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Abstract:	Background Extended-spectrum cephalosporin resistance (ESC-R) in Escherichia coli and Klebsiella pneumoniae is a healthcare threat; high gastrointestinal carriage rates are reported from South-east Asia. Colonisation prevalence data in Cambodia are lacking. The aim of this study was to determine gastrointestinal colonisation prevalence of ESC-resistant E. coli (ESC-R-EC) and K. pneumoniae (ESC-R-KP) in Cambodian children/adolescents and associated socio-demographic risk factors; and to characterise relevant resistance genes, their genetic contexts, and the genetic relatedness of ESC-R strains using whole genome sequencing (WGS). Results Faeces and questionnaire data were obtained from individuals <16 years in north-western Cambodia, 2012. WGS of cultured ESC-R-EC/KP was performed (Illumina). Maximum likelihood phylogenies were used to characterise relatedness of isolates; ESC-R-associated resistance genes and their genetic contexts were identified from de novo assemblies using BLASTn and automated/manual annotation. 82/148 (55%) of children/adolescents were ESC-R-EC/KP colonised; 12/148 (8%) were co-colonised with both species. Independent risk factors for colonisation were hospitalisation (OR: 3.12, 95%, CI [1.52-6.38]) and intestinal parasites (OR: 3.11 [1.29-7.51]); school attendance conferred decreased risk (OR: 0.44 [0.21-0.92]. ESC-R strains were diverse; the commonest ESC-R mechanisms were blaCTX-M 1 and 9 sub-family variants. Structures flanking these genes were highly variable, and for blaCTX-M-15, -55 and -27, frequently involved IS26. Chromosomal blaCTX-M integration was common in E. coli. Conclusions Gastrointestinal ESC-R-EC/KP colonisation is widespread in Cambodian children/adolescents; hospital admission and intestinal parasites are independent risk factors. The genetic contexts of blaCTX-M are highly mosaic, consistent with rapid horizontal exchange. Chromosomal integration of blaCTX-M may result in stable propagation in these community-associated pathogens.
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Response to Reviewers:	28th February, 2019 Dear Dr Devoto, RE: Epidemiology of paediatric gastrointestinal colonisation by extended spectrum cephalosporin-resistant Escherichia coli and Klebsiella pneumoniae isolates in north-west Cambodia. Revised manuscript BMC Microbiology (MCRO-D-17-00672) Thank you for informing us that our article is potentially acceptable for publication, pending essential editorial revisions. We have carefully addressed the editorial revisions as follows: _____ ___ 1. & 2.Funding / competing interests sections amended. Please note, there are no financial competing interests. 3.As requested, methods section has been moved to after Conclusions 4.Details of consent process now included in the Methods under "Patients and setting". In the “ethical approval and consent to participate” section it is now clearly states that all included participants gave informed consent and that samples were excluded from the study if this had not been obtained. 5.Done. 6.Individual contributions of all authors to the manuscript have now been specified. 7.We have uploaded the data to NCBI BioProject (PRJNA391054), and referenced this in our manuscript.

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	8.Done. 9.Done. _____ We hope that you find our revisions satisfactory and that the manuscript is now acceptable for publication in BMC Microbiology. Yours sincerely, Jon van Aartsen MBChB, BSc, PhD, MRCP Phone + 447955541677 jjvanaartsen@outlook.com

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Epidemiology of paediatric gastrointestinal colonisation by extended spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in north-west Cambodia

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ABSTRACT

Background

Extended-spectrum cephalosporin resistance (ESC-R) in *Escherichia coli* and *Klebsiella pneumoniae* is a healthcare threat; high gastrointestinal carriage rates are reported from South-east Asia. Colonisation prevalence data in Cambodia are lacking. The aim of this study was to determine gastrointestinal colonisation prevalence of ESC-resistant *E. coli* (ESC-R-EC) and *K. pneumoniae* (ESC-R-KP) in Cambodian children/adolescents and associated socio-demographic risk factors; and to characterise relevant resistance genes, their genetic contexts, and the genetic relatedness of ESC-R strains using whole genome sequencing (WGS).

Results

Faeces and questionnaire data were obtained from individuals <16 years in north-western Cambodia, 2012. WGS of cultured ESC-R-EC/KP was performed (Illumina). Maximum likelihood phylogenies were used to characterise relatedness of isolates; ESC-R-associated resistance genes and their genetic contexts were identified from *de novo* assemblies using BLASTn and automated/manual annotation. 82/148 (55%) of children/adolescents were ESC-R-EC/KP colonised; 12/148 (8%) were co-colonised with both species. Independent risk factors for colonisation were hospitalisation (OR: 3.12, 95%, CI [1.52-6.38]) and intestinal parasites (OR: 3.11 [1.29-7.51]); school attendance conferred decreased risk (OR: 0.44 [0.21-0.92]. ESC-R strains were diverse; the commonest ESC-R mechanisms were $bla_{\text{CTX-M}}$ 1 and 9 sub-family variants. Structures flanking these genes were highly variable, and for $bla_{\text{CTX-M-15, -55 and -27}}$, frequently involved IS*26*. Chromosomal $bla_{\text{CTX-M}}$ integration was common in *E. coli*.

Conclusions

Gastrointestinal ESC-R-EC/KP colonisation is widespread in Cambodian children/adolescents; hospital admission and intestinal parasites are independent risk factors. The genetic contexts of $bla_{\text{CTX-M}}$ are highly mosaic, consistent with rapid horizontal exchange. Chromosomal integration of $bla_{\text{CTX-M}}$ may result in stable propagation in these community-associated pathogens.

MAIN TEXT

BACKGROUND

Escherichia coli and *Klebsiella pneumoniae* are two bacterial pathogens of the Enterobacteriaceae family that can cause a wide spectrum of clinical disease, ranging from cystitis and intra-abdominal abscesses to sepsis. Both species also asymptomatically colonise the gastrointestinal tract, a reservoir that assists in the acquisition and spread of antimicrobial

resistance (AMR)[1, 2]. The increasing prevalence of AMR worldwide is reducing the efficacy of our limited armamentarium of empirical broad-spectrum antibiotics, such as extended-spectrum cephalosporins (ESCs), resulting in increased healthcare costs and mortality[3-5].

Recent reports from South-east Asia show substantial variation between country and cohort in gastrointestinal colonisation by Enterobacteriaceae possessing Ambler class A extended spectrum beta-lactamases (ESBLs) and/or class C AmpC enzymes, which can hydrolyse third and fourth generation cephalosporins. In the Lao People's Democratic Republic, for example, 23% of pre-school children carried these strains, in contrast to a much higher prevalence of 65.7% in a rural Thai adult population[6-8]. Data describing the prevalence and mechanisms of antibiotic resistance in Cambodia are limited to only a few studies. Vlieghe and colleagues found 49.7% of Enterobacteriaceae from blood cultures in Phnom Penh from 2007-2010 were cefotaxime-resistant, mostly due to CTX-M-15 and CTX-M-14 enzymes[9]. Studies from 2004/5 and 2007-2011 identified ESC resistance in 36-44% of urinary tract infection isolates[10, 11]. Recent data from a Cambodian reference laboratory suggests that the prevalence of ESBL resistance amongst Enterobacteriaceae isolated from hospitalised and community-based patients increased from 23.8% in 2012 to 38.4% in 2015[12]. The gastrointestinal colonisation prevalence of ESC-resistant (ESC-R) *E. coli* and *K. pneumoniae* in Cambodia has previously only been investigated in hospitalised neonates[13], where on initial admission 21% were colonised with ESC-R *E. coli* (ESC-R-EC) and 33% with ESC-R *K. pneumoniae* (ESC-R-KP), increasing to 34% and 42% respectively on repeat admissions. Our study aimed to expand on this work by: (i) estimating the prevalence of gastrointestinal colonisation with ESC-R-EC and ESC-R-KP in Cambodian children and adolescents, and the molecular mechanisms responsible; (ii) investigating socio-demographic risk factors for ESC-R colonisation; (iii) determining genetic relatedness of ESC-R strains.

RESULTS

Sampling, culture and basic demographics

In total, 196 faecal samples were obtained from a consecutive subset of children/adolescents enrolled in an intestinal parasite prevalence study. 48 samples were excluded from this study because of: (i) lack of specific consent for wider use of the faecal samples beyond the faecal parasite survey (n=36); (ii) no epidemiological data records (n=1); (iii) no (n=3) or poor (n=5) growth on culture; or (iv) replicate samples for the same patient (n=3), leaving 148 samples/individuals for analysis.

Overall, 184 distinct colony types grew within the cefpodoxime inhibition zones; 141 were pink (presumed *E. coli*) and 43 were blue (presumed *Klebsiella* spp., *Enterobacter* spp. or *Citrobacter* spp.). All pink colonies but only 22/43 (54%) blue colonies were confirmed as phenotypically ESC-R using BSAC methods. All 163 confirmed ESC-R isolates were sequenced; two failed and were excluded from further analysis. Of the 161 sequences, *in silico* species identification confirmed 135 (84%) isolates were *E. coli*, 18 (11%) *K. pneumoniae*, and 8 (5%) *Enterobacter* spp. 38 *E. coli* isolates and one *K. pneumoniae* isolate were genetically sufficiently closely related to another isolate obtained from the same patient sample to be considered as the same strain (defined as ≤5 chromosomal SNVs); these were also excluded leaving 122 isolates for analysis. None of the 148 faecal samples yielded imipenem resistant colonies.

Participants were median 4.2 years old (interquartile range: 1.1-8.8) at sample collection; 70/148 (47%) were male. 70/147 (48%; 1 missing) were inpatients at sample collection. Although most were from Siem Reap province (99/148 [67%]), the hospital catchment is such that the remainder were recruited from 10 other provinces. 16/148 (11%) were clinically

malnourished, and 23/148 (16%) had ≥1 underlying chronic medical condition including HIV (n=5), haematological disease (n=3), congenital cardiac disease (n=5), tuberculosis (n=4), and asthma (n=2)(Table 1).

Prevalence of and risk factors for colonisation with ESC-R EC and/or ESC-R-KP

A total of 114 confirmed ESC-R-EC (n=97) and ESC-R-KP (n=17) remained in the analysis and were carried by 82/148 participants, giving a combined ESC-R-EC/KP prevalence of 55% (95% CI: 47%-64%); 53% for ESC-R EC (79/148 patients; 95% CI: 45%-62%) and 10% for ESC-R KP (15/148 patients; 95% CI: 6%-16%). Co-colonisation with both ESC-R-EC and ESC-R-KP was observed in 12/82 (15%). Independent risk factors for ESC-R-EC/KP colonisation included being a current inpatient (OR=3.64; 95% CI [1.71-7.74), p=0.001) and the presence of faecal parasites (OR=3.96 [1.55-10.12], p=0.004). ESC-R-EC/KP colonisation was lower in males (OR=0.39 [0.18-0.84], p=0.015) and in those attending school (OR=0.39 [0.18-0.83], p=0.015)(Table 1).

Sequence type, Ambler class and genetic mechanisms of ESC-R

The 97 ESC-R-EC isolates came from 33 known and 6 novel STs (Fig.1, for details see Table S1). 22% (17/79) of patients were colonised by at least two different ESC-R-EC STs, although this may underestimate diversity as only a small number of colonies (≤3) were sampled per patient [14]. The 17 ESC-R-KP strains came from 11 known and 3 novel STs (n=4 isolates) (Fig.2, Table S2). Two patients were colonised by two different ESC-R *K. pneumoniae* STs (2/15, 13%).

In total, 77% (88/114) and 23% (26/114) of isolates displayed Ambler class A or C phenotypes, respectively (Table 2). Neither species were associated with Ambler class A (76% [74/97] versus 82% [14/17]) or class C (24% [23/97] versus 18% [3/17]; Fishers exact test; p=0.759). In all class A isolates the phenotype could be explained by the presence of one (84/88, 95%) or two (4/88, 5%) *bla*$_{\text{CTX-M}}$ genes; *bla*$_{\text{SHV}}$ (12/88, 14%) and *bla*$_{\text{VEB}}$ (1/88, 1%) occurred less commonly. Class C gene families were only identified in 38% (10/26) of phenotypically class C isolates: specifically *bla*$_{\text{CMY-2}}$ (8/26, 31%) or *bla*$_{\text{DHA}}$ (2/26, 8%). In the remaining 16 isolates, the genetic basis for the class C phenotype was unclear; of note, however, *ampC* promoter mutations were not assessed.

111 *bla*$_{\text{CTX-M}}$ genes were found in 94% (107/114) of ESC-R-EC/KP, with two separate alleles identified in 4% of isolates (4/114). The most frequently identified allele was *bla*$_{\text{CTX-M-15}}$ (53/111, 48%), followed by: *bla*$_{\text{CTX-M-55}}$ (24/111, 22%), bla$_{\text{CTX-M-14}}$ (17/111,15%), *bla*$_{\text{CTX-M-27}}$ (14/111, 13%) and *bla*$_{\text{CTX-M-24}}$ (3/111, 3%). Two different *bla*$_{\text{CTX-M}}$ alleles were found in 21% (18/82) of individuals carrying ESC-R-EC/KP.

All 15 identified *bla*$_{\text{SHV}}$ genes were found only in ESC-R-KP. Of these, 3/15 (20%) are likely to confer ESC-R: *bla*$_{\text{SHV-27-like}}$ (1/15, 7%), 1/15 *bla*$_{\text{SHV-28}}$ (1/15, 7%), *bla*$_{\text{SHV-99-like}}$ (1/15, 7%); the remaining possess either narrow-spectrum beta-lactamase activity (11/15, 73%: 3/15 *bla*$_{\text{SHV-1/ SHV-1-like}}$, 20%; 4/15 bla$_{\text{SHV-11/ SHV-11-like}}$, 27%; 3/15 *bla*$_{\text{SHV-33}}$, 20.0%; and 1/15 *bla*$_{\text{SHV-83}}$, 7%) or their beta-lactamase phenotype is unknown (1/15, 7%: 1/15 *bla*$_{\text{SHV-142}}$, 7%). All *bla*$_{\text{SHV-}}$positive ESC-R-KP possessed other genes that could explain their ESC-R phenotype: *bla*$_{\text{CTX-M-14}}$ (2/15, 13%), *bla*$_{\text{CTX-M-15}}$ (10/15, 67%), *bla*$_{\text{CTX-M-27}}$ (2/15, 13%), or *bla*$_{\text{DHA}}$ (1/15, 7%). The study population carriage prevalence of common ESC-R conferring

genetic mechanisms encoded by ESC-R-EC/KP was therefore: 53% *bla*$_{\text{CTX-M}}$ (78/148), 2% *bla*$_{\text{SHV}}$ (3/148), 1% *bla*$_{\text{VEB}}$ (1/148), 5% *bla*$_{\text{CMY-2}}$ (8/148), 1% *bla*$_{\text{DHA}}$ (2/148). Two individuals (1%) carried isolates with *bla*$_{\text{OXA-48}}$ (one *K. pneumoniae* ST48 [56B1] and one *E. coli* ST648 [94P1]); no other carbapenem resistance mechanisms were identified. Both isolates were resistant to ertapenem with a minimum inhibitory concentration (MIC) of >1µg/ml); 56B1 had intermediate resistance to imipenem (MIC 4µg/ml) and meropenem (MIC 8µg/ml), whilst 94P1 was sensitive to both with MICs of 1µg/ml and 0.25µg/ml, respectively.

Genetic context of bla$_{CTX\text{-}M}$

For the 41 *E. coli* harbouring *bla*$_{\text{CTX-M-15}}$, it was chromosomally located in five cases (12%), and likely in plasmid contexts in two; in the remaining cases it was not possible to determine wider chromosomal/plasmid location (Table 3). One isolate (38P1) harboured short contigs containing truncated *bla*$_{\text{CTX-M-15}}$, leaving 40 cases in which to evaluate the immediate flanking contexts surrounding the *bla*$_{\text{CTX-M}}$ gene. All contained IS*Ecp1* upstream of *bla*$_{\text{CTX-M-15}}$, but with considerable evidence of additional mobilisation events/mosaicism (Table 3). In particular, IS*Ecp1* was truncated by IS*26* at 24, 497, 524, 1067, 1173, 1421, or 1489bp in 13 isolates, consistent with at least seven IS*26*-associated insertion events within IS*Ecp1*(Fig.3). Another 13 IS*Ecp1* elements were truncated by contig breaks, without any specific associated genetic signatures, although contig breaks are frequently due to repeat structures and may therefore have represented additional disruption events. One isolate had an intact IS*Ecp1* element, without any wider flanking upstream context. The 13 cases with an intact IS*Ecp1* were consistently flanked by variable lengths of Tn*2*, which was truncated by an IS*26* right IRR in 2/7 evaluable cases (and by an unknown sequence in the other 5/7). Two isolates had a complete Tn*2* structure interrupted by IS*Ecp1*-*bla*$_{\text{CTX-M-15}}$ (TCTCA-TCTCA and TTTTA-TAAAA target site sequences [TSSs] respectively)(Fig.3). Overall, genetic contexts of

bla$_{\text{CTX-M-15}}$ were consistent with integration and mobilisation of IS*Ecp1*-*bla*$_{\text{CTX-M-15}}$ within a Tn*2* element, as previously described(26), with subsequent rearrangement events facilitated by IS*26* and perhaps other ISs(27)(Table S3).

For the 24 *E. coli* harbouring *bla*$_{\text{CTX-M-55}}$, it was chromosomally located in 4 (17%), plasmid in 4 (17%) and unknown in 16 (67%). One contig contained a truncated *bla*$_{\text{CTX-M-55}}$, leaving 23 evaluable contexts. Similar to *bla*$_{\text{CTX-M-15}}$, it was invariably associated with IS*Ecp1* upstream of *bla*$_{\text{CTX-M-55}}$ (Fig.4), which was often incomplete, representing at least 3 different IS*26*-associated IS*Ecp1* disruption events (Table 3). Intact IS*Ecp1* were flanked by variable lengths of Tn*2* sequence, apart from 120P1 where the contig was truncated immediately at the 5' end of IS*Ecp1*. One isolate (2P1) had the same *bla*$_{\text{CTX-M}}$/Tn*2* unit as for *bla*$_{\text{CTX-M-15}}$ (but with TACTC-TAAAA), consistent with the evolution of *bla*$_{\text{CTX-M-55}}$ from *bla*$_{\text{CTX-M-15}}$ (1 SNV difference) within this unit (Figs.3, 4).

For the 15 *E. coli* harbouring *bla*$_{\text{CTX-M-14}}$, it was chromosomally located in 2 (13%) cases, plasmid-associated in 5 (33%), and unknown in 8 (53%). Again, it was invariably associated with IS*Ecp1*, but more often complete and with different mechanisms of disruption (2 IS*Vsa5-like* sequence, one IS*1S* R IRR). All cases had an IS*903* element at the 3' end of *bla*$_{\text{CTX-M-14}}$; this had been disrupted in 6 cases, with additional contig breaks in 5 cases (Fig.5). Two of three *E. coli bla*$_{\text{CTX-M-24}}$ contexts were chromosomal, with flanking contexts similar to *bla*$_{\text{CTX-M-14}}$ (Fig.S1). In the 12 *bla*$_{\text{CTX-M-27}}$ cases, the *ISEcp1* element had been disrupted by an IS*26* L IRR in all contexts, at 149, 192, 208 and 388bp, but the wider genetic context of this structure was indeterminable in all cases (Fig.S2).

Overall, *bla*$_{\text{CTX-M}}$ was chromosomal in 13/92 cases (14%; 13/25 [52%] cases where plasmid versus chromosomal location could be assessed), suggesting that CTX-M genes may be incorporated chromosomally and indiscriminately in significant numbers of colonising *E. coli*, with possible implications for their stable propagation within the wider *E. coli* population.

For *K. pneumoniae*, 12 isolates harboured *bla*$_{\text{CTX-M-15}}$, in a plasmid-associated context in 9/12 cases, and an unknown context in 3/12 cases. Three isolates harboured a complete *bla*$_{\text{CTX-M-15}}$/Tn*2* complex with GTTAA-GTTAA TSS, most consistent with a direct transposition of this element into a plasmid context. In the other isolates, the IS*Ecp1*-*bla*$_{\text{CTX-M-15}}$-ORF477 was flanked by variable stretches of Tn*2*-associated sequence identical to that found in the *E. coli* isolates, and similarly truncated either as a result of contig breaks, or by IS*26* inverted repeats, consistent with between species and within species mobilisation (Fig.S3).

Four *K. pneumoniae* isolates harboured *bla*$_{\text{CTX-M-9}}$ group genes; two of these (*bla*$_{\text{CTX-M-14}}$) shared the same IS*Ecp1* (Fig.5) and ~18kb upstream flanking plasmid sequence; and two (*bla*$_{\text{CTX-M-27}}$) an IS*Ecp1* element truncated at position 1499 by an IS*26* L IRR (Fig.S2).

DISCUSSION

We observed significant gastrointestinal carriage prevalence of both ESC-R-EC and ESC-R-KP in Cambodian children sampled in 2012; approximately one in twelve children was co-colonised with ESC-R strains of both species. A wide diversity of ESC-R strain types was observed, including several genotypes categorised as “high risk” clones, such as *E. coli* STs 38, 405, 131, 354 and 648[15]. The predominant ESC-R genotypic mechanism was *bla*$_{\text{CTX-M}}$,

with the major allelic variants being those widely described elsewhere in Asia (Group 1: *bla*$_{\text{CTX-M-15, -55}}$, Group 9: *bla*$_{\text{CTX-M-14, -24, -27}}$). Approximately one-third of the Cambodian population is <18 years old, so this group may be acting as a significant reservoir for the spread of antimicrobial resistant organisms.

We did not identify any carbapenem-resistant isolates using our imipenem-based screening method; however, two (1%) individuals were colonised with transmissible carbapenemase genes identified on sequencing of cultured isolates. These isolates were phenotypically susceptible or intermediately-susceptible to imipenem, which has since been shown to be a less sensitive indicator of carbapenemase - particularly *bla*$_{\text{OXA-48}}$ - presence, and we may therefore have been underestimating the prevalence of these genes [16]. Nevertheless, our results are consistent with other Cambodian datasets, including an earlier study (2007-2010) where no carbapenemase genes were identified in 183 Enterobacteriaceae blood culture isolates by means of PCR and a more recent study (2013-2014) where only 2 of 289 (0.7%) hospitalised neonates were found to harbour imipenem-resistant isolates [9, 13]. This could be explained by a much lower antibiotic selection pressure resulting from poor local availability of carbapenems compared with other neighbouring countries such as Thailand at the time of the study. Of note, the *bla*$_{\text{OXA-48}}$-*E. coli* isolate from this study was isolated from an outpatient without any known chronic health problems, suggesting there may be some carriage of carbapenem-resistant isolates in the community (or that we missed a healthcare exposure for this individual). Repeat assessment of the extent of carbapenem-resistant EC/KP in both community and hospitalised individuals in Cambodia is warranted.

Independent risk factors for colonisation by ESC-R-EC/KP included inpatient status, consistent with transmission within hospital, and/or selection of these organisms from low-level carriage by the use of antibiotics on admission given the high burden of infectious diseases in this region. Infection control in resource-limited settings remains challenging, and despite improvements within the study hospital[17], recent longitudinal surveillance within the neonatal care unit identified high rates of import of ESC-R-KP (62% colonised on admission) as well as nosocomial acquisition (23%)[13]. Inpatient acquisition of ESC-R-EC/KP has also been identified as a major problem in other low/middle-income settings[18]. The specific effect of faecal parasites on gut microbiota is not well-studied, but they are thought to significantly perturb microbial diversity[19]. Parasite infestation may also result in inappropriate antimicrobial use, including antibiotics, perhaps leading to secondary colonization with drug-resistant commensals. The decreased risk associated with school attendance has been observed in a previous study in Spain[20], and may represent a proxy marker for increased socio-economic status, and parental levels of education, which were not evaluated here, but may translate into better awareness of appropriate antibiotic use[21, 22]. The decreased risk associated with male gender is unexplained; but independent associations for ESBL-EC/KP colonization have been described for both genders in previous studies[18, 23, 24].

Of particular importance was the high prevalence of chromosomal integration of *bla*$_{\text{CTX-M}}$ in *E. coli* in this study (>14%), perhaps contributing to the stable propagation of this resistance gene family within certain strains. Whilst the previously reported chromosomal integration of *bla*$_{\text{CTX-M}}$ in Spanish *K. pneumoniae* isolates was not observed in our study, this could not be excluded for three isolates in which the *bla*$_{\text{CTX-M}}$ genetic location (i.e. either plasmid or chromosomal) remained indeterminate due to the limitations of the genetic assemblies [25].

In addition, despite the limitations of short-read assemblies, the genetic contexts of *bla*$_{\text{CTX-M}}$ suggested high levels of genetic plasticity in flanking structures, and significant associations with IS*26* for *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-55}}$, and *bla*$_{\text{CTX-M-27}}$. IS*26* has been previously hypothesised to facilitate the mobility of *bla*$_{\text{CTX-M}}$ and genetic rearrangement of resistance gene plasmids, and is likely contributing to the dissemination of these resistance genes within the human gastrointestinal reservoir[26-28].

This study has several limitations. Our survey dates from 2012, and the epidemiology of ESC-R EC/KP carriage may have changed in the intervening timeframe. We only included up to three bacterial colonies per faecal sample, likely resulting in significant under-estimation of the diversity present at the population level[14]. Also, storage conditions for faecal samples may have impacted on the isolates that were cultured. Short-read sequencing resulted in limited information regarding the wider genetic context of important resistance genes conferring ESC-R; nevertheless, we were still able to ascertain that the genetic contexts of these resistance genes are extremely diverse. Our outpatient study population may not be truly representative of healthy children in the community, given that these were individuals that had presented to the outpatient department for some form of medical review. Lack of more detailed information on some potential risk factors meant we were unable to fully assess the specific mechanisms promoting ESC-R EC/KP colonisation. Further work characterising the role of healthcare admissions, socio-economic factors and intestinal parasites on the acquisition and long-term carriage dynamics of these strains would be valuable. In addition, our sample size was too small and sparse to investigate geographical clustering of strain types, and to investigate specific risk factors for colonisation with common strain types or resistance gene alleles. Despite these limitations, our data are important as they represent the

largest molecular epidemiological study of gastrointestinal ESC-R-EC/KP colonisation in Cambodia and form a useful benchmark for future studies.

CONCLUSION

This study adds to the growing body of literature demonstrating widespread gastrointestinal colonisation with ESC-R-EC and ESC-R KP in Southeast Asia[8], and showing that exposure to this reservoir may in turn act as a source for the wider, global transfer of these strains[29]. The genetic contexts of important resistance genes are highly mosaic, consistent with rapid exchange of resistance genes within and between bacterial hosts. Significant levels of chromosomal integration of the most important ESC-R gene family, $bla_{\text{CTX-M}}$, were also observed, and may result in these genes being stably maintained and propagated in one of the most common community-associated pathogens, namely *E. coli*. Our observations are alarming and, in the context of widespread, unregulated and often inappropriate antibiotic use, as seen in Cambodia, these selection pressures are likely to further facilitate the dissemination of AMR genes.

METHODS

Patients and setting

Faecal samples were obtained from a consecutive subset of children/adolescents (<16 years) who had been enrolled in a prospective study that aimed to identify the prevalence of intestinal parasites in children/adolescents attending Angkor Hospital for Children in Siem Reap, Cambodia, from 3rd April 2012 to 29th June 2012, as described previously[30]. Informed consent was obtained by explaining the study to children/adolescents and their

caregivers, and confirmed by the caregiver's signature or a witnessed thumbprint if they were illiterate.

Microbiological methods

Samples were frozen at -80°C as aliquots homogenised in 0.9% sterile saline with 10% glycerol within an hour of receipt in the laboratory. For this study, faecal samples were thawed, and aliquots diluted 1:10 in saline and incubated for 16 hours at 37°C on Orientation CHROMagar (BD, Oxford, United Kingdom) with 10 µg cefpodoxime and 10 µg imipenem discs (Oxoid, Basingstoke, United Kingdom). For each faecal sample, up to three pink and/or dark blue colonies with different colonial morphotypes that grew within the cefpodoxime zone of inhibition (presumed ESC-R-EC and ESC-R-KP respectively) were selected for further analysis. Each selected colony was tested using the British Society of Antimicrobial Chemotherapy (BSAC) combination disc method to identify whether cefpodoxime (ESC) resistance was mediated via ESBLs (Class A: cefpodoxime-resistant, and cefpodoxime+clavulanic acid-sensitive) or via non-ESBL mechanisms (e.g. Class C AmpC beta-lactamases: cefpodoxime-resistant, and cefpodoxime+clavulanic acid-resistant)[31]. All identified ESC-R colonies were stored frozen at -80°C in nutrient broth with 10% glycerol. Carbapenem susceptibility testing was performed via BD Phoenix automated susceptibility testing (microbroth dilution method; Becton Dickinson, Franklin Lakes, NJ, USA).

Whole genome sequencing and sequence data processing

DNA was extracted from sub-cultured ESC-R isolates using a commercial kit (Fujifilm Quickgene, Japan) with an additional mechanical lysis step (Fastprep MP Biomedicals, USA). All isolates were sequenced using the Illumina HiSeq 2500, generating 150bp paired-

end reads. Sequence data have been deposited in GenBank (project accession: PRJNA391054).

To identify single nucleotide variants (SNVs) reads were mapped to species-appropriate reference genomes (*E. coli* CFT073 [GenBank: AE014075.1] and *K. pneumoniae* MGH78578 [GenBank: CP000647.1]), and variants called as described previously[32]. Alignments of variable sites were padded to the length of the reference genome using bases with the same %GC content as that observed within each dataset. Bootstrapped, maximum-likelihood phylogenies were reconstructed for each species using RaxML version 7.7.6[33], using a generalised time-reversible model and four categories of rate heterogeneity (./RAxML-7.7.6/raxmlHPC-PTHREADS-SSE3 -f a -s <input_alignment.phy> -m GTRGAMMA -p 12345 -c 4 -x 12345 -# 100 -n <output_raxml_rapid_bootstrap>). Phylogenies have been deposited as projects in MicroReact to enable an interactive assessment of geographic distribution of genotypes (*E. coli*: https://microreact.org/project/By8bf5ajg; *K. pneumoniae*: https://microreact.org/project/Hy_yQcaog[34].

Contigs were assembled using Velvet/VelvetOptimiser (hash value range: 75-149)[35, 36]. *In silico* MLST was determined by BLASTn[37] matches (100% match) to the Achtman/Pasteur MLST schemes for *E. coli* and *K. pneumoniae*[38, 39], and supported correct species identification. The presence/absence of resistance genes was determined using BLASTn and an in-house curated resistance gene database of over 60 gene families[40]. Genes were considered present if a blast match of ≥80% of the query sequence was identified at ≥80% sequence identity using the *de novo* assemblies as blast databases. Ambler class genotype

was class A if *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$ and/or *bla*$_{\text{VEB}}$ were present, and/or class C if *bla*$_{\text{CMY-2}}$, *bla*$_{\text{DHA}}$ and *bla*$_{\text{ACT-like}}$ genes were present. Where patient faecal samples yielded ≥2 strains, all resistance genes were treated as a single entity within the individual's profile.

The genetic context of *bla*$_{\text{CTX-M}}$ was examined by extracting the contigs containing these genes, and annotating these using PROKKA[41], combined with BLASTn and manual annotation with reference to mobile genetic elements in the ISFinder database[42]. Gene locations were characterised as "chromosomal" if other annotations on the contig were only found in chromosomal contexts in the top 20 BLASTn hits when the contig was compared with bacterial sequences available in GenBank (using default parameters); "plasmid" if the other annotations matched only plasmid sequences; or unknown if these conditions were not met e.g. the assembled contigs were too short to verify this.

Epidemiological analyses

Information regarding putative socio-demographic risk factors for ESC-R EC/KP colonisation (collected on a standardised form) included details on: gender, age, hospitalisation status, residence in Siem Reap province versus elsewhere, water source (river, rain, well, bottled, piped, boiled), domestic animals (cats, dogs, birds), livestock (chickens, ducks, pigs, cows or water buffalo), toilet availability, malnutrition, co-morbidities, presence/absence of diarrhoea, presence/absence of parasites (assessed within [30]), soap usage for hand-washing and school attendance. No details regarding antibiotic consumption were ascertained within the study, but previous work locally has shown that individuals are often ill-informed about the nature of any medications used and that 32% of outpatient attendees have evidence of urinary antimicrobial activity[43].

Statistical analyses

Independent risk factors for carriage were identified from a multivariable, stepwise, logistic regression model based on complete cases and initially including all factors (backwards elimination using exit $p<0.1$ to reduce over-fitting). A final multivariable logistic model was then fitted including all cases for which complete information was available for the retained risk factors. Statistical analyses were performed using STATA version 14 (StataCorp, College Station, USA).

DECLARATIONS

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB), Angkor Hospital for Children, and the Oxford Tropical Research Ethics Committee (OXTREC 12–12). Caregivers of all included participants gave informed consent for their child to participate in the intestinal parasite survey, and for the samples to be used more widely in additional studies approved by the IRB. For patients who did not provide consent, their samples were excluded from the study.

Consent for publication

Not applicable.

Availability of data and material

The data have been deposited as part of BioProject accession number PRJNA391054 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

This project was conceived and designed by NS, TEAP, NPJD, DWC, ASW, CEM and JJvA. CEM, CMP, PT, NP, SM, and KS collected patient data and faecal samples. JJvA and NS conducted faecal screening for ESC-R-EC/KP in addition to preparation of isolates for whole genome sequencing. NS, TD, AG and AES developed and executed bioinformatics analysis of the sequence data. NS, JJvA and ASW performed the statistical analysis. NS and JJvA

interpreted the patient and bioinformatics data sets, and wrote the manuscript. All the authors have read, revised and approved the final manuscript.

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FIGURE LEGENDS

Figure 1.

Phylogeny of study *Escherichia coli* isolates. Interactive map of geographic locations and genetic attributes can be visualised at: https://microreact.org/project/By8bf5ajg

Figure 2.

Phylogeny of study *Klebsiella pneumoniae* isolates. Interactive map of geographic locations and genetic attributes can be visualised at: https://microreact.org/project/Hy_yQcaog

Figure 3. Schematic of aligned genetic contexts for $bla_{\text{CTX-M-15}}$ in study *Escherichia coli*. Features of interest are highlighted in the figure key. White numbers within open reading frames denote truncated sequence length (bp). Isolates harbouring this genetic context are listed to the left of the figure. "x" denotes contig breaks. [P] denotes plasmid contexts; [c] chromosomal contexts.

Figure 4. Schematic of aligned genetic contexts for *bla*$_{\text{CTX-M-55}}$ in study *Escherichia coli*. Features of interest are highlighted in the figure key. White numbers within open reading frames denote truncated sequence length (bp). Isolates harbouring this genetic context are listed to the left of the figure. "x" denotes contig breaks. [P] denotes plasmid contexts; [c] chromosomal contexts.

Figure 5. Schematic of aligned genetic contexts for *bla*$_{\text{CTX-M-14}}$ in study *Escherichia coli* and *Klebsiella pneumoniae*. Features of interest are highlighted in the figure key. White numbers within open reading frames denote truncated sequence length (bp). Isolates harbouring this genetic context are listed to the left of the figure. "x" denotes contig breaks. [P] denotes plasmid contexts; [c] chromosomal contexts.

TABLES

Table 1. Clinical and epidemiological details of all 148 participants, also categorised by presence/absence of gastrointestinal colonisation with ESC-resistant *E. coli* and/or *K. pneumoniae*, and multivariable logistic regression outcomes

	Overall (n=148)	ESC-R *E. coli*/ *K. pneumoniae* colonised (n=82; 55%)	ESC-R *E. coli*/ *K. pneumoniae* non-colonised (n=66; 45%)	Univariable logistic regression for ESC-R carriage		Multivariable logistic regression for ESC-R carriage[¥]	
	Number (%) unless otherwise specified	**n (%)**	**n (%)**	**OR [95% CI]**	**p**	**OR [95% CI]**	**p**
Median age [IQR], years	4.24 [1.10 - 8.82]	3.07 [0.97 - 7.21]	6.23 [1.32 - 9.23]	1.00 [1.00-1.00]	0.712		
Male	70 (47)	35 (43)	35 (53)	0.66 [0.34-1.27]	0.211	0.39 [0.18-0.84]	**0.015**
Inpatient[*]	70 (48)	49 (60)	20 (32)	3.28 [1.66-6.50]	**0.001**	3.64 [1.71-7.74]	**0.001**
Province							
Siem Reap	99 (67)	51 (62)	48 (72)	1			
Other (versus Siem Reap)[¶]	49 (33)	31 (38)	18 (28)	1.06 [0.72-1.58]	0.716		

Malnutrition	16 (11)	12 (15)	4 (6)	2.66 [0.81-8.67]	0.105		
Co-morbidities[§]	25 (17)	19 (23)	6 (9)	3.01 [1.13-8.06]	**0.028**		
Diarrhoea present[&]	70 (48)	44 (54)	26 (40)	1.78 [0.92-3.46]	0.086		
Water sources							
Well	123 (83)	64 (78)	59 (89)	0.42 [0.16-1.08]	0.073		
Bottled	17 (11)	13 (16)	4 (6)	2.92 [0.90-9.43]	0.073		
River	5 (3)	3 (4)	2 (3)	1.22 [0.20-7.49]	0.834		
Rain	8 (5)	5 (6)	3 (5)	1.36 [0.31-5.93]	0.680		
School attendance	71 (48)	32 (39)	39 (59)	0.44 [0.23-0.86]	**0.016**	0.39 [0.18-0.83]	**0.015**
All animals	119 (80)	65 (79)	54 (82)				
Domestic animals	113 (76)	61 (74)	50 (79)	0.78 [0.36-1.69]	0.532		
Cat	51 (34)	32 (39)	19 (29)	1.58 [0.79-3.17]	0.194		
Dog	100 (68)	57 (70)	43 (65)	1.22 [0.61-2.43]	0.573		

Birds	24 (16)	12 (15)	12 (18)	0.77 [0.32-1.85]	0.561
Livestock/food animals	89 (60)	54 (66)	35 (53)	1.71 [0.88-3.32	0.114
Water buffalo	4 (3)	3 (4)	1 (1)	2.47 [0.25-24.3]	0.439
Chickens	80 (54)	49 (60)	31 (47)	1.67 [0.87-3.23]	0.122
Pigs	23 (16)	14 (17)	9 (14)	1.30 [0.53-3.23]	0.567
Ducks	2 (1)	2 (2)	0	1 (omitted)	
Cattle	24 (16)	15 (18)	9 (14)	1.42 [0.58-3.48]	0.446
Use of toilet for defecation	88 (59)	45 (54)	42 (65)	0.65 [0.33-1.27]	0.207
Use of soap[#]					
Never	34 (23)	18 (23)	16 (25)	1	

Some use (versus Never)	111 (77)	62 (76)	49 (75)	1.12 [0.52-2.43]	0.765		
Presence of intestinal parasites	36 (24)	25 (30.49)	11 (17.19)	2.19 [0.99-4.88]	0.054	3.96 [1.55-10.12]	**0.004**

[*] missing one datapoint (n=147)

[&] missing two datapoints (n=146)

[#] missing three datapoints (n=145)

[¥] Backwards elimination performed using 144 cases for which complete information available, on all predictors, using exit $p \leq 0.1$. Final model then incorporated 147 cases for which complete information available on included predictors (gender, inpatient status, presence of intestinal parasites and school attendance)

[¶] "Other province" category includes: Banteay Meanchey (n=21), Oddar Meanchey (8), Kampong Thom (6), Battambang (5), Preah Vihear (3), Kampong Cham (2), Kampong Chhang (1), Pursat (1), Other (2)

[§] Includes: HIV (n=5), blood dyscrasia (3), Down's syndrome (2), congenital heart disease (6), tuberculosis (4), asthma (2), other (8); five individuals had multiple co-morbidities

p-values <0.05 in bold

Table 2. Summary of Ambler Class A and C phenotypes and genotypes in ESC-resistant *E. coli* and *K. pneumoniae* isolates

	Amber Class Phenotype						
	***E. coli* (n = 97)**		***K. pneumoniae* (n = 17)**		**Both species (n = 114)**		**Total**
	A (n = 74)	C (n = 23)	A (n = 14)	C (n = 3)	A (n = 88)	C (n = 26)	(n = 114)
Ambler Class A genes							
bla$_{CTX-M}$ positive †	73 (99%)	18 (78%)	14 (100%)	2 (67%)	87 (99%)	20 (77%)	107 (94%)
CTX-M-14	14	1	2	0	16	1	17
CTX-M-15	28	13	12	0	40	13	53
CTX-M-24	2	1	0	0	2	1	3
CTX-M-27	12	0	0	2	12	2	14
CTX-M-55	20	4	0	0	20	4	24
Total	76	19	14	2	90	21	111
bla$_{SHV}$ positive	0 (0%)	0 (0%)	12 (86%)	2 (67%)	12 (14%)	2 (8%)	14 (12%)

SHV-1/1-like**	0	0	2	1	2	1	3
SHV-11/11-like**	0	0	2	1	2	1	3
SHV-27*	0	0	1	0	1	0	1
SHV-28*	0	0	1	0	1	0	1
SHV-33**	0	0	3	0	3	0	3
SHV-83**	0	0	1	0	1	0	1
SHV-99/99-like*	0	0	1	0	1	0	1
SHV-142¥	0	0	1	0	1	0	1
Total	0	0	12	2	12	2	14
bla$_{VEB}$ positive	1 (1%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)	1 (1%)
None identified	0	5 (22%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (4%)
Ambler Class C genes							
bla$_{CMY-2}$ positive	0 (0%)	8 (35%)	0 (0%)	0 (0%)	0 (0%)	8 (31%)	8 (31%)

bla$_{DHA}$ positive	0 (0%)	1 (4%)	0 (0%)	1 (33%)	0 (0%)	2 (8%)	2 (8%)
bla$_{ACT\text{-}like}$ positive	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
None identified	0 (0%)	14 (61%)	0 (0%)	2 (67%)	0 (0%)	16 (62%)	16 (62%)

† Isolates with two separate *bla*$_{CTX\text{-}M}$ alleles were identified in 4% of isolates (4/114)

* ESBL

** not ESBL

¥ Unknown beta-lactamase phenotype

Table 3. Summary of genetic contexts of *bla*$_{CTX-M}$ in ESC-resistant *E. coli* and *K. pneumoniae*

	***bla*$_{CTX-M-15}$**		***bla*$_{CTX-M-55}$**		***bla*$_{CTX-M-14}$**		***bla*$_{CTX-M-27}$**		***bla*$_{CTX-M-24}$**	
	EC (n=41)	*KP* (n=12)	EC (n=24)	KP (n=0)	*EC* (n=15)	*KP* (n=2)	*EC* (n=12)	*KP* (n=2)	*EC* (n=3)	*KP* (n=0)
Location (chromosome versus plasmid)										
Chromosomal	5 (12%)	0	4 (17%)	-	2 (13%)	0	0	0	2 (67%)	-
Likely plasmid	2 (5%)	9 (75%)	4 (17%)	-	5 (33%)	2 (100%)	0	0	1 (33%)	-
Not determined	34 (83%)	3 (25%)	16 (67%)	-	8 (53%)	0	12 (100%)	2 (100%)	0	-
Evaluable immediate flanking context	**n=40***	**n=12**	**n=23¥**	-	**n=15**	**n=2**	**n=12**	**n=2**	**n=3**	-
IS*Ecp1* upstream	**40 (100%)**	**12 (100%)**	**23 (100%)**	-	**15 (100%)**	**2 (100%)**	**12 (100%)**	**2**	**3 (100%)**	-

3’ GACTA target site sequence (TSS) at 48bp upstream of *bla*$_{\text{CTX-M}}$	36 (90%)	12 (100%)	23 (100%)	-	0	0	0	0	0	-
3’ TTTCA TSS at 127bp upstream of *bla*$_{\text{CTX-M}}$	4 (10%)	0	0	-	0	0	0	0	0	-
3’ GAATA TSS at 42bp	0	0	0	-	15 (100%)	2 (100%)	12 (100%)	2 (100%)	3 (100%)	-
IS*Ecp1* incomplete	**26 (65%)**	**0**	**14 (61%)**	-	**6 (40%)**	**0**	**12 (100%)**	**2 (100%)**	**1 (100%)**	-
due to IS*26* element	13** (32%) L IRR - 11	0	12† (52%) L IRR - 1	-	0	0	12 (100%) L IRR - 12	2 (100%) L IRR - 2	0	-

	R IRR - 2		R IRR - 11							
due to contig break	13 (32%)	0	2 (9%)	-	3 (20%)	0	0	0	1 (33%)	-
due to IS*Vsa5*-like	0	0	0	-	2 (13%)	0	0	0	0	-
due IS*1S* R IRR	0	0	0	-	1 (7%)	0	0	0	0	-
IS*Ecp1* complete	**13 (32%)**	**12 (100%)**	**9 (39%)**	-	**9†† (60%)**	**2 (100%)**	**0**	**0**	**2 (67%)**	-
5’ TCATA TSS	9 (22%)	12 (100%)	4 (17%)	-	0	0	0	0	0	-
5’ TAATA TSS	4 (10%)	0	3 (13%)	-	0	0	0	0	0	-
5’ TAACA TSS	0	0	2 (14%)	-	0	0	0	0	0	-
5’ CATTA TSS	0	0	0	-	4 (44%)	0	0	0	1 (33%)	-
5’ AAATA TSS	0	0	0	-	2 (22%)	0	0	0	0	-
5’ TAAAA TSS	0	0	0	-	1 (11%)	0	0	0	0	-
5’ GCCGA TSS	0	0	0	-	1 (11%)	0	0	0	0	-

5’ TATAT TSS	0	0	0	-	0	0	0	0	1 (33%)	-
5’ TAGCA TSS	0	0	0	-	0	2 (100%)	0	0	0	-

EC = *E. coli*, KP = *K. pneumoniae*

* excluding one isolate with short contigs harbouring truncated *bla*$_{\text{CTX-M-15}}$

** IS*Ecp1* truncated at 24, 497, 524, 1067, 1173, 1421, or 1489bp.

¥ excluding one isolate with short contig harbouring truncated *bla*$_{\text{CTX-M-55}}$

† IS*Ecp1* truncated at 267, 309 or 497bp

†† For one only 1 bp of 5’ TSS evaluable

¶ IS*Ecp1* truncated at 149, 192, 208 and 388bp

TSS=target site sequence

L IRR=left inverted repeat region

R IRR=left inverted repeat region

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ADDITIONAL FILES

Additional File 1:

- File: bmcmicro_camb_esbl_figS1_23122017.pdf

- Title: Figure S1

- Description: Schematic of aligned genetic contexts for $bla_{CTX\text{-}M\text{-}24}$ in study Escherichia coli. Features of interest are highlighted in the figure key. White numbers within open reading frames denote truncated sequence length (bp). Isolates harbouring this genetic context are listed to the left of the figure. "x" denotes contig breaks. ^{P} denotes plasmid contexts; ^{c} chromosomal contexts.

Additional File 2:

- File: bmcmicro_camb_esbl_figS2_23122017.pdf

- Title: Figure S2

- Description: Schematic of aligned genetic contexts for *bla*$_{CTX\text{-}M\text{-}27}$ in study *Escherichia coli* and *Klebsiella pneumoniae*. Features of interest are highlighted in the figure key. White numbers within open reading frames denote truncated sequence length (bp). Isolates harbouring this genetic context are listed to the left of the figure. "x" denotes contig breaks. ^{P} denotes plasmid contexts; ^{c} chromosomal contexts.

Additional File 3:

- Figure: bmcmicro_camb_esbl_figS3_23122017.pdf

- Title: Figure S3

- Description: Schematic of aligned genetic contexts for *bla*$_{CTX\text{-}M\text{-}15}$ in study *Klebsiella pneumoniae*. Features of interest are highlighted in the figure key. White numbers within

open reading frames denote truncated sequence length (bp). Isolates harbouring this genetic context are listed to the left of the figure. "x" denotes contig breaks. [P] denotes plasmid contexts; [c] chromosomal contexts.

Additional File 4:

- File: bmcmicro_camb_esbl_supplementary_info_17022019.docx

- Title: Supplementary Tables

- Description: **Table S1.** Sequence type distribution of *E. coli* isolates obtained in this study.

Table S2. Sequence type distribution of *K. pneumoniae* isolates obtained in this study

Additional File 5:

- File: bmcmicro_camb_esbl_tableS3_23122017.xlsx

- Title: Table S3

- Description: Detail of genetic contexts for $bla_{CTX\text{-}M}$ in sequenced isolates.

Figure 2

Click here to access/download;Figure;bmcmicro_camb_esbl_fig2_23122017.pdf

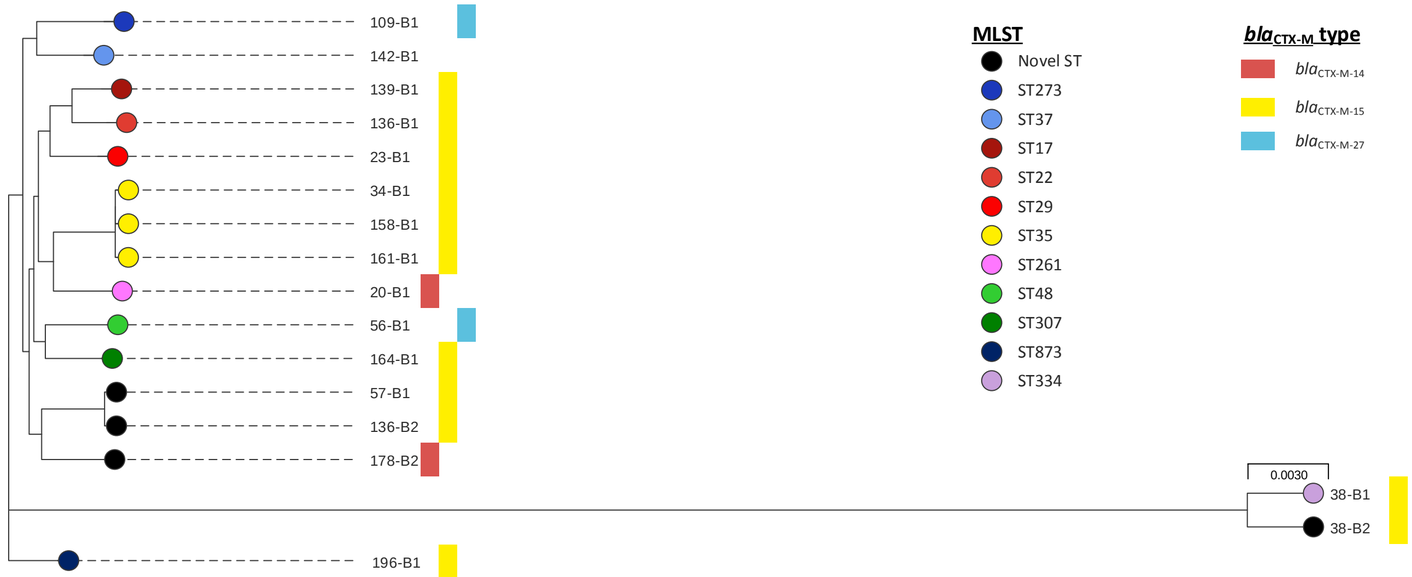
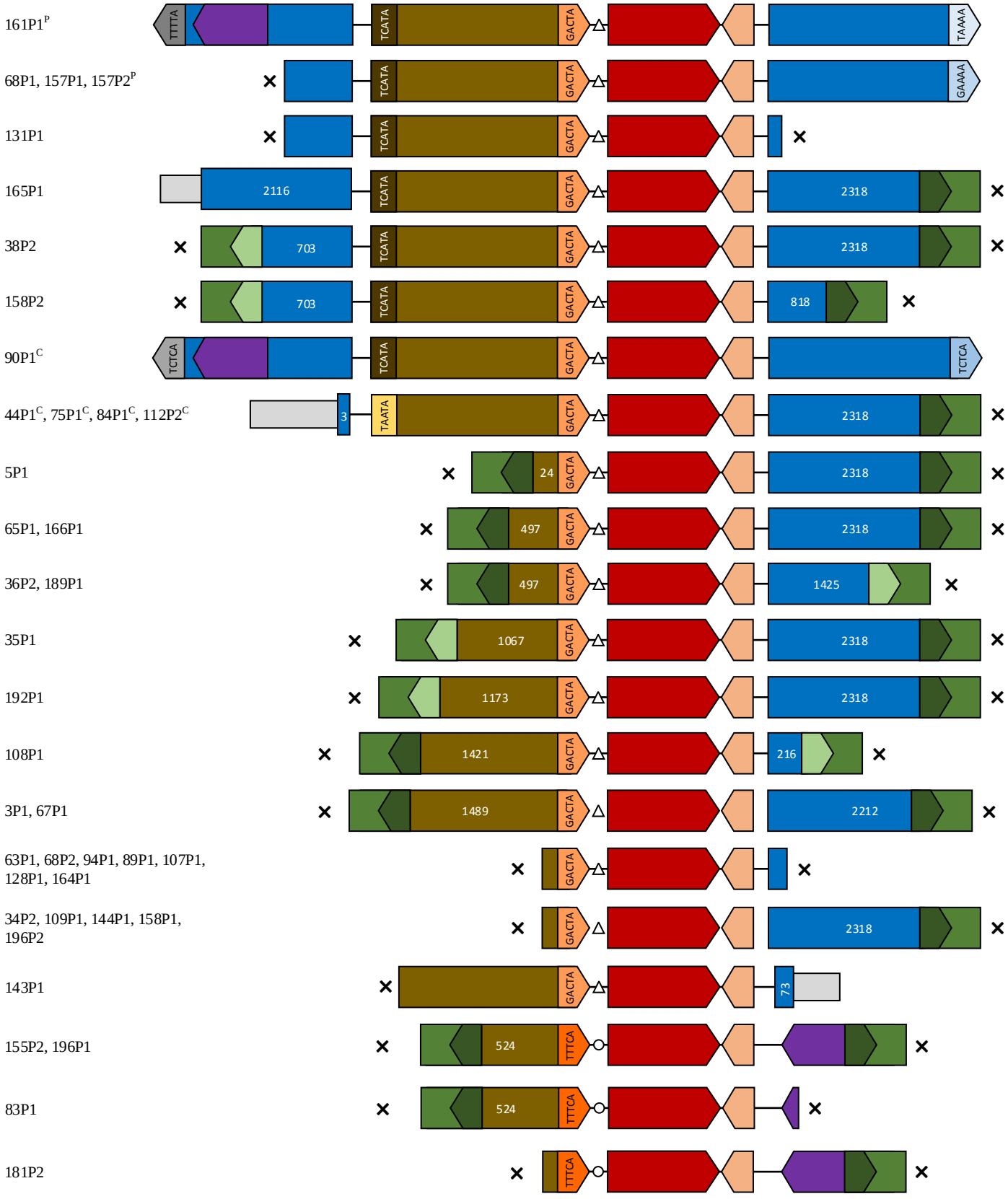


Figure 3

Click here to access/download;Figure;bmcmicro_camb_esbl_fig3_23122017



Key

IS*26*

Unknown flanking sequence

bla_{TEM-1}

Sequence upstream of $bla_{CTX-M-15}$

Tn2 flanking sequence and target site sequences (TSS)

IS*Ecp1* and target site sequences (TSS)

$bla_{CTX-M-15}$ and ORF477

Figure 4

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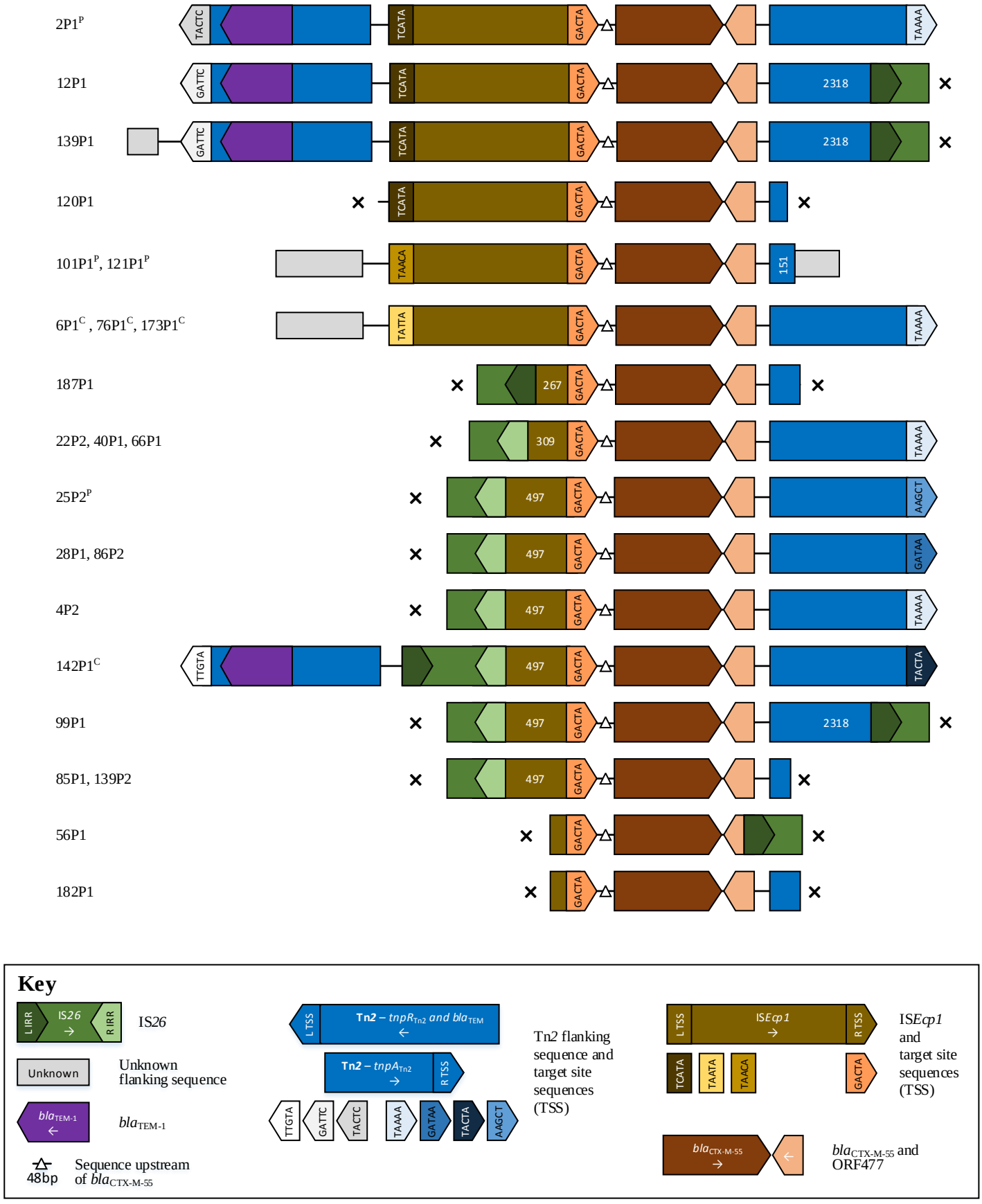

Figure 5

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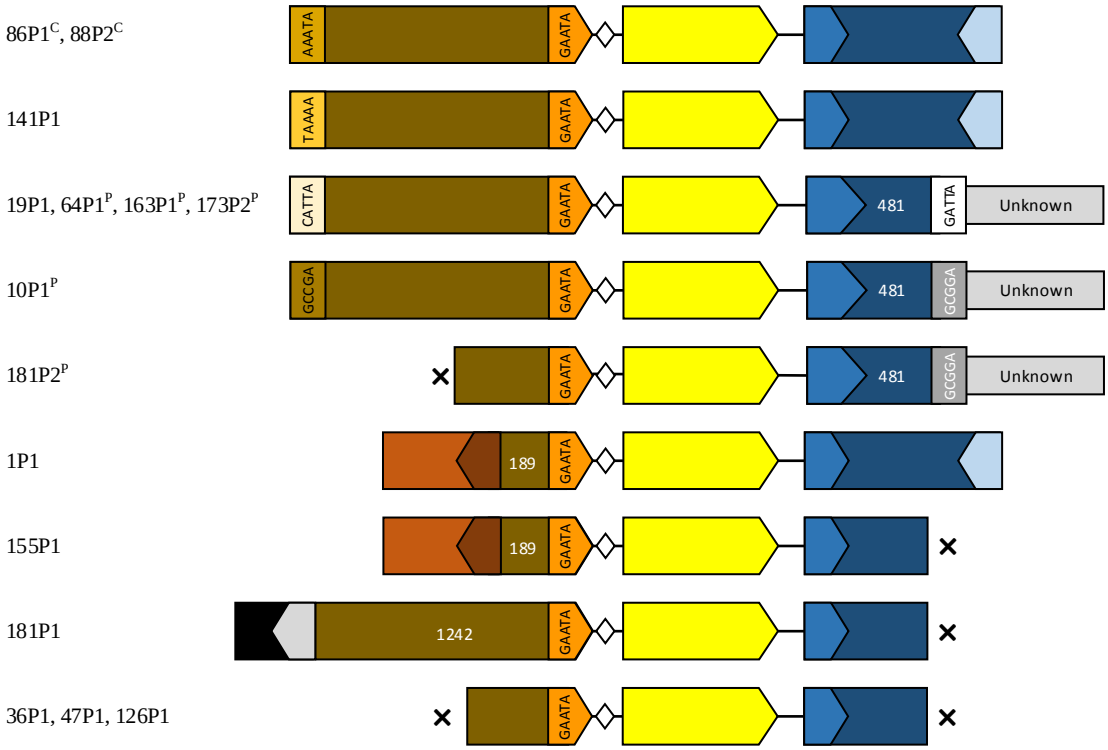
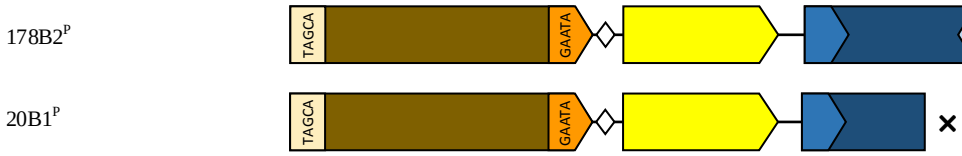
a) ***E. coli***



b) ***K. pneumoniae***



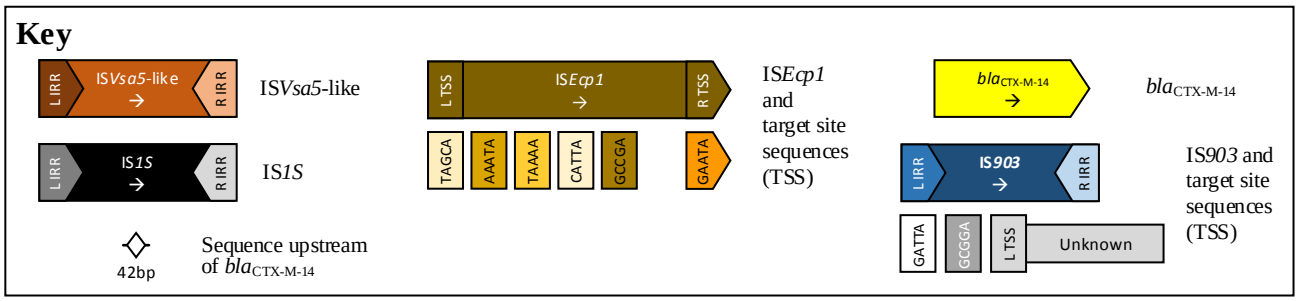


Figure 1

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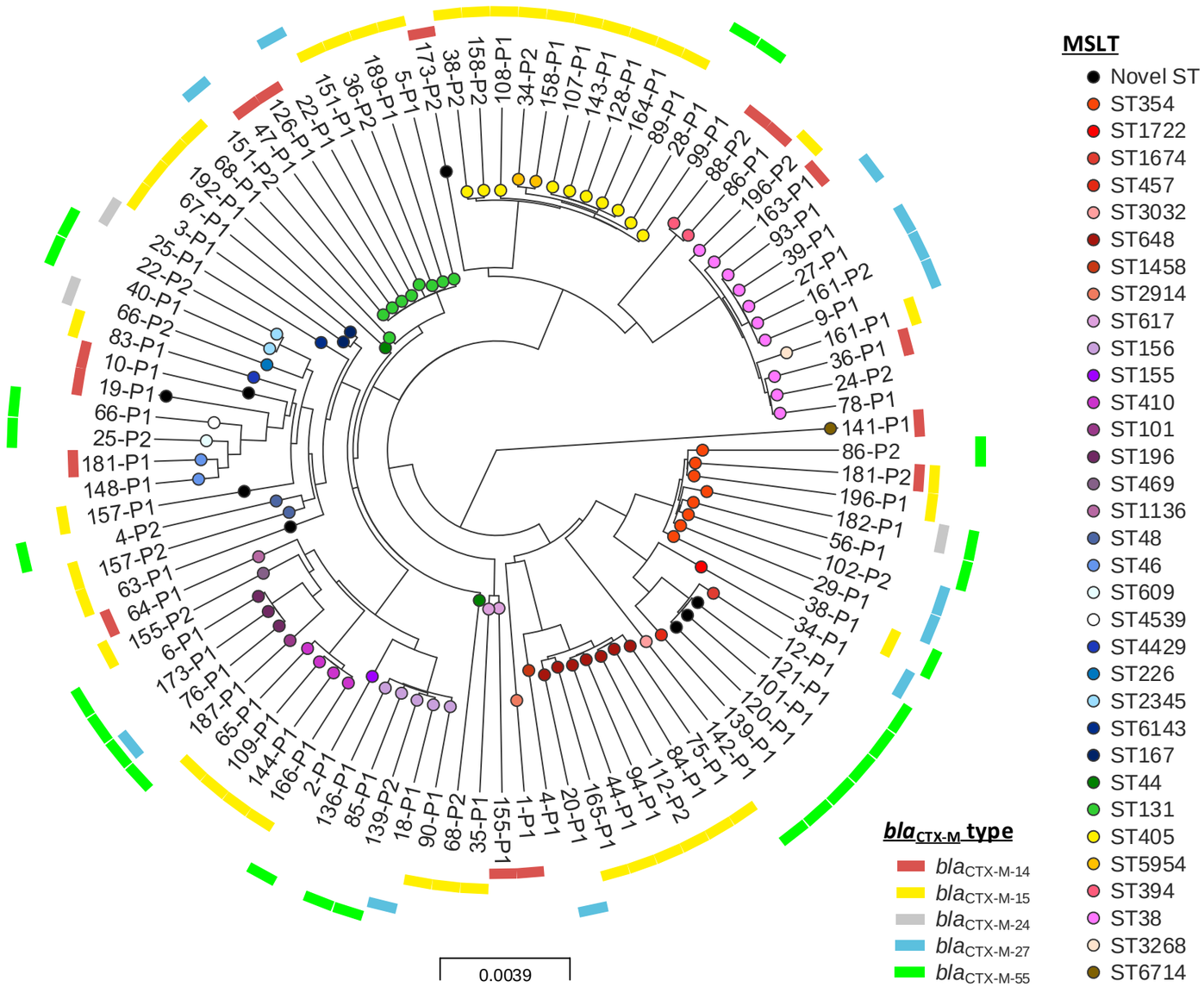

MSLT

- Novel ST
- ST354
- ST1722
- ST1674
- ST457
- ST3032
- ST648
- ST1458
- ST2914
- ST617
- ST156
- ST155
- ST410
- ST101
- ST196
- ST469
- ST1136
- ST48
- ST46
- ST609
- ST4539
- ST4429
- ST226
- ST2345
- ST6143
- ST167
- ST44
- ST131
- ST405
- ST5954
- ST394
- ST38
- ST3268
- ST6714

bla_{CTX-M} type

- $bla_{CTX-M-14}$
- $bla_{CTX-M-15}$
- $bla_{CTX-M-24}$
- $bla_{CTX-M-27}$
- $bla_{CTX-M-55}$

Table S3

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Supplementary Tables

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