


Emergence of colistin-resistant gram-negative rods in intensive care units: A cross-sectional study from a developing country

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Abstract

Objectives: To determine the clinical features, outcomes, and factors associated with the emergence of colistin-resistant gram-negative rods isolated from patients admitted to intensive care units.

Methods: This cross-sectional study was conducted at the intensive care units of Liaquat National Hospital, from April 2019 to February 2020. Gram-negative rods resistant to colistin with minimum inhibitory concentrations ≥ 4 mcg/mL according to Clinical and Laboratory Standards Institute criteria as reported in cultures were included. Clinical, demographical data and treatment given were recorded and analyzed using SPSS version 25.

Results: A total of 93 patients were included; 58.1% were males. The mean age of patients was 59.48 ± 18.36 years. The most common organism isolated was *Klebsiella pneumoniae* (91.4%). The most common specimen was the tracheal (62.4%). Ventilator-acquired pneumonia was seen in 38.7%. The most common co-morbid disease seen in patients was diabetes (41%); 77% had a symptomatic infection and were treated with a combination of 2 or more antibiotics, most commonly meropenem plus fosfomycin. The most common susceptible antibiotics were fosfomycin (72%) and tigecycline (50.5%). Mean intensive care unit stay and total duration of hospital stay were prolonged (16.83 ± 12.93 and 23.34 ± 17.52 days, respectively). Forty-eight (62.3%) patients with symptomatic infection with colistin-resistant isolates were treated and discharged, and mortality was seen in 23 (29.9%). A significant association was found between mortality and symptomatic infection, endotracheal intubation with mechanical ventilation ($p = 0.003$), and a prolonged hospital stay of >20 days ($p = 0.041$).

Conclusion: Colistin-resistant gram-negative rods pose a significant problem especially in developing countries because of limited therapeutic options. Stringent infection control and comprehensive antimicrobial stewardship programs are needed to overcome this challenge.

Keywords

Colistin resistance, gram-negative rods, extensively drug-resistant

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Introduction

Multi-drug-resistant (MDR) gram-negative rods (*GNRs*), including *Pseudomonas*, *Acinetobacter*, *Klebsiella pneumoniae*, and other *enterobacterales* resistant to carbapenems, have emerged as a significant threat worldwide, especially in hospital-acquired infections (HAIs).^{1–3} Colistin is regarded as the last resort against these organisms.^{1,2,4} But due to the injudicious use of this antibiotic especially in intensive care settings, *GNRs* showing resistance to colistin are increasingly encountered due to selective antibiotic pressure and horizontal transmission.^{4,5} Colistin resistance is often associated with carbapenem resistance, and such

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organisms are classified as extensively drug-resistant (XDR).⁶ Reports of colistin plus carbapenem-resistant cases have emerged from different parts of the world and because of limited therapeutic options available, is becoming a major global health concern.^{2,7-9}

In Europe, detection of colistin-resistant strains was reported as early as 2000.^{10,11} Monaco et al.¹² reported 43% of their carbapenemase-producing carbapenem-resistant *K. pneumoniae* isolates to be colistin-resistant as well. Colistin resistance in other *enterobacterales*, for example, *E. coli* and *non-enterobacterales* like *Acinetobacter* and *Pseudomonas*, has also been reported in the literature.^{1,3,8,13} The highest rate of colistin resistance is being reported in South East Asia, followed by Europe and America. Although the overall prevalence is less than 10% worldwide, it is continually increasing.¹⁴ In Pakistan, a laboratory-based study by Qamar et al.¹⁵ identified 40 (15.9%) out of 251 *enterobacterales* to be colistin-resistant. Another study from Pakistan by Asif et al.¹⁶ identified only 3 colistin-resistant isolates, and all of them were non-fermenters. Colistin resistance is associated with increased morbidity, mortality, and more extended hospital stays and costs.^{1,7,8} Factors related to the emergence of colistin resistance include prior hospitalization, prolonged use of carbapenems and colistin, indwelling catheters, multiple co-morbidities, and increasing age as identified in previous literature.¹⁷ Available antimicrobial options against colistin-resistant *GNRs* are minimal. Current therapeutic choices are restricted, and novel antimicrobials with adequate coverage are not widely known or very expensive to be considered an option, especially in resource-limited settings. They are often used as combination therapy with at least 2 or 3 antibiotics given together.^{7,8,18,19}

Since colistin resistance can be one of the most severe and life-threatening issues encountered in healthcare settings, it is essential to know baseline clinical and epidemiological characteristics and associated factors that lead to its emergence. To the best of our knowledge, very sparse literature regarding the prevalence and related factors for colistin resistance exists in Pakistan.

Our study aimed to gather more information involving all *GNRs*, including *non-enterobacterales* so that the increasing drug resistance in healthcare settings can be curtailed through judicious use of antibiotics and other appropriate measures.

Methodology

Study design and setting

This cross-sectional study was conducted in intensive care units (ICUs) at one of the largest multidisciplinary, tertiary care hospitals in Karachi, from April 2019 to February 2020.

Study population and sampling technique

Patients aged 13 years and above with a positive culture (tracheal, blood, urine) showing gram-negative rods with a

colistin minimum inhibitory concentration (MIC) ≥ 4 plus carbapenem resistance, admitted only in ICUs were included through non-probability consecutive sampling. Patients were followed up till their stay in the ICUs.

Patients having cultures sent from outside the hospital, transferred-in patients with a colistin-resistant *GNR* from the previous hospitalization, a positive culture from an earlier admission, and gram-negative rods intrinsically resistant to colistin (*Burkholderia sp.*, *Stenotrophomonas sp.*, *Proteus sp.*, *Providencia sp.*) were excluded.

Sample size calculation

By taking the prevalence of colonized patients by colistin-resistant gram-negative bacteria, $p=52\%$ ¹ using a margin of error $d=10\%$, the total calculated sample size was 96 patients, taking a 95% confidence level using the World Health Organization (WHO) sample size calculation formula. The final number of patients included in the study was 93 as data collection was stopped due to the COVID-19 pandemic. The margin of error was also increased to 10% to reduce the sample size because of the reason stated above.

Data collection methods and instruments

After approval from the Institutional Review Board (IRB) and the Ethical Review Committee of the hospital (ERC no #0476-2019-LNH-ERC), data were collected on a pre-formed proforma by the investigator. Data were collected after getting informed written consent from the patient, or an attendant (in case the patient was unable to give consent), admitted in ICUs. Patients' confidentiality was maintained strictly by not revealing their identities on the proforma and only using coded medical record numbers. This information was also only disclosed to the primary investigator. Positive culture reports showing colistin-resistant (MIC ≥ 4) *GNRs* were collected from the microbiology department. Information regarding the following variables from patients with positive cultures were noted: medical record number, age, gender, and other demographics, and the total number of days in hospital/ICU. Clinical data included co-morbid conditions, source of isolates, symptoms, and signs, invasive devices (indwelling catheters and endotracheal intubation), previous antibiotic regimen, microorganisms isolated, drug sensitivity data including colistin MIC, targeted therapy is given, and outcome of patients. Culture identification (phenotype only) and sensitivity testing were performed using routine laboratory protocols at the microbiology laboratory. The MIC of colistin was determined using the broth microdilution method and VITEK II automated system. Colistin MIC of $\leq 2 \mu\text{g/mL}$ was considered sensitive and $\geq 4 \mu\text{g/mL}$ as resistant according to the Clinical and Laboratory Standards Institute (CLSI) 2019 criteria.²⁰

A positive culture showing *GNRs* accompanied by signs and symptoms related to the site of infection involved, as detailed in the proforma, was considered an *infection*.

Fever and leukocytosis were also indicative of infection. Colonization was a presence of a *GNR* in an isolate but no signs and symptoms related to the site. HAIs were defined as nosocomially acquired infections that were not present at the time of admission and manifested after 48 h of hospitalization. These included ventilator-acquired pneumonia (VAP) defined as the onset of signs and symptoms of pneumonia including new infiltrates on chest X-ray with tracheal culture positive for colistin-resistant *GNR*; hospital-acquired pneumonia (HAP) had similar criteria but without invasive mechanical ventilation; bloodstream infection (BSI) was defined as isolation of colistin-resistant *GNR* in blood specimen with fever and with or without leukocytosis. Catheter-related bloodstream infection (CRBSI) was defined as the isolation of colistin-resistant *GNR* simultaneously from a blood sample drawn from a central line (intravascular catheter) and the catheter tip or from a peripheral vein, and catheter-associated urinary tract infection (CAUTI) was defined as isolation of colistin-resistant *GNR* in a urine specimen obtained from a catheter with the proper septic technique with fever and/or leukocytosis.²¹

Statistical analysis

Patient data were compiled and analyzed through a statistical package for Social Sciences (SPSS) Version 25. Frequencies and percentages were computed for qualitative variables like gender, admitting diagnosis, source of isolates, co-morbid and risks, clinical characteristics, previous antibiotic regimen, site of sample collection, isolated microorganisms, sensitive antibiotics, and colistin MICs. Mean \pm standard deviation (SD) was calculated for quantitative variables, that is, age, duration of ICU/hospital before enrollment, and the total time of ICU/hospital. The chi-square test was used to analyze the qualitative variables while the Student t-test was used for the quantitative variables. Stratification was done for age and gender to see the effect of these modifiers on the outcome using the chi-square test. P-value \leq 0.05 was considered significant.

Results

The study was completed with 93 patients having colistin-resistant *GNRs* in their isolates. Male predominance with 54 (58.1%) patients was seen. The mean age of the patients was 59.48 ± 18.36 years. The most common isolate was tracheal aspirate, $n=58$ (62.4%). The most common co-morbid were diabetes, $n=39$ (41.9%), and chronic kidney disease, $n=22$ (23.7%). Fever was found in 46 (49.5%) patients, while 83 (89.2%) had leukocytosis. Respiratory tract infections were the most common infections distributed as HAP in 39 (41.9%) and VAP in 36 (38.7%) patients. Other sites of infections are given in Table 1. The most common previously used antibiotic regimen was a combination of meropenem and colistin. Other antibiotics used are described in Table 2.

Table 1. Clinical characteristics of population under study ($n=93$).

	Frequency (%)
Gender	
Male	54 (58.1)
Female	39 (41.9)
Source of isolate	
Tracheal aspirate	58 (62.4)
Blood	20 (21.5)
Urine	12 (12.9)
Other	6 (6.6)
Co-morbid conditions	
Diabetes mellitus	39 (41.9)
Chronic kidney disease	22 (23.7)
Malignancy	1 (1.1)
Autoimmune disease	3 (3.2)
Immunosuppressive therapy	4 (4.3)
HIV	0 (0)
Fever	
Yes	46 (49.5)
No	47 (50.5)
Leukocytosis	
Yes	83 (89.2)
No	10 (10.8)
Respiratory tract infection	
Hospital-acquired pneumonia	39 (41.9)
Ventilator-acquired pneumonia	36 (38.7)
Urinary infection	
Urinary tract infection	7 (7.5)
Catheter-associated urinary tract infection	5 (5.4)
Bloodstream infection	
Bacteremia/bloodstream infection	16 (17.2)
Catheter-related bloodstream infection	5 (5.4)
Indwelling devices and antibiotic use	
Endotracheal intubation and mechanical ventilation	41 (44.1)
Urinary catheter	81 (87.1)
Central line	71 (76.3)
Any other instrumentation or surgical intervention	33 (35.5)
Broad spectrum antibiotics for more than 7 days	82 (88.2)
Colistin MIC 50	4 mcg/MI
Colistin MIC 90	8 mcg/mL
Duration of ICU stay (mean \pm SD)	15.98 ± 12.59
Duration of hospital stay (mean \pm SD)	21.70 ± 16.83
Outcome	
Treated and discharged	48 (51.6)
Transferred/left against medical advice (LAMA)	16 (17.2)
Expired	28 (30.1)

HIV: human immunodeficiency virus; MIC: minimum inhibitory concentration; ICU: intensive care unit.

Previous cultures were not included so we were unable to determine whether the previous treatment regimen was empirical or targeted. The detailed baseline clinical characteristic of the population under study is presented in Table 1.

Table 2. Previous antibiotic regimens used.

Name of antibiotics	n (%)
Meropenem plus colistin	84 (90)
Pipercillin Tazobactam	19 (20.4)
Ceftriaxone sodium	10 (10.8)
Moxifloxacin	10 (10.8)
Minocycline	10 (10.8)
Cotrimoxazole	8 (8.6)
Fosfomycin	8 (8.6)
Gentamycin	7 (7.5)
Ciprofloxacin	6 (6.5)
Cefoperazone-Sulbactam	3 (3.2)
Amikacin	3 (3.2)

Minocycline and fosfomycin were mostly used in combination with meropenem and/or colistin.

K. pneumoniae was the most frequent pathogen isolated, others are shown in Figure 1. The major sensitive antibiotics against the isolates were fosfomycin 67 (72%), tigecycline 47 (50.5%), chloramphenicol 12 (12.9%), aminoglycosides 11 (11.8%), co-trimoxazole 8 (8.6%), and tetracycline 4 (4.3%). None of the isolates were sensitive to carbapenems.

Seventy-seven (82.8%) patients had a symptomatic infection, out of which 50 (64.9%) were males. A comparison of clinical characteristics and associated factors related to symptomatic infection versus colonization in patients with colistin-resistant *GNRs* isolates is given in Table 3. Male gender ($p=0.005$) and total duration of hospital stay ($p=0.039$) were significantly associated with infection by a colistin-resistant *GNR*, respectively. Patients with positive cultures not representative of infection of the site from which they were collected were not treated and regarded as colonizers. In contrast to patients with symptomatic infection, mean ICU stay and total hospital stay were shortened in colonized cases and the difference was found to be statistically significant ($p=0.039$).

Overall in-hospital mortality was 28/93 (30%) out of which 23 (82.1%) had a symptomatic infection and 5 (17.9%) were colonizers. So, a significant association was found between mortality and symptomatic disease ($p<0.001$). We also found a significant association between overall mortality and mechanical ventilation ($p=0.003$) and a prolonged hospital stay of >20 days ($p=0.041$) when compared between survivors versus non-survivors. However, there was no significant association between mortality and the presence of other invasive devices, BSI or CAUTI, and co-morbidities like diabetes and chronic kidney among the survivors versus non-survivors.

Meropenem and fosfomycin in combination were the most common antibiotics used for the treatment in 46 (59.7%) patients. The details of the combinations of antibiotics used to treat patients and their outcomes are presented in Table 4.

Discussion

This cross-sectional study was conducted in the ICUs of one of the largest tertiary care centers in the city for almost 1 year. We found a total of 93 patients with colistin-resistant *GNR* isolates. The most common site of infection was the respiratory tract and the most common organism identified as *K. pneumoniae*. The most common susceptible antibiotic was fosfomycin which was used in combination with other antibiotics to treat symptomatic infections. A positive association was seen between symptomatic infections with a colistin-resistant *GNR* and mortality.

The number of isolates with colistin-resistant *GNRs* reported in our study was higher as compared to previous studies that mainly reported outbreaks.^{1,2,22–24} A study from India by Arjun et al.⁴ showed 24 patients identified over 18 months with colistin-resistant organisms. This shows that colistin resistance among the *GNRs* is on the rise in our region, though data supporting this are limited.^{4,15}

Our study showed *K. pneumoniae* to be the predominant organism, similar to a study by Arjun et al.⁴ in India and by Qamar et al.¹⁵ in Pakistan. Studies done internationally in Europe and Brazil also showed *K. pneumoniae* as the most common organism.^{12,25,26} However, few Indian investigators found *E. coli* to be the predominant organisms in their isolates.^{27,28} We also found a minimal number of non-enterobacterales: *Pseudomonas* and *Acinetobacter* in our isolates corroborating the findings by Rossi et al.²⁶ In contrast, earlier studies have reported *Acinetobacter sp.* as the most common *GNR* to be colistin-resistant.^{10,29,30}

Our patient population showed a predominance of males, a finding similar to studies by Qamar et al.¹⁵ (56.8%) and the EUSCAPE project (60%).¹² The mean age of our patients also coincided with the findings of other studies.^{4,15,31} This could simply be reflective of the fact that the elderly population with multiple co-morbidities is more likely to be admitted to ICUs because of severe disease.

The most common isolate in our study was tracheal aspirate, followed by blood and urine. Consequently, the most common infections identified were also respiratory tract infections involving ventilated and non-ventilated patients. However, several other studies have shown the urinary tract to be the most common site of infection.^{4,12,15,31} Arjun et al.⁴ identified 33% of colistin-resistant *GNRs* in urine. Capone et al.³² found BSIs to be the most common, followed by urinary tract and respiratory tract infections. This could be attributable to the variable patient population in these studies ranging from general ward settings to ICUs, whereas our study comprised patients admitted to the ICUs only, hence the increased frequency of VAP.

Studies have reported several factors to be associated with the emergence of colistin resistance, most significantly previous exposure to colistin alone or in combination with broad-spectrum antibiotics, especially carbapenems.^{5,12} In our study, the majority of patients were treated with a

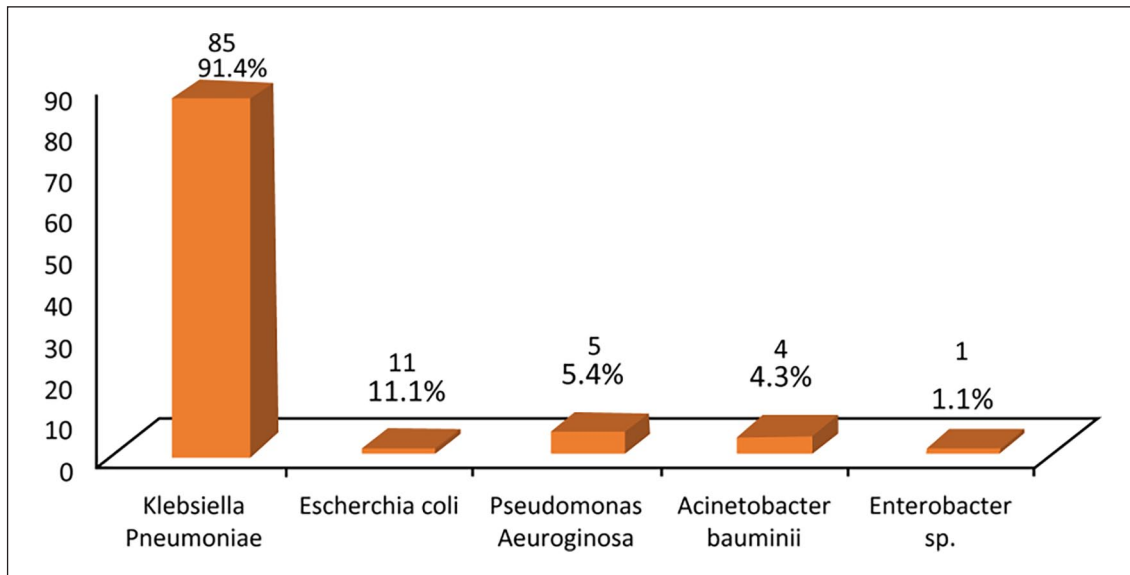


Figure 1. Frequency of colistin-resistant gram-negative rods.

combination of meropenem and colistin, frequently with another antimicrobial from a different class for more than 7 days before developing a colistin-resistant isolate. Similar findings were reported by Arjun et al.,⁴ where the most common previously used antibiotics were carbapenems followed by colistin and combinations of beta lactam-beta lactamase inhibitors. Other studies have also reported exposure to colistin therapy as a risk factor for developing resistance in *GNRs* especially *K. pneumoniae* and *Acinetobacter bauminii*.^{29,33} However, some recent studies have also reported the emergence of colistin resistance without any prior use of the antibiotic.^{3,31} This shows that although regarded as a weak therapeutic agent with significant toxicity, the increased use of colistin either as monotherapy or in combination for the treatment of *CREs* and other *GNRs* has led to the development of resistance against it.¹⁴ Other risk factors as described in previous studies^{4,9,17} include previous prolonged duration of the hospital or ICU stay, presence of multiple invasive devices, and presence of co-morbidities especially diabetes and chronic kidney disease were also found in a majority of our patients but on further analysis, not all were found to be of statistical significance.

The majority of our patients, similar to Arjun et al.,⁴ had evidence of symptomatic infection while the rest were regarded as colonizers and did not receive treatment. The distinction between actual infection and colonization is important as unnecessary use of antibiotics can be avoided. This, in turn, can lead to lower costs, shorter duration of hospital stay, and prevent the development of antimicrobial resistance. Furthermore, Arjun et al.⁴ also noted a prolonged median duration of hospital stay, a finding similar to our study where the total duration of hospital stay was significantly longer in symptomatic patients than in those with colonization. Other important associations seen with

symptomatic infections as compared to colonization, were male gender, previous exposure to a combination of meropenem plus colistin for >7 days, and the outcome. Similar to Arjun et al.,⁴ symptomatic infections were positively associated with increased mortality in our study.

In our study, the majority of isolates were sensitive to fosfomycin, followed by tigecycline. Similar findings were noted by Arjun et al. where tigecycline was the most common antibiotic with 75% of isolates sensitive to it. They only tested four isolates against fosfomycin and found all of them to be sensitive.⁴ Another study reported 72% of their isolates to be sensitive to tigecycline.³⁴ Similar to our study, Falagas et al.³⁵ found 92.8% of their MDR isolates to be sensitive to fosfomycin. This shows that intravenous fosfomycin is fast emerging as a valid therapeutic option for XDR organisms in combination with other antibiotics, though data regarding its use are limited, and monotherapy can lead to the development of resistance.^{36,37}

As in previous studies, our patients were also treated with a combination of two or three antibiotics with at least one susceptible antibiotic being part of the regimen. We have minimal options in terms of the availability of antibiotics to treat XDR and pan-drug-resistant (PDR) *GNRs*, as newer generation antibiotics are not readily available in Pakistan. Antibiotics currently being used include tigecycline, intravenous fosfomycin, aminoglycosides, and minocycline showing variable efficacy against XDR and PDR *GNRs*.^{34,38-40} The most common regimen used in our setting was meropenem and intravenous fosfomycin, for a variable duration depending on the site of infection. A study assessing the synergism of fosfomycin and meropenem against colistin-resistant *K. pneumoniae* found the combination to be more effective than a combination of fosfomycin and colistin.³⁷ Few patients in our study also received

Table 3. Comparison of clinical characteristics and associated factors of patients with symptomatic infections versus colonization with colistin-resistant GNRs.

S. no.	Demographics	Symptomatic infections n = 77	Colonizers n = 16	p value
1	Gender			
	1a. Male	50 (64.9)	4 (25)	0.005*
	1b. Female	27 (35.1)	12 (75)	
2	Mean ICU stay (days)	16.83 ± 12.93	11.60 ± 10.47	0.153**
3	Total duration of hospital stays (days)	23.34 ± 17.52	13.60 ± 10.41	0.039*
4	Source of isolate			
	4a. Tracheal			
	Yes	46 (59.7)	12 (75)	0.395**
	No	31 (40.3)	4 (25)	
	4b. Urine			
	Yes	10 (13)	2 (12.5)	1.000**
	No	67 (87)	14 (87.5)	
	4c. Blood			
	Yes	18 (23.4)	2 (12.5)	0.508**
	No	59 (76.6)	14 (87.5)	
5	Co-morbidities			
	5a. Diabetes			
	Yes	30 (39)	9 (56.3)	0.267**
	No	47 (61)	7 (43.8)	
	5b. Chronic kidney disease			
	Yes	17 (22.1)	5 (31.3)	0.519**
	No	60 (77.9)	11 (68.8)	
	5c. Malignancy			
	Yes	1 (1.3)	0 (0)	1.000**
	No	76 (98.7)	16 (100)	
	5d. Autoimmune disease			
	Yes	2 (2.6)	1 (6.3)	0.436**
	No	75 (97.4)	15 (93.8)	
6	Invasive devices			
	6a. Endotracheal intubation and mechanical ventilation			
	Yes	34 (44.2)	7 (43.8)	1.000**
	No	43 (55.8)	9 (56.3)	
	6b. Central line			
	Yes	59 (77.6)	12 (75)	0.755**
	No	17 (22.4)	4 (25)	
	6c. Urinary catheter			
	Yes	67 (87)	14 (87.5)	1.000**
	No	10 (13)	2 (12.5)	
7	Site of infection			
	7a. Respiratory tract infection			
	7a1. Hospital-acquired pneumonia	32 (52.5)	7 (50)	0.868**
	7a2. Ventilator-acquired pneumonia	29 (47.5)	7 (50)	
	7b. Bloodstream infection			
	7b1. Bacteremia/bloodstream infection			
	Yes	15 (19.5)	1 (6.3)	0.289**
	No	62 (80.5)	15 (93.8)	
	7b2. Catheter-related bloodstream infection			
	Yes	4 (5.2)	1 (6.3)	1.000**
	No	73 (94.8)	15 (93.8)	

(Continued)

Table 3. (Continued)

S. no.	Demographics	Symptomatic infections n = 77	Colonizers n = 16	p value
	7c. Urinary tract infection			
	7c1. Urinary tract infection			
	Yes	6 (7.9)	1 (6.3)	1.000**
	No	70 (92.1)	15 (93.8)	
	7c2. Catheter-associated urinary tract infection			
	Yes	5 (6.6)	0 (0)	0.583**
	No	71 (93.4)	16 (100)	
8	Outcome			
	8a. Expired	23 (29.9)	5 (33.3)	<0.001*
	8b. Discharged	48 (62.3)	0 (0)	
	8c. LAMA	6 (7.8)	10 (66.7)	
9	Prolonged hospital stay > 20 days			
	Yes	35 (45.5)	3 (18.8)	0.055**
	No	42 (54.5)	13 (81.3)	
10	Previous antibiotics use (for > 7 days)			
	10a. Meropenem alone			
	Yes	0 (0)	0 (0)	NA
	No	77 (100)	16 (100)	
	10b. Colistin alone			
	Yes	0 (0)	0 (0)	NA
	No	77 (100)	16 (100)	
	10c. Meropenem + Colistin			
	Yes	73 (94.8)	11 (68.8)	0.007*
	No	4 (5.2)	5 (31.3)	

ICU: intensive care unit; LAMA: left against medical advice; NA: not applicable.

Chi-square test and Student t-test is applied.

*Significant at p value < 0.05, **insignificant at p value > 0.05.

Table 4. Combination of antibiotics used to treat patients and their outcomes.

S. no.	Combination therapy	Total number of patients treated with the regimen (%)	Number of patients discharged (%)	Number of patients who left against medical advice (LAMA)	Number of patients expired (%)
1	Meropenem + Fosfomycin	46 (49.4)	25 (26.8)	5 (5.37)	16 (17.2)
2	Meropenem + Ertapenem	4 (4.30)	2 (2.15)	0 (0)	2 (2.15)
3	Fosfomycin + Colistin	2 (2.15)	1 (1.07)	0 (0)	1 (1.07)
4	Meropenem + Tigecycline	2 (2.15)	2 (2.15)	0 (0)	0 (0)
5	Fosfomycin + Ertapenem	1 (1.07)	1 (1.07)	0 (0)	0 (0)
6	Fosfomycin + Tigecycline	1 (1.07)	1 (1.07)	0 (0)	0 (0)
7	Meropenem + Minocycline	1 (1.07)	0 (0)	0 (0)	1 (1.07)
8	Fosfomycin + Ciprofloxacin	1 (1.07)	1 (1.07)	0 (0)	0 (0)
9	Fosfomycin + Co-triamoxazole	1 (1.07)	1 (1.07)	0 (0)	0 (0)
10	Meropenem + Co-triamoxazole	1 (1.07)	1 (1.07)	0 (0)	0 (0)
11	Minocycline + Fosfomycin	1 (1.07)	0 (0)	0 (0)	1 (1.07)
12	Amikacin + Ciprofloxacin	1 (1.07)	1 (1.07)	0 (0)	0 (0)
13	Meropenem + Fosfomycin + Colistin	3 (3.22)	3 (3.22)	0 (0)	0 (0)
14	Fosfomycin + Minocycline + Septran	2 (2.15)	0 (0)	0 (0)	2 (2.15)
15	Meropenem + Fosfomycin + Minocycline	1 (1.07)	1 (1.07)	0 (0)	0 (0)

LAMA: left against medical advice.

a combination of ertapenem and meropenem given as a prolonged infusion, especially in cases with *K. pneumoniae* bacteremia. This combination has shown both bactericidal and synergistic action against colistin-resistant *GNRs*, especially *K. pneumoniae*.⁴¹ Previous studies have also shown a similar trend of using combination antibiotics, most commonly colistin with tigecycline, fosfomycin, and a carbapenem and in some cases an aminoglycoside.^{4,33,42} A study by Pontikis et al.⁴³ showed a successful outcome in 54.2% of patients treated with fosfomycin combined with colistin or tigecycline. Tigecycline in combination with gentamicin also showed lower mortality rates in a study by Gonzalez-Padilla et al.³⁴ In contrast, Rojas et al. reported minimal use of colistin as part of their combination regimen but reported using amikacin most frequently followed by tigecycline. They also reported using a combination of two and three antibiotics in 27% and 49% of their patients, respectively.³¹ Combination of antibiotics has been reported to provide synergism and also reduce the risk of development of resistance.^{34,37,38}

The overall mortality was increased in our patients with symptomatic infections, which is also seen in previous studies.^{4,31,32} In the study by Arjun et al.,⁴ overall mortality rate was 56.5% and Capone et al.³² also found high mortality (40.6%) when compared to patients with colistin susceptible isolates (20.3%, $p=0.04$). This suggests a positive association between infection with a colistin-resistant organism and increased mortality. Similar to findings in previous studies, we also found a significant association between mortality and prevalence of invasive devices, that is, endotracheal intubation along with mechanical ventilation and a lengthy hospital stay of >20 days.^{4,17,23,31–33,42} An increase in mortality was also seen with BSIs, as reported by Arjun et al.⁴ and Capone et al.,³² associated with the use of central and peripheral venous catheters and arterial lines, but we failed to find a similar association in our study. This shows that colistin resistance is readily transmitted in hospitals especially in intensive care settings due to multiple invasive devices and is associated with worse outcomes.⁴

Limitations

There are several limitations to our study:

1. Study design: A prospective cohort study design would have been better as we would have followed our patients for outcomes in the long term.
2. Sample size: A smaller sample size and a 10% margin of error. Due to the emergence of the COVID-19 pandemic, we were unable to continue our data collection, so had to limit our sample and increase our margin of error to 10%.
3. Failure to include APACHE or SAPS score for assessment of mortality/probability of survival.
4. Failure of the study methodology to calculate an overall frequency of colistin-resistant organisms in the hospital setting.
5. Failure to document microbiologic clearance in patients.
6. The lack of a control group consisting of non-colistin-resistant associated diseases limits the value of this study.
7. Failure to include a genetic or molecular component to determine mechanisms of resistance to colistin because of resource limitations.

Conclusion

Our study demonstrated the increasing emergence of resistance against colistin in gram-negative rods, especially *K. pneumoniae*. Infection with such XDR organisms is associated with invasive devices and prolonged hospital stay and leads to increased mortality. This poses a significant therapeutic challenge due to the limited antimicrobial options. Strict infection control measures and comprehensive antimicrobial stewardship programs along with steps to create awareness about adverse outcomes of antimicrobial resistance, are essential to overcome this epidemic of resistant gram-negative bacteria.

Further prospective, multi-center, or surveillance studies are required to better document the increasing rate of emergence of these organisms in our region. Additional molecular studies detecting genetic mutations responsible for colistin resistance also need to be done. Studies highlighting the synergistic effects of multiple combination therapies are also required to identify better therapeutic options.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval was obtained from the Institutional Review Board and Ethical Review Committee of Liaquat National Hospital and Medical College with ERC no #0476-2019-LNH-ERC.

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Informed consent

Written informed consent was obtained from all subjects or their legally authorized representatives (in case the patient was unable to give consent) before study initiation.

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Supplemental material

Supplemental material for this article is available online.

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