Title page

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Anti-microbial resistance genes, virulence genes and associated mobile genetic elements of multidrug- resistant *Enterobacterales* isolated from hospital acquired urinary tract infections in Sri Lanka

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Abstract

Background

Antimicrobial resistant and virulent strains of *Enterobacterales* are frequent causes of hospital-acquired urinary tract infections (HA-UTI). This study explores the virulence and antibiotic resistance determinants, associated mobile genetic elements (MGE) and sequence types of six multidrug-resistant (MDR) *Klebsiella pneumoniae* (Kp1-Kp6), one *Escherichia coli* (Esc) and one *Enterobacter cloacae* strain (ECC) from a collection of 120 uropathogenic *Enterobactericeae* isolated from HA-UTI in Sri Lanka using genomic sequencing and comparative genomics.

Results

This study describes the first isolation of MDR *K. pneumoniae* ST16, *E. coli* ST131 and *E. hormaechei* subsp. *steigerwaltii* ST93 in Sri Lanka. All isolates carried genes conferring resistance to six or more different classes of antibiotics. Only five carried known carbapenemase genes with the others harbouring multiple AmpC and ESBL (extended-spectrum β-lactamase) genes. ECC manifested both $bla_{\text{NDM-4}}$ and $bla_{\text{OXA-181}}$ together with the *ble* gene which encodes resistance to bleomycin. The *K. pneumoniae* strains harbored fimbrial genes (*fim*, *mrk*) that play a role in the pathogenesis of UTI. Several extra-intestinal pathogenic *Escherichia coli* associated virulence genes were identified in Esc. The efflux pump gene, *acrA* and the T6SS gene cluster were detected in ECC. Many antimicrobial resistance (AMR) and virulence genes were identified associated with MGE. In all isolates IS*Ecp*1 flanked upstream of $bla_{\text{CTX-M-15.}}$ The $bla_{\text{OXA-48-like}}$ carbapenemase genes, $bla_{\text{OXA-181 and}}$ $bla_{\text{OXA-232}}$, were carried on ColKP3 plasmids and were associated with IS*Ecp*1. In Esc, the AMR gene, $bla_{\text{TEM-1B}}$ and virulence gene, *tra*T were found on an IncF plasmid replicon.

In Kp2, Kp4 and Kp6 the AMR genes *sul1* and *tetB* present on IncR plasmid replicons were associated with the insertion sequence IS6100. In Kp5, $bla_{\text{LAP-2}}$ and *qnr*S1co-existed and were flanked by IS*Ecl*. AMR gene clusters of 3 to 4 genes, conferring resistance to multiple antimicrobial classes, flanked by mobile elements were identified in seven isolates.

Conclusions

The concurrent presence of resistance to multiple antibiotics and a variety of virulence factors facilitates pathogenicity in hospital acquired infections. This study revealed a diversity of AMR genes, virulence genes, associated MGE and sequence types among MDR uropathogenic *Enterobacterales* isolated from HA-UTI in Sri Lanka.

Keywords

Anti-microbial resistance genes, Virulence genes, Mobile genetic elements, *Enterobacterales*, Hospital-acquired urinary tract infections, Sri Lanka

Anti-microbial resistance genes, virulence genes and associated mobile genetic elements of multidrug- resistant *Enterobacterales* isolated from hospital acquired urinary tract infections in Sri Lanka

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Background

The spread of multidrug resistant **(**MDR) *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* complex (ECC) strains resulting in treatment failure and increased mortality has become a global health problem [1, 2]. In particular, extended spectrum β-lactamase (ESBL) and carbapenemase producing strains of *K. pneumoniae*, *E. coli* and ECC are of concern due to their association with transferable multidrug resistance and the establishment of successful clones [2, 3, 4]. Such strains pose a potential threat to hospitals as they are known to cause hospital outbreaks [4]. There have been several reports in Sri Lanka of outbreaks due to MDR *K. pneumoniae* and *E. coli* strains resulting in closure of wards [5, 6].

Several sequence types (STs) of MDR *K. pneumoniae*, such as ST14, 15, 16, 17, 23, 29, 101 and 147, are widely distributed across the globe [7, 8]. *E. coli* ST131 is notorious as a globally

disseminated cause of difficult to treat urinary tract infections (UTI) and sepsis [9]. *E. xiangfangensis* ST114, *E. hormaechei* subsp. *steigerwaltii* ST90 and ST93 and *E. cloacae* cluster III ST78 are widely disseminated, MDR strains of the ECC [2]. The presence of a variety of antimicrobial resistance (AMR) genes, conferring resistance to several antimicrobial classes, in combination with genes coding for a range of virulence factors, often carried on the same plasmid, enhance the pathogenic potential of these MDR clones [10, 11, 12].

K. pneumoniae serotypes K1, K2, O1, O2 and O3 and *E. coli* ST131, serotype O25:H4 are associated with high pathogenicity [13, 14]. However, the virulence correlates of MDR ECC have not been described in detail [2, 15].

The increasing ease of whole genome sequencing (WGS) has permitted detailed investigation of such strains in high resolution. This study aims to identify the STs, serotypes, AMR genes, virulence genes and associated mobile genetic elements (MGE) of six MDR *K. pneumoniae* strains, one *E. coli* strain and one ECC strain isolated from hospital acquired UTI (HA- UTI) in Sri Lanka using comparative genomics.

Results

STs and serotypes

The six *K. pneumoniae* isolates belonged to three STs; Kp2, Kp4 and Kp6 were ST147, Kp1 and Kp3 were ST16 and Kp5 was ST15, respectively. Multiple K and O loci were seen in these strains (Table 1). Esc was identified as *E.coli* ST131 with a predicted serotype of O25:H4. The single ECC isolate was identified as *E. hormaechei* subsp. *steigerwaltii* ST93. (Table 1).

AMR genes

A total of 37 AMR genes conferring resistance to different antimicrobial classes were identified with all isolates harboring genes coding for resistance to six or more antibiotic classes (Fig. 1).

Resistance to aminoglycosides [*aac(6')-Ib-cr*, *aph(3')-Ia*, *aac(3)-IIa*, *aph(3'')-Ib*, *aph(6)-Id*, *aac(3)-IId*, *aadA5*], β-lactams (*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{SHV-1}}$, *bla*$_{\text{SHV-11}}$, *bla*$_{\text{SHV-28}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{DHA-1}}$, *bla*$_{\text{ACT-7}}$, *bla*$_{\text{LAP-2}}$, *bla*$_{\text{OXA-181}}$, *bla*$_{\text{OXA-232}}$, *bla*$_{\text{NDM-4}}$), fosfomycin (*fosA*), macrolides [*erm(B), mph(A), mdh (A)*], chloramphenicol (*catA2, catB3*), sulphonamides (*sul1, sul2*), trimethoprim (*dfrA1, dfrA14, dfrA17*), quinolones [*aac(6')-Ib-cr, oqxA, qnrS1, qnrB4*], rifampicin (*ARR-3*) and tetracycline [*tet(A), tet(B), tet(D)*] was seen.

Kp3, Kp4 and Kp6 harbored *bla*$_{\text{OXA-181}}$ and Kp1, *bla*$_{\text{OXA-232}}$. The ECC strain carried both *bla*$_{\text{OXA-181}}$ and *bla*$_{\text{NDM-4}}$ together with the *ble* gene which encodes resistance to bleomycin. Kp5 showed a multiple ESBL / AmpC β-lactamase combination of *bla*$_{\text{CTX-M-15,}}$ *bla*$_{\text{SHV-28,}}$ *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{DHA-1.}}$ This isolate also carried the acquired narrow spectrum *bla*$_{\text{LAP-2}}$ gene. Kp2 and Esc carried only multiple ESBL genes (*bla*$_{\text{CTX-M-15,}}$ *bla*$_{\text{SHV-11}}$ and *bla*$_{\text{TEM-1B}}$ and *bla*$_{\text{CTX-M-}}$15, *bla*OXA$_{\text{-1}}$ and *bla*$_{\text{TEM-1B}}$, respectively) (Table 1, Fig. 1).

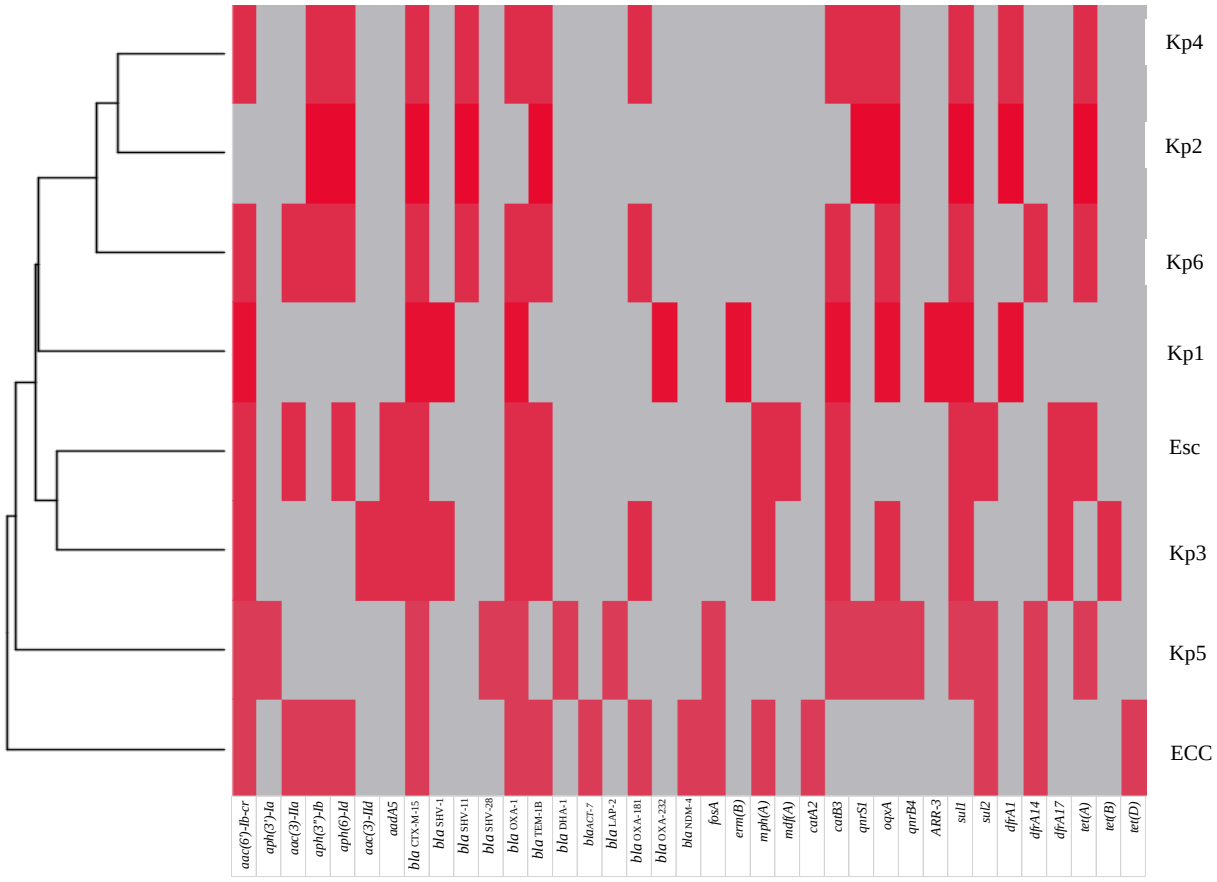


Fig. 1. The dendrogram and heat map generated using the presence and absence of AMR genes (red boxes indicate the presence of genes and grey boxes indicate the absence of genes).

Aminoglycoside resistance genes: *aac(6')-Ib-cr, aph(3')-Ia, aac(3)-IIa, aph(3'')-Ib, aph(6)-Id, aac(3)-IId, , aadA5*; β-lactam resistance genes:, $bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-1}}$, $bla_{\text{SHV-11}}$, $bla_{\text{SHV-28}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$, $bla_{\text{DHA-1}}$, $bla_{\text{ACT-7}}$, $bla_{\text{LAP-2}}$, $bla_{\text{OXA-181}}$, $bla_{\text{OXA-232}}$, $bla_{\text{NDM-4}}$; fosfomycin resistance genes: *fosA*; macrolide resistance genes: *erm(B), mph(A), mdh (A)*; phenicol resistance genes: *catA2, catB3*; quinolone resistance genes: *aac(6')-Ib-cr, oqxA, qnrS1, qnrB4*; rifampicin resistance genes: *ARR-3*; sulphonamide resistance genes: *sul1, sul2*; trimethoprim resistance genes: *dfrA1, dfrA14, dfrA17*; tetracycline resistance genes: *tet(A), tet(B), tet(D)*.

Virulence genes

Virulence genes coding for several functional proteins were identified (Fig. 2). Kp1-4 and Kp6 exhibited the yersiniabactin locus (*ybt*) encoding yersiniabactin siderophores while Kp5

manifested the *kfu* iron uptake system gene. While all *K. pneumoniae* strains harbored the Type 2 fimbrial gene cluster *mrk*, Kp1 and Kp3 showed additional *fim*A genes. The serum resistance-associated outer membrane lipoprotein *traT* gene was seen in *K. pneumoniae* Kp1 and Kp3 strains and in the *Esc* strain. Genes coding for adherence factors (*afa*, *iha*, *yfc*V), toxins (*sat*) and iron acquisition systems (*iut*A, *sit*A, *chu*A) and several other virulence genes were present in the *Esc* strain. The curli fiber gene cluster *csg*, efflux transporter periplasmic adaptor subunit *acr*A, bacteriocins gene cluster *ent* and Type VI secretion systems locus (T6SS) were found in the ECC strain (Table 1, Fig. 2).

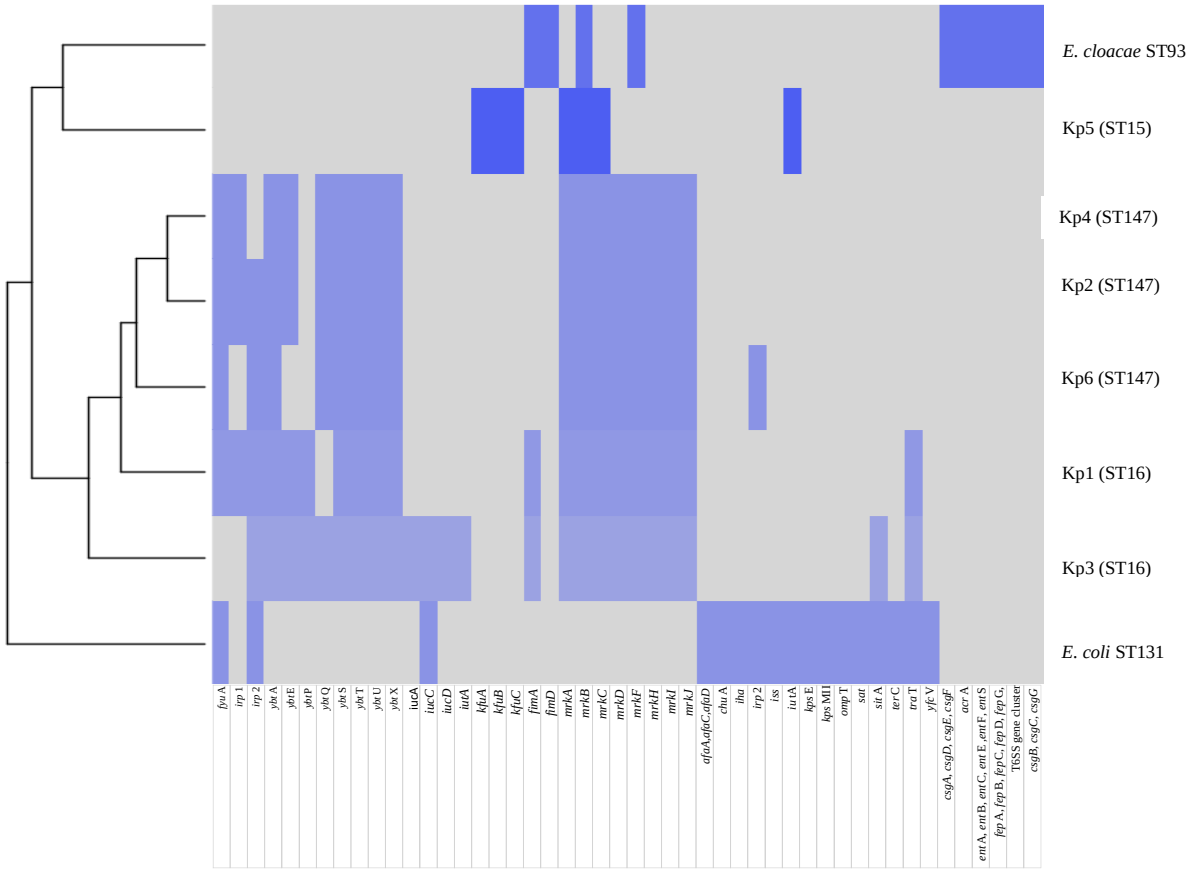


Fig.2. The dendrogram and heat map generated using the presence and absence of virulence genes (blue boxes indicate the presence of genes and grey boxes indicate the absence of genes).

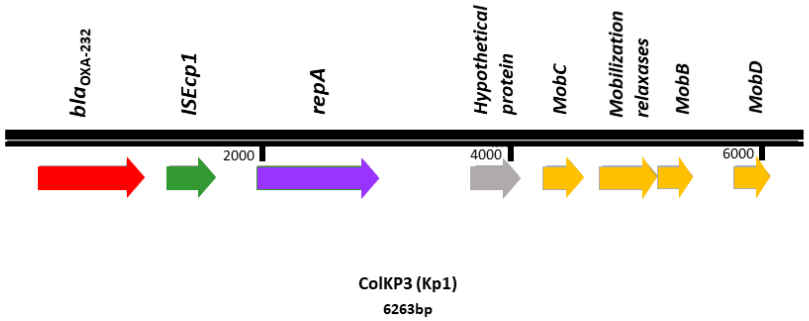
Siderophore genes: *fyu*A , *irp*1, *irp*2, *iuc*A, *iuc*D, *iut*A, *ybt*A, *ybt*E, *ybt*P, *ybt*Q, *ybt*S, *ybt*T, *ybt*U, *ybt*X, *chu*A, *fep*A; iron uptake genes: *kfu*A, *kfu*B, *kfu*C, *irp*, sitA; fimbrial adherence determinant genes: *fimA*, *fimD*, *mrk*A, *mrk*B, *mrk*C, *mrk*D, *mrk*F, *mrk*H, *mrk*I, *mrk*J, *afa*A, *afa*C, *afa*D, *iha*, yfcV; serum survival gene: *iss*; capsular polysaccharide gene: *kps*; outer membrane proteas: *omp*T; secreted autotransporter toxin: *sat*; tellurium ion resistance protein: *ter*C; serum resistance-associated outer membrane lipoprotein: *tra*T; Curli fiber gene: *csg*A, *csg*B, *csg*C, *csg*D, *csg*E, *csg*F, *csg*G; efflux transporter periplasmic adaptor subunit: *acr*A; bacteriocins genes: *ent*A, *ent*B, *ent*C, *ent*E,*ent*F, *ent*F; Type VI secretion systems locus: T6SS.

AMR and virulence genes associated MGE

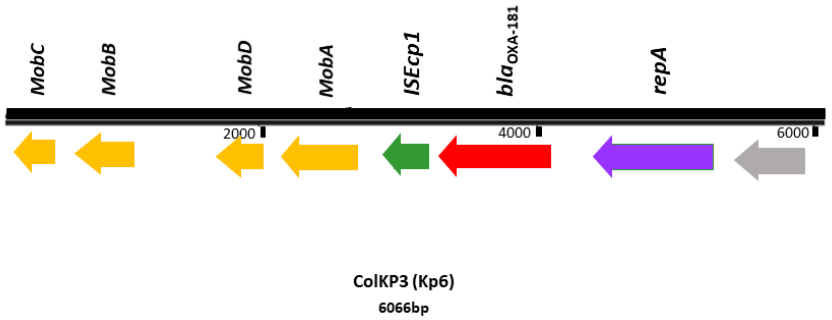
Several AMR genes and virulence genes were associated with MGE (Table 2). In all isolates IS*Ecp*1 flanked upstream of $bla_{\text{CTX-M-15}}$. In Kp1, Kp3 and Kp6, ColKP3(size ~ 6.1kb) plasmids carried the $bla_{\text{OXA-48-like}}$ genes flanked by IS*Ecp*1(IS1380 family) (Fig. 3). In Kp2 and Kp4, IncR plasmids (size ~ 31kb and ~ 42kb) carried *sul1 and tet(A)* flanked by IS6100 (Table 2). In Kp1, Kp3 and Esc, IncF plasmids (size ~ 59kb) carried virulence genes (eg. *tra*T, *sit*A). In Kp1 and Esc strain the IncF plasmid (size ~ 65kb) carried both an AMR gene ($bla_{\text{TEM-1B}}$) and a virulence gene (*tra*T) (Fig. 3). Clusters of AMR genes, encoding resistance to multiple antimicrobial classes associated with MGE were identified in seven of the eight strains (Kp1, Kp3-Kp6, Esc, ECC) (Fig. 4).

Table 2 MGE associated with AMR and virulence genes

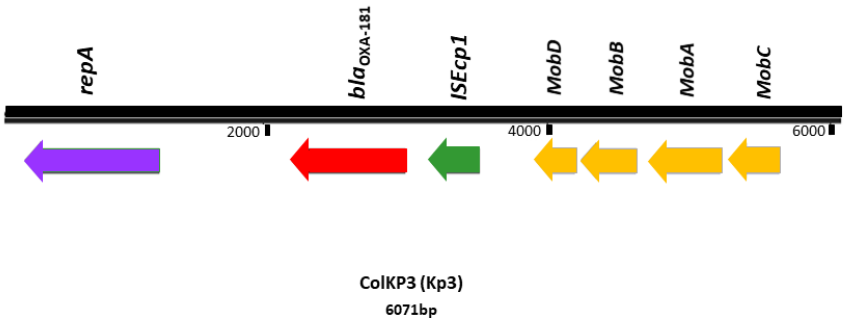
(a)


blaOXA-232
ISEcp1
repA
Hypothetical protein
MobC
Mobilization relaxases
MobB
MobD
2000
4000
6000
ColKP3 (Kp1)
6263bp

(b)


MobC
MobB
MobD
MobA
ISEcp1
blaOXA-181
repA
2000
4000
6000
ColKP3 (Kp6)
6066bp

(c)


repA
blaOXA-181
ISEcp1
MobD
MobB
MobA
MobC
2000
4000
6000
ColKP3 (Kp3)
6071bp

(d)

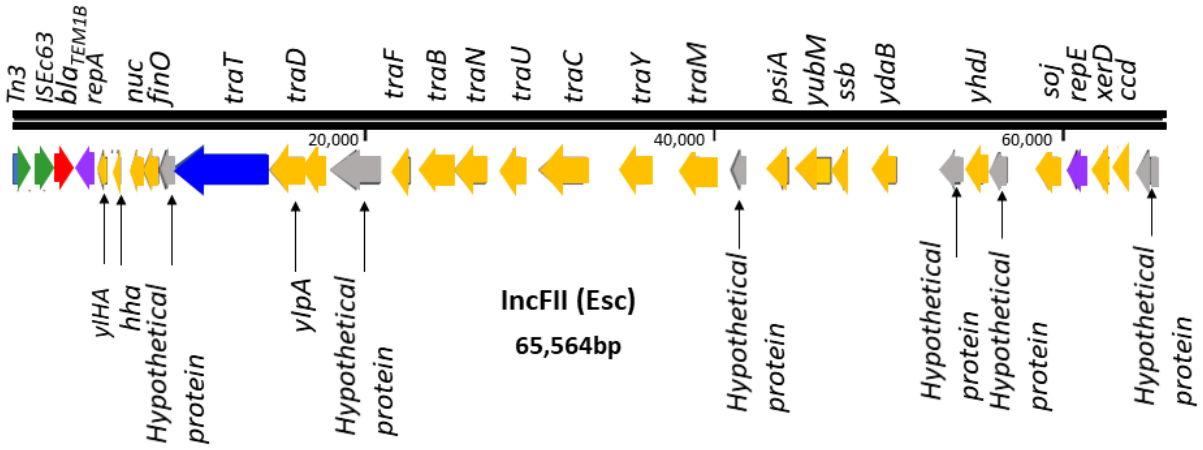
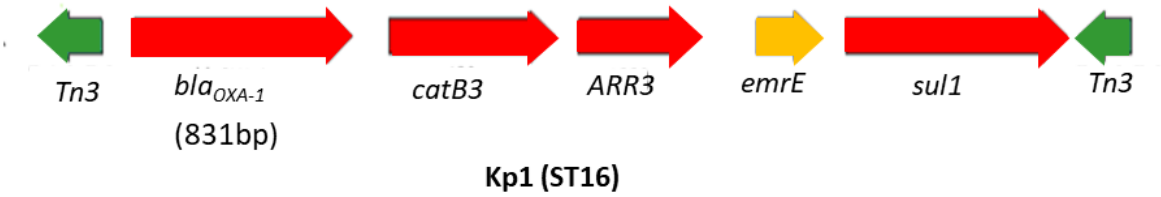


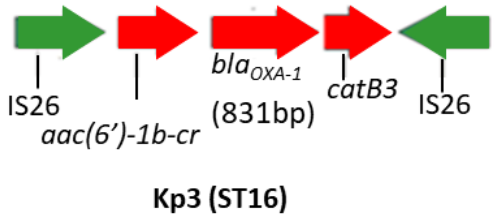
Fig. 3. Plasmid maps of AMR plasmids identified in *K. pneumoniae*.

ColKP3 plasmids; (a), ColKP3 carrying $bla_{\text{OXA-232}}$ in Kp1; (b), ColKP3 carrying $bla_{\text{OXA-181}}$ in Kp3; (c), ColKP3 carrying $bla_{\text{OXA-181}}$ in Kp6. (d), IncF plasmid carrying $bla_{\text{TEM-1B}}$ AMR gene (red) and *tra*T virulence gene (blue)

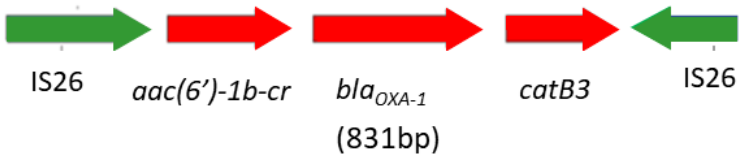
(a)



(b)



(c)

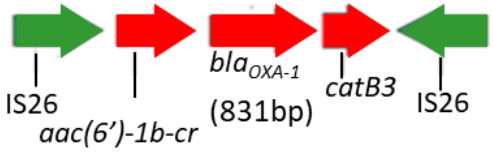


Kp4 (ST147)

(d)

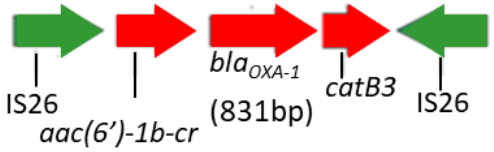
Kp5 (ST15)

(e)



Kp6 (ST147)

(f)



E.coli **(ST131)**

(g)

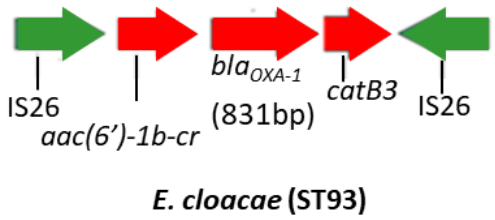


Fig. 4. Clusters of AMR genes encoding resistance to multiple antimicrobial classes (*aac*(6')-Ib-cr, ARR3, *bla*OXA-1, *cat*B3 and *sul*), associated with MGE .

(a)-(e), clusters of AMR genes associated with MGE in *K. pneumoniae*; (f), cluster of AMR genes associated with MGE in *E. coli*; (g), cluster of AMR genes associated with MGE in *E. cloacae*.

Discussion

We studied six MDR *K. pneumoniae* (Kp1-Kp6), one *E. coli* (Esc) and one *Enterobacter cloacae* (ECC) strain from a collection of 120 uropathogenic *Enterobactericeae* isolated from HA-UTI in two hospitals in the Western Province of Sri Lanka during 2015–2016.

While *K. pneumoniae* ST15 and ST147 have been reported in Sri Lanka previously [16,17], this paper describes the isolation of *K. pneumoniae* ST16, *E. coli* ST131 and *E. hormaechei* subsp. *steigerwaltii* ST93 for the first time in Sri Lanka. These STs are globally successful clones, known to disseminate multidrug resistance and associated with hospital outbreaks in many countries [2, 7, 9].

The isolates harboured genes coding for resistance to 6 or more antibiotic classes exemplifying co-selection of resistance genes. The co-occurrence of multiple AMR genes in Sri Lanka has been reported previously only in *K. pneumoniae* [17]. This paper broadens this to include both *E. coli* and *E. hormaechei.* Although all the isolates were carbapenem resistant by disc diffusion, three

strains, Kp2, Kp5 and Esc, did not carry any known carbapenemase genes. The combination of multiple Amp C / ESBL production and other resistance mechanisms may have resulted in phenotypic carbapenem resistance in these isolates [18]. The *bla*$_{NDM}$ gene in the ECC strain was found together with the *ble* gene which confers resistance to bleomycin. This association has been identified regularly in *bla*$_{NDM}$ producing isolates of *Enterobacterales* [19, 20]. Both the *bla*$_{NDM}$ and the *ble* gene are believed to have originated from the same progenitor [19]. In Kp5, *bla*$_{LAP-2}$ and *qnr*S1, which are known to co-exist, were found together, flanked by IS*Ecl*. The *bla*$_{LAP-2}$ gene has been found on unknown plasmids in *Enterobacterales* from China, Tunisia, Norway and the Netherlands and its close association with *qnr*S1 flanked by IS*Ecl* has been reported [21]. However, in our study we could not identify any plasmid associated with these genes.

The *K. pneumoniae* strains harbored fimbrial genes (*fim, mrk*) that play a role in the pathogenesis of UTI [22]. The type 3 fimbrial adhesins gene cluster *mrk,* which is associated with biofilm formation, was present in all the *K. pneumoniae* strains. In Kp1, Kp3 and Esc the serum resistance-associated outer membrane lipoprotein (*tra*T) which has a predominant role in pathogenicity was identified. Several extraintestinal pathogenic *Escherichia coli* (ExPEC) associated virulence genes (*afa*A, *afa*C, *afa*D, *chu*A, *irp*2, *iut*A, *kps*M, *omp*T, *sit*A, *ter*C , *tra*T, and *yfc*V) previously described in highly pathogenic *E.coli* isolated from hospital-acquired infections in different countries were seen in Esc [22]. The virulence genes *iha* and *iss* which are frequently reported in *E. coli* ST131 were also present in our isolate [14]. Overexpression of the efflux pump gene *acr*A has been observed to increase antibiotic resistance and virulence in *E. cloacae* [23]. The T6SS gene cluster coding for the type VI secretory system, which confers the ability to survive in a range of environments, was seen in the ECC strain [15].

The *bla*$_{\text{CTX-M-15}}$ gene in all the isolates had the international IS*Ecp*1-*bla*$_{\text{CTX-M}}$ type genetic context [24]. *Bla*$_{\text{OXA48-like}}$ carbapenemase genes (*bla*$_{\text{OXA-181}}$ and *bla*$_{\text{OXA-232}}$) in three of our isolates (Kp1, Kp3 and Kp6) were carried by ColKP3 plasmids and were associated with IS*Ecp*1. This association has been reported previously both globally and locally [17, 25, 26]. Although plasmids of the IncF group are some of the most frequent resistance plasmids found in *Enterobacterales*, none of the *K. pneumoniae* strains in our study carried AMR genes on IncF plasmid replicons [27]. However, these plasmids carried virulence genes such as *tra*T and *sit*A in some strains (Kp1 and Kp3). In Esc an AMR gene (*bla*$_{\text{TEM-1B}}$) and a virulence gene (*tra*T) were found in combination on an IncF plasmid replicon. High rates of IncF plasmids co-harboring resistant and virulence genes have been reported in China [28]. In Kp2, Kp4 and Kp6 the AMR genes present on IncR plasmid replicons (*sul1*, *tetB*) were associated with insertion sequence IS6100. Although IncR plasmids are believed to be nontransferable, association with such transposable elements may allow the spread of these genes [29].

We found that AMR gene clusters of 3 to 4 genes conferring resistance to multiple antimicrobial classes were flanked by mobile elements in almost all the strains which may indicate co-transmission of these genes. The most common AMR genes located in these clusters were *aac(6')-Ib-cr*, *bla*$_{\text{OXA-1}}$ and *catB3* flanked by IS26 (composite transposon). This cassette is widely distributed in *Enterobacterales* in China [30].

Conclusion

The concomitant presence of resistance against multiple antibiotics and virulence factors facilitates bacterial pathogenicity in hospital-acquired infections. We studied eight MDR uropathogenic *Enterobacterales* isolated from HA-UTI in Sri Lanka using genomic sequencing and comparative

genome analysis. A diverse variety of antibiotic resistance genes, virulence genes and associated MGE were identified that indicate the presence of highly adapted strains of MDR *Enterobacterales* in the hospital setting in Sri Lanka.

Methodology

Study setting

Eight *Enterobacterales* strains found to be multidrug resistant by disc diffusion [31] were selected from a collection of 120 uropathogenic *Enterobacterales* from HA-UTI from two tertiary care hospitals, Sri Jayawardenepura General Hospital (SJGH) and Neville Fernando Teaching Hospital (NFTH), in the Western Province of Sri Lanka in 2015/2016. Six *K. pneumoniae* strains (Kp1-Kp6), one *E. coli* strain (Esc) and one *Enterobacter cloacae* strain (ECC) were included in the study. (Table 1).

Table 1 Clinical data and characteristics of strains under study

WGS and comparative genome analysis

WGS was performed by the MicrobesNG service (http://www.microbesng.uk). Comparative genome analysis of WGS data was done using bioinformatics tools. The species of the ECC strain and the STs of the isolates were identified by the MLST tool provided by the Centre for Genomic Epidemiology (CGE) [32] and the Institut Pasteur BIGSdb database [33, 34]. Capsule synthesis (K) and lipopolysaccharide (O) loci of *K. pneumoniae* were determined using Kaptive2 [35]. *E. coli* serotype was determined using SerotypeFinder 2.0 [36] and the FimH type was identified by FimTyper 1.0 [37].

The presence of virulence genes in the *K. pneumoniae* isolates was investigated using the Institut Pasteur BIGSdb database [33, 34]. Virulence genes of *E. coli* were investigated using virulencefinder 2.0 at Center for Genomic Epidemiology (CGE) [37] and the virulence genes of ECC were queried from the virulence factor database (VFDB) using reference genomes [38]. The National Center for Biotechnology Information (NCBI) GenBank Basic Local Alignment Search Tool (BLAST) tool [39] was used to find the corresponding regions with the highest similarity scores (100% coverage, 99% identity) to confirm identification. Antibiotic resistance genes were recognized using Resfinder 2.1 provided by the CGE [40].

MGE flanking AMR genes and virulence genes were identified by MobileElementFinder [41]. Plasmid replicons and MGE were further identified by PlasmidFinder 1.3 [42] and by ISFinder [43]. The NCBI GenBank BLAST tool [39] was used to confirm the corresponding regions with the highest similarity scores (100% coverage, 99% identity) [44]. Schematic maps of the plasmids and the flanking regions of the *bla* genes were created using snapgene.

Whole-genome sequences of the six Sri Lankan *Enterobacterales* strains have been submitted to the GenBank at National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA717825. The genome sequence accession numbers are JAGKSN000000000, JAGKSM000000000, JAGRPG000000000, JAGKSL000000000, JAGKSK000000000, JAGKSJ000000000, JAGKSH000000000 and JAGKSI000000000

Abbreviations

AMR: Antimicrobial resistance

BLAST: Basic Local Alignment Search Tool

CGE: Center for Genomic Epidemiology

ECC: *Enterobacter cloacae* complex

ESBL: Extended spectrum β-lactamase

Esc: *Escherichia coli*

HA-UTI: Hospital acquired urinary tract infections

K: Capsule synthesis loci

Kp: *Klebsiella pneumoniae*

MDR: Multidrug resistant

MGE: Mobile genetic elements

NCBI: National Center for Biotechnology Information

NFTH: Neville Fernando Teaching Hospital

O: Lipopolysaccharide loci

SJGH: Sri Jayawardenepura General Hospital

ST: Sequence types

UTI: Urinary tract infections

VFDB: Virulence factor database

WGS: Whole genome sequencing

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Ethics Review Committee of the University of Colombo, Faculty of Medicine [EC-14-143].

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the GenBank at National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA717825, the genome sequence accession numbers are JAGKSN000000000, JAGKSM000000000, JAGRPG000000000, JAGKSL000000000, JAGKSK000000000, JAGKSJ000000000, JAGKSH000000000 and JAGKSI000000000

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

VP, EC, KJ and ND designed the study. VP, SG and SD collected samples, the laboratory analyses and the phenotypic characterization and antimicrobial susceptibility testing .VP, VE and AA carried out WGS analysis. VP drafted the manuscript. All authors read, critically reviewed and approved the final manuscript.

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Authors' information (optional)

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Tables

Table 1 Clinical data and characteristics of strains under study

				Molecular typing					**Resistance Profile CLSI disc diffusion**										
Strain ID	**Hospital**	**Unit**	**Sample**	**Date**	**ST**	***Serotypes***	**AMR genes**	**Virulence genes**	**CPD**	**CAZ**	**CTX**	**CRO**	**AZM**	**FEP**	**FOX**	**CTT**	**IPM**	**EMM**	**ETP**
Kp1	SJGH	MW	Urine	Oct-2015	ST16	*K15, K17, K51, K52 O3b*	*bla*$_{CTX-M-15,}$ *bla*$_{SHV-1,}$ *bla*$_{OXA-1,}$ *bla*$_{OXA-232,}$ *aac(6')-Ib-cr, erm(B), catB3, aac(6')-Ib-cr, oqxA, ARR-3, sul, dfrA1*	*fyuA, irp1, irp2, ybtA, ybtE ybtP, ybtS, ybtT, ybtU, ybtX, traT, fimA, mrkA,mrkB, mrkC,mrkD ,mrkF,mrkH , mrkI, mrkJ*	R	R	R	R	R	R	R	R	R	R	R
Kp2	SJGH	ICU	Urine	Oct-2015	ST147	*K64 O2v1*	*bla*$_{CTX-M-15,}$ *bla*$_{SHV-11,}$ *bla*$_{TEM-1B,}$ *aph (6)-Id, aph (3'')-Ib, qnrS1, oqxA, sul1, dfrA1, tet(A)*	*fyuA, irp1, irp2, ybtA, ybtE, ybtQ, ybtS, ybtT, ybtU, ybtX, mrkA,mrkB, mrkC,mrkD mrkF,mrkH, mrkI, mrkJ*	R	R	R	R	R	R	R	R	R	R	R
Kp3	NFTH	MW	Urine	Oct-2015	ST16	*K15, K17, K51, K52 O3b*	*bla*$_{CTX-M-15,}$ *bla*$_{SHV-1,}$ *bla*$_{OXA-1,}$ *bla*$_{TEM-1B,}$ *bla*$_{OXA-181,}$ *aac (6')-Ib-cr, aac (3)-IId aadA5,mph(A)*	*irp2, iucA, iucC, iucD, iutA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, traT*	R	R	R	R	R	R	R	R	R	R	R

							,catB3,oqxA, sul1, dfrA17, tet(B)	*fimA, mrkA, mrkB,mrkC, mrkD,mrkF, mrkH, mrkI, mrkJ*													
Kp4	NFTH	MW	Urine	Oct-2015	ST147	*K64 O2v1*	*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{SHV-11}}$ *bla*$_{\text{OXA-1}}$, *bla*$_{\text{TEM-1B}}$ *bla*$_{\text{OXA-181}}$ *aac(6')-Ib-cr, aph(3')-Ib, aph(6)-Id, catB3, oqxA, qnrS1, sul1, dfrA1, tet(A)*	*fyuA , irp1, ybtA, ybtE, ybtQ, ybtS, ybtT, ybtU, ybtX,mrkA, mrkB,mrkC, mrkD,mrkF, mrkH, mrkI, mrkJ*	R	R	R	R	R	R	R	R	R	R	R
Kp5	SJGH	MW	Urine	Nov-2015	ST15	*K112 O1v1*	*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{SHV-28}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{DHA-1}}$, *bla*$_{\text{LAP-2}}$ *aac(6')-Ib-cr, aph(3')-Ia, fosA, catB3, oqxA, qnrS, qnrB4, sul1, sul2, dfrA14, tet(A)*	*kfuA, kfuB, kfuC,mrkA, mrkB, mrkC*	R	R	R	R	R	R	R	R	I	S	S
Kp6	SJGH	ICU	Urine	Nov-2015	ST147	*K64 O2v1*	*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{SHV-11}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{OXA-181}}$, *aac(6')-Ib-cr, aac(3)-IIa, aph(3'')-Ib, aph(6)-Id, catB3, oqxA, qnrS1, sul1, dfrA14, tet(A)*	*fyuA , irp2, ybtA, ybtQ, ybtS, ybtT, ybtU, ybtX mrkA,mrkB, mrkC,mrkD ,mrkF,mrkH ,mrkI, mrkJ*	R	R	R	R	R	R	R	R	R	R	R

Esc	NFTH	MW	Urine	Oct-2015	ST131	*O25:H4*	*bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *bla*$_{OXA-1}$, *aac(6')-Ib-cr*, *aph(6)-Id*, *aac(3)-IIa*, *aadA5*, *mph(A)*, *mdf(A)*, *catB3*, *sul1*, *sul2*, *dfrA17*,*tet(A)*	*afaA*,*afaC*, *afaD*,*chuA*, *fyuA*,*iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII*, *ompT*, *sat*, *sitA*,*terC*, *traT*, *yfcV*	R	R	R	R	R	R	R	R	S	S	R
ECC	SJGH	ICU	Urine	Oct-2015	ST93	-	*bla*$_{ACT-7}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *bla*$_{OXA-1}$, *bla*$_{NDM-4}$, *bla*$_{OXA-181}$, *aac(3)-IIa*, *aac(6')-Ib-c*, *aph(3'')-Ib*, *aph(6)-Id*, *fosA*, *mph(A)*, *aac(6')-Ib-cr*, *sul2*, *dfrA14*, *catA2*,*tet(D)*	*mrkB*, *mrkF*, *fimA*, *fimD*, *csgA*, *csgD*, *csgE*, *csgF*, *acrA*, *entA*, *entB*, *entC*, *entE* ,*entF*, *entS* *fepA*, *fepB*, *fepC*, *fepD*, *fepG*, *T6SS gene cluster*,*csgB* , *csgC*, *csgG*	R	R	R	R	R	R	R	R	R	R	R

MW, Medical Ward; ICU, intensive care unit; R, resistant; I, intermediate; S, susceptible; CPD, cefpodoxime; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; AZM, aztreonam; FEP, cefepime; FOX, cefoxitin; CTT, cefotetan; IPM, imipenem; MEM, meropenem; ETP, ertapenem

Table 2 MGE associated with AMR and virulence genes

	AMR gene	**Virulence Gene**	**Plasmid replicons**	**MGE**
Kp1 (ST16)	*erm*(B)			IS26-IS26 composite transposon
	$bla_{\text{CTX-M-15}}$			ISEcp1, Tn3-like transposon
	$bla_{\text{OXA-1-}}$ *catB3- ARR3- emrE- sul*1			Tn3-like transposon
	$bla_{\text{OXA-232}}$		ColKP3	ISEcp1
		traT	IncFII(K)	
Kp2 (ST147)	$bla_{\text{CTX-M-15}}$			ISEcp1, Tn3-like transposon
	*sul*1		IncR	IS6100
		fyuA		ISEc33
Kp3 (ST16)	$bla_{\text{CTX-M-15}}$			ISEcp1, Tn3-like transposon
	aac(6')-Ib-cr - $bla_{\text{OXA-1}}$ - *cat*B3			ISEc9, IS26-IS26 composite transposon

	bla$_{TEM-1B}$	*traT*	IncFII	
	bla$_{OXA-181}$		ColKP3	ISEcp1
		sitA	IncFIA	ISKpn8
Kp4 (ST147)	*bla*$_{CTX-M-15}$			ISEcp1, Tn3-like transposon
	aac(6')-Ib-cr - *bla*$_{OXA-1}$ - *cat*B3			IS26-IS26 composite transposon
	tet(A)		IncR	IS6100
Kp5 (ST15)	blaCTX-M-15	*iutA*		ISEcp1, Tn3-like transposon
	aac(6')-Ib-cr - *bla*$_{OXA-1}$- *catB- sul*			ISEc33, ISVsa3
	aph(3')-Ia			IS26-IS26 composite transposon
	*qnrS*1			ISKpn19, ISEcl1
	bla$_{Lap-2}$			ISEcl1

Kp6 (ST147)	*bla*$_{\text{CTX-M-15}}$			ISEcp1, Tn3-like transposon
	aac(6')-Ib-cr - *bla*$_{\text{OXA-1}}$ - catB3			IS26-IS26 composite transposon
	tet(A)			IS6100
	bla$_{\text{TEM-1B}}$			ISKpn19
		irp2		ISEc33
	bla$_{\text{OXA-181}}$		ColKP3	ISEcp1
Esc	*bla*$_{\text{CTX-M-15}}$			ISEcp1, Tn3-like transposon
	aac(6')-Ib-cr - *bla*$_{\text{OXA-1}}$ - catB3			IS26-IS26 composite transposon
	*dfr*A17			IS6100
	mdf(A)	*ompT*		MITEEc1/ IS630
	tet(A)	*sitA*		Tn5403, Unit transposon

	bla$_{TEM-1B}$	*traT*	IncFII	ISEc63, Tn3-like transposon
		iutA		IS30, IS629
		fyuA		ISEc38
		yfcV		MITEEc1/ IS630
		kpsMII		IS4
		afaC		IS640/ IS21
		Usp		ISEc53 & ISEc52
		terC		MITEEc1/ IS630
ECC	*bla*$_{CTX-M-15}$			ISEcp1, Tn3-like transposon
	aac(6')-Ib-cr - *bla*$_{OXA-1}$ - *cat*B3			IS26-IS26 composite transposon
	*sul*2			IS5075/ IS110
	bla$_{NDM-4}$ - *ble*			IS5