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Outcome, risk factors and therapeutic strategies in carbapenem-resistant Gram-negative bacteraemia from Pakistan

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Background: Carbapenem-resistant Gram-negative (CRGN) bacteraemia has high mortality and limited therapeutic options. We assessed the risk factors and outcome of CRGN bacteraemia treated with limited options.

Methods: A prospective cohort study done at a tertiary care hospital in Pakistan, from October 2021 to August 2022. All patients >18 years with CRGN bacteraemia were assessed for demographics, source, risk factors and treatment received. Outcome was assessed as bacterial clearance and all-cause mortality at Day 14 of bacteraemia.

Results: We included 175 patients. Median age was 45 years (IQR 30–58) and the majority of our patients were on haemodialysis (75%). We found 14 day mortality in 26.8% of our patients; in addition, microbiological clearance was achieved in 95%. The central line (49.7%) was the most common source and *Klebsiella* spp. (47%) the most common organism. On multivariate analysis, risk factors for mortality were Foley's catheter [aOR 2.7 (95% CI 1.1–6.5)], mechanical ventilation [aOR 5.1 (95% CI 1.6–15.8)] and Pitt bacteraemia score >4 [aOR 3.48 (95% CI 1.1–10.5)]. Source control was a significant protective factor [aOR 0.251 (95% CI 0.09–0.6)]. The majority received a colistin-based regimen with no difference in mortality between monotherapy and combination therapy.

Conclusions: Our cohort of CRGN bacteraemia is unique, comprising younger patients mostly on haemodialysis with a central line as the source of bacteraemia and we have found 14 day mortality of 27%. Colistin with various combinations can be an effective option in patients with renal failure having prompt source control.

Introduction

Carbapenem-resistant Gram-negative (CRGN) infections have emerged as a global emergency and a major concern for practising clinicians.[1] CRGN bacteraemia accounts for increases in mortality, length of hospital stay, ICU stay and cost.[2,3] The WHO generated a priority pathogen list of highly resistant Gram-negative pathogens for which there are very limited treatment options. These include *Acinetobacter*, *Pseudomonas* and various Enterobacterales (including *Klebsiella*, *Escherichia coli*, *Serratia* and *Proteus*).[4] The most important mechanism of resistance among these organisms is the production of carbapenem-hydrolysing enzymes called carbapenemases. The prevalence of carbapenemase production is variable in different regions of the world, with variation of gene expression in these organisms. For example, the most prevalent enzymes produced by these organisms are KPC in the USA, MBL in South-East Asia, and OXA-48 in the Middle East.[5–7] This difference has major implications in choosing appropriate antibiotics. The newer antibiotics like ceftazidime/avibactam have limited coverage for MBL producers, which are highly prevalent in our part of the world.[8] In Pakistan, the overall pooled proportion of MBL-producing CRGN is around 34%.[9] Among MBLs, NDM production is the most prevalent in Pakistan, with around 40% positivity in different samples.[10]

A systematic review on risk factors for CRGN infections found previous carbapenem use, colonization with carbapenem-resistant organisms, ICU stay and mechanical ventilation, presence of catheter, length of hospital stay and high APACHE score as significant factors.[11] From Pakistan, risk factors identified for

CRGN bacteraemia were age >50 years, ICU stay, septic shock on presentation, diabetes, tracheostomy and presence of a central line.[12,13] Babar *et al.*[14] noted that inappropriate choice of empirical antibiotics is associated with significant mortality among patients with CRGN bacteraemia.

With the high prevalence of MBL-producing CRGN infections and very limited treatment options in Pakistan, our aim was to find out the risk factors, outcome and bacterial clearance for CRGN bacteraemia.

Methods

Ethics

Approval was taken from the Ethics Review Committee of SIUT and written informed consent was obtained from all participants. The study was approved by the Ethical Review Committee of the hospital (approval number SIUT-ERC-2021/A-350). Consent for publication was taken from the institutional research advisory board (CRP No. 313).

Study site

This prospective cohort study was conducted at the Sindh Institute of Urology and Transplantation (SIUT) in Karachi, Pakistan from October 2021 till August 2022. SIUT is the largest hospital for renal diseases and transplantation in Pakistan. It also caters for urology, oncology, hepatobiliary, gastroenterology and general surgery patients.

Inclusion criteria

All hospitalized patients aged >18 years with a positive blood culture for *Klebsiella* spp., *Pseudomonas aeruginosa, Pseudomonas* spp., *Escherichia coli* and *Acinetobacter* that were resistant to carbapenems (Imipenem and/or Meropenem) were included in this study. In cases where multiple cultures were positive, only the first positive blood culture was included. Each patient was enrolled only once in the study period.

Exclusion criteria

Patients receiving haemodialysis on an outpatient basis were excluded. Patients who met the eligibility criteria but died or were transferred or discharged prior to a positive culture report, and were no longer inpatients, were excluded from the study.

Data collection

The proformas were filled in for those patients who met the eligibility criteria after informed consent. Data were collected for demographics, clinical features, laboratory parameters, recent hospitalization, recent exposure to carbapenems, empirical choice of antibiotic and ICU stay. The Charlson comorbidity index and Pitt bacteraemia score were calculated for all the patients at baseline. APACHE II score and quick SOFA (q-SOFA) score were documented. The most likely source of bacteraemia was determined on the basis of clinical and laboratory evaluation. The presence of a central line, a peripheral vascular catheter, a urethral catheter, nephrostomy tubes, drains, endotracheal intubation, total parenteral nutrition, any recent surgical procedure and haemodialysis were noted. Source control was documented, such as removal of dialysis lines, urinary catheters, abscess drainage etc.

Microbiological tests

Speciation and drug susceptibility testing of isolates were performed as per CLSI guidelines. Drug susceptibility was performed using a disc diffusion method according to CLSI breakpoints and later tested for susceptibility against second-line available antimicrobial agents such as tigecycline, minocycline and fosfomycin. Susceptibility to tigecycline was determined according to FDA criteria for Enterobacteriaceae.[15] Colistin MICs were determined by a broth microdilution method, (MICs ≤2 mg/L as intermediate and ≥4 mg/L as resistant) as per CLSI guidelines (edition 31).[16] We did not check molecular resistance mechanism for carbapenemase production.

Antimicrobial therapy

Treatment was prescribed by the primary physicians in consultation with infectious disease (ID) physicians. Treatment strategies were divided into non-colistin-based and colistin-based therapy, which was further divided into colistin monotherapy and colistin combination therapy. Duration of definitive antimicrobial therapy was noted. Appropriate empirical antibiotic meant the patient received the antibiotic within 48 h from onset of bacteraemia, to which the organism was later found to be susceptible from the reported culture results.

Follow-up and outcomes

The patients were followed at Day 3 with repeat blood culture to document bacterial clearance. Fever, total leucocyte counts (TLCs) and hypotension were also documented. Patients were then followed till Day 14 for all-cause mortality. If discharged by the primary team before the end of 14 days, telephone contact was made to determine whether the patient was alive or dead.

The primary outcomes were bacteriological clearance and all-cause mortality at Day 14. The secondary outcomes were treatment strategies received with mortality and duration of hospital stay.

Statistical analysis

Statistical package for the social sciences (SPSS) version 22.0 was used for data entry and analysis. Frequencies and percentages were reported for categorical variables. Mean with standard deviation or median with IQR were used for continuous variables, as appropriate. Bivariate and multivariate analysis were used for identifying the independent risk factors for carbapenem-resistant bacteraemia and mortality outcome. Multivariate analysis was done as stepwise logistic regression using a forward logistic regression method. For all tests, $P < 0.05$ was considered as significant for univariate analysis. Crude ORs and adjusted ORs (aORs) with 95% CIs were reported.

Results

A total of 175 patients were included in the study. The median age was 45 years (IQR 30–58) and no difference was found between survivors versus the non-survivors group. Table 1 summarizes baseline demographics, risk factors, clinical and laboratory parameters of all patients and comparison between survivors and non-survivors.

A total of 47 (26.8%) patients had died by Day 14 of bacteraemia. The most common comorbidity in our cohort was renal failure patients on haemodialysis (131; 75%). The other comorbidities are shown in Table S1, available as Supplementary data at *JAC-AMR* Online.

Risk factors, on univariate analysis were: ICU stay [OR 5.77 (95% CI 2.72–12.2)], high APACHE score (26 versus 22; $P <$ 0.001), having a Foley's catheter [OR 3.48 (95% CI 1.63–7.44)], being on a mechanical ventilator [OR 7.96 (95% CI 3.75–16.9)], q-sofa score >2 [OR 7.98 (95% CI 3.79–16.8)] and Pitt bacteraemia score ≥4 [OR (8.67 (95% CI (3.98–18.8)] were significantly associated with mortality. On multivariate analysis (Table 2), the

Table 1. Demographics and risk factors for mortality

Characteristics	Total ($n=175$)	Survivors ($n=128$; 73%)	Non-survivors ($n=47$; 27%)	*P* value	OR
Age (years), median (IQR)	45 (30–58)	45 (30–57)	50 (30–61)	0.499	—
Female, *n* (%)	62 (35.4)	44 (34.4)	18 (38.3)	0.631	—
Comorbidities, *n* (%)					
CCI >5	23 (13.3)	17 (13.3)	6 (12.8)	0.929	—
Ischaemic heart disease	9 (5.1)	4 (3.1)	5 (10.6)	0.046	3.69 (0.95–14.4)
Renal failure on haemodialysis	131 (74.9)	97 (75.8)	34 (72.3)	0.642	—
Risk factors					
ICU stay at time of bacteraemia, *n* (%)	78 (44.6)	43 (33.6)	35 (74.5)	<0.001	5.77 (2.72–12.2)
APACHE score at time of bacteraemia, median (IQR)	24 (19–27)	22 (16–25)	26 (23–30)	0.001	—
q-SOFA score >2, *n* (%)	56 (32)	25 (19.5)	31 (66.0)	<0.001	7.98 (3.79–16.8)
Pitt bacteraemia score ≥4, *n* (%)	42 (24)	16 (12.5)	26 (55.3)	<0.001	8.67 (3.98–18.8)
Mechanical ventilation, *n* (%)	48 (27.4)	20 (15.6)	28 (59.6)	<0.001	7.96 (3.75–16.9)
Recent hospitalization (within 1 month), *n* (%)	108 (61.7)	83 (64.8)	25 (53.2)	0.160	—
Surgery, *n* (%)	53 (30.3)	39 (30.5)	14 (29.8)	0.931	—
Central line, *n* (%)	134 (76.6)	95 (74.2)	39 (83.0)	0.225	—
Foley's catheter, *n* (%)	98 (56.0)	62 (48.4)	36 (76.6)	0.01	3.48 (1.63–7.44)
Percutaneous nephrostomy tube, *n* (%)	21 (12.0)	17 (13.3)	4 (8.5)	0.389	—
Neutropenia, *n* (%)	2 (1.1)	0 (0)	2 (4.3)	0.019	—
Recent exposure to a carbapenem (>5 days), *n* (%)	28 (16.0)	19 (14.8)	9 (19.1)	0.491	—
Clinical signs and symptoms and laboratory parameters, *n* (%)					
Fever	140 (80)	103 (80.5)	37 (78.7)	0.798	—
Hypotension	49 (28)	27 (21.1)	22 (46.8)	0.001	3.29 (1.61–6.72)
Altered mental status	55 (31.4)	25 (19.5)	30 (63.8)	<0.001	7.27 (3.47–15.2)
Leucopenia	6 (3.4)	2 (1.6)	4 (8.5)	0.025	—
Thrombocytopenia	55 (31.4)	33 (25.8)	22 (46.8)	0.08	—
Source of bacteraemia, *n* (%)					
Central line	87 (49.7)	61 (47.7)	26 (55.3)	0.369	—
Urine	52 (29.7)	43 (33.6)	9 (19.1)	0.064	—
Pneumonia	1 (0.6)	1 (0.8)	0 (0.0)	0.543	—
Surgical site infection	6 (3.4)	6 (4.7)	0 (0.0)	0.131	—
Skin and soft tissue infection	12 (6.9)	6 (4.7)	6 (12.8)	0.061	—
Abdomen	11 (6.3)	6 (4.7)	5 (10.6)	0.151	—
No source identified	6 (3.4)	5 (3.9)	1 (2.1)	0.567	—
Source control	127 (72.6)	100 (78.1)	27 (57.4)	0.007	0.38 (0.18–0.77)
Duration of hospital stay from bacteraemia, days, median (IQR)	12 (8–18)	14 (10–22)	8 (5–11)	<0.001	—

CCI, Charlson comorbidity index; thrombocytopenia: $<100\times10^9$/L; leucopenia: $<4\times10^9$/L.

most significant risk factors for mortality were having a Foley's catheter [aOR 2.7 (95% CI 1.1–6.5)], being on a mechanical ventilator [aOR 5.1 (95% CI 1.6–15.8)] and Pitt bacteraemia score ≥4 [aOR 3.48 (95% CI 1.1–10.5)]. Source control was found to be a significant protective factor for mortality in our cohort [aOR 0.251 (95% CI 0.09–0.6)].

The most common source of bacteraemia was a central line (49.7%). However, there was no statistically significant difference between survivors and non-survivors regarding the source of bacteraemia.

Table 3 shows microbiological data and treatment received for survivors and non-survivors. The most common causative organism was *Klebsiella* spp. (83; 47%). *Klebsiella* spp. were significantly associated with mortality (59.6% versus 43%; $P=0.051$) and comparisons with other pathogens are shown in Figure S1. A colistin-based regimen was received by 149 (85%) patients. There was no difference in mortality between those who received colistin monotherapy versus combination therapy. In non-colistin-based regimens, other different antibiotics were used (Table S2).

The susceptibility patterns of different organisms are shown in Figure 1. The carbapenem zone size was zero for 67 (38%) isolates. Colistin MICs were determined for a total of 160 isolates; the median MIC was 1 mg/L (IQR 0.5–2). Four (2.5%) isolates

had colistin MICs of >2 mg/L; all of them were *Klebsiella* spp. There was no difference in outcome on the basis of colistin MICs (3% versus 0%; $P=0.57$).

Discussion

Our study was a single-centre experience from a low middle-income country (LMIC), where we are facing a high number of CRGN organisms as a major public health crisis on the one hand, and lack of availability and development of new antimicrobials, especially for NDM-producing organisms, on the other hand.[17] We looked into the risk factors and the outcome of different treatment regimens for CRGN bacteraemia in the scenario of very limited treatment options. In our cohort, the all-cause mortality was 27% which is comparable with that reported in the literature. Shah *et al.*[18] from India reported a mortality of 20%–30% among patients with CRGN bacteraemia. Similarly, a large multinational cohort study (Panorama) reported a crude mortality of 35% among CRGN bacteraemia.[17]

We found that being on a mechanical ventilator, having a Pitt bacteraemia score of >4 and having a Foley's catheter are significant risk factors for mortality among patients with CRGN bacteraemia. Ahmed *et al.*[12] reported ICU stay and central venous catheters as significant risk factors for mortality. A study from our centre found ICU stay, septic shock on presentation and immunosuppressive medications as risk factors.[13] Urinary catheterization, high Pitt bacteraemia score and inappropriate empirical antibiotics were reported from Turkey.[19] Similarly, a retrospective study from Malaysia found ICU stay, septic shock and raised serum creatinine as risk factors for mortality among CRGN bacteraemia.[20] Hence, we can predict that patients with CRGN bacteraemia who have prolonged ICU stay, requiring central lines, urinary catheterization or mechanical ventilation have a major risk of morbidity and mortality.

Klebsiella spp. were the most common isolates found in our study having significant association with mortality. Four isolates of *Klebsiella* spp. were found to be XDR with colistin MICs of >2 mg/L. *Klebsiella* spp. are one of the most common nosocomial pathogens harbouring highly infectious plasmid-mediated resistance genes.[21] A study from Pakistan found the presence of highly resistant *Klebsiella pneumoniae* in different ecological niches, with 43% expressing the bla_{NDM-1} gene.[22] Furthermore, the most important area of concern is the higher MIC of colistin in 2.5% of the isolates in our study. Colistin resistance has been reported from Pakistan and South Asia. Imtiaz *et al.*[23] found colistin resistance to be as high as 15% in various clinical isolates; the majority were from urine samples.[23] Increased mortality with *Klebsiella* spp. was likely due to high resistance to almost all antibiotics including colistin. Strict infection control measures along with antibiotic stewardship are stressed to prevent the spread of this highly virulent and resistant organism.

Our main objective is to find the outcome of CRGN bacteraemia in the scenario of very limited treatment options. A combination of ceftazidime/avibactam and aztreonam is found to be efficacious for CRGN producing MBL enzymes.[24] However, in Pakistan we do not have these two antibiotics available. In this prospective observational study, we used a variety of combinations. The majority received colistin-based therapy. We found that the bacterial clearance was adequate (95%) and the mortality rate was found to be 27%. Previous studies from our institute by Babar *et al.* and Kalam *et al.* reported mortality of 19%–46% in CRGN bacteraemia.[13,14] There are scant data other than the above studies for mortality of carbapenem-resistant Enterobacteriaceae (CRE) in haemodialysis patients. Eilertson *et al.*[25] reported higher mortality with CRE infections in patients on renal replacement therapy, although only one-quarter of patients had bacteraemia and the median age was 62 years. We did not see a different or higher mortality rate for CRE bacteraemia in our cohort comprising mainly haemodialysis patients, due in part to the younger median age and prompt source control. Almangour *et al.*[26] from Saudi Arabia reported similar mortality

Table 2. Multivariate logistic regression for risk factors of mortality

Variables	*P* value	OR	95% CI
Foley's catheter	0.024	2.731	(1.139–6.548)
Mechanical ventilation	0.004	5.135	(1.663–15.855)
Pitt bacteraemia score ≥4	0.027	3.486	(1.155–10.519)
Source control	0.004	0.251	(0.099–0.635)

Table 3. Microbiological data and antibiotic regimens among total patients, survivors and non-survivors

Treatment options	Total ($n=175$)	Survivors ($n=128$)	Non-survivors ($n=47$)	*P* value
Microbiological data				
Polymicrobial	13 (7.4)	10 (7.8)	3 (6.4)	0.749
Acinetobacter spp.	44 (25.1)	37 (28.9)	7 (14.9)	0.058
Klebsiella spp.	83 (47.4)	55 (43.0)	28 (59.6)	0.051
Microbiological clearance	167 (95.4)	123 (96.1)	44 (93.6)	0.487
New bacteraemia	23 (13.1)	14 (10.9)	9 (19.1)	0.154
Treatment				
Colistin-containing regimen	149 (85.1)	105 (82.0)	44 (93.6)	0.056
Colistin monotherapy	20 (11.3)	16 (12.5)	4 (8.5)	0.462
Colistin combinations	129 (73.7)	89 (69.5)	40 (85.1)	
Duration of therapy (days), median (IQR)	9 (7–13)	10 (7.25–14)	8 (4–9)	<0.001

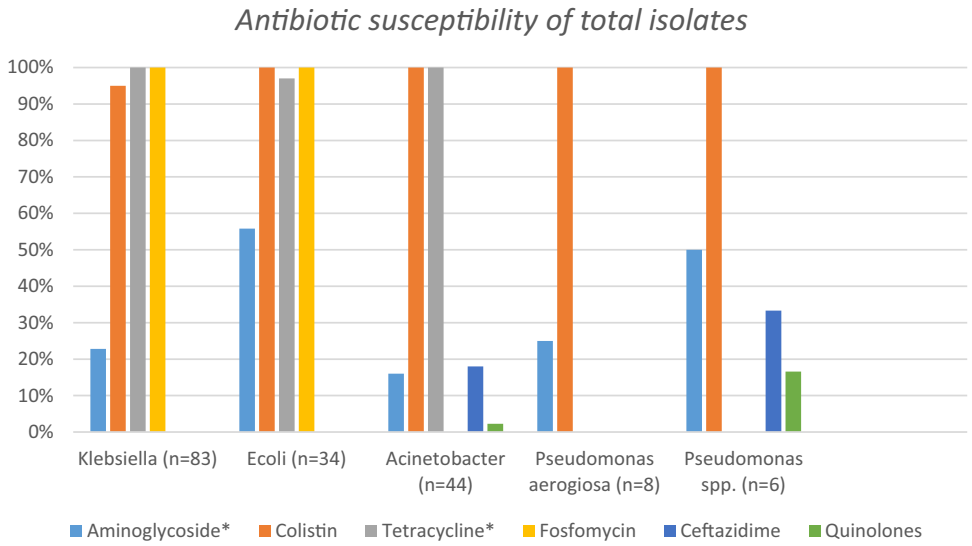

Figure 1. Antibiotic susceptibility patterns of all isolates. *Aminoglycosides: susceptibility was checked for amikacin, gentamicin and tobramycin. *Tetracyclines: susceptibility was checked for doxycycline, minocycline and tigecycline.

rates for colistin and ceftazidime/avibactam in their patient cohort where the majority were infected with OXA-48-producing organisms. However, Chen *et al.*[27] from China used carbapenems with different combinations in CRGN bloodstream infections and found a very high mortality rate of more than 50%. The majority of our cohort were on haemodialysis, with 50% having central line infection. Prompt removal of dialysis lines and good source control may be the reason for better outcomes. Furthermore, we can assume that renal failure patients were found to achieve higher serum concentrations of colistin because of decreased renal excretion leading to a larger fraction of prodrug being converted to colistin.[28] This may be the reason for the good bacterial clearance and lower mortality in our cohort.

Moreover, we found no difference in outcome between colistin monotherapy versus combination therapy. Our findings are consistent with two recent randomized trials. A multinational European trial found combination therapy to not be superior to monotherapy.[29] Similarly, the Overcome trial, an international randomized placebo-controlled trial, found no difference in outcome between colistin monotherapy and combination therapy.[30] In both these trials, the majority of the patient population had pneumonia and the most common organism was *Acinetobacter*. However, in our study, all of our patients had bacteraemia, with 45% having *Klebsiella* spp. We can conclude that colistin monotherapy is equally as effective as combination therapy against Enterobacterales, with good bacterial clearance and no difference in mortality.

The limitations of our study were: firstly, it was an observational study. Secondly, due to resource constraints, we relied on zone size with the disc diffusion method for the estimation of MICs for carbapenems and did not have molecular genetic data for carbapenemase-producing Enterobacterales in our cohort. Furthermore, we do not have colistin drug level monitoring available. We need more studies on the epidemiology based on molecular genetic analysis and treatment outcomes with options like aztreonam if available.

In conclusion, the outcome of CRGN bacteraemia in our patients is comparable with that in the literature. In places where newer options are very expensive or unavailable, colistin with various combinations can be a safe and effective option, particularly in patients with renal failure and prompt source control.

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This was a prospective observational cohort study that was done as a part of routine work.

Transparency declarations

None to declare.

Author contributions

Mehreen Fatima contributed to study conceptualization, data collection, manuscript writing, analysis and interpretation; Sunil Kumar Dodani contributed to the study conceptualization, methodology, data analysis and

interpretation; Asma Nasim contributed to study conceptualization, data interpretation, manuscript writing, review and overall study supervision; Zaheer Udin Babar contributed to methodology review and data interpretation; Beena Rani, Sanjay Badlani and Maryam Mushtaq contributed to methodology and data collection; Ali Nadeem contributed to microbiological data collection, interpretation and methodology review.

Data and material availability

Data and material will be provided if required for publication.

Supplementary data

Figure S1 and Tables S1 and S2 are available as Supplementary data at *JAC-AMR* Online.

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