Genomic insights into the mechanism of carbapenem resistance dissemination in Enterobacterales from a tertiary public heath setting in South Asia

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Running title: CRE in a clinical setting of Bangladesh

Summary: 10.6% patients were CRE-positive. Only 27% patients were prescribed at least one antibiotic to which infecting pathogen was susceptible. Several clinically important antibiotics exposures was the risk for CRE acquisition. *E. coli* ST167 was the dominant CRE clone.

ABSTRACT

Background

Given the high prevalence of multidrug resistance (MDR) across South Asian (SA) hospitals, we documented the epidemiology of carbapenem resistant Enterobacterales (CRE) infections at Dhaka Medical College Hospital between October 2016 to September 2017.

Methods

We enrolled patients and collected epidemiology and outcome data. All Enterobacterales were characterised phenotypically and by whole genome sequencing. Risk assessment for the patients with CRE were performed compared to patients with carbapenem-susceptible Enterobacterales (CSE).

Results

10.6% of all 1831 patients with a clinical specimen collected had CRE. In-hospital 30-day mortality was significantly higher with CRE [50/180 (27.8%)] than CSE [42/312 (13.5%)] (p=0.001); however, for blood-stream infections, this was insignificant. Out of 643 Enterobacterales isolated, 210 were CRE. bla_{NDM} was present in 180 isolates, $bla_{\text{OXA-232}}$ in 26, $bla_{\text{OXA-181}}$ in 24 and $bla_{\text{KPC-2}}$ in 5. Despite this, ceftriaxone was the most commonly prescribed empirical antibiotic and only 27% patients were prescribed at least one antibiotic to which their infecting pathogen was susceptible. Significant risk factors for CRE isolation included burns unit and ICU admission, and prior exposure to levofloxacin, amikacin, clindamycin and meropenem. *E. coli* ST167 was the dominant CRE clone. Clustering suggested clonal transmission of *K. pneumoniae* ST15 and the MDR hyper-virulent clone, ST23. The major trajectories involved in horizontal gene transfer were IncFII and IncX3, IS*26*, and Tn*3*.

Conclusion

This is the largest study from a SA public hospital combining outcome, microbiology and genomics. The findings indicate the urgent implementation of targeted diagnostics, appropriate antibiotic use and infection control interventions in SA public institutions.

Keywords: Carbapenem-resistant Enterobacterales, nosocomial infections, outbreak, plasmid-mediated resistance, Bangladesh, South Asia

Introduction

Carbapenem-resistant Enterobacterales (CRE) are one of the World Health Organisation (WHO) listed critical priority pathogens [1]. The emergence and spread of CRE in a clinical setting drastically limit therapeutic options increasing mortality and morbidity [2-4]. Whilst the clonal expansion of multidrug resistant (MDR) pathogens in nosocomial infections frequently occurs in settings with poor infection prevention and control (IPC) policies, horizontal gene transfer (HGT) plays a pivotal role in the spread of antimicrobial resistance (AMR) [3,5].

Several studies have documented the burden of carbapenem resistance in healthcare associated infections in South Asia (SA) [6-8] and the genes, bla_{NDM} and $bla_{\text{OXA-181}}$ have been shown to be the predominant mechanisms of carbapenem resistance in the region [9-11]. However, there are significant data gaps and lack of antimicrobial resistance (AMR) surveillance programs in SA (Supplementary Table 1) [6-8,12-13]. None of the previous studies in SA have described the impact, burden, and transmission dynamics of CRE by combining epidemiological, clinical, and genomic data (Supplementary Table 2).

This study was designed to better understand the molecular epidemiology of carbapenem resistance mechanism at Dhaka Medical College Hospital (DMCH). The combination of whole genome-based analysis with rigorous epidemiological data provides a powerful spatiotemporal assessment to explore the mechanisms and drivers of AMR in a Bangladeshi hospital setting.

Methods

Study design, hospital setting, participants, and sampling

We performed an observational cohort study at DMCH, the largest public hospital setting of Bangladesh containing 2600 allocated beds, from October 2016 to September 2017. This study was approved by the Ethical Review Committee of DMCH (Supplementary methods) [14].

Specimens referred to the DMCH microbiology laboratory for culture and sensitivity based on local physicians' clinical judgement on suspected infections were included [15]. Participants' demography and clinical information were recorded (Supplementary methods). All isolates recovered at the DMCH microbiology laboratory were transferred to Cardiff University and investigated further (n=643) (Figure 1). Patients were enrolled for this study if at least one of their specimens was positive for Enterobacterales.

Case definition

Isolates were categorised as carbapenem-susceptible Enterobacterales (CSE) if sensitive to both imipenem and meropenem and CRE if resistant or increased exposure to either. For *Proteus*, *Providencia* and *Morganella*, imipenem was excluded from the definitions because of intrinsic resistance [16]. The participants, with at least one positive culture of CRE were considered as CRE cases. Any patient with a positive CSE culture was regarded as a CSE case.

Phenotypic characterization of Enterobacterales

Blood specimens were cultured using BacT/ALERT 3D (bioMerieux, North Carolina, USA) at DMCH. Isolates were sub-cultured onto chromogenic UTI agar (E&O Laboratories Ltd, Scotland, UK). The species were identified by Matrix-Assisted Laser Desorption/Ionization-

Time of Flight mass spectrometry (Bruker Daltonics, Bremen, Germany). Minimum inhibitory concentrations (MIC) to clinically relevant antimicrobials were determined by agar dilution and interpreted according to European Committee on Antimicrobial Susceptibility Testing breakpoints (v10.0) [17-18].

Whole-genome sequencing (WGS)

All Enteribacterales isolated were sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA) and a set of $bla_{\text{NDM-positive}}$ isolates by minION sequencing (Oxford Nanopore technologies, Oxford, UK). Details of the bioinformatics analysis are described in Supplementary Methods. Briefly, Kmer database (v3.0.2) [available in Center for Genomic Epidemiology (CGE)] was deployed for species identification, the Clermont *Escherichia coli* phylotyping (v1.4.0), Kaptive (v0.7.3) for *Klebsiella pneumoniae* capsular typing. Comprehensive Antibiotic Resistance Database (CARD) and PlasmidFinder were deployed for antimicrobial resistance genes (ARGs) and plasmid replicon types with a cut off of ≥95% coverage and ≥95% identity, respectively using ABRicate (v0.9.7). Multilocus sequence type (MLST) was assigned based on seven loci MLST databases in CGE (v2.0.0), where appropriate. Time-calibrated evolutionary analysis was performed using the BEAST package (v1.10.4) to estimate the date of the most recent common ancestor (MRCA).

Scrutinizing 'high-risk' clones for carbapenem resistance in Bangladeshi hospital

The clonal relatedness of isolates was assessed using core-genome alignment following clustering for the presence and absence of genes, MLST profiling and pairwise SNP distances. A cut-off of ≤10 SNPs combining with epidemiological information was used to define possible

clonal transmission clusters using the R library iGRAPH [19]. Clusters were removed if they did not contain any carbapenemase producer.

Statistical analysis

Binary logistic regression was used to analyse the association of CRE with categorical variables of interest. The *p* values were adjusted by the Benjamini-Hochberg procedure when repetitive variable types were tested. A Cox proportional hazards model was used to compare all-cause in-hospital 30-day mortality between CRE and CSE cases. Patients discharged alive or with an in-hospital mortality over 30 days were used as the competing variable for outcome analysis. Statistical analyses were conducted using IBM SPSS (v26) and Tableau (v2020.4).

Results

Prevalence of carbapenem resistance in clinical Enterobacterales

A total of 1893 clinical specimens from 1831 patients were included. 58% (1098/1893) of the specimens were culture positive and 1583 isolates were recovered (Supplementary Table 3-5). The proportion of Enterobacterales isolated was 33.9% (643/1893) and CRE comprised 11.1% (210/1893). The prevalence of CRE cases was 10.6% (194/1831) (Figure 1).

Out of 643 Enterobacterales, 210 were CRE (12.6% were recovered form wound swabs, 7.8% from urine and 7.5% from blood) and 433 were CSE (28.5% form wound swabs, 24.1% from urine and 10.1% from blood) (Figure 1) (Supplementary Table 4).

The predominant species were *E. coli* (226/1583, 14.3%), *K. pneumoniae* (221/1583, 14%), *Proteus mirabilis* (64/1583, 4%) and *Enterobacter cloacae* complex (39/1583, 2.5%) (Supplementary Table 5).

Antimicrobial's resistance profile

We found a high frequency of resistance in Enterobacterales against both β-lactam and non-β-lactam antibiotics except colistin and fosfomycin. Excluding intrinsically resistant species, 4.9% (31/636) was resistant to fosfomycin and 0.9% (5/534) to colistin (Figure 2). CRE exhibited significantly higher resistance rates to ciprofloxacin, levofloxacin, amikacin, gentamicin, and sulfamethoxazole-trimethoprim than CSE (p<0.0001) (Table 1).

Carbapenemases identified were bla_{NDM} (180/643, 28%) [predominantly $bla_{\text{NDM-5}}$ (97/643, 15.1)], $bla_{\text{OXA-232}}$ (26/643, 4%), $bla_{\text{OXA-181}}$ (24/643, 3.7%) and $bla_{\text{KPC-2}}$ (5/643, 0.8%) (Figure 3). A small number of phenotypically carbapenem susceptible isolates were positive for $bla_{\text{OXA-232}}$ (n=9) and $bla_{\text{OXA-181}}$ (n=1) (Supplementary Table 9).

The clinically important resistance genes, *aadA2*, *APH*, *armA*, $bla_{\text{CTX-M-15}}$, and $bla_{\text{TEM-1}}$ were associated with bla_{NDM}-positive isolates (p<0.05) (Table 1).

Risk and outcome analysis

Out of 534 clinical cases, 194 (36.3%) were CRE cases and 340 (63.7%) were CSE cases. Our data indicated that 94.1% (503/534) of the patients with Enterobacterales infections were treated with empirical antibiotics on admission to DMCH. Ceftriaxone was the most commonly prescribed antimicrobial (328/534, 61.4%) among the participants followed by metronidazole (159/534, 29.8%), ciprofloxacin (123/534, 23%) and amikacin (103/534, 19.3%). Carbapenem usage was 13.1% (meropenem) and 1.5% (imipenem).

Being part of the 6 to 25 years age group (p=0.041), being female (p=0.029), burns unit (p<0.0001) and ICU admission (p=0.001) and exposure to certain antibiotics [levofloxacin

(p<0.0001), amikacin (p=0.008), clindamycin (p=0.008) and meropenem (p=0.044)] were associated with increased risk of CRE infections. Statistical associations remained unchanged in the adjusted models (Table 2).

Excluding patients discharged against medical advice (DAMA), all-cause in-hospital 30-day mortality was significantly associated with CRE cases, occurring in 50/180 (27.8%) CRE and 42/312 (13.5%) CSE cases (p=0.001). Significant associations were also observed after adjusting with the confounders (Table 3). No significant association of mortality was observed with CRE for the cohort of patients with Enterobacterales bloodstream infections (BSIs) (Table 4).

Based on the available data, only 27% (144/534) of the patients were prescribed at least one antibiotic to which their infecting pathogens were susceptible (Supplementary Table 10), however, data was not available regarding dosage, duration and indication of antibiotics therapy.

'High-risk' clones for carbapenem resistance

E. coli

The prevalent sequence types (STs) among clinical *E. coli* included ST131 (23/226, 10.2%), ST405 (21/226, 9.3%), ST648 (21/226, 9.3%), ST410 (21/226, 9.3%) and ST167 (18/226, 8%) (Figure 4A). ST167 was significantly associated with carbapenem resistance (p=0.004). The majority of *E. coli* belonged to phylogroup A (53/226, 23.5%) and D (49/226, 21.7%) followed by others (Supplementary Table 11-12).

Bayesian phylogenetic analysis suggests that the populations of major *E. coli* clones had the MRCA between 1978 and 2007, including isolates from outside the hospital (taken from NCBI). Dates of MRCAs for hospital-only subclades ranged from 2004-2015 for ST167, 2008-2017 for

ST448, 2010-2013 for ST8346, 1990-1999 for ST405 and 2006-2013 for ST648 (Figure 5A, Supplementary Figure 2-5). The average median substitution rate of *E. coli* was 3.11 SNPs per genome/year (Supplementary Table 13).

K. pneumoniae

Predominant *K. pneumoniae* STs included ST23 (35/221, 15.8%) and ST15 (29/221, 13.1%) (Figure 4B). All isolates belonging to ST16 and ST515 were resistant to carbapenems. The prevalent clinical clone, ST23 having KL1 capsular type did not have a significant association with carbapenem resistance, however, 68.6% (24/35) of isolates of ST23 were found to be carbapenem resistant (OR: 0.478, 95% CI: 0.222-1.033) (Supplementary Table 14-15).

The dates for the MRCAs of *K. pneumoniae* including NCBI isolates were between 1932 and 1980. DMCH's subclades were emerged between 1998 and 2016 for ST15 and 2014 and 2017 for ST16 (Figure 5B, Supplementary Figure 5). The average median substitution rate was 2.22 SNPs per genome/year (Supplementary Table 13).

Clonal transmission of carbapenem resistance

We identified five clusters of *E. coli* (EC1, EC2 and EC4 to EC6), 12 of *K. pneumoniae* (KP1 to KP12) and one of *E. cloacae* complex (EnC1) using a 10 SNPs threshold between isolates with common carbapenemase alleles and none of cluster contained isolates outside DMCH included in our analysis (Figure 6). We found linkage between isolates in the clusters at ≤2 SNPs threshold using overlapping of patients' hospital stays and common wards of isolation. These connections were observed in the burns unit, fistula unit, ICU, NICU and urology. The largest cluster was KP12 (ST23) followed by KP8 (ST15) (Figure 6C). The dated phylogenetic tree of

major clones revealed that the putative transmission clusters were predicted to have been introduced into DMCH between 2013 and 2016 (Supplementary Table 13).

Investigating transmission of carbapenem resistance due to horizontal transfer of plasmids

A total of 125 isolates were characterized by hybrid assembly of short-reads and long-reads sequence data, yielding complete, circular plasmids harbouring *bla*$_{\text{NDM-5}}$ (n=74), *bla*$_{\text{NDM-1}}$ (n=37), *bla*$_{\text{NDM-7}}$ (n=6), *bla*$_{\text{NDM-4}}$ (n=3), *bla*$_{\text{OXA-232}}$ (n=7), and *bla*$_{\text{OXA-181}}$ (n=4). The major Inc types in association with different carbapenemase alleles were the following: IncFII (n=41), IncX3 (n=9) and IncFIA (n=9) in associations with *bla*$_{\text{NDM-5}}$, IncC (n=10) and IncFIB & IncHI1B (n=6) in associations with *bla*$_{\text{NDM-1}}$, IncX3 (n=6) in associations with *bla*$_{\text{NDM-7}}$, and ColKP3 (n=6) in associations with *bla*$_{\text{OXA-232}}$ (Table 5). Plasmids of different Inc types harbouring *bla*$_{\text{NDM}}$ typically carried multiple ARGs except IncX3 (Supplementary Figure 6). The clinically important ARGs in associations with *bla*$_{\text{NDM}}$-positive plasmids were: *bla*$_{\text{TEM-1}}$ (70/120, 58.3%) and *bla*$_{\text{CTX-M-15}}$ (21/120, 17.5%) for β-lactams; *aadA* (73/120, 60.8%), *rmt* (72/120, 60%), *armA* (19, 15.8%), *AAC(6')-Ib* (38/120, 31.7%), *APH(3'')* (20/120, 16.7%), *ANT(3'')-IIa* (5/120, 4.2%), *AAC(3)* (3/120, 2.5%), *APH(6)-Id* (3/120, 2.5%) for aminoglycosides; *sul* (93/120, 77.5%) for sulphonamides; *dfrA* (76/120, 63.3%) for trimethoprim; *qnrB* (8/120, 6.7%) and *qnrS1* (5/120, 4.2%) for quinolones.

To investigate the plasmid mediated dissemination of carbapenem resistance, plasmids characterized in this study were grouped based on common carbapenemase allele, common Inc type, similar molecular weight, and similarities at ≥99% identity with >80% coverage between plasmids at the nucleotide level. Accordingly, NDM-5-positive plasmids fell into 21 groups, NDM-1-positive plasmids into 21 groups, OXA-181-positive plasmids into 3 groups, OXA-232-positive plasmids into 2 groups, one group of NDM-7 and one group of NDM-4-positive

plasmids. Plasmid mediated horizontal transfer of carbapenem resistance were predicted for the groups designated as FII_N5_2 (n=7), FII_N5_3 (n=31), and X3_N5_1 (n=9), FIB&HI1B_N1_1 (n=6), FIB(pQil)_N1_1 (n=2), FIA_N1_1 (n=2), X3_N7_1 (n=6), A/C2_O181_1 (n=2) and ColKP3_O232_1 (n=6), based on the distribution of plasmids within a wide range of bacterial host (Table 5, Supplementary Figure 7-14).

Investigating possible role of mobile genetic elements in the spread of carbapenem resistance

Based on the variation of genes immediate to *bla*$_{\text{NDM-5}}$, plasmids were divided into nine groups, designated as N5G1 to N5G9 (Figure 7). A conserved region (incomplete IS*Aba125*, *bla*$_{\text{NDM-5}}$, *ble, trpF*, *dsbD*, IS*91*) was common across all plasmids harbouring *bla*$_{\text{NDM-5}}$ expect N5G4 (IncX3) and N5G6 (IncFIB(pQil)) which lacked IS*91*. The conserved region of *bla*$_{\text{NDM-5}}$ in association with complex class 1 integron [(*sul1-qacE-aadA-dfrA- intI*), or (*sul1-qacE-*Δmaturase-*aadA-dfrA- intI*)], flanked by intact IS*26* at both 3' and 5' end in same orientation was found among plasmids of N5G1 and N5G2 (Figure 7).

NDM-1-positive plasmids were divided into eight groups (designated as NIG1 to N1G8) based on the genetic environment around *bla*$_{\text{NDM-1}}$. The genetic structure, Tn*125* (*bla*$_{\text{NDM-1}}$-*ble-trpF-dsbD-cutA-groES-groEL*-IS*91*), bordered by intact IS*Aba125* at the upstream and downstream] was observed in plasmids of IncC (n=5) (Figure 8). The insertion sequence, IS*Aba125* was absent at the downstream of the plasmids of N1G2 to N1G8 and an incomplete IS*Aba125* was present at the upstream among plasmids of N1G3 to N1G8. Variation of genes in the conserved region was observed among the plasmids of N1G4 to N1G8. Plasmids of N1G4 were flanked by Tn*3* at both 3' and 5' ends in the same orientation (Figure 8).

Discussion

The recent article by Naghavi et al., provides a sobering analysis on the burden caused by common MDR/XDR infections and a warning, that as a global community, we are rapidly surrendering any advantage we had on treating infections such as pneumonia and sepsis. Furthermore, Naghavi et al., highlights significant gaps, not least from LMICs and advocates the acute need for large LMIC clinical studies that combines detailed microbiology (and genomics) with recorded outcome data [20]. In this study, we present the largest dataset comprising clinical outcome, microbiology and genomic data from SA and in particular, based in a large public hospital where such datasets capture different socio-economic cohorts to previous studies (Supplementary Table 2). Public hospitals in SA are grossly oversubscribed (typically 4-5 times the #inpatient/bed), antibiotics are delivered empirically and a limited number of clinical specimens are only sent for culture sensitivity (Supplementary Table 16) [21-22]. The problem of AMR is considerably higher in health settings with unsubscribed antimicrobial usage [23]. Our data revealed that bla_{NDM}, $bla_{\text{OXA-181}}$/$bla_{\text{OXA-232}}$ and $bla_{\text{KPC-2}}$ were main carbapenem resistance determinants in CRE in Bangladesh. NDM-positive plasmids except IncX3 commonly coharboured multiple ARGs (Supplementary Figure 6). The association of CRE acquisition with the usage of multiple antibiotic classes may be explained by co-selection in hospital settings, both of MDR CRE clones and their key MDR plasmids (Table 2) [9,23-24]. Moreover, several factors such as introduction of artificial devices, long-term antibiotic exposure, prolonged hospital stays and clinical comorbidities can be responsible for CRE acquisition among patients in burns and ICU [25-27].

Previous reports suggest that CRE was associated with a 3-fold greater mortality than CSE cases [2-4]. This study recorded substantially higher mortality with CRE (27.8%) than CSE cases

(13.5%) (p=0.001) including cohort with positive culture of Enterobacterales from both sterile (blood) and non-sterile sampling sites (wound swabs, tracheal aspirates etc.). Given the level of statistical significance in adjusted models, it was possible that comorbidities might influence mortality among patients in ICU and burn (Table 3) [25-27]. Worse patients' outcome are invariably associated with limited therapeutic options [2,4,28-29]. We demonstrated that 73% of the patients did not receive at least one appropriate antibiotic (Supplementary Table 10). However, this study was unable to conclude whether ineffective antibiotic therapy or any other confounders influenced mortality among the patients with CRE due to the limited number of BSIs and limited clinical information (e.g. comorbidities or antibiotic therapy).

To date, the presence of *bla*$_{NDM}$ in different clonal lineages has mainly been reported from China, Europe, or USA and, as such, there is a bias in the global reporting of their geographical distribution [30]. This study documented numerous prevalent clones with *bla*$_{NDM}$ (ST167, ST648, ST448, ST405 of *E. coli* and ST15, ST23, ST147, ST16 of *K. pneumoniae*) (Figure 4-5, Supplementary Figure 1-5). Additionally, *E. coli* ST8346 was recognised as newly emerging clone carrying *bla*$_{NDM-1}$ (Supplementary Figure 2, Supplementary Table 11).

Based on spatiotemporal analysis, it is possible that CRE clones had been established at DMCH over an extended period of time. The average substitution rates of these clones (3 SNPs/genome/year) were in-line with previous reports (Supplementary Table 13) [31-32]. We observed the presence of common carbapenemases among the isolates differing by ≤10 SNPs, some of which represented tight clades (0-2 SNPs threshold), combined with evidence of common ward of isolation and overlapping of patients' hospital stay (Figure 6), suggesting potential recent transmission or acquisition of clones from a common source. The number of such events was considerably higher among patients with *K. pneumoniae* infections compared to

other species (Figure 6), indicating higher transmissibility of *K. pneumoniae* [33]. Of particular concern was the spread of $bla_{\text{NDM-5}}$ via a highly virulent *K. pneumoniae* ST23 (KP12) clone having the KL1 locus [35].

This study documented the plasmid mediated horizontal dissemination of bla_{NDM} among different species of Enterobacterales, mostly by IncFII and IncX3 (Table 5, Supplementary Figure 9, Supplementary Figure 13). IncX3 is widely spread throughout China and SA and associated bla_{NDM}, particularly, $bla_{\text{NDM-5}}$ [36-37]. However, we found plasmids of a wide variety of Inc types (IncFII, IncFIA, IncR, IncFIB & IncFII, IncFIB(pQil), and IncFIB(pQil) & IncFII) harbouring $bla_{\text{NDM-5}}$ yet had an identical conserved regions followed by class 1 integron together flanked by IS*26* (Figure 7-8). It can therefore be hypothesized that transposition of the IS*26*-flanked segment occurred via two-steps recombination where IS*26* released DNA segment from donor plasmid and IS*91* facilitated its insertion into the recipient plasmid by rolling circle replication [38-39].

A limitation of this study was the inability to capture all possible Enterobacterale infections due to the practice norms at DMCH in terms of microbiological sampling. These are typical of many public hospitals in SA. However, this study represents the most comprehensive report on the epidemiology and mechanism of CRE in Enterobacterales in a SA. This study has also: 1. Evidenced the high burden of CRE compared to previously reported studies. 2. Provided data on outcome and inappropriate antibiotic use that will inform better antibiotic stewardship programs including antibiotic access and affordability in the public sector. 3. Demonstrated genomic evidence on the clonal spread of virulent CRE prioritising infection control programs in limited financial settings. Whilst this study was taken in the largest public hospital in Bangladesh, many

of these findings can be extrapolated across SA encompassing a population of nearly 2 billion and signals the need for greater engagement and targeted investment.

NOTES

Author contributions: R. F. and T. R. W contributed equally to the work. R.F., L.S.J. and T.R.W designed the study, R.F. and T.W.R. obtained funding, R.F., M.A.R. M.P. and M. A. K were involved with project administration and data collection, R.F., K.S., E.P., I.B., B.H. and J.M. performed laboratory works, R.F. and W.J.W. did the statistical analysis, R.F., K.S., A.J.V.T., J.M.C., J.P. and M.F.G. did the bioinformatic analysis, R.F., L.S.J., A.J.V.T., M. A. K and T.R.W. verified the data and drafted the manuscript. All authors critically reviewed the manuscript and approved the final report before submission.

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Data availability: The study methods, statistical and bioinformatics analysis are available in detail in the main text and supplementary material. Genomes of Enterobacterales have been deposited in NCBI under the BioProject number of PRJNA722682, PRJNA719593, and PRJNA714521.

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Conflict of interest disclosures: We have no conflict to declare.

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Figure Legends:

Figure 1. Flowchart diagram of participants included in this study.

Figure legend. *Multiple clinical specimens were collected from 61 patients (blood & wound swab, n=51; blood & urine, n=3; blood & tracheal aspirate, n=2; wound swab & urine, n=2; blood & catheter tip, n=1; urine & tracheal aspirates, n=1; blood, urine & catheter tip, n=1).**35 Patients had multiple culture positive samples (blood & wound swab, n=26; blood & urine, n=2; blood & tracheal aspirate, n=2; wound swab & urine, n=2; blood & catheter tip, n=1; urine & tracheal aspirates, n=1; blood, urine & catheter tip, n=1).

Figure 2. **Antimicrobial susceptibility patterns of different species of Enterobacterales.**

Figure legend. AMC, amoxicillin-clavulanic acid; AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CST, colistin; CTX, cefotaxime; FEP, cefepime; FOF, fosfomycin; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; SXT, sulfamethoxazole-trimethoprim; TZP, piperacillin-tazobactam. Values in parentheses indicate row percentage. Data on *Salmonella* spp. (n=5), *P. anthophila* (n=1), *L. adecarboxylata* (n=1), and *E. hermannii* (n=1) are not included in this table. **Klebsiella* species other than *K. pneumoniae*. The upper cells against each species represent the frequency of resistance and the lower cells represent percentage. Heatmap indicates higher (●) to lower (●) percentage of resistance. Cells are highlighted grey (●) if the respective organism is intrinsically resistant to pertinent antibiotic.

Figure 3. Sankey diagram representing the distribution of carbapenemase alleles among different species of Enterobacterales.

Figure 4. ML tree generated from core-genome analysis of *E. coli* and *K. pneumoniae* isolated in this study.

Figure legend. A. ML tree generated from core-genome analysis of *E. coli*. **B.** ML tree generated from core-genome analysis of *K. pneumoniae*. Core-genome alignment was performed using roary (v3.12.0). The ML trees from the core genome were built with RAxML-ng (v0.9.0.git-mpi) using a GTR evolutionary model and gamma correction with bootstrapping. Isolates retrieved from NCBI for the phylogenetic analysis in this figure are stated in Supplementary Table 17.

Figure 5. Time calibrated phylogenetic tree generated from of *E. coli* and *K. pneumoniae* genomes.

Figure legend. A. Phylogenetic tree generated from of E. coli genomes belonged to ST167. Total number of isolates in this analysis was 97. Closely related isolates from other STs (ST10, ST1702, and novel allele) identified by core-genome phylogeny and pair-wise SNPs count (if isolates were differed by ≤100 SNPs from any isolate of ST167) were included in this analysis. **B.** Phylogenetic tree generated from of K. pneumoniae genomes belonged to ST15. Total number of isolates in this analysis was 54. Closely related isolate from a novel allele identified by core-genome phylogeny and pair-wise SNPs count (if isolates were differed by ≤100 SNPs from any isolate of ST15) were included in this analysis. Putative transmission clades (0-10 SNPs differences) are highlighted by green. MRCA and clock rate are stated in Supplementary Table 13. Isolates retrieved from NCBI for the phylogenetic analysis in this figure are stated in Supplementary Table 17. PSU, paediatric surgery.

Figure 6. Spatiotemporal assessment to investigate putative clonal transmission of carbapenem resistance.

Figure legend. A. Putative transmission clusters of *E. coli* at ≤10 SNPs threshold between the isolates in the respective clusters. **B.** Putative transmission clusters of *K. pneumoniae* at ≤10 SNPs threshold between the isolates in the respective clusters. The ancestral sequence at each node including the root was inferred using pyjar and the pairwise SNP distance between the roots and each isolate was calculated using pairsnp (v0·0·7). Pairwise SNPs between isolates were generated using pairsnp (v0·0·7). **C.** Diagram representing number of linkages among the isolates in the clusters by 0 to 2 SNPs threshold, aligning with epidemiological data. Isolates differed by 0 to 2 SNPs without overlapping of pertinent patients' hospital stay are represented as common group in the figure using Tableau (v2020.4).

Figure 7. Schematic layout of genetic context around *bla*$_{\text{NDM-5}}$ in different plasmid backgrounds.

Figure legend. Arrows represent the position and transcriptional direction of the open reading frames. Truncated genes are denoted by "*". Accession numbers of specific plasmids' sequences will be provided upon acceptance of the article. The layout of genetic context has been outlined using Geneious (v11.0.2).

Figure 8. Schematic layout of genetic context around *bla*$_{\text{NDM-1}}$ in different plasmid backgrounds.

Figure legend. Arrows represent the position and transcriptional direction of the open reading frames. Truncated genes are denoted by "*". Accession numbers of specific plasmids' sequences will be provided upon acceptance of the article. The layout of genetic context has been outlined using Geneious (v11.0.2).

Table Titles:

Table 1. Phenotypic and genomic resistance profile of CRE.

Table 2. Descriptive statistics for risk assessment of CRE clinical cases compared to CSE cases.

Table 3. Cox proportional hazards models to analyse for the impact carbapenem resistance on patients' outcome.

Table 4. Cox proportional hazards models to analyse the impact of carbapenem resistance and mortality among the patients with positive blood culture of Enterobacterales.

Table 5. Stratification of plasmids based on resistance patterns, Inc types, and plasmid size.

Table 1. Phenotypic and genomic resistance profile of CRE.

Antimicrobial groups	Phenotypic resistance of Enterobacterales, n (%) (n=643)	Associations between phenotypic resistance and CRE, n (%)*			Carbapenemase alleles identified in this study	ARGs, significantly associated with carbapenem resistant genes[a]	
		CRE (n=210)	CSE (n=433)	*p* value		bla_{NDM-5}	bla_{NDM-1}
AMC	617 (96)	210 (100)	407 (93.9)	-	-	bla_{TEM-1}	
TZP	342 (53.2)	208 (99)	134 (30.9)	-	-	bla_{OXA-1}	bla_{OXA-1}, bla_{OXA-9}
CRO	531 (82.6)	210 (100)	321 (74.1)	-	-	bla_{CMY-59}, $bla_{CTX-M-15}$, bla_{VEB-5}	
CAZ	543 (84.4)	210 (100)	333 (76.9)	-	-		
CTX	539 (83.8)	210 (100)	329 (76)	-	-		
FEP	517 (80.4)	210 (100)	307 (70.9)	-	-		
IPM	248 (38.6)	166 (79)	82 (18.9)	-	bla_{NDM-5}, bla_{NDM-5}, bla_{NDM-7}, bla_{NDM-4}, $bla_{OXA-181}$, $bla_{OXA-232}$	-	-
MEM	203 (31.6)	203 (96.7)	0 (0)	-			
CIP	559 (86.9)	206 (98.1)	353 (81.5)	<0.0001	-	*qnrS1*	*qnrA1*, *qnrB17*, *qnrD1*
LVX	511 (79.5)	191 (91)	320 (73.9)	<0.0001	-		
AMK	305 (47.4)	190 (90.5)	115 (26.5)	<0.0001	-	*aadA2*, *APH(3'')-Ib*, *APH(3'')-Ia*, *APH(6)-Id*, *armA*, *rmtB*	*AAC(2')-Ia*, *aadA2*, *APH(3')-Ia*, *APH(3')-VI*, *armA*, *rmtF*
GEN	407 (63.3)	197 (93.8)	210 (48.5)	<0.0001	-		
SXT	550 (85.5)	200 (95.2)	350 (80.8)	<0.0001	-	*dfrA12*, *sul1*, *sul2*	*dfrA14*, *sul1*
FOF	38 (5.9)	18/210 (8.6)**	13/426 (3.1)**	0.002	-	-	-
CST	97 (15.1)	1/194 (0.5)***	4/340 (1.2)***	0.446	-	-	-

Legend. AMC, amoxicillin-clavulanic acid; AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CST, colistin; CTX, cefotaxime; FEP, cefepime; FOF, fosfomycin; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; SXT,

sulfamethoxazole-trimethoprim; TZP, piperacillin-tazobactam; CRE, carbapenem-resistant Enterobacterales; CSE, carbapenem-sensitive Enterobacterales. *To compare the differences of resistance to non-β-lactams between CRE and CSE, *p* values were calculated. ***M. morganii* (n=6) and *L. adecarboxylata* (n=1) were excluded from the analysis as the species are intrinsically resistant to fosfomycin. ****Proteus* spp. (n=67), *Providencia* spp. (n=23), *S. marcescens* (n=13) and *M. morganii* (n=6) were excluded from the analysis as the species are intrinsically resistant to colistin. [a]As $bla_{\text{NDM-5}}$ and $bla_{\text{NDM-1}}$ are the major carbapenemases in this study, ARGs significantly associated with $bla_{\text{NDM-5}}$ and $bla_{\text{NDM-1}}$ compared to $bla_{\text{NDM-5}}$-negative and $bla_{\text{NDM-1}}$-negative Enterobacterales were included in this table. Details about the analysis are described in Supplementary Table 5 and Supplementary Table 6.

108 **Table 2. Descriptive statistics for risk assessment of CRE clinical cases compared to CSE cases.**

Attributes		CRE (n=194)	CSE (n=340)	Unadjusted logistic regression			Adjusted with patients admitted to burns unit			Adjusted with patients infected with CSE with carbapenemases producers*		
				p value	OR	95% CI	*p* value	OR	95% CI	*p* value	OR	95% CI
[b]Age (years)	0 to 5	27 (13.9)	49 (14.4)	0.875	0.960	0.578-1.594	0.976	0.992	0.592-1.662	0.918	0.973	0.584-1.622
	6 to 25	67 (34.5)	89 (26.2)	0.041	1.488	1.015-2.180	0.231	1.274	0.857-1.893	0.033	1.521	1.034-2.235
	26 to 50	66 (34)	130 (38.2)	0.331	0.833	0.576-1.204	0.514	0.882	0.605-1.285	0.307	0.824	0.569-1.194
	>50	34 (17.5)	73 (21.5)	0.273	0.777	0.495-1.221	0.542	0.866	0.546-1.374	0.238	0.761	0.484-1.198
[a]Gender	Female	81 (41.8)	110 (32.4)	0.029	0.667	0.463-0.961	0.052	0.692	0.477-1.003	0.027	0.660	0.457-0.953
	Male	113 (58.2)	230 (67.6)									
[b]SE group	BPL	84 (43.3)	161 (47.4)	0.366	1.178	0.826-1.680	0.412	1.164	0.810-1.671	0.415	1.160	0.812-1.657
	Poor	74 (38.1)	134 (39.4)	0.773	1.055	0.734-1.515	0.671	1.083	0.749-1.568	0.725	1.067	0.742-1.536
	LM	34 (17.5)	40 (11.8)	0.064	0.627	0.382-1.030	0.062	0.618	0.372-1.024	0.070	0.631	0.383-1.038
	UM[c]	2 (1)	4 (1.2)	-	-	-	-	-	-	-	-	-
	UH[c]	0 (0)	1 (0.3)	-	-	-	-	-	-	-	-	-
[b]Admitting wards	Burns	74 (38.1)	69 (20.3)	<0.0001	0.413	0.279-0.611	-	-	-	<0.0001	0.368	0.246-0.549
	Surgery	14 (7.2)	73	<0.0001	3.515	1.925-	-	-	-	<0.0001	3.588	1.963-

			(21.5)			6.420						6.559
	Urology	23 (11.9)	64 (18.8)	0.036	1.724	1.032-2.880	-	-	-	0.027	1.789	1.070-2.990
	ICU	33 (17)	27 (7.9)	0.001	0.421	0.245-0.724	-	-	-	0.003	0.435	0.253-0.749
	Other wards	50 (25.8)	107 (31.5)	0.165	1.323	0.891-1.963	-	-	-	0.126	1.363	0.917-2.025
[a]Comorbidity (DM)	Yes	15 (7.7)	58 (17.1)	0.003	2.454	1.350-4.463	0.015	2.127	1.160-3.901	0.003	2.492	1.369-4.536
	No	179 (92.3)	282 (82.9)									
[a]Comorbidity **(malignancy)**	Yes	7 (3.6)	15 (4.4)	0.653	1.233	0.494-3.078	0.935	1.039	0.411-2.625	0.606	1.272	0.509-3.177
	No	187 (96.4)	324 (95.6)									
[b]Antibiotics exposure during hospital stay before sampling**	Ceftriaxone	128 (66)	200 (58.8)	0.102	0.737	0.510-1.063	0.625	0.908	0.617-1.336	0.058	0.700	0.484-1.012
	Metronidazole	49 (25.3)	110 (32.4)	0.085	1.415	0.953-2.102	0.889	1.031	0.671-1.584	0.072	1.440	0.968-2.142
	Ciprofloxacin	27 (13.9)	96 (28.2)	<0.0001	2.434	1.521-3.894	0.007	1.958	1.202-3.191	<0.0001	2.500	1.561-4.006
	Amikacin	49 (25.3)	54 (15.9)	0.008	0.559	0.362-0.863	0.021	0.592	0.380-0.923	0.006	0.541	0.348-0.840
	Meropenem	33 (17)	37 (10.9)	0.044	0.596	0.359-0.989	0.017	0.532	0.317-0.894	0.061	0.616	0.371-1.023
	Flucloxacillin	27 (13.9)	70 (20.6)	0.054	1.604	0.988-2.602	0.064	1.594	0.974-2.608	0.080	1.546	0.949-2.519
	Levofloxacin	45 (23.2)	35 (10.3)	<0.0001	0.380	0.234-0.616	0.116	0.615	0.336-1.128	<0.0001	0.343	0.209-0.565
	Clindamycin	28 (14.4)	25 (7.4)	0.008	0.471	0.266-0.833	0.075	0.585	0.324-1.056	0.009	0.465	0.261-0.828
[a]Number of antibiotics	Monotherapy	19 (10.3)	50 (15.7)	0.093	0.620	0.353-1.087	0.315	0.745	0.419-1.324	0.072	0.596	0.340-1.048

prescribed***	More than one drug	165 (89.7)	269 (84.3)									
[a]Hospital stay before sampling	≤7 days	68 (35.1)	136 (40)	0.258	0.810	0.561-1.167	0.590	0.902	0.619-1.313	0.184	0.780	0.540-1.126
	>7 days	126 (64.9)	204 (60)									

Values in parentheses indicate column percentage. SE, socioeconomic; BPL, below the poverty level; P, poor; LM, lower middle; UM, upper middle; UH, upper high; ICU, intensive care unit; DM, diabetes mellitus; CRE, carbapenem-resistant Enterobacterales; CSE, carbapenem-sensitive Enterobacterales. Cells are highlighted if any variable was significantly associated with CRE. Binary logistic regression was performed to assess risks, and to calculate OR and 95% CI. *We found the presence of $bla_{\text{OXA-232}}$ (n=9) and $bla_{\text{OXA-181}}$ (n=1) in phenotypically carbapenem-susceptible isolates. **Eight common antibiotics prescribed at DMCH are included in this descriptive analysis. ***Patients without any antibiotic (n=31) were excluded from the analysis. [a]Attributes with two categories such as sex, number of antibiotics prescribed, hospital stay before sampling and comorbidity, the logistic regressions had one of the categories as reference value. [b]The attributes having more than two possible values, each value was compared with all the others combined, e.g. for age group 0 to 5, a binary variable 0 to 5 against all other age groups was used, for 6 to 25, the binary age variable was 6 to 25 verses all other age bands. [c]Statistical analysis was not performed due to low frequency of cases.

Table 3. Cox proportional hazards models to analyse the impact of carbapenem resistance and mortality among the patients with positive culture of Enterobacterales.

Cohort		All-cause in-hospital 30-day mortality	Discharged alive/in-hospital mortality after 30 days	*p* value[a]	SHR[a]	95% CI[a]
Patients with positive culture of Enterobacterales (n=492)	CRE (n=180)	50 (27.8)	130 (72.2)	0.001	0.491	0.325-0.741
	CSE (n=312)	42 (13.5)	270 (86.5)			
	Model 1: Adjusted by age and gender			0.001	0.510	0.337-0.771
	Model 2: Model 1+ adjusted by admission to burn unit[b]			0.007	0.561	0.367-0.855
	Model 3: Model 1+ adjusted by admission to ICU[b]			0.051	0.654	0.428-1.001
	Model 4: Model 1+ adjusted by exposure to amikacin[b]			0.004	0.537	0.354-0.816
	Model 5: Model 1+ adjusted by exposure to meropenem[b]			0.003	0.535	0.353-0.812
	Model 6: Model 1+ adjusted by exposure to levofloxacin[b]			0.008	0.562	0.368-0.859
	Model 7: Model 1+ adjusted by exposure to clindamycin[b]			0.003	0.529	0.349-0.803

Values in parenthesis indicate row percentage. SHR, subdistribution hazard ratio. Patients with DAMA (n=40) and outlier cases (n=2) (hospital stay >100 days from 'time from infection' to outcome) were excluded from the outcome analysis. [a]A Cox proportional hazards model was fitted with time points 'time from Enterobacterales isolation to outcome' as 'time-to-event' and 'time from admission to Enterobacterales isolation' as 'covariate'. [b]Confounders such as age, gender, admission to burn unit and ICU, exposure to amikacin, meropenem, levofloxacin and clindamycin were adjusted to understand the changes of *p* values (level of statistical significance) in the adjusted model.

Table 4. Cox proportional hazards models to analyse the impact of carbapenem resistance and mortality among the patients with positive blood culture of Enterobacterales.

Cohort		All-cause in-hospital 30-day mortality	Discharged alive/in-hospital mortality after 30 days	*p* value	SHR	95% CI
Patients with positive blood culture of Enterobacterales (n=83)	CRE (n=38)	19 (50)	19 (50)	0.571	0.834	0.445-1.562
	CSE (n=45)	22 (48.9)	23 (51.1)			

Values in parenthesis indicate row percentage. DAMA, discharge against medical advice. SHR, subdistribution hazard ratio. Patients with DAMA (n=40) and outlier cases (n=2) (hospital stay >100 days from 'time from infection' to outcome) were excluded from the outcome analysis. A Cox proportional hazards model was fitted with time points 'time from Enterobacterales isolation to outcome' as 'time-to-event' and 'time from admission to Enterobacterales isolation' as 'covariate'.

140 **Table 5. Stratification of plasmids based on resistance patterns, Inc types, and plasmid size.**

NDM-5-positive plasmids				
Plasmid Inc type	**Size of plasmid**	**Similarity of plasmids in a group at the nucleotide level**	**Group designation for this study**	**Bacterial host (n*)**
IncFII	~71 kb	-	FII_N5_1	*E. coli*: ST410 (1)
	~80 to ~87 kb	Coverage: 87% to 100%; Identity: ≥99%	**FII_N5_2**	*E. coli*: ST101 (2), ST405 (2), ST2083 (1), ST617 (1) *K. pneumoniae*: ST11 (1)
	~91 to ~99 kb	Coverage: 83% to 100%; Identity: ≥99%	**FII_N5_3**	*K. pneumoniae*: ST23 (11), ST515 (3), ST147 (2), ST48 (2), ST11 (1), ST16 (1), ST490 (1) *E. coli*: ST5954 (1), ST10820 (1), ST2659 (1), ST405 (1), ST448 (1), ST8346 (1) *E. cloacae* (1) *C. rodentium* (2)
	~127 kb	-	FII_N5_4	*E. coli* ST648 (1)
	~238 kb	-	FII_N5_5	*K. pneumoniae* ST23 (1)
IncX3	~45 to ~49 kb	Coverage: 100%; Identity: ≥99%	X3_N5_1	*E. coli*: ST448 (3), ST167 (2) *E. cloacae* ST113 (3) *K. pneumoniae* ST16 (1)
IncFIA	~106 kb	-	FIA_N5_1	*E. coli* ST648 (1)
	~118 kb	-	FIA_N5_2	*E. coli* ST167 (1)
	~127 to ~128 kb	Coverage: 100%; Identity: ≥99%	FIA_N5_3	*E. coli* ST167 (4)
	~131 kb	-	FIA_N5_4	*E. coli* ST131 (1)
	~152 kb	-	FIA_N5_5	*E. coli* ST405 (1)
	~159 kb	-	FIA_N5_6	*E. coli* ST167 (1)

IncFIB(pQil)	~134 kb	Coverage: 99% to 100%; Identity: ≥99%	FIB(pQil)_N5_1	*K. pneumoniae* ST231 (3)
	~163 kb	-	FIB(pQil)_N5_2	*K. pneumoniae* ST16 (1)
IncR	~112 kb	-	R_N5_1	*E. coli* ST410 (1)
	~143 kb	Coverage: 99% to 100%; Identity: ≥99%	R_N5_2	*K. pneumoniae* ST23 (3)
IncFIB	~123 to ~128 kb	Coverage: 100%; Identity: ≥99%	FIB_N5_1	*E. coli* ST405 (2)
IncFIB & IncFII	~190 kb	Coverage: 100%; Identity: ≥99%	FIB&FII_N5_1	*K. pneumoniae* ST11 (2)
IncC	~196 kb	-	C_N5_1	*K. pneumoniae* ST515 (1)
IncFIB(pQil) & IncFII	~203 kb	-	FIB(pQil)&FII_N5_1	*K. pneumoniae* ST11 (1)
IncFII & IncC	~275 kb	-	FII&C_N5_1	ST515 (1)
NDM-1-positive plasmids				
IncC	~72 kb	-	C_N1_1	*K. pneumoniae* ST11 (1)
	~154 to ~174 kb	Coverage: 99% to 100%; Identity: ≥99%	C_N1_2	*K. pneumoniae* ST395 (5)
	~287 to ~296 kb	Coverage: 97% to 100%; Identity: ≥99%	C_N1_3	*P. stuartii* (4)
IncFIB & IncHI1B	~279 to ~345 kb	Coverage: 84% to 100%; Identity: ≥99%	**FIB&HI1B_N1_1**	*K. pneumoniae*: ST15 (3), ST15 (2), ST1998 (1)

IncFIB(pQil)	~119 kb	Coverage: 100%; Identity: ≥99%	**FIB(pQil)_N1_1**	*K. variicola* (1), *K. pneumoniae* ST14 (1)
	~135 kb	Coverage: 100%; Identity: ≥99%	FIB(pQil)_N1_2	*K. pneumoniae* ST15 (2)
	~163 kb	Coverage: 100%; Identity: ≥99%	FIB(pQil)_N1_3	*K. pneumoniae* ST16 (2)
IncFIA	~141 kb	Coverage: 97%; Identity: ≥99%	**FIA_N1_1**	*K. pneumoniae*: ST152 (1), ST16 (1)
IncR	~70 kb	-	R_N1_1	*K. pneumoniae* ST572 (1)
	~152 kb	-	R_N1_2	*K. pneumoniae* ST17 (1)
IncFIB& IncFII	~215 kb	-	FIB&FII_N1_1	*K. pneumoniae* ST147 (1)
	~206 kb	-	FIB&FII_N1_2	*K. pneumoniae* ST16 (1)
IncFIB	~150 kb	-	FIB_N1_1	*K. pneumoniae* ST16 (1)
IncFIB & IncC	~304 kb	-	FIB&C_N1_1	*K. pneumoniae* ST15 (1)
IncFII	~158 kb	-	FII_N1_1	*E. cloacae* (1)
IncHI1A	~182 kb	-	HI1A_N1_1	*E. cloacae* (1)
IncHI1B	~242 kb	-	HI1B_N1_1	*E. cloacae* (1)
IncHI2	~276 kb	-	HI2_N1_1	*E. coli* ST38(1)
IncX3	~58 kb	-	X3_N1_1	*E. cloacae* (1)
Unknown	~100 kb		un_N1_1	*Providencia stuartii* (1)
	~100 kb		un_N1_2	*S. marcescens* (1)
NDM-7-positive plasmids				
IncX3	~46 kb	Coverage: 97% to 100%; Identity: ≥99%	**X3_N7_1**	*E. coli*: ST101 (2), ST448 (1) *C. farmeri* (1) *E. cloacae* (1) *S. marcescens* (1)
NDM-4-positive plasmids				

IncFIA	~79 kb	Coverage: 99%; Identity: ≥99%	FIA_N4_1	*E. coli* ST648 (3)
OXA-181-positive plasmids				
IncX3	~51 kb	-	X3_O181_1	*E. coli* ST410 (1)
IncA/C2	~182 kb	Coverage: 100%; Identity: ≥99%	**A/C2_O181_1**	*E. coli*: ST2659 (1), ST8346 (1)
IncFIC(FII)	~79 kb	-	FIC(FII)_O181_1	*E. coli* ST448 (1)
OXA-232-positive plasmids				
ColKP3	~61 kb	Coverage: 100%; Identity: ≥99%	**ColKP3_O232_1**	*K. pneumoniae*: ST231 (3), ST15 (3)
IncFIB(pQil)	~134 kb	-	FIB(pQil)_O232_1	*K. pneumoniae* ST231 (1)

*n indicates the number of isolates from which plasmids were characterized. Groups are 'underlined' and 'bold' whether horizontal transfer of plasmids were predicted for any group based on similarities of plasmids in a group and distribution of plasmids in wide range of species or wide clonal types.

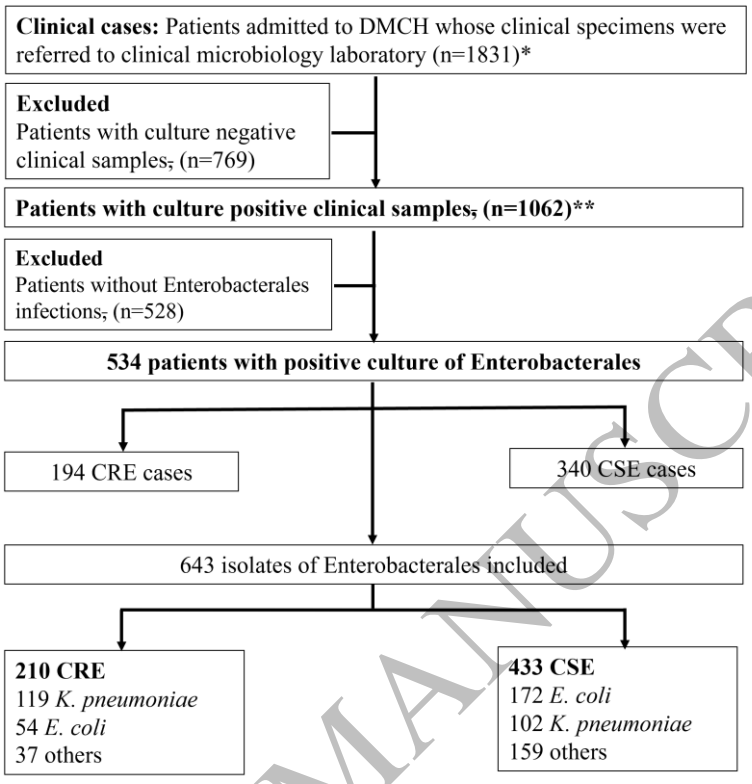
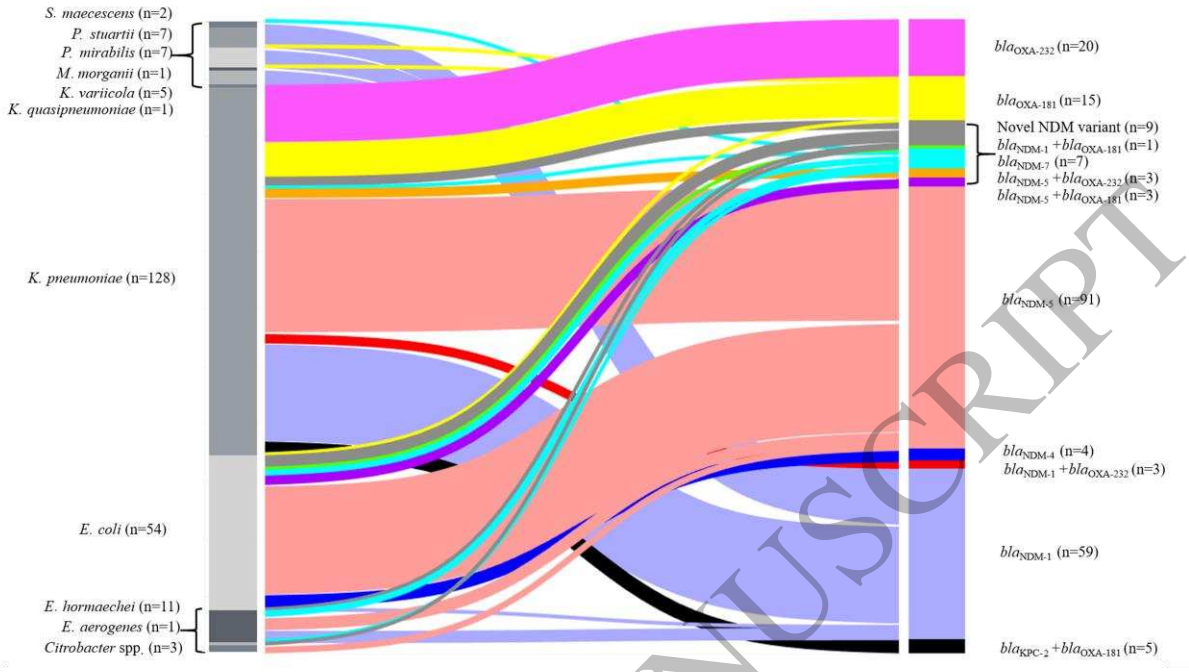


Figure 1
98x103 mm (6.2 x DPI)

Organisms	Resistance to respective antibiotics														
	AMC	TZP	CRO	CAZ	CTX	FEP	IPM	MEM	CIP	LVX	AMK	GEN	SXT	FOF	CST
E. coli (n=226)	217 96.0%	129 57.1%	192 85.0%	192 85.0%	192 85.0%	186 82.3%	38 16.8%	53 23.5%	202 89.4%	202 89.4%	65 28.8%	114 50.4%	158 69.9%	0 0.0%	0 0.0%
K. pneumoniae (n=221)	218 98.6%	151 68.3%	206 93.2%	209 94.6%	207 93.7%	201 91.0%	94 42.5%	118 53.4%	211 95.5%	176 79.6%	150 67.9%	170 76.9%	218 98.6%	16 7.2%	4 1.8%
Other *Klebsiella* spp. (n=26)	26 100.0%	11 42.3%	20 76.9%	20 76.9%	20 76.9%	20 76.9%	4 15.4%	6 23.1%	21 80.8%	13 50.0%	7 26.9%	5 19.2%	24 92.3%	0 0.0%	0 0.0%
Proteus spp. (n=67)	61 91.0%	8 11.9%	44 65.7%	50 74.6%	49 73.1%	48 71.6%	67 100.0%	3 4.5%	60 89.6%	60 89.6%	46 68.7%	60 89.6%	65 97.0%	6 9.0%	67 100.0%
Enterobacter spp. (n=42)	42 100.0%	19 45.2%	34 81.0%	36 85.7%	35 83.3%	31 73.8%	11 26.2%	12 28.6%	28 66.7%	23 54.8%	8 19.0%	24 57.1%	34 81.0%	7 16.7%	1 2.4%
Citrobacter spp. (n=11)	11 100.0%	3 27.3%	7 63.6%	7 63.6%	7 63.6%	7 63.6%	3 27.3%	2 18.2%	7 63.6%	6 54.5%	4 36.4%	7 63.6%	8 72.7%	0 0.0%	0 0.0%
Providencia spp. (n=23)	22 95.7%	17 73.9%	20 87.0%	22 95.7%	21 91.3%	17 73.9%	23 100.0%	7 30.4%	22 95.7%	22 95.7%	19 82.6%	19 82.6%	23 100.0%	2 8.7%	23 100.0%
Serratia spp. (n=13)	12 92.3%	3 23.1%	3 23.1%	2 15.4%	3 23.1%	2 15.4%	2 15.4%	2 15.4%	1 7.7%	2 15.4%	1 7.7%	2 15.4%	12 92.3%	0 0.0%	13 100.0%
M. morganii (n=6)	6 100.0%	1 16.7%	3 50.0%	3 50.0%	3 50.0%	3 50.0%	6 100.0%	0 0.0%	6 100.0%	6 100.0%	4 66.7%	4 66.7%	6 100.0%	6 100.0%	6 100.0%

Figure 2

159x83 mm (6.2 x DPI)

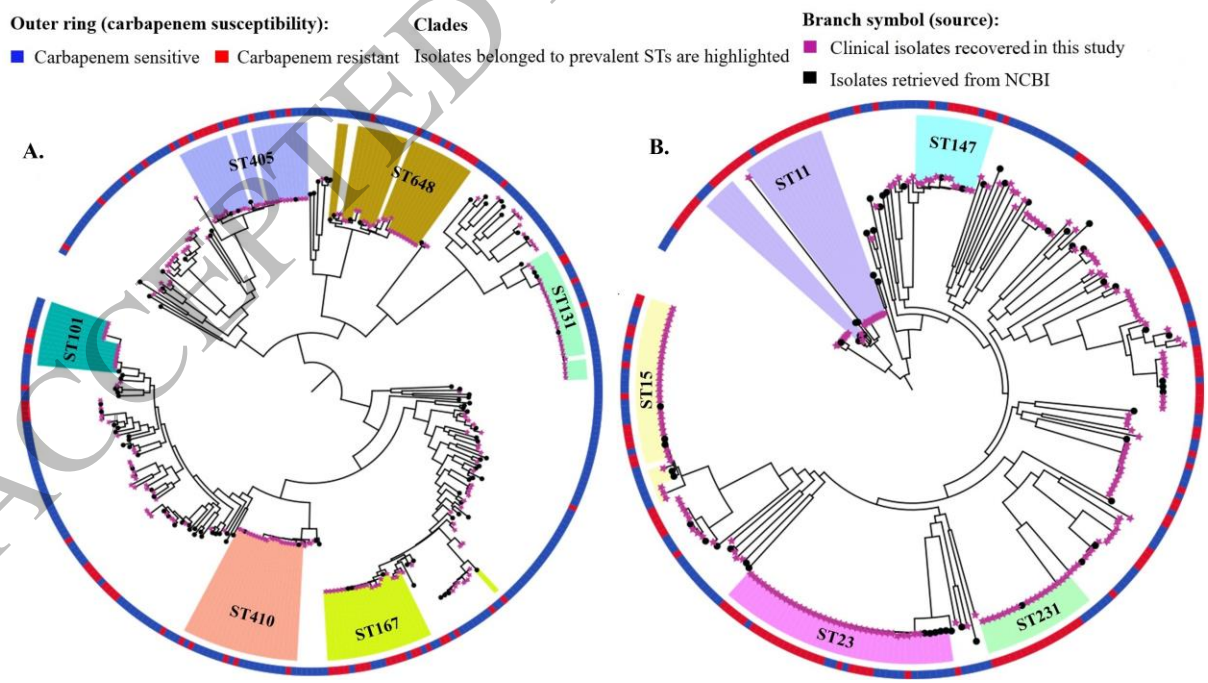


154

155 **Figure 3**
156 **159x89 mm (6.2 x DPI)**

157

158



159

160 **Figure 4**
161 **159x89 mm (6.2 x DPI)**

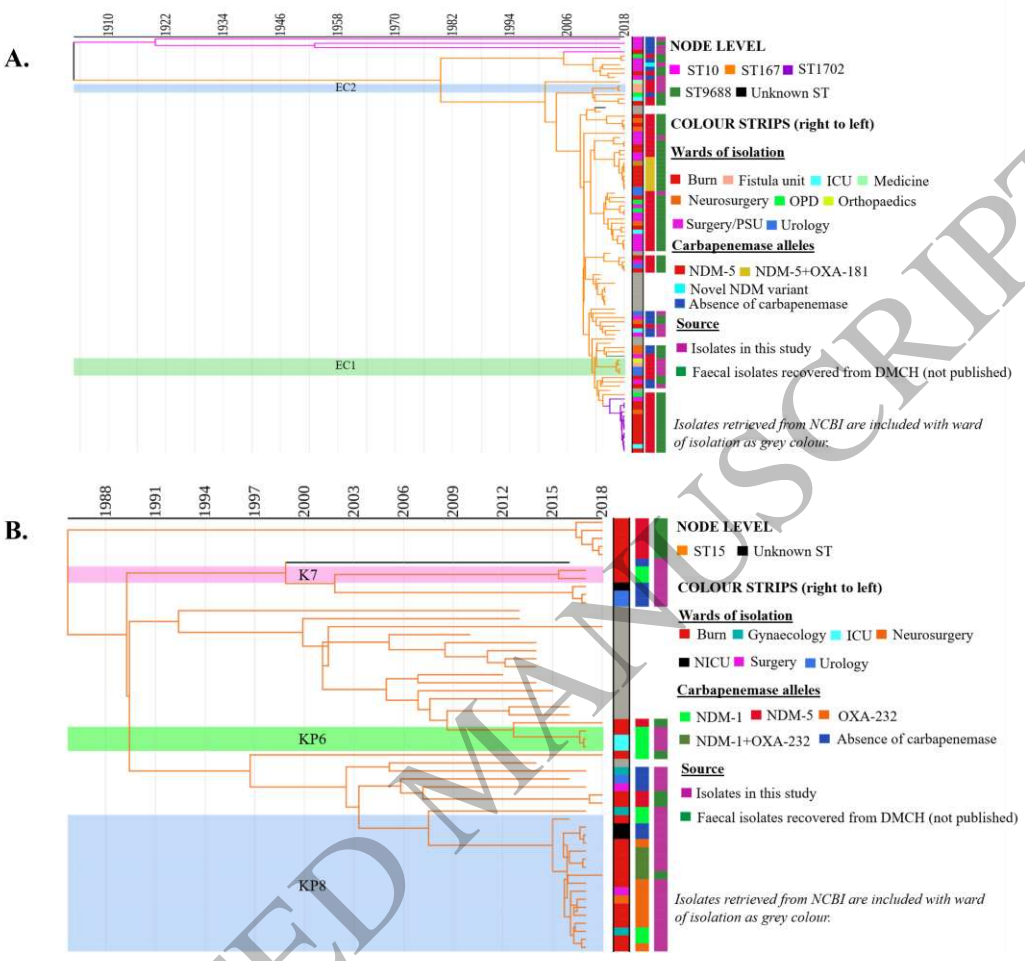


Figure 5
137x126 mm (6.2 x DPI)

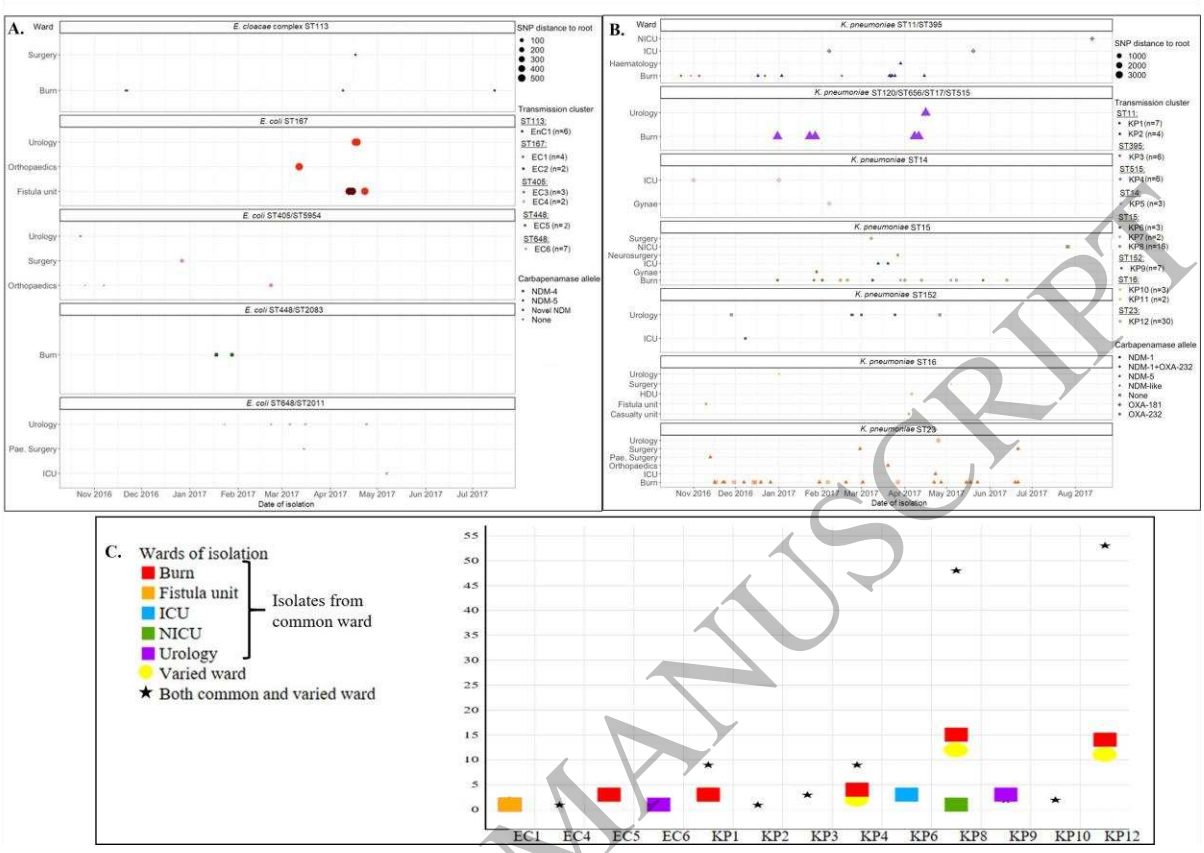


Figure 6

159x112 mm (6.2 x DPI)

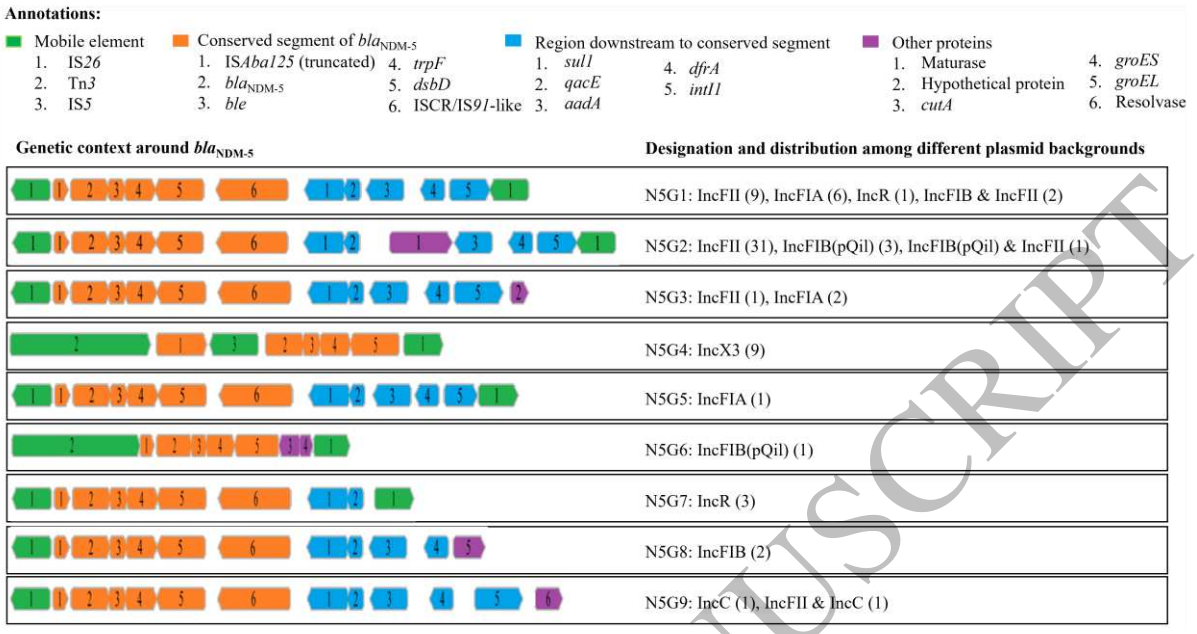


Figure 7
159x84 mm (6.2 x DPI)

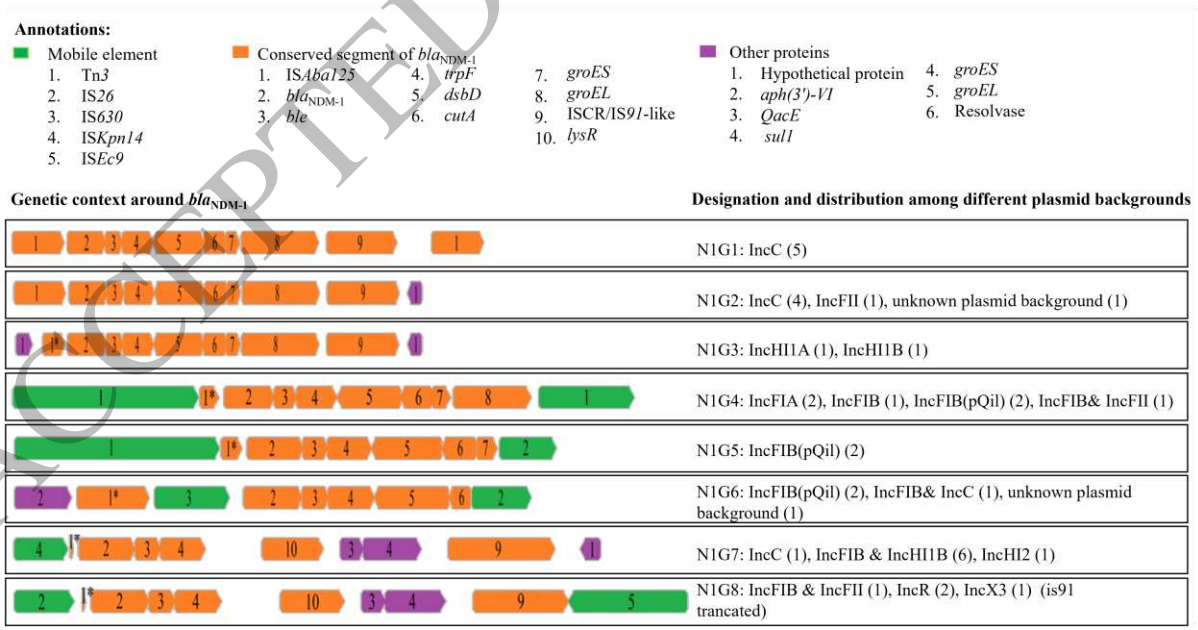


Figure 8
159x83 mm (6.2 x DPI)