Effect of carbapenem resistance on outcomes of bloodstream infection caused by Enterobacteriaceae in low-income and middle-income countries (PANORAMA): a multinational prospective cohort study

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SUMMARY

Background
Low-income and middle-income countries (LMICs) are under-represented in reports on the burden of antimicrobial resistance. We aimed to quantify the clinical effect of carbapenem resistance on mortality and length of hospital stay among inpatients in LMICs with a bloodstream infection due to Enterobacteriaceae.

Methods
The PANORAMA study was a multinational prospective cohort study at tertiary hospitals in Bangladesh, Colombia, Egypt, Ghana, India, Lebanon, Nepal, Nigeria, Pakistan, and Vietnam, recruiting consecutively diagnosed patients with carbapenem-susceptible Enterobacteriaceae (CSE) and carbapenem-resistant Entero-bacteriaceae (CRE) bloodstream infections. We excluded patients who had previously been enrolled in the study and those not treated with curative intent at the time of bloodstream infection onset. There were no age restrictions. Central laboratories in India and the UK did confirmatory testing and molecular characterisation, including strain typing. We applied proportional subdistribution hazard models with inverse probability weighting to estimate the effect of carbapenem resistance on probability of discharge alive and in-hospital death, and multistate modelling for excess length of stay in hospital. All patients were included in the analysis.

Findings
Between Aug 1, 2014, and June 30, 2015, we recruited 297 patients from 16 sites in ten countries: 174 with CSE bloodstream infection and 123 with CRE bloodstream infection. Median age was 46 years (IQR 15–61). Crude mortality was 20% (35 of 174 patients) for patients with CSE bloodstream infection and 35% (43 of 123 patients) for patients with CRE bloodstream infection. Carbapenem resistance was associated with an increased length of hospital stay (3·7 days, 95% CI 0·3–6·9), increased probability of in-hospital mortality (adjusted subdistribution hazard ratio 1·75, 95% CI 1·04–2·94), and decreased probability of discharge alive (0·61, 0·45–0·83). Multilocus sequence typing showed various clades, with marginal overlap between strains in the CRE and CSE clades.

Interpretation
Carbapenem resistance is associated with increased length of hospital stay and mortality in patients with bloodstream infections in LMICs. These data will inform global estimates of the burden of antimicrobial resistance and reinforce the need for better strategies to prevent, diagnose, and treat CRE infections in LMICs.

INTRODUCTION

Antimicrobial resistance (AMR) represents a substantial global health and economic threat, with resistance to so-called last-line antibiotics, such as carbapenems, of most concern.[1, 2] An accurate estimate of the burden of AMR is needed to inform decisions about allocation of resources towards strategies such as infection control measures, antimicrobial stewardship, and development of new antimicrobials.[3, 4] To date, only a few small studies have assessed the effect of carbapenem resistance on health outcomes, with most studies done in high-income settings.[5, 6, 7, 8, 9, 10] Yet, it is likely that the burden is greatest in low-income and middle-income countries (LMICs).[11] A 2011 meta-analysis of health-care-associated infections in LMICs reported an almost complete absence of data regarding the effect of AMR,[12] and WHO has identified a knowledge gap on the burden of AMR in this setting.[2]

We aimed to describe the clinical features, outcomes, and molecular epidemiology of bloodstream infections caused by carbapenem-resistant Enterobacteriaceae (CRE) compared with carbapenem-susceptible Enterobacteriaceae (CSE) in LMICs. Our primary objective was to quantify the effect of carbapenem resistance on in-hospital mortality and length of hospital stay among inpatients diagnosed with a bloodstream infection due to a member of the Enterobacteriaceae family in LMICs.

METHODS

Study design and participants

We did a multinational prospective cohort study, the PANORAMA study. We established the PANORAMA network of local investigators from hospitals that met the following criteria: location in a country with a low-income or middle-income economy (at the time of patient recruitment),[13] tertiary-level hospital according to WHO definition,[14] and microbiology laboratory able to detect CRE.[15] Patients were eligible if diagnosed with a bloodstream infection caused by a member of the Enterobacteriaceae family. We excluded patients who had previously been enrolled in the study and those not treated with curative intent at the time of bloodstream infection onset. Patients were recruited from hospitals linked to the local investigators. The exposure of interest was carbapenem resistance. We used an apportionment ratio of 1:1 to improve study precision.[16] Local investigators enrolled consecutive eligible patients during a 6-month study period up to an initial maximum of five patients with CSE bloodstream infections and five patients with CRE bloodstream infections (established by the local laboratory in each hospital). After recruiting the initial ten patients, centres were invited to include ten more patients (five CRE and five CSE) in an ongoing stepwise manner for a maximum of 6 months. Patients were followed-up until discharge or in-hospital death. Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Melbourne, VIC, Australia.[17] This study was approved by the human research ethics committee at each institution. In some cases, consent was obtained. More frequently, the need for consent was waived according to local human research ethics guidance.

Outcomes

The primary outcomes were in-hospital mortality and length of hospital stay. As in-hospital mortality and discharge alive are competing outcomes, we modelled the effect of carbapenem resistance on both.[18]

Procedures

Between Aug 1, 2014, and June 30, 2015, the local investigators collected the following patient-level covariates: age, sex, referral source (admission from long-term care facility, nursing home, or transfer from another hospital), severity of underlying illness (measured using the Charlson comorbidity index[19]), Pitt bacteraemia score[20] at bloodstream infection onset, and intensive care unit admission or surgical procedure before the bloodstream infection. Sanitation was categorised by household latrine facilities (ie, open defecation), and education used as a surrogate for socioeconomic status. We collected the following information about bloodstream infections: onset date (first positive culture), Enterobacteriaceae species, polymicrobial infection, source of infection (ie, primary, central-line associated, or secondary bloodstream infection), and epidemiological attribution (ie, hospital acquired, community acquired, or health-care associated).[21] Antibiotic treatment was recorded. Agents used from day 0 (onset date) to day 2 of infection were defined as initial treatment. Persistent bloodstream infection was defined as a positive follow-up blood culture containing the same Enterobacteriaceae after more than 48 h of active antimicrobial treatment, or in the case of a catheter-associated bloodstream infection, a positive blood-culture result more than 3 days after removing the catheter. Recurrent bloodstream infection was defined as bacteraemia due to the same microbiologically documented Enterobacteriaceae (ie, species and antimicrobial susceptibility pattern), developing after the discontinuation of treatment. Follow-up blood cultures were taken at the discretion of the treating clinicians.

All blood culture isolates were processed in the clinical microbiology laboratory of each participating hospital for identification and antibiotic susceptibility testing, using standard microbiology methods. Strains were screened for carbapenem non-susceptibility according to recommendations of the International Working Group.[15] All laboratories participated in an established external quality assurance programme. When possible, bacterial strains were sent to one of two central study laboratories (Cardiff, UK, and New Delhi, India) for confirmatory testing and molecular characterisation.

Species identification of bacterial isolates was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF; Bruker Daltonik GmbH, Coventry, UK), according to manufacturer's instructions, and the acquired mass spectra were compared with mass spectra in the Bruker database using MALDI Biotyper 3.1 software. Antibiotic resistance profiles against different antibiotics were established by disc diffusion, and minimum inhibitory concentrations of ertapenem, meropenem, imipenem, and colistin were established by agar dilution.[22] Tests and interpretation were done according to the European Committee on Antimicrobial Susceptibility Testing guidelines version 7.1 (2017), and *Escherichia coli* ATCC25922 was used as the control.

Bacterial isolates were screened for the presence of bla_{NDM}, bla_{KPC}, $bla_{oxa-48-like}$, and bla_{VIM} by PCR, and for the presence of bla_{IMP-1}, bla_{IMP-2}, and bla_{IMP-3} by multiplex and individual PCR; a subset was confirmed by sequencing. See the appendix for primer sequences, target genes, and PCR conditions.

Multilocus sequence typing (MLST) was done following published protocols for (1) *E coli* and (2) *Klebsiella pneumoniae*. eBURST analysis was done to assess relationships between the different isolates on the basis of their sequence types (ST), and associated epidemiological data using PHYLOViZ.

Statistical analysis

The sample size estimate was based on an expected in-hospital mortality of 40% among exposed (CRE) patients and 20% among non-exposed (CSE) patients.[6] Given an α value of 0·05 and power of 80%, we required 91 patients in each group to test the hypothesis that mortality is higher among exposed patients than among non-exposed patients. We assumed an institution-level intra-cluster correlation of 0·01 and a sample size of ten from each institution; therefore, we needed an actual sample size of 100 patients in each group to reach an effective sample size of 91.

The effect of carbapenem resistance on the probability of mortality and discharge alive was estimated using proportional subdistribution hazards models (Fine and Gray models[23]), with time from infection as the timescale (time 0 is infection, with infection therefore included as a time-fixed covariate). Hospital stays were censored at 90 days. For both outcomes (mortality and discharge alive), two models were fitted. The base model adjusted only for time from admission to infection. We applied inverse probability weighting to this model to account for confounding.[24] We computed a propensity score for each patient, representing their probability of being infected with a carbapenem-resistant strain, using boosted regression trees that included time from admission to infection, age, sex, Charlson comorbidity index, location before admission, Pitt bacteraemia score, bacterial organism, education, and sanitation as predictors.[25] We then used Cox proportional subdistribution hazards models weighted by 1 divided by the propensity score for patients with CRE blood-stream infection and 1 divided by (1 minus propensity score) for patients with CSE bloodstream infection. Time from admission to infection was included as an independent variable for doubly robust estimation. To account for missing values, we imputed 50 datasets using multiple imputation by chained equations (mice package), did the analysis described above with each dataset, and summarised results using Rubin's rule (appendix).[26, 27, 28]

We used multistate modelling to estimate excess length of hospital stay associated with carbapenem resistance.[29] We considered time from admission as the timescale. All patients entered the model at the time of infection in one of two states: CSE bloodstream infection or CRE bloodstream infection. Left truncation was taken into account by the estimators.[30] Length of hospital stay is determined by moving into a final (ie, absorbing) state representing the day of discharge or in-hospital death. The excess length of hospital stay was based on non-parametric estimators of the transition probabilities associated with the multistate model (appendix).[30] SEs and CIs were derived by bootstrap re-sampling runs.

We did several sensitivity analyses. First, to evaluate the effect of timescale and to supplement the Fine and Gray models, we used Cox proportional hazards models to estimate the effect of carbapenem resistance on instantaneous risk of mortality, discharge alive, and a combined endpoint (all-cause end-of-stay) using two timescales: time from admission and time from infection. Second, to address the possibility of misclassification bias, in addition to the primary analysis including all recruited patients (ie, complete cohort) all analyses described were repeated on the subset of patients whose bloodstream infection strain was transported to one of two central laboratories for confirmatory testing (ie, confirmed cohort). The analysis of the complete cohort, which could be considered an intention-to-treat analysis, is presented in the main text of this paper, whereas the results for the confirmed cohort and other statistical sensitivity analyses are presented in the appendix. Statistical analyses were done using R, version 3.3.2, including the survival, etm, mvna, twang, and mice packages.

RESULTS

16 hospitals agreed to participate in the study (appendix), comprising ten public and six private facilities, with a median of 400 acute care beds (IQR 270–1100). Hospitals were located in two countries with low-

income economies (Bangladesh and Nepal), six with lower-middle-income economies (Egypt, Ghana, India, Nigeria, Pakistan, and Vietnam), and two with upper-middle-income economies (Colombia and Lebanon). 14 (88%) of 16 hospitals had specialist units for neonates, 12 (75%) for obstetrics, ten (63%) for burns, nine (56%) for solid-organ transplantation, and six (38%) for bone-marrow transplantation.

We recruited 297 patients between Aug 1, 2014, and June 30, 2015: 174 with CSE bloodstream infection and 123 with CRE bloodstream infection (figure 1). 297 patients were included in the complete cohort (ie, primary analysis). There were no dropouts. Missing data are described for each variable in table 1. Median age of all patients was 46 years (IQR 15–61); 182 (61%) participants were male and 115 (39%) were female (table 1). Open defecation at home was reported by 20 (8%) of 256 patients. Median age-adjusted Charlson comorbidity index was 2·0 (IQR 0·0–5·0), and median Pitt bacteraemia score was 2·0 (IQR 0·5–4·0)

In-hospital mortality occurred in 35 (20%) of 174 patients with CSE bloodstream infection and in 43 (35%) of 123 patients with CRE bloodstream infection. Carbapenem resistance was associated with increased probability of in-hospital mortality and decreased probability of discharge alive in both the base and inverse probability weighted models (table 2). From the multistate model, carbapenem resistance was associated with 3·7 days (95% CI 0·3–6·9) excess length of stay in hospital. The results of the confirmed cohort and sensitivity analyses are consistent with those of the primary analysis in the complete cohort (appendix). From the isolates sent for confirmatory testing, positive predictive values for local laboratory designation of carbapenem resistance were 87% (84 of 97) and negative predictive values for local laboratory designation of carbapenem resistance were 96% (106 of 110).

Exposure to health care before bloodstream infection onset was more frequent among patients with CRE bloodstream infection than those with CSE bloodstream infection. The median length of stay in hospital before bloodstream infection onset was 1 day (IQR 0–5) for patients with CSE bloodstream infection and 4 days (0–14) for patients with CRE bloodstream infection (figure 2). Furthermore, more patients with CRE bloodstream infection had been exposed to antibiotics, intensive care, and surgery within the 30 days before bloodstream infection onset than had the patients with CSE bloodstream infection (table 1)

Antibiotic treatment is summarised in the appendix. Among 110 patients with CRE bloodstream infection, 36 (33%) were prescribed a polymyxin as part of initial treatment. The antibiotic classes most frequently prescribed to patients with CRE bloodstream infection from day 3 onwards were carbapenems (55 [59%] of 93) and polymyxins (48 [52%] of 93), with 35 (38%) of 93 patients in this group receiving an antibiotic from both of these classes. Carbapenems were the most commonly used antibiotic class for CSE bloodstream infection, with 65 (39%) of 165 patients prescribed an agent from this class during the first 3 days of infection (ie, initial treatment period) and 63 (47%) of 135 patients prescribed an agent from this class on subsequent days.

Although the incidence of septic shock among patients with CSE bloodstream infection (46 [26%] of 174 patients) and CRE bloodstream infection (33 [27%] of 123 patients) was similar, persistent bacteraemia was more frequently detected among patients with CRE bloodstream infection (21 [17%] of 123 patients) than CSE bloodstream infection (14 [8%] of 174 patients). Recurrent bacteraemia was also more frequently detected among patients with CRE bloodstream infection (eight [7%] of 123 patients) than CSE bloodstream infection (five [3%] of 174 patients).

Antibiotic susceptibility against 14 distinct antibiotics was established for 101 *K pneumoniae* isolates, 65 *E coli* isolates, and 25 other Enterobacteriacae isolates (appendix). The antibiotic to which carbapenem-

resistant isolates were most frequently susceptible was colistin: 36 (83%) of 41 *K pneumoniae* isolates, all 15 *E coli* isolates, and six (75%) of eight isolates of other Enterobacteriaceae. All CRE isolates were non-susceptible to cephalosporins and aztreonam. Resistance to non-carbapenem antibiotics was also widespread among CSE, particularly *K pneumoniae*. All carbapenem-susceptible *K pneumoniae, E coli*, and other Enterobacteriaceae were susceptible to colistin, and amikacin (21 [57%] of 37) and tigecycline (18 [53%] of 34]) were active against more than half of the carbapenem-susceptible *K pneumoniae* isolates.

In total, 109 *K pneumoniae* and 66 *E coli* isolates were subjected to MLST. We identified 48 *K pneumoniae* STs. The most common were ST14 (n=15), a non-virulent clone associated with capsular serotype K2, and ST231 (n=14). 14 isolates belonged to clonal complex 258, including ST258, ST11, ST340, and ST2516. 35 STs were uniquely represented by one isolate. Additionally, five new allelic profiles (figure 3) were identified and have been added to the *K pneumoniae* MLST database at Institut Pasteur (Paris, France). Among the *E coli* isolates, 34 STs were identified. The most common was ST131 (n=nine), with 20 STs represented by one isolate. eBURST diagrams based on the STs obtained for *K pneumoniae* and *E coli* isolates are shown by carbapenem susceptibility in figure 3 and by geographical region in the appendix.

Among the 208 available isolates, bla_{NDM} was the most commonly identified carbapenemase-encoding gene; it was present in 35 carbapenem-resistant *K pneumoniae* isolates (21 from south Asia, ten from Africa, and four from west Asia), ten carbapenem-resistant *E coli* isolates (seven from south Asia, two from Africa, and one from west Asia), two other carbapenem-resistant Enterobacteriaceae, and one carbapenem-susceptible *Klebsiella oxytoca* (from Pakistan). $bla_{OXA-48-like}$ genes were identified in 34 carbapenem-resistant *K pneumoniae* isolates (29 from south Asia, four from Africa, and one from west Asia) and one carbapenem-susceptible *K pneumoniae* isolate (Africa). 11 carbapenem-resistant *E coli* isolates carried $bla_{OXA-48-like}$ genes (nine from south Asia and two from west Asia). bla_{KPC} was detected in eight carbapenem-resistant *K pneumoniae* isolates (seven from South America and one from south Asia) and one carbapenem-susceptible *K pneumoniae* isolate from South America. bla_{KPC} was also found in six carbapenem-resistant *E coli* isolates from south Asia and five carbapenem-resistant *Enterobacter cloacae* isolates. bla_{VIM} genes were detected in three carbapenem-resistant *K pneumoniae* isolates from Egypt. No bla_{IMP} genes were detected. Coexistence of two or more carbapenemase genes was identified in 22 isolates. Carbapenemase-encoding genes were not found in one carbapenem-resistant *E coli* isolate (Ghana), 11 carbapenem-resistant *K pneumoniae* isolates (six from Ghana, three from India, and two from Egypt), and one *K oxytoca* isolate from Ghana.

DISCUSSION

These data show that in LMICs, patients with bloodstream infection caused by CRE have increased risk of death and length of stay in hospital compared with patients with bloodstream infection caused by CSE. Although this finding is not unexpected, data for the burden of AMR in LMICs are scarce, despite long-standing recognition of this knowledge gap.[31, 32] Such information is essential to justify the allocation of the resources required to strengthen laboratory, infection control, and antimicrobial stewardship capacity, and other strategies to address AMR in LMICs.[33, 34]

Estimates of the global clinical burden of AMR need to include data from LMICs rather than being extrapolated from high-income countries for three reasons. First, there is an inverse relationship between national income and prevalence of drug-resistant infections.[35] Potential reasons for this association include high population density, poor sanitation, contaminated water, environmental reservoirs, suboptimal implementation of infection control and antimicrobial stewardship interventions, self-medication with over-the-counter antibiotics, and greater use of antibiotics for food production.[32, 35] Second, the clinical impact of each infection might be greater in LMICs because of barriers to health-care

access, diagnostic testing, and therapeutic options.[36] Finally, the molecular basis of carbapenem resistance varies by geographical region,[37] so regional molecular data are required to evaluate the potential use of newer antimicrobial agents with activity against specific carbapenemase types.

The findings of this study are consistent with, and extend, the existing literature regarding the health impact and microbiology of carbapenem resistance among Enterobacteriaceae causing bloodstream infection in LMICs.[38, 39, 40, 41] Villegas and colleagues[41] recruited patients from 11 hospitals in seven Latin American countries and found that CRE bloodstream infection was associated with a four-times increase in the odds of in-hospital mortality compared with CSE bloodstream infection.[42] In this study, in-hospital mortality occurred in 35 (20%) of 174 patients with CSE bloodstream infection and in 43 (35%) of 123 patients with CRE bloodstream infection. We caution against directly comparing this result with recent studies from high-income settings without considering other differences in patient cohorts, including time period, patient acuity, and antibiotic susceptibilities. The INCREMENT study,[43] done in 26 tertiary hospitals in ten countries, reported 30-day all-cause mortality of 43% (189 of 437) in predominantly southern European patients with bloodstream infection due to carbapenemase-producing Enterobacteriaceae. The higher mortality than in our study might relate to changes in the management of carbapenemase-producing Enterobacteriaceae over time; 404 (92%) of 437 patients in the INCREMENT study had infections from 2004 to 2011, and mortality was significantly higher among those patients than among those with infections from 2012 to 2013 (adjusted hazard ratio 1·43, 95% CI 1·02–2·01). Although several other factors are likely to be relevant, this secular trend is supported by smaller studies in high-income settings.[6, 7, 44] Additionally, INCREMENT excluded patients who had received appropriate antibiotics before bloodstream infection onset and used a different outcome measure to that used in our study (30-day *vs* in-hospital mortality).

The most common carbapenemase genes detected in this study were bla_{NDM} and $bla_{OXA-48-like}$, consistent with the large number of isolates from south Asia, which is the recognised epicentre of bla_{NDM} and a region noted for more recent spread of OXA-48-like producing Enterobacteriaceae.[45] As previously described, we found bla_{KPC} in isolates from South America,[45] but also in those from south Asia. No carbapenemase-encoding genes were identified in 13 CRE, suggesting the presence of other carbapenem resistance mechanisms.

The most common *K pneumoniae* STs were ST14 and ST231. *K pneumoniae* ST14 is a non-virulent ST that has been associated with capsular serotype K2 and bla_{NDM},[46, 47] a trend we also noted: eight of 15 *K pneumoniae* ST14 isolates from south Asia in this study carried bla_{NDM}. Similarly, we found $bla_{OXA-48-like}$ genes in 12 of 14 *K pneumoniae* ST231 isolates (all from south Asia), as previously described.[48] *K pneumoniae* ST258 is considered a high-risk international clone that is associated with clonal expansion of clades producing *K pneumoniae* carbapenemase.[45] In this study, five *K pneumoniae* ST258 isolates carrying bla_{KPC} genes were responsible for bloodstream infection cases in South America. 14 of 109 *K pneumoniae* isolates belonged to the clonal complex 258 (including ST258, ST11, ST340, and ST2516), which is associated with multidrug resistance and hospital dissemination.[49] The most common *E coli* ST was ST131, a high-risk international clone associated with $bla_{CTX-M-15}$.[45]

A strength of this study is its prospective design, which facilitated accurate data collection. We also sought to provide valid estimates by explicitly accounting for competing outcomes and time-varying exposures. We did sensitivity analyses to explore the potential effect of misclassification of carbapenem susceptibility. To address the possibility of misclassification bias, we did all analyses on a subset of patients whose bacterial strains were characterised using phenotypic and molecular methods in one of two central study laboratories (ie, confirmed cohort). The multicentre nature of the study supports

external validity. Finally, the molecular characterisation of dominant clades and carbapenemase genes adds detailed genetic information that is often lacking in clinical studies.

We acknowledge that this study has limitations. First, by fixing an equal ratio of susceptible and resistant infections, we are unable to directly establish the incidence of our exposure. This concession was made to increase the efficiency of our study (ie, to produce more precise estimates for a given sample size). Second, we restricted this study to tertiary institutions in large metropolitan areas to minimise information bias, assure feasibility, and reduce heterogeneity, but this strategy limits the generalisability of our findings. In rural hospitals, the clinical effect might be even greater due to the absence of adequate diagnostic and therapeutic infrastructure. Third, there is a substantial degree of heterogeneity present in this study with regard to hospital resources, patients (neonates *vs* adults), bacterial pathogens (*E coli* and *K pneumoniae*), and resistance mechanisms. We did not power this study to do subgroup analyses stratified by these parameters. Finally, by comparing patients with bloodstream infection due to CSE with those with bloodstream infection due to CRE, we have estimated the effect of carbapenem resistance among patients with bloodstream infection. We are unable to estimate the effect of Enterobacteriaceae bloodstream infection (susceptible or resistant) in the absence of an uninfected group.

This study contributes to an improved understanding of the scale of the emerging threat of carbapenem resistance in LMICs. We provide detailed clinical data and molecular characterisation of strains, including high-risk multidrug-resistant clones with few remaining therapeutic options. We have produced a robust estimate of the mortality and excess length of hospital stay attributable to carbapenem resistance. When combined with incidence data, this study will inform estimates of the global health and economic burden of carbapenem resistance. In the future, affordable surveillance mechanisms, interventions to prevent infection, and management strategies should be developed to reduce the burden of bloodstream infections caused by CRE in LMICs.

RESEARCH IN CONTEXT

Evidence before this study
We were provided with the results of an unpublished systematic review done by Pezzani and colleagues commissioned by WHO on the clinical burden of multidrug-resistant bacteria, which included studies published before July 15, 2017. This included six studies reporting the effect of carbapenem resistance on mortality and length of hospital stay among patients with bloodstream infection caused by Enterobacteriaceae spp in low-income and middle-income countries (LMICs). We added one publication by searching the PubMed database for studies published from July 1, 2007, to Nov 6, 2017, assessing the clinical effect of carbapenem resistance in LMICs. We used the search terms "Carbapenems" AND "Drug Resistance, Bacterial" OR "carbapenem-resistant" OR "carbapenemase-producing" OR "carbapenemase" AND "Enterobacteriaceae" AND "Bacteremia" OR "bloodstream infection" AND "Developing countries" OR "low-income" OR "middle-income". Altogether, this approach yielded seven publications: three were case series without a comparator group, three were single-centre studies (two in Taiwan and one in Brazil) with 75 participants or fewer, and one was a multicentre cohort study in seven Latin American countries. Although most studies reported higher mortality and increased length of hospital stay among patients with carbapenem-resistant bloodstream infections, existing studies were underpowered and not able to account adequately for confounding and time-dependent bias. No published data were identified from low-income countries or from Africa, the Middle East, or south Asia.

Added value of this study
We add to previous evidence by including patients from a broader range of LMICs, and by using an analytical approach to account for the fact that discharge alive is a competing risk for in-hospital mortality and differences in follow-up between patients. We found that carbapenem resistance is associated with increased mortality and length of hospital stay among patients with bloodstream infections due to Enterobacteriaceae in LMICs. To our knowledge, these data represent the most detailed description to date of clinical and microbiological features of bloodstream infections due to carbapenem-resistant versus carbapenem-susceptible Enterobacteriaceae in LMICs. We also show the feasibility of studies to generate estimates of antimicrobial resistance in LMICs.

Implications of all the available evidence
The prevalence of resistance to third-generation cephalosporins among Enterobacteriaceae is unacceptably high, and carbapenems are increasingly being used to treat bloodstream infections. Carbapenem resistance is resulting in excess deaths and health expenditure in LMICs. In the absence of coordinated surveillance, this burden might go unrecognised, and appropriate interventions will be delayed. Although there is a need to improve surveillance of antimicrobial resistance in LMICs, this must be accompanied by strategies to limit the effect of resistance on patients with infections. Future research should develop affordable mechanisms for surveillance, evaluate the clinical effectiveness of interventions for infection prevention and antimicrobial stewardship, and optimise the management of bloodstream infections caused by carbapenem-resistant Enterobacteriaceae in LMICs.

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Figure 1. Trial profile
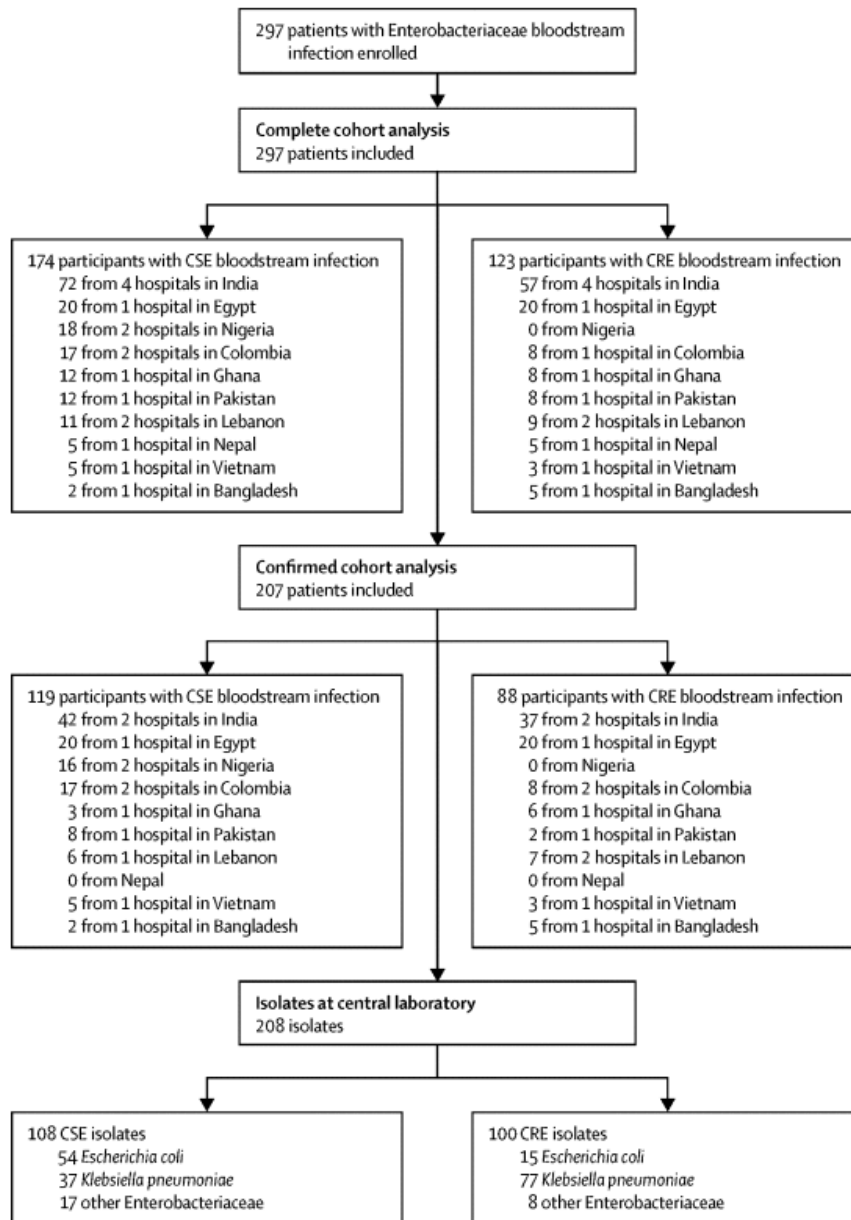
More than one isolate was available from some participants in the confirmed cohort. The complete cohort analysis included all patients. The confirmed cohort analysis included the subset of patients whose bloodstream infection strain was transported to a central study laboratory for confirmatory testing. CRE=carbapenem-resistant Enterobacteriaceae. CSE=carbapenem-susceptible Enterobacteriaceae.

Figure 2. Time from hospital admission to bloodstream infection and outcome, stratified by carbapenem susceptibility
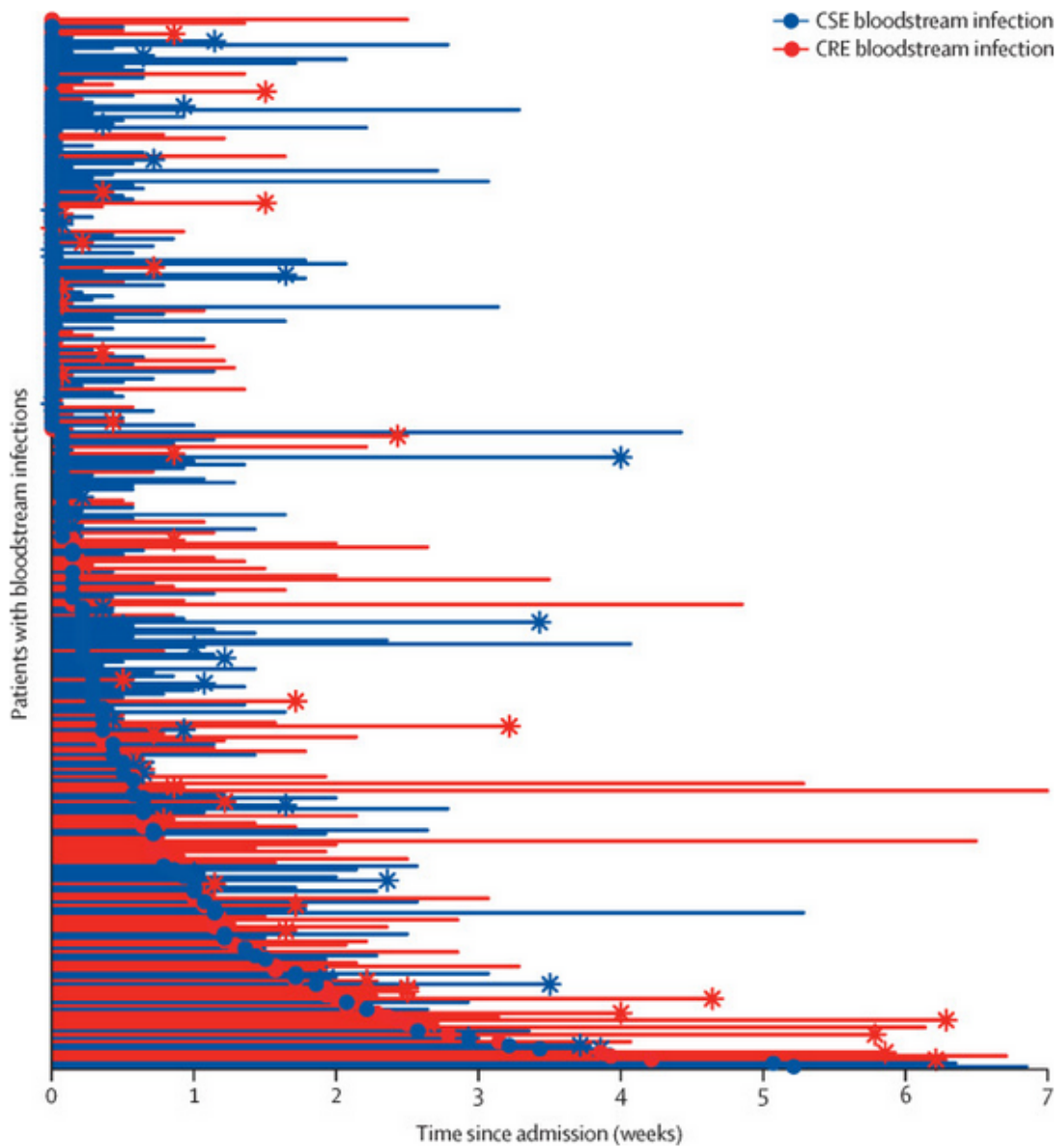
Each line represents one patient admission. Admissions are arranged vertically according to time from admission to bloodstream infection onset. Filled circles indicate bloodstream infection onset. Stars at the end of bars show mortality. CRE=carbapenem-resistant Enterobacteriaceae. CSE=carbapenem-susceptible Enterobacteriaceae.

Figure 3. eBURST diagrams of Klebsiella pneumoniae (A) and *Escherichia coli* (B) isolates showing the relationship between isolates on the basis of their multilocus sequence typing and carbapenem susceptibility
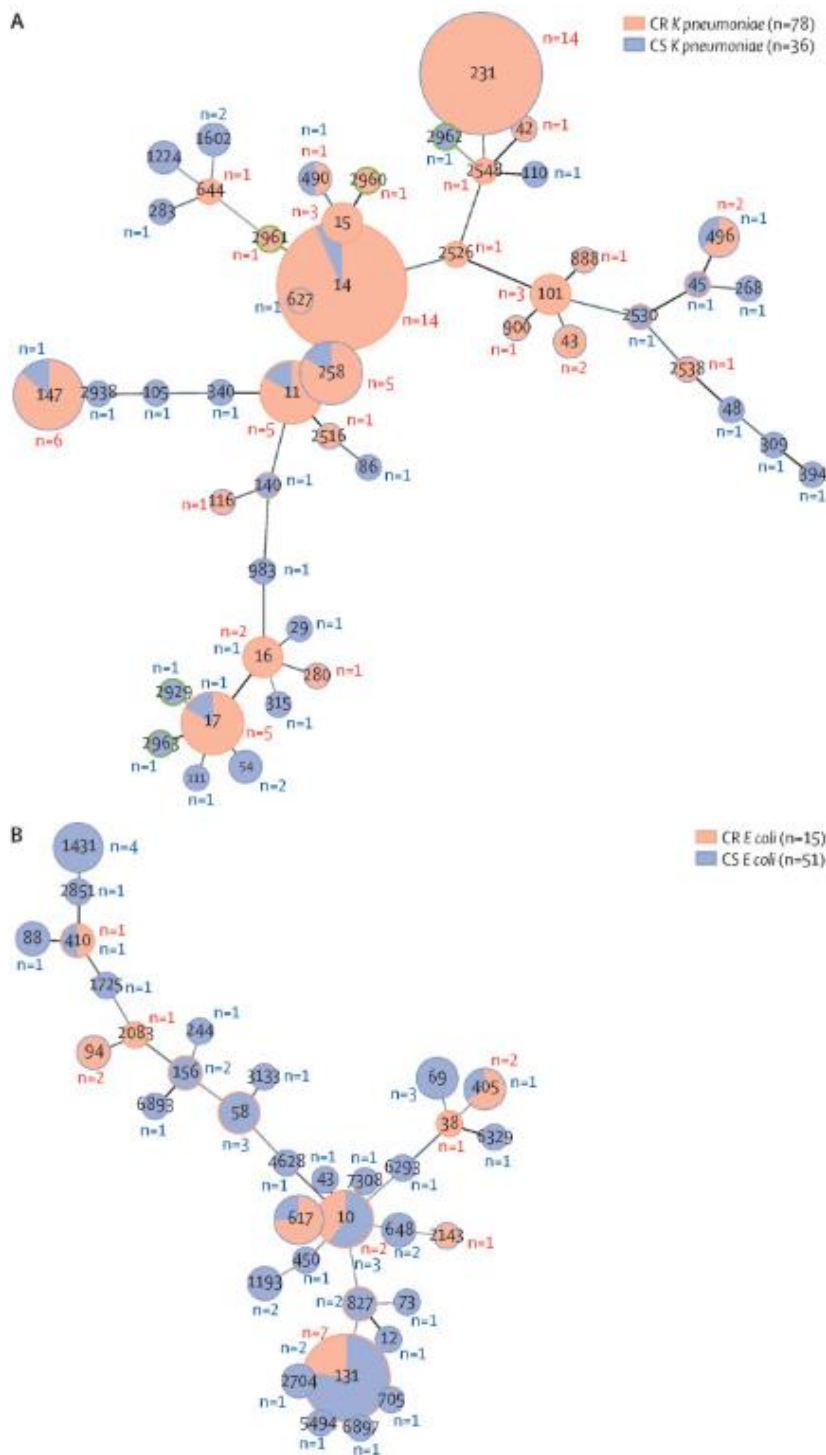
Each node within the tree represents a single ST. The size of the nodes is proportional to the number of isolates represented by said node. Within each node, the area of blue and orange represents the number of carbapenem-susceptible and carbapenem-resistant strains. Nodes are labelled with corresponding ST. Nodes representing new STs are delineated with a green circle. ST=sequence type. CR=carbapenem-resistant. CS=carbapenem-susceptible.

Table 1. Patient characteristics

	CSE BSI (n=174)	CRE BSI (n=123)
Demographics		
Age (years)	46·0 (9·0–64·8)	46·0 (21·5–60·0)
Sex		
Female	74 (43%)	41 (33%)
Male	100 (57%)	82 (67%)
Education among participants aged at least 18 years*		
None	9 (8%)	13 (14%)
Primary	10 (8%)	10 (11%)
Secondary	28 (24%)	20 (21%)
Tertiary	42 (35%)	25 (36%)
Not available	30 (25%)	27 (28%)
Open defecation at home		
Yes	14 (8%)	6 (5%)
No	139 (80%)	97 (79%)
Not available	21 (12%)	20 (16%)
Location before admission		
Home	117 (67%)	62 (50%)
Transfer from other hospital	40 (23%)	48 (39%)
Long-term care		1 (1%)
Born this episode	14 (8%)	5 (4%)
Not available	3 (2%)	7 (6%)
Age-adjusted Charlson comorbidity index	2·0 (0·0–5·0)	2·0 (0·0–4·5)
Health-care exposures before BSI onset		
Antibiotic exposure (within 30 days)		
Yes	76 (44%)	70 (57%)
No	80 (46%)	34 (28%)
Not available	18 (10%)	19 (15%)
ICU admission (within 30 days)		
Yes	51 (29%)	54 (44%)
No	123 (71%)	69 (56%)
Surgery (within 30 days)		
Yes	26 (15%)	27 (22%)
No	148 (85%)	96 (78%)
Immunosuppressive therapy (within 3 months)		
Yes	27 (16%)	18 (15%)
No	143 (82%)	103 (84%)
Not available	4 (2%)	2 (2%)
Previous hospitalisation (within 12 months)		

	CSE BSI (n=174)	CRE BSI (n=123)
Yes	96 (55%)	77 (63%)
No	77 (44%)	46 (37%)
Not available	1 (1%)	0 (0%)
Qualifying Enterobacteriaceae		
Escherichia coli	83 (48%)	20 (16%)
Klebsiella species	69 (40%)	85 (69%)
Other Enterobacteriaceae	19 (11%)	10 (8%)
More than one Enterobacteriaceae	3 (2%)	8 (7%)
ICU at time of BSI onset	73 (42%)	68 (55%)
Pitt bacteraemia score at BSI onset[‡]	2·0 (0·0–4·0)	2·5 (1·0–5·0)
Epidemiological classification		
Community acquired	60 (34%)	11 (9%)
Hospital acquired	69 (40%)	65 (53%)
Health-care associated with community onse	25 (14%)	29 (24%)
Not available	20 (11%)	18 (15%)
Source		
Primary	99 (57%)	59 (48%)
Central-line associated	18 (10%)	26 (21%)
Secondary[‡]	57 (33%)	38 (31%)
CNS	1 (2%)	2 (5%)
Cardiovascular system infection	0	1 (3%)
Gastrointestinal system infection	14 (25%)	8 (21%)
Lower respiratory infection, other than pneumo	6 (11%)	3 (8%)
Pneumonia or ventilator-associated event	12 (21%)	6 (16%)
Reproductive tract infection	1 (2%)	0
Surgical site infection	3 (5%)	4 (11%)
Skin and soft tissue infection	0	9 (24%)
Urinary tract infection	20 (35%)	5 (13%)
Total length of stay (days)	11·0 (6·0–24·0)	19·0 (10·0–31·5)

Data are n (%) or median (IQR). CSE=carbapenem-susceptible Enterobacteriaceae. BSI=bloodstream infection. CRE=carbapenem-resistant Enterobacteriaceae. ICU=intensive care unit.

- 119 patients with CSE BSI and 95 patients with CRE BSI were aged at least 18 years.
- † Not available for one patient with CSE BSI and one patient with CRE BSI.
- ‡ For sources of secondary BSI, percentages represent the proportion of all secondary BSIs accounted for by that source (stratified by carbapenem susceptibility).

Table 2. Effect of carbapenem resistance on in-hospital death and discharge alive from proportional subdistribution hazards models*

	Subdistribution HR (95% CI)
In-hospital mortality	
Base analysis	1·68 (1·07–2·64)
Inverse probability weighted analysis	1·75 (1·04–2·94)
Alive at discharge	
Base analysis	0·63 (0·48–0·84)
Inverse probability weighted analysis	0·61 (0·45–0·83)

HR=hazard ratio.

* Analysis done on the complete cohort; all models include time from admission to infection as a covariate

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