conjugative plasmids – are predictably very frequent among *E. coli* in different habitats (4, 5). The transmission of $bla_{\text{CTX-M}}$-harbouring *E. coli* ($bla_{\text{CTX-M}}$-Ec) between contaminated food-animals and humans has been suggested by several reports (6-8).

Vietnam bears a heavy burden of infectious diseases with an increasing prevalence of ESBL-producing *E. coli* (ESBL−Ec) from 34.4% in 2007 to 48.1% during 2009-2011 and a predominance of $bla_{\text{CTX-M}}$-Ec across habitats (9-11). Use of antibiotics, available over-the-counter for both humans and animals, is concordantly high accounting for >70% of pharmaceuticals used in livestock farming (12-15). Further, close proximity of small-scale food-animal processing facilities to populated areas that practise manual slaughtering coupled with weak biosecurity and food-safety controls (13) poses a high risk to the local population.

Recent studies utilizing whole-genome sequencing (WGS) have, however, reported a limited potential of clonal transmission of ESBL-Ec between habitats and implied plasmids as vehicles of disseminating $bla_{\text{CTX-M}}$ genes (16, 17). Thus, in this study, we aimed to

investigate the transmission dynamics of *bla*$_{\text{CTX-M}}$ genes, whether mediated by successful *E. coli* clones using multilocus sequence-typing and WGS, or by plasmids and smaller mobile genetic elements (MGEs) using plasmid-based sequencing. The overarching aim was to understand the contribution of food-animal sources in causing community-acquired urinary tract infections (CA-UTIs). We carried out this study in the Hanoi metropolitan area in northern Vietnam, a heavily populated region with high-density livestock production, demonstrating a high antibiotic consumption in livestock farming in the country (18), and a recognized hotspot of animal-associated AMR.

METHODS

A priori One Health study design and sample/strain collection

This study, approved by the Ethics Committee of Hanoi School of Public Health (registration number 262/2014/YTCC-HD3), was conducted over two years (February 2014–December 2015). Following written informed consent from patients or their legal guardians, urines were collected from 8,564 outpatients consulting for symptomatic urinary tract infections (UTIs) in three hospitals in Hanoi: National Hospital of Paediatrics (NHP), Military Hospital (MH), and Vietnam-Cuba Hospital (VNCB) (Figure 1).

During the same time period and within a 5-33 km distance from the three hospitals, samples from live (n=48) and slaughtered chickens (n=228), live (n=27) and slaughtered pigs (n=145), and their environment in slaughterhouses (n=78) were collected from four farms and eight small-scale slaughterhouses in Hung Yen, Hoai Duc, and Hanoi (Figure 1).

Inclusion criteria for patients, animal sampling, microbiological and sequencing analyses are detailed in Additional File 1.

ESBL-producing *E. coli* (ESBL-Ec)

A total of 2,084 *E. coli*, isolated from urine and non-human samples (Table 1), were phenotyped for ESBL production. ESBL-Ec (n=235) and $bla_{\text{CTX-M}}$–Ec (n=198) were genotyped for $bla_{\text{CTX-M}}$ and multilocus sequence types (MLST), respectively, by PCR/Sanger sequencing (Figure 2). The 198 $bla_{\text{CTX-M}}$-Ec were further screened for susceptibility to 16 antibiotics from 10 classes (Additional file 2).

Sequencing and Bioinformatics analyses

Plasmids isolated from 198 $bla_{\text{CTX-M}}$-Ec (Zyppy™ Plasmid MiniPrep kit, Zymo Research, USA), were sequenced (2x250 bp, MiSeq, Illumina Inc., USA). Plasmid and chromosomal raw reads were separated and subjected to a secondary analysis workflow (Additional file 1).

The mean sequencing coverage obtained for plasmids of 198 *bla*$_{CTX-M}$-Ec strains was 90X (median: 72X, range: 22-455X, IQR: 50-102X).

Long-read sequencing (MinION, Oxford Nanopore Technology, UK) was performed on plasmid DNA of 11 *bla*$_{CTX-M}$ –Ec representing *bla*$_{CTX-M}$ variants present in more than one sample source. Three strains found to harbour chromosomal *bla*$_{CTX-M}$ by short-read sequencing were whole-genome long-read sequenced. *De novo* hybrid assembled plasmid scaffolds were used for subsequent reference-mapping analysis. Plasmid similarity was defined on a >90% sequence coverage and identity with the reference plasmid.

ST10 *bla*$_{CTX-M}$–Ec comparative genomic analysis

ST10 *bla*$_{CTX-M}$–Ec were recovered from both humans, animal and environmental samples. To understand clonal relatedness, genomic DNAs from ST10 strains recovered from this study (n=4) were also subjected to whole-genome short-read sequencing. In addition, sequences from 18 ST10 *E. coli* from different sample sources and geographical regions were retrieved from publicly available databases. Genomic diversity between these strains was studied using a gene-by-gene approach (core-and whole-genome MLST, cg/wgMLST) and pan-genome analysis (Additional file 1).

Statistical analyses

Normal distribution of the sample groups and equality of the variances were assessed by the Kolmogorov-Smirnov, chi-square test and Hartley's f test. Fisher's exact, ANOVA and non-parametric Kruskal-Wallis tests were used to compare the prevalence and the resistance levels (i.e., number of antibiotic categories that a strain exhibited resistance to and expressed as medians and ranges) of the studied groups. OpenEpi (http://www.openepi.com) (19) and R v.3.5.1 were used for analyses (95% confidence interval and $P < 0.05$).

RESULTS

ESBL-producing and multidrug-resistant *bla*$_{CTX-M}$-Ec across sample sources

From all sample sources, 9,090 samples were screened; *E. coli* was identified in 2,084 (23%); ESBL production was confirmed in 235/2,084 (11%) *E. coli* (Table 1). Compared to urine (152/1,705, 9%), the prevalence of ESBL−Ec was higher in live chickens (11/28, 39%, P = 4e-05), chicken carcasses (42/187, 22%, P = 3e-07), and in pigs (10/20, 50%, P = 7e-6), but not in the environmental (9/48, 19%, P = 0.054) or pig carcass (11/96, 11%, P = 0.49) samples.

Among the 235 ESBL−Ec, *bla*$_{CTX-M}$ −Ec (n=198, 84%) were predominant, ranging from 27% to 91% across sample sources (Table 1). Of the 137 patients with CA-UTIs caused by *bla*$_{CTX-M}$ −Ec, average age was 3.7 years (range 0.08−42 years). Majority of *bla*$_{CTX-M}$ −Ec (n=192, 97%) were multidrug-resistant (MDR, i.e., resistant to ≥1 agent in ≥3 classes) harbouring a rich pool of 69 acquired antimicrobial resistance genes (ARGs) (Additional file 3). Six non-MDR strains were isolated from humans and resistant to 2 drug classes. Strains from non-human sources (n=61) were resistant to more antibiotic categories (median: 5 vs 4, range: 3-7 vs 2-8, P = 0.0015) and carried more acquired ARGs (median: 13 vs 10, range: 2-20 vs 1-18, P <0.001) than 137 strains from human sources.

Distinct sequence types indicate lack of clonal transmission across sample sources

Among the 198 *bla*$_{CTX-M}$ −Ec, we identified 48 different sequence types (STs). Most STs, except for ST10 and ST155, were source-specific i.e., found in strains from one sample origin only. ST155 was a chicken-specific lineage found in live chickens (7/10, 70%), chicken carcasses (16/34, 47%), and the environment in chicken slaughterhouses (2/7, 29%).

ST10 was found in 1 urine and in non-human samples i.e., from chicken carcasses (n=2) and pig slaughterhouse environment (n=1). A comparative analysis on the 22 ST10 genomes showed 12 distinct typable O:H serotypes (Additional file 4: Table S1) and cgMLST allelic

158 loci distance ranging from 275 to 1,108 between the ST10 *E. coli* isolated from humans and
159 non-human sources. Human ST10 (n=9) clustered distinctly from the other 13 non-human
160 strains (Additional file 5: Figure SF3). Amongst these 22 ST10 genomes, of the 13,152 genes
161 identified as the pan-genome, only 2,744 genes formed the core-genome, indicating highly
162 varied accessory genomes carried by ST10 across sample sources. These results suggest
163 distinct animal- and human-adapted sub-lineages of ST10.

164 In the 137 patients with CA-UTIs due to *bla*$_{\text{CTX-M}}$−Ec, 31 STs were found, with the most
165 prevalent being ST1193 (28%, n=38), ST131 (22%, n=30) and ST12 (9%, n=12). Non-human
166 samples yielded 61 *bla*$_{\text{CTX-M}}$−Ec, wherein the human STs were not identified (Figure 3A),
167 and were divided into 18 STs among which ST155 (41%, n=25), ST7176 (11%, n=7) and
168 ST93 (10%, n=6) were predominant.

169 **Distinct dominant *bla*$_{\text{CTX-M}}$ variants across sample sources suggest limited sharing**
170 **between the studied niches**

171 The 198 *bla*$_{\text{CTX-M}}$−Ec harboured several plasmid incompatibility (Inc) types (Additional file
172 3), on average three per strain (range: 1−6, IQR: 2−3). IncF was the most prevalent Inc type
173 (191/198, 96%) found in all sample sources.

174 Eight *bla*$_{\text{CTX-M}}$ variants belonging to CTX-M group 1 and CTX-M group 9 were identified. In
175 human CA-UTI strains, six *bla*$_{\text{CTX-M}}$ variants were found (Figure 3B); and *bla*$_{\text{CTX-M-27}}$ was
176 predominant (105/137, 77%) while it was found in only five non-human strains. *bla*$_{\text{CTX-M-27}}$
177 was associated mostly with ST1193 (36/137, 26%) and ST131 (19/137, 14%) in human
178 samples. These two combinations were not observed in non-human sources.

179 Of the six *bla*$_{\text{CTX-M}}$ variants identified in non-human sources, *bla*$_{\text{CTX-M-55}}$, although present
180 only in 5 human strains, was the most dominant variant (22/61, 36%). The most common ST-
181 *bla*$_{\text{CTX-M}}$ combination identified in non-human sources was ST155-*bla*$_{\text{CTX-M-65}}$ (12/61, 20%).

The only combination overlapping between human and non-human samples was ST10-*bla*$_{CTX-M-27}$, uniquely found in one human (U-NHP-95) and one environmental strain from a pig slaughterhouse surface (E11). However, the ST10 comparative genomic analysis showed 899 cgMLST allelic loci differences between these two strains.

Limited genetic similarity suggests a restricted horizontal transmission of *bla*$_{CTX-M}$-harbouring plasmids from food-animals to humans

The mobile genetic elements adjoining *bla*$_{CTX-M}$ were specific to CTX-M groups regardless of the sample origins or the plasmid/chromosomal locations of the genes (Figure 4A). IS*Ecp1* element, either intact or truncated, with a standard promoter region, was found upstream of all *bla*$_{CTX-M}$ genes in both CTX-M groups. Downstream of *bla*$_{CTX-M}$ genes, *orf477* sequence was found in CTX-M group 1, and IS*903B* was found in CTX-M group 9.

Fourteen strains carrying five *bla*$_{CTX-M}$ variants (*bla*$_{CTX-M-15}$, *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-27}$, and *bla*$_{CTX-M-65}$) commonly found in more than one sample origin were selected for long-read sequencing. These *bla*$_{CTX-M}$ located on 13 plasmids belonging to 5 incompatibility types (IncFII, IncFIB, IncHI2, IncI1, IncN) and ranging from 75 kb to 257 kb in size (Additional file 4: Table S3). Three *bla*$_{CTX-M}$ chromosomal locations were also identified.

Between strains from human urine and food-animals in either farms or slaughterhouses, we did not identify similar genetic structures of plasmids harbouring shared *bla*$_{CTX-M}$ genes, suggesting a limited contribution of *bla*$_{CTX-M}$-harbouring plasmids to disseminating *bla*$_{CTX-M}$ directly between humans and food-animals.

On the contrary, between strains from the environment of the slaughterhouses and strains from slaughtered food-animals or humans, highly similar plasmids were observed (Figure 4B). A multi-replicon IncHI2–IncN plasmid (pE64, 256,080 bp), co-harbouring *bla*$_{CTX-M-14}$ and *mcr-1.1,* was identified from an ST48 chicken carcass strain (Additional 5: Figure SF4). The detailed descriptions of this multi-replicon plasmid was provided in Additional file 3.

Highly similar plasmids (pE40, pE46, pE70, and pE78, Additional file 5: Figure SF5) sharing 99-100% identity to 97-99% the length of pE64 plasmid were isolated from two environmental strains and two chicken carcass strains from different slaughterhouses (Additional file 4: Table S3).

On the other hand, between strains isolated from the slaughterhouse environment and humans, similar *bla*$_{\text{CTX-M-55}}$-IncFII plasmids (pE10 and pUNHP31) sharing 99% homology were also observed (Figure 4B, Additional file 4: Table S3). The two plasmids co-harboured *cmlA* encoding for chloramphenicol resistance.

We also performed long-read sequencing on the two ST10–*bla*$_{\text{CTX-M-27}}$ strains (urine strain: U-NHP-95 and environmental strain: E11). The results showed that *bla*$_{\text{CTX-M-27}}$ were located on IncFII plasmids in both strains; however, the two plasmids shared only 69% similarity. While the genes surrounding *bla*$_{\text{CTX-M-27}}$ were highly similar: *mph(A)*−IS*26*−*erm(B)*−Δ*TnAs3*−IS*26*−ΔIS*Ecp1*−*bla*$_{\text{CTX-M-27}}$−ΔIS*903B* and the IS*Ecp1* element (1,656 bp) was truncated in both structures, the lengths of the truncated IS*Ecp1* elements were essentially different (149 bp in U-NHP-95 vs 540 bp in E11). This divergence indicated that these two genetic structures were not originated from the same origin or underwent distinct genetic rearrangements.

Intriguingly, we found IS*6* family elements (IS*26,* IS*15DIV* and IS*15DI)* flanking the *bla*$_{\text{CTX-M}}$ genetic unit (IS*Ecp1*–*bla*$_{\text{CTX-M}}$ – *orf477/*IS*903B*) in both CTX-M group 1 (*bla*$_{\text{CTX-M-55}}$) and group 9 (*bla*$_{\text{CTX-M-27}}$) in 34 clinical, 8 chicken carcass and 1 environmental strain belonging to diverse STs. This frequent occurrence of IS*6* flanking *bla*$_{\text{CTX-M-27}}$ and *bla*$_{\text{CTX-M-55}}$ observed in humans and non-human sample sources suggested that the IS*6* family, in particular IS*26,* IS*15DIV* and IS*15DI*, using replicative transposition mechanism, might play an important role in mediating the mobilization of *bla*$_{\text{CTX-M}}$. However, it did not necessarily prove that *bla*$_{\text{CTX-M}}$ genes were directly transmitted between food-animals and humans.

DISCUSSION

In this study, we collected *bla*$_{CTX-M}$−*Ec* from sympatric populations of patients with CA-UTI, from food-animals and from their immediate environment in slaughterhouses to delineate whether transmission events occurred and whether these were mediated by *E. coli* clones, or conjugative plasmids or MGEs flanking *bla*$_{CTX-M}$.

Food-borne acquisition of multi-drug resistant *E. coli* has been much-studied during the last decade albeit with varying results. Evidence of transmission of whole-bacteria and plasmids from poultry and their contribution to human extraintestinal infections has been reported, while other studies, utilizing similar typing methods, did not find any evidence of such transmission events (8). These discrepancies could be attributed to the low granularity of the available typing approaches (single gene amplification or restriction mapping) when these studies were carried out. The recent utilization of whole-genome sequencing did not support ESBL-Ec transmission (16, 20), although low-level transmission of *bla*$_{CTX-M-1}$-harbouring plasmids between humans and livestock was observed (16).

We also identified ST (ST10), plasmid types (IncF, IncHI2, InI1) and *bla*$_{CTX-M}$ variants (*bla*$_{CTX-M-15}$, *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-27,}$ and *bla*$_{CTX-M-65}$) that were shared between chickens/pigs and humans. Even though we had enriched for plasmid sequences by plasmid sequencing, plasmid reconstruction based on short reads was challenging mainly due to large MDR plasmids containing repeated sequences and MGEs (21). Consequently, conferring plasmid transmission based on the presence of shared plasmid replicon types resulted from short-read sequencing might produce erroneous results. Long-read sequencing of plasmids and of whole genomes that enabled clear delineation and assembly of the genetic context of shared *bla*$_{CTX-M}$ genes, also carried on the same plasmid Inc types, gave no evidence of horizontal transmission between sources. Importantly, several IS*6*-family elements flanking

256 *bla*$_{CTX-M}$ were commonly found across sample sources, and lend support to growing evidence
257 of IS-mediated transfer as a plausible means of *bla*$_{CTX-M}$ dissemination.

258 Key strengths of our study was a focus on CA-UTI, an infection with a direct causal
259 association with the gut flora (22, 23), which also constitutes a potential reservoir of ESBL-
260 Ec acquired via food. In addition to a prospective One Health study design, we also ensured
261 that the sampled food-animal sources were geographically proximal to the hospitals
262 recruiting CA-UTI patients. Finally, our study location was optimal to delineate the burden of
263 livestock-associated ESBL-Ec in human infections: (i) Cephalosporins are one of the most
264 commonly-used antibiotics in Vietnam (15, 24); (ii) Hanoi locates in the Red River Delta,
265 one of the hotspots of resistance in animals where cephalosporin is also used in animal
266 farming, albeit at lower levels than in humans (1, 25, 26); and (iii) Both sectors report a high
267 prevalence of ESBL-Ec (10, 18).

268 In concordance with a previous report (27), we found a similar prevalence of ESBL-Ec
269 among non-human samples. However, the small number of poultry and pig farms sampled
270 might not be entirely representative of the diversity of *bla*$_{CTX-M}$-Ec harboured in food animals
271 in Vietnam. The origin of slaughtered animals, currently untraceable due to the lack of record
272 systems and the involvement of multiple stakeholders in the food value chain (28), further
273 hampered our efforts to define an epidemiological network. Another potential study
274 limitation was the high proportion of children/infants among the recruited patients: 114/137
275 were <5-years-old and 41/137 were <1-year-old. In Vietnam, however, it is common practice
276 for adults with mildly symptomatic UTI to self-medicate with over-the-counter bought
277 antibiotics. Whereas, parents of children/infants with UTI symptoms are more likely to seek
278 professional advice. While intake of animal protein by infants is likely limited, recent studies
279 have demonstrated a high abundance of antibiotic resistance genes in the infant gut

microbiota even in the absence of antibiotic exposure with potential sources being breast milk, and the maternal gut microbiota (29, 30).

In conclusion, we found distinct *bla*$_{CTX-M}$–Ec and *bla*$_{CTX-M}$-harbouring plasmids in *E. coli* among food-animals and in humans with CA-UTI. These data suggest evolutionary bottlenecks to the adaptation of strains and plasmids across co-habiting host species. Presence of common IS*6*-family elements flanking *bla*$_{CTX-M}$ variants in different plasmid backbones, however, highlights these transposable elements as potential AMR transmission vehicles either within or across habitats.

Additional files

Additional file 1 (docx, 41 kb): Supplementary Methods.

Additional file 2 (xlsx, 37 kb): Strain details.

Additional file 3 (docx, 36 kb): Supplementary Results.

Additional file 4 (docx, 37 kb): Supplementary Tables.

Additional file 5 (docx, 3803 kb): Supplementary Figures.

Transparency declaration

Consent for publication

Not applicable.

Availability of data and materials

The sequencing data of this study have been deposited in GenBank database under BioProject accession number PRJNA614455.

Conflict of interest

The authors declare that they have no competing interests.

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The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Authors' contribution

SMK and TTHH designed the study and experiments. SMK, THH, HG, ADD were responsible for managing the project. SMK, TTHH, CL and TNP designed the sampling framework. MNN, CL, TTHH, THL, TBNH, TNP, ADD, TSN collected samples and strains, performed laboratory work and contributed to the acquisition of the data. MNN and BBX performed bioinformatics analyses. MNN, BBX, and SMK interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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References

1. Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, et al. Global trends in antimicrobial resistance in animals in low- and middle-income countries. Science (New York, NY). 2019;365(6459).
2. Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, et al. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2011;17(6):873-80.
3. Thorsteinsdottir TR, Haraldsson G, Fridriksdottir V, Kristinsson KG, Gunnarsson E. Prevalence and genetic relatedness of antimicrobial-resistant Escherichia coli isolated from animals, foods and humans in Iceland. Zoonoses and public health. 2010;57(3):189-96.
4. Doi Y, Paterson DL, Egea P, Pascual A, López-Cerero L, Navarro MD, et al. Extended-spectrum and CMY-type b-lactamase-producing Escherichia coli in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. Clinical Microbiology and Infection. 2010;16(1):33-8.
5. Canton R, Gonzalez-Alba JM, Galán JC. CTX-M Enzymes: Origin and Diffusion. Frontiers in Microbiology. 2012;3(110).
6. Kluytmans JA, Overdevest IT, Willemsen I, Kluytmans-van den Bergh MF, van der Zwaluw K, Heck M, et al. Extended-spectrum beta-lactamase-producing Escherichia coli from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013;56(4):478-87.
7. Hammerum AM, Larsen J, Andersen VD, Lester CH, Skovgaard Skytte TS, Hansen F, et al. Characterization of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins. The Journal of antimicrobial chemotherapy. 2014;69(10):2650-7.
8. Lazarus B, Paterson DL, Mollinger JL, Rogers BA. Do human extraintestinal Escherichia coli infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review.

Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(3):439-52.
9. Hawser SP, Bouchillon SK, Hoban DJ, Badal RE, Hsueh PR, Paterson DL. Emergence of high levels of extended-spectrum-beta-lactamase-producing gram-negative bacilli in the Asia-Pacific region: data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program, 2007. Antimicrob Agents Chemother. 2009;53(8):3280-4.
10. Biedenbach DJ, Bouchillon SK, Hoban DJ, Hackel M, Phuong DM, Nga TTT, et al. Antimicrobial susceptibility and extended-spectrum beta-lactamase rates in aerobic gram-negative bacteria causing intra-abdominal infections in Vietnam: report from the Study for Monitoring Antimicrobial Resistance Trends (SMART 2009–2011). Diagnostic Microbiology and Infectious Disease. 2014;79(4):463-7.
11. Yamasaki S, Le TD, Vien MQ, Van Dang C, Yamamoto Y. Prevalence of extended-spectrum beta-lactamase-producing Escherichia coli and residual antimicrobials in the environment in Vietnam. Animal health research reviews. 2017;18(2):128-35.
12. Dang Pham Kim, Claude Saegerman, Caroline Douny, Ton Vu Dinh, Bo Ha Xuan, Binh Dang Vu, et al. First Survey on the Use of Antibiotics in Pig and Poultry Production in the Red River Delta Region of Vietnam. Food and Public Health. 2013;3(5):247-56.
13. Coyne L, Arief R, Benigno C, Giang VN, Huong LQ, Jeamsripong S, et al. Characterizing Antimicrobial Use in the Livestock Sector in Three South East Asian Countries (Indonesia, Thailand, and Vietnam). Antibiotics (Basel, Switzerland). 2019;8(1).
14. Carrique-Mas JJ, Choisy M, Van Cuong N, Thwaites G, Baker S. An estimation of total antimicrobial usage in humans and animals in Vietnam. Antimicrobial Resistance & Infection Control. 2020;9(1):16.
15. Nguyen KV, Thi Do NT, Chandna A, Nguyen TV, Pham CV, Doan PM, et al. Antibiotic use and resistance in emerging economies: a situation analysis for Viet Nam. BMC Public Health. 2013;13:1158.
16. de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, et al. Dissemination of cephalosporin resistance genes between Escherichia coli strains from farm animals and humans by specific plasmid lineages. PLoS genetics. 2014;10(12):e1004776.
17. Ben Sallem R, Ben Slama K, Rojo-Bezares B, Porres-Osante N, Jouini A, Klibi N, et al. IncI1 plasmids carrying bla(CTX-M-1) or bla(CMY-2) genes in Escherichia coli from healthy humans and animals in Tunisia. Microbial drug resistance (Larchmont, NY). 2014;20(5):495-500.
18. Dang STT, Bortolaia V, Tran NT, Le HQ, Dalsgaard A. Cephalosporin-resistant Escherichia coli isolated from farm workers and pigs in northern Vietnam. Tropical Medicine & International Health. 2018;23(4):415-24.
19. Sullivan KM, Dean A, Soe MM. OpenEpi: A Web-based Epidemiologic and Statistical Calculator for Public Health. Public Health Reports. 2009;124(3):471-4.
20. Ludden C, Raven KE, Jamrozy D, Gouliouris T, Blane B, Coll F, et al. One Health Genomic Surveillance of Escherichia coli Demonstrates Distinct Lineages and Mobile Genetic Elements in Isolates from Humans versus Livestock. mBio. 2019;10(1):e02693-18.
21. Arredondo-Alonso S, Willems RJ, van Schaik W, Schürch AC. On the (im)possibility of reconstructing plasmids from whole-genome short-read sequencing data. Microbial genomics. 2017;3(10).
22. Moreno E, Andreu A, Pigrau C, Kuskowski MA, Johnson JR, Prats G. Relationship between Escherichia coli strains causing acute cystitis in women and the fecal E. coli population of the host. Journal of clinical microbiology. 2008;46(8):2529-34.
23. Magruder M, Sholi AN, Gong C, Zhang L, Edusei E, Huang J, et al. Gut uropathogen abundance is a risk factor for development of bacteriuria and urinary tract infection. Nature Communications. 2019;10(1):5521.
24. Vu TVD, Do TTN, Rydell U, Nilsson LE, Olson L, Larsson M, et al. Antimicrobial susceptibility testing and antibiotic consumption results from 16 hospitals in Viet Nam: The VINARES project 2012–2013. Journal of global antimicrobial resistance. 2019;18:269-78.
25. Cuong NV, Phu DH, Van NTB, Dinh Truong B, Kiet BT, Hien BV, et al. High-Resolution Monitoring of Antimicrobial Consumption in Vietnamese Small-Scale Chicken Farms Highlights Discrepancies Between Study Metrics. Frontiers in Veterinary Science. 2019;6(174).
26. Nguyen NT, Nguyen HM, Nguyen CV, Nguyen TV, Nguyen MT, Thai HQ, et al. Use of Colistin and Other Critical Antimicrobials on Pig and Chicken Farms in Southern Vietnam and Its Association with Resistance in Commensal Escherichia coli Bacteria. Applied and environmental microbiology. 2016;82(13):3727.
27. Nguyen VT, Jamrozy D, Matamoros S, Carrique-Mas JJ, Ho HM, Thai QH, et al. Limited contribution of non-intensive chicken farming to ESBL-producing Escherichia coli colonization in humans in Vietnam: an epidemiological and genomic analysis. The Journal of antimicrobial chemotherapy. 2019;74(3):561-70.
28. Nguyen Thi Thuy M, Dorny P, Lebailly P, Le Thi Minh C, Nguyen Thi Thu H, Dermauw V. Mapping the pork value chain in Vietnam: a systematic review. Tropical animal health and production. 2020;52(6):2799-808.

29. Pärnänen K, Karkman A, Hultman J, Lyra C, Bengtsson-Palme J, Larsson DGJ, et al. Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. Nature Communications. 2018;9(1):3891.
30. Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, et al. The human milk microbiota: Origin and potential roles in health and disease. Pharmacological Research. 2013;69(1):1-10.

Tables

Table 1. Prevalence of ESBL–*E. coli* and *bla*$_{CTX-M}$ –*E. coli* across samples sources

Sample source	Number of samples	*E. coli* n (%)	ESBL-*E. coli* n (%)	*bla*$_{CTX-M}$–*E. coli* n (%)
Humans – CA-UTI patients	8564	1705 (20%)	152 (9%)	137 (90%)
Chickens	48	28 (58%)	11 (39%)	10 (91%)
Chicken carcasses	228	187 (82%)	42 (22%)	34 (81%)
Pigs	27	20 (74%)	10 (50%)	7 (70%)
Pig carcasses	145	96 (66%)	11 (11%)	3 (27%)
Slaughterhouse environment	78	48 (62%)	9 (19%)	7 (78%)
Total	9090	2084 (23%)	235 (11%)	198 (84%)

418 **Figure legends**

419 **Figure 1. Sampling site co-ordinates in Hanoi metropolitan area.** Urine samples from patients
420 with UTIs were collected from 3 studied hospitals: National Hospital of Paediatrics (NHP; n=4,608),
421 Military Hospital (MH; n=2,606), and Vietnam-Cuba Hospital (VNCB; n=1,350). Food-animal
422 samples from live/slaughtered chickens and pigs were collected from two chicken farms (CF.1 and
423 CF.2), two pig farms (PF.1 and PF.2), six chicken slaughterhouses (CS.A – CS.F) and two pig
424 slaughterhouses (PS.A and PS.B). All sampling sites were in the radius of ~30 km from the hospitals,
425 mapped using Microreact tool at https://microreact.org/. Sample types collected from animals
426 included swabs of carcasses, caecum/cloaca, and faeces. Environmental samples included swabs
427 from the floors of the slaughter areas and the lairage areas and water samples from water sources
428 used for the slaughtering process.

429 **Figure 2. Schematic workflow describing the experimental study design**.

430 **Figure 3. (A) The association and distribution of dominant *bla*$_{\text{CTX-M}}$–Ec clones across sample**
431 **sources. (B) Prevalence of *bla*$_{\text{CTX-M}}$ variants in each sample origin.** Three clinical strains each
432 carried two *bla*$_{\text{CTX-M}}$ genes.

433 **Figure 4. (A) Schematic representations of typical genetic structures flanking *bla*$_{\text{CTX-M}}$ genes**
434 involving IS*Ecp1*, putative promoter and *orf477*/IS*903B* elements adjoining *bla*$_{\text{CTX-M}}$ genes in group
435 1 and group 9. The spacer between IS*Ecp1* and *bla*$_{\text{CTX-M}}$ group 1 genes were varied from 45 bp to
436 127 bp. While the spacers between IS*Ecp1* or *orf477*/IS*903B* and *bla*$_{\text{CTX-M}}$ group 9 were consistent.
437 **(B) Shared plasmids and genetic context of *bla*$_{\text{CTX-M}}$ genes between sample sources identified by**
438 **long-read sequencing.**

439

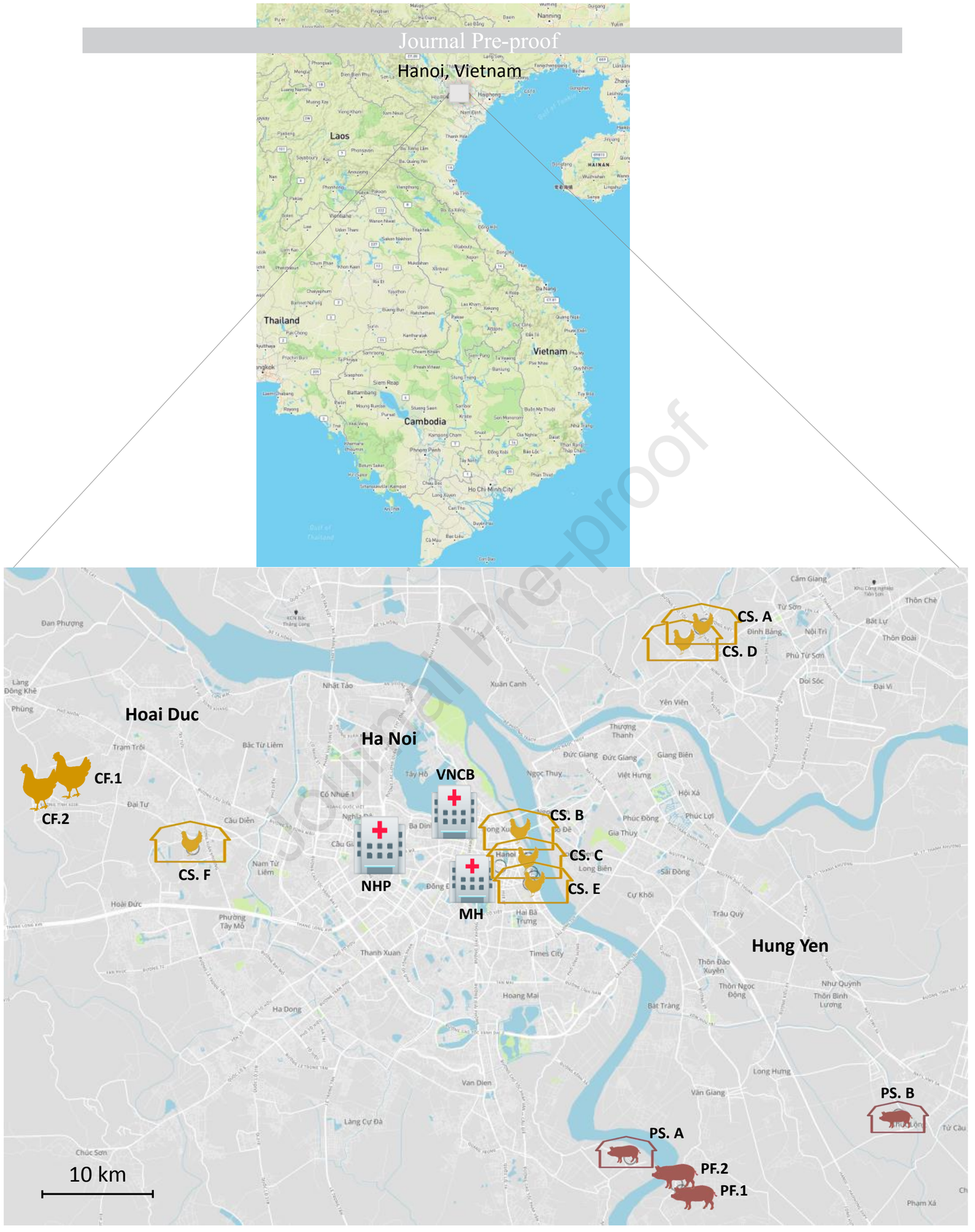
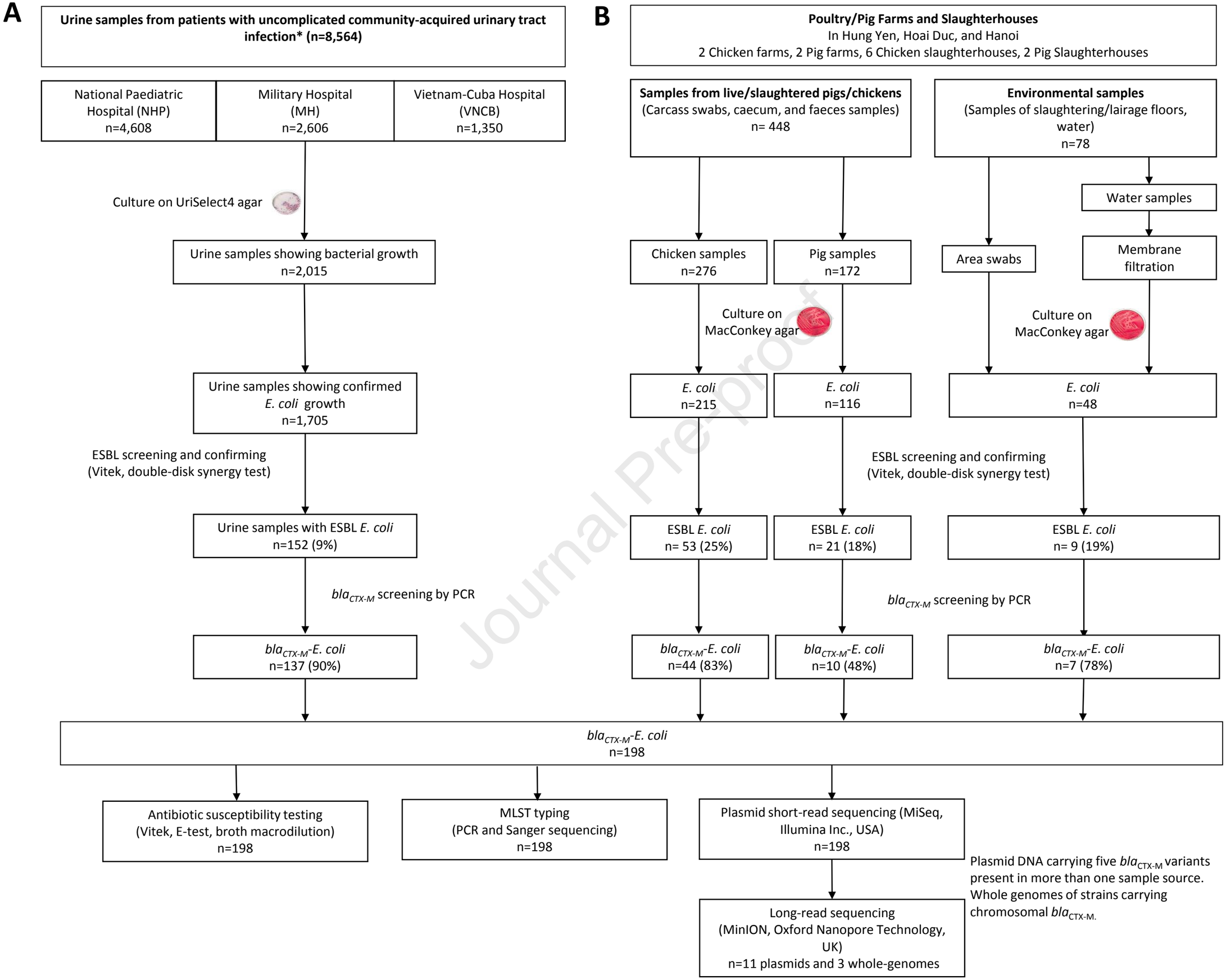


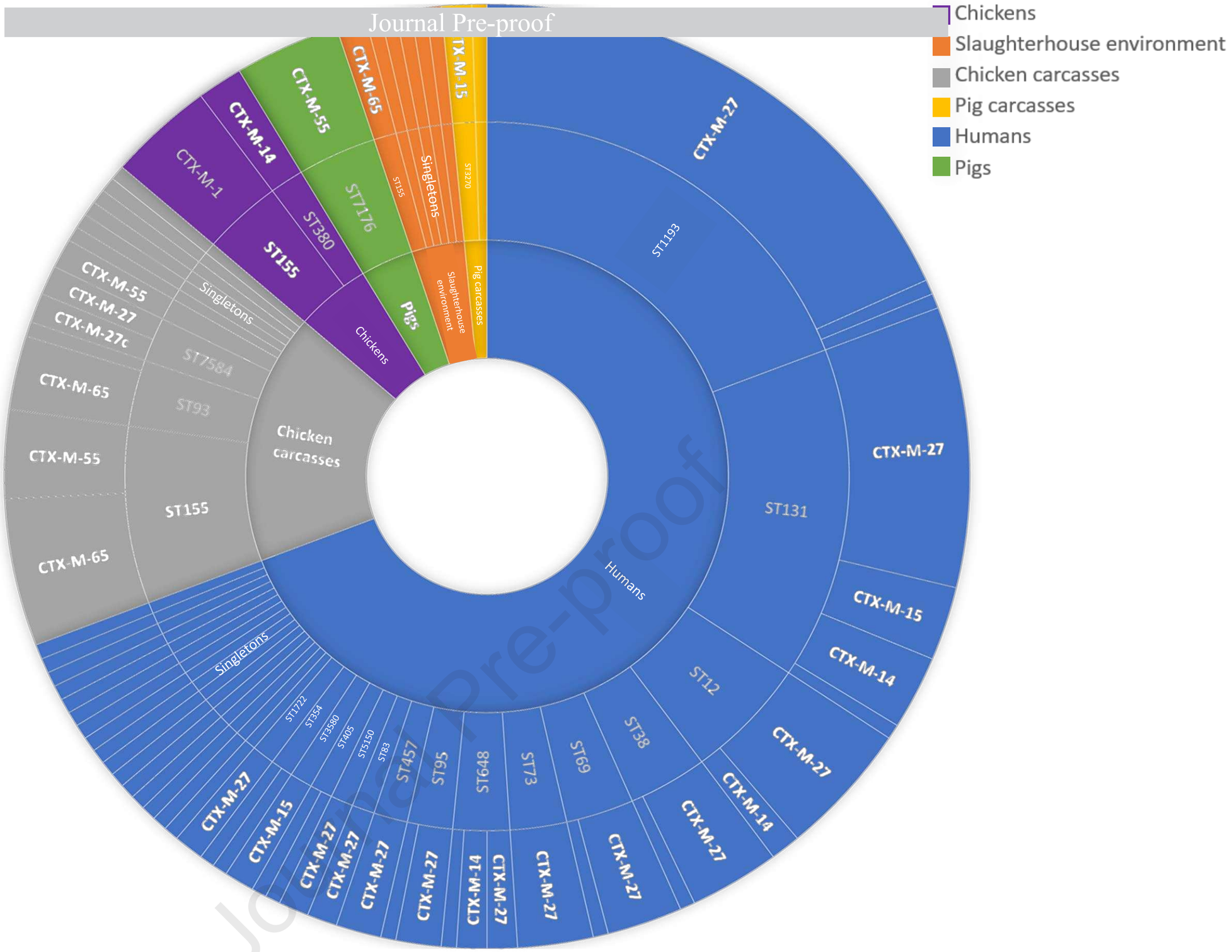
Figure 1



*Uncomplicated urinary tract infections were defined as infections without underlying renal, structural or neurological diseases based on clinical signs and symptoms

Figure 2

A



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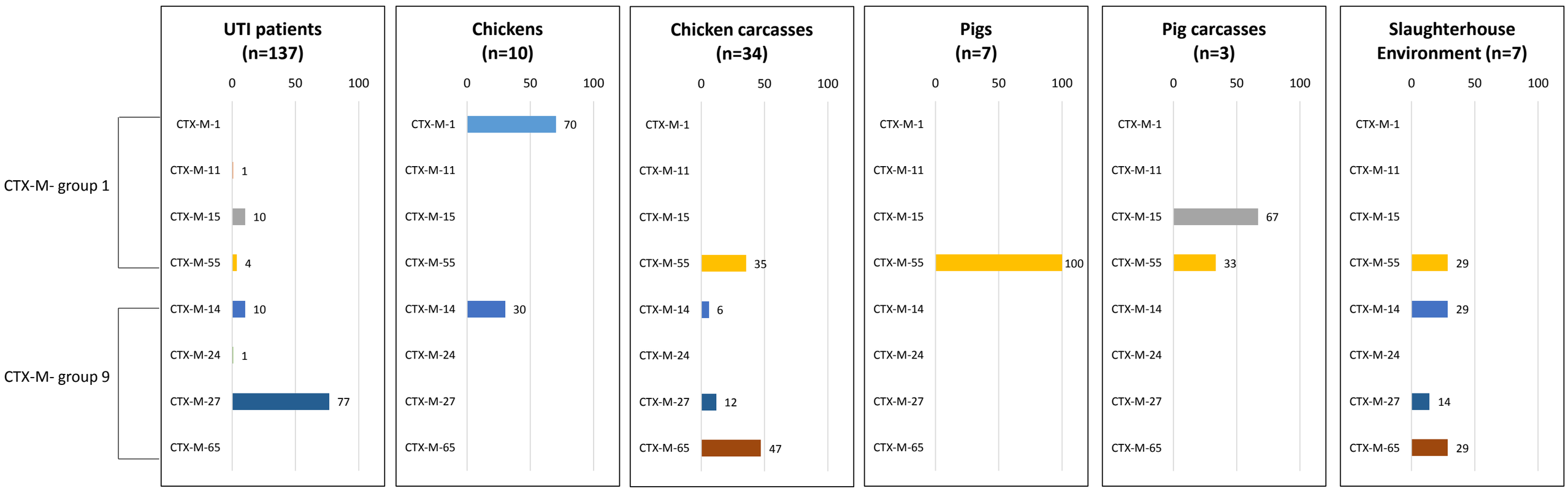
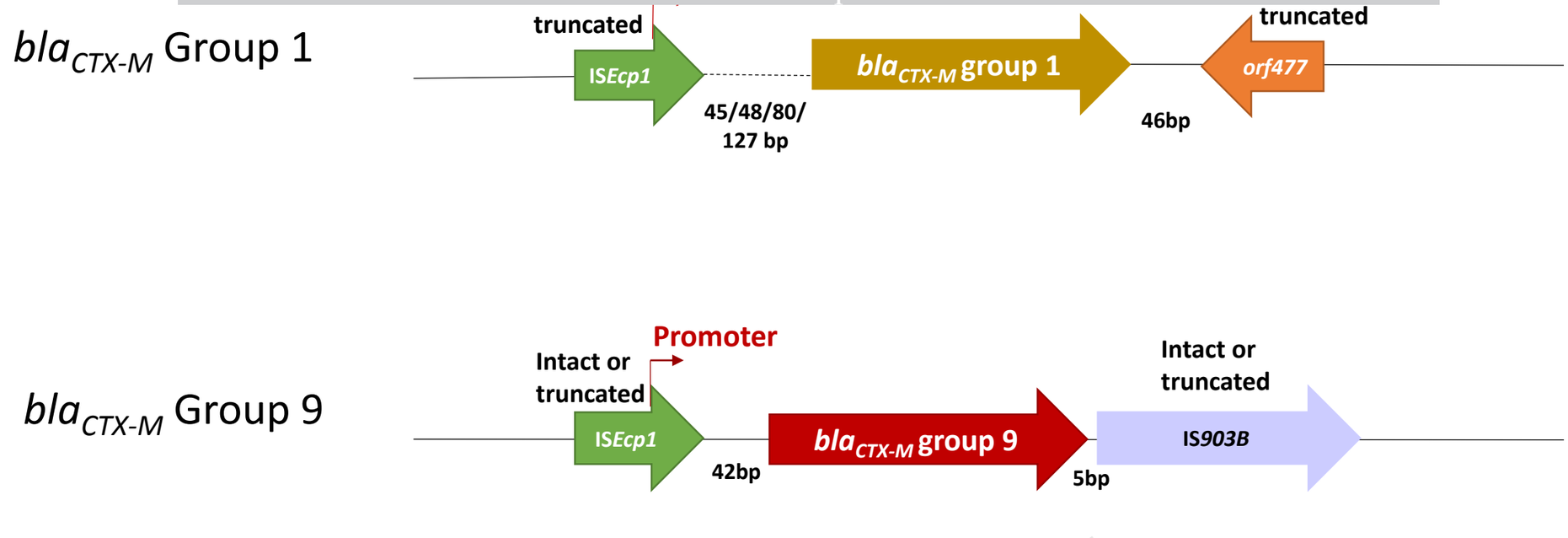


Figure 3

A



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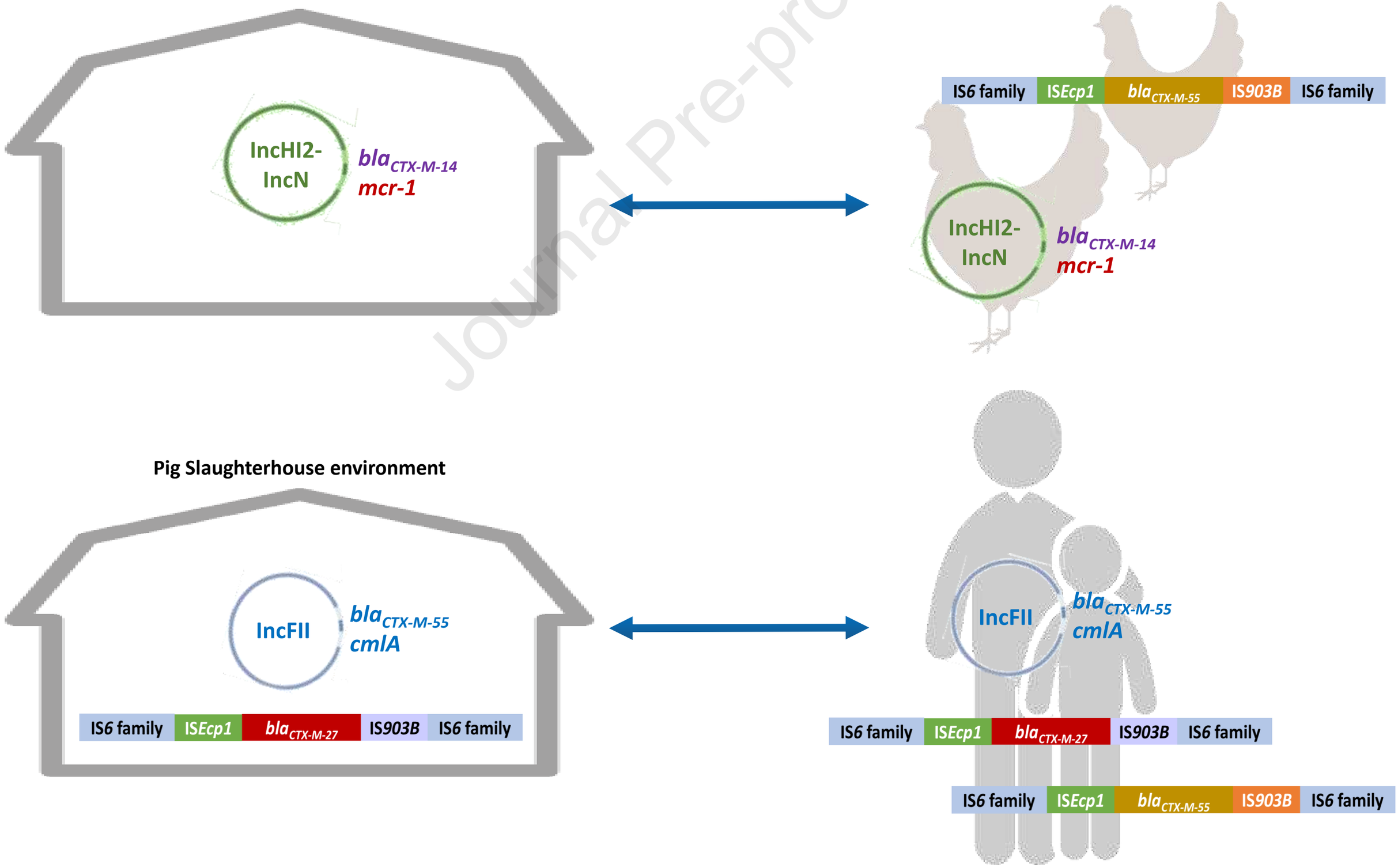

Figure 4