# Unveiling colistin resistance in extensively drug-resistant microorganisms among ICU patients of tertiary care hospital Karachi, Pakistan

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## ABSTRACT

**Objective:** To determine the prevalence of extensively drug-resistant (XDR) microorganisms in the intensive care unit (ICU) of a tertiary care hospital in Karachi and to identify the presence of colistin resistance (CLR) among these XDR isolates.

**Material and Methods:** A cross sectional study was carried out in the ICU of a tertiary care hospital from August 2022 to February 2023 and various clinical samples of XDR Gram negative bacilli (GNB) were collected from ICU. These specimens were processed by following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI), Agar and broth microdilution methods were used to assess the colistin susceptibility of XDR isolates.

**Results:** The study focused on 100 extensively drug-resistant (XDR) specimens. *K. Pneumoniae* dominated (32%), followed by *E. coli* (23). Among the 100 XDR, 89% were colistin susceptible, and 11% were resistant, tested by agar and broth microdilution. Of the 11% CLR, *P. aeruginosa* was most common, with the highest resistance in urine specimens. Colistin resistance was highest for *P. aeruginosa* (45%), followed by *A. baumannii* (18%), *E. coli* (18%), *K. Pneumoniae* (9%), and *K. oxytoca* (9%). CLR isolates were mainly (27%) from urine.

**Conclusion:** Prolonged hospital stays and antibiotic pressure can lead to CLR development. Labs should monitor XDR closely, implementing measures to minimize antibiotic resistance. Controlling colistin resistance through stewardship is crucial.

**Keywords:** Extensively drug resistant**,** Colistin resistant, Minimum inhibitory concentration, Nasobronchial lavage

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## INTRODUCTION

Antimicrobial resistance is escalating globally, in both hospital and community communities. ICUs are particularly prone to nosocomial infections, with the majority of infections caused by gram-negative bacteria, including *ESBL-E* and/ or *CPE*, *P. aeruginosa*, and *A. baumannii* [1]. Nosocomial infections typically involve gram-negative bacteria and these bacteria may become (XDR) extensively drug-resistant due to their propensity to acquire antibiotic resistance [2]. Gram-negative XDR bacteria offer a significant threat to the economy of developing nations like Pakistan, where rates of antibiotic resistance are significantly greater as a result of the overuse and improper prescription of antibiotics [3]. Strict monitoring of antibiotic resistance is necessary to create prompt management strategies and remedies to this grave public health issue [4]. Clinicians have a tremendous challenge when treating infections brought on by some infectious agents, such as extensively drug-resistant (XDR) Gram-negative bacteria notably in situations with limited resources like in most labs of Pakistan. In order to track the increasing incidence of antibiotic

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resistance and create effective strategies for the management and control of these infections, there are still little national data available on the epidemiologic traits of CR and XDR Gram-negative bacilli in Pakistan. For this purpose, the phenotypic characteristics of XDR strains of GNB isolates obtained from different samples were studied [5]. A polymyxin E called colistin was discovered in Japan in 1949. Bacillus polymyxa is the true source of polymyxin E [6].

Not because of an improved safety profile, polymyxins had returned as a last-resort treatment against MDR and XDR Gram-negatives by the mid-1990s due to the emergence of XDR Gram-negative superbugs, particularly *P. aeruginosa*, *A. baumannii, and K. pneumonia,* which are resistant to all other available antibiotics, as well as the lack of novel antimicrobials available to treat MDR bacterial infections. Unfortunately, colistin has been over- and misused in animal and human medicine, leading to the widespread evolution of infections resistant to it [7]. A quick and accurate approach for testing colistin's antimicrobial susceptibility is required due to the rise in multi-resistant gram-negative bacterial infections and concurrent rise in colistin resistance [8].

A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The broth or agar dilution are the most popular and fundamental techniques [9].

The micro- or macro-dilution of broth is one of the most straightforward methods for assessing an antimicrobial's susceptibility. The agar dilution method is one of the pioneering AST approaches used by researchers to establish the MIC of an antimicrobial agent accessible since the 1940s, along with the broth dilution method. “The MIC, which is typically given in mg/L (ug/mL), is the lowest concentration of a medicine that suppresses the growth of visible bacteria on agar or in broth [10]. It is still used nowadays, especially for newly developed drugs, as a quantitative measure in AST [11].

Hence, there is a need to delineate the prevalence of XDR microorganisms in ICUs and further investigate the occurrence of colistin resistance in these isolates. By studying the prevalence and identification of CLR in XDR isolates collected from a tertiary care hospital in Karachi, this research aims to provide valuable insights into the resistance patterns of these microorganisms and contribute to the understanding of antimicrobial resistance in ICU settings.

## MATERIAL AND METHODS

This study was carried out in the Microbiology Department of the Combined Military Hospital (CMH) Karachi from August 2022 to March 2023 with authorization from the Institutional Ethical Review Board of CMH Mair Cantt (File No. 85/2020/Trg/ERC). We collected 100 specimens from CMH Malir Cantt patients in the intensive care unit (ICU) for the cross-sectional investigation. Using openepi.com, the sample size for 85 isolates was calculated while taking into account the 5.81 percent CT-resistant bacterial frequency in Pakistan.[11] Achieving a 95% confidence interval with a 0.5% margin of error served as the foundation for the computation. Consecutive non-probability sampling was the technique used for sampling.

The XDR gram-negative rods were selected during a three-month sampling process. With the exception of urine samples, which were placed on cled agar, all ICU specimens were placed on 5% Sheep Blood Agar (SBA) and MacConkey agar. After overnight incubation, samples showing growth of Gram-negative bacilli were identified using standard laboratory protocols, including Gram staining and API 20E. XDR isolates from various sources were confirmed using the Kirby-Bauer method. The research tool, Performa, was designed for reliability. Colistin sulfate, known as Polymyxin E, was used in the study, and its potency was determined following CLSI guidelines. Colistin susceptibility was assessed by agar and broth microdilution, with MICs expressed as the lowest concentration preventing visible growth under controlled conditions. The CLSI guidelines were followed in order to evaluate both approaches using the quality-control (QC) strain of *P. aeruginosa* with ATCC 27853. A range of 0.5 to 2µg/ml was

deemed appropriate for quality control.[12]Data were statistically analyzed using SPSS software (version 24), The Categorical data is reported as Frequencies and percentages and quantitative in mean ± SD or Median (IQR). The significance level was set at a p-value of ≤0.05, and the Cohen's kappa test was applied for agreement determination. For difference among the nature of specimen and microbe Fishers exact test was applied.

## RESULTS

A total of 100 XDR (resistant to at least one drug in all classes except one or two drugs) isolates from various clinical specimens were examined in the current investigation. Urine samples from intensive care unit patients included the bulk of the isolates. The polymyxin propensity of these bacteria was evaluated using two methods (AD & BMD), and the findings were compared to one another using BMD as the reference method, as indicated in table-I. The most prevalent isolates was *K. pneumoniae* [32]. 1 ug/ml and 2 ug/ml, respectively, were corresponding MIC 50 and MIC 90 values. (Table-I). A comparison between the broth and agar dilution procedures was carried out on 100 XDR isolates.

The isolates with the highest frequencies that were relevant to the sample were *K. pneumonia* in pus (31%), and *E. Coli* in urine (83%) *P.aeruginosa* in urine (35%), A. baumanii in NBL(35%) . E. cloacae in sputum (50%), *K. oxytoca* in pus (60%), and *C. ferrundii* in NBL (100%). Agar dilution technique revealed that two of the 32 *K.pneumoniae* strains were resistant. Out of the 23 isolates of *E. Coli* tested in the present study, two were resistant. This study included 17 *A.baumannii*, 4 of which were resistant. There were two resistant *P. aeruginosa* among the 14 isolates examined in this study. *E. cloacae* were 6 in this study, none of them was resistant. There were five *K.oxytoca* in this research, and one of them was resistant. Of the three *C. freundii* none were resistant, (Table-II) The p-value shows statistical significance difference, among the distribution of microbes in specimen type, (<0.001).

The study on CLR isolates indicates a diverse range of bacterial strains from different clinical samples (Table 1.3). P. aeruginosa comprises 45% of the total isolates, with two from double lumen, and one each from urine, sputum, and pus. *A.baumannii* represents 18% with one isolate from tissue and another from NBL. *E. coli* contributes 18%, originating primarily from urine. *K.pneumoniae and K. oxytoca* each accounts for 9% from NBL and urine samples respectively. This comprehensive analysis highlights the varying frequencies of bacterial strains in clinical specimens, providing crucial insights into microbial prevalence in healthcare settings.

**Table-I: Clinical sources of XDR (n=100) isolates.**

| Clinical samples% N=100 | | MIC Range (≤0.5-32µg/ml | Isolates (n=100) % |
|---|---|---|---|
| Urine | 31 (31%) | | *K. Pneumonia* 32 (32%) |
| Pus | 22 (22%) | MIC 50 = 1 | *E. Coli* 23 (23%) |
| Blood | 15 (15%) | MIC 90= 2 | *A. Baumannii* 17 (17%) |
| NBL | 14 (14%) | Susceptibility% = 90% | *P. Aeruginosa* 14 (14%) |
| Tissue | 6 (6%) | | *E. Cloacae* 6 (6%) |
| Sputum | 3 (3%) | | *K. Oxytoa* 5 (5%) |
| Tip for C/S | 3 (3%) | | *C. Ferundii* 3 (3%) |
| **CVP** | 4 (4%) | | |

**Table-II: Analysis of nature of specimens.**

| Name of microbe | Nature of specimen (N %) | | | | | | | P-Value |
|---|---|---|---|---|---|---|---|---|
| | Urine | Blood | I/V Catheter | NBL | Pus | Sputum | Tissue | |
| *Klebsiella pneumoniae* | 5 (16%) | 8 (25%) | 4 (12%) | 5 (15%) | 10 (31%) | | | |
| *E.coli* | 19 (83%) | 2 (8.7%) | | | 2 (8.7%) | | | **<0.001** |
| *Acenito bacterbaumani* | | 2(11.8%) | | 6 (35%) | 3 (17.6%) | | 6 (35%) | |

| | | | | | |
|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 5 (35%) | 3 (21%) | | 4 (28.6%) | 2 (14%) |
| *Enterobacter cloacae* | | | | | 3 (50%) |
| *Klebsiella oxytoca* | 2 (40%) | | | 3 (60%) | |
| *Citrobacter ferundi* | | | 3(100%) | | |

**Table-III: Colistin resistant isolates isolated from various specimens.**

| Name of CLR isolate | No. of CLR isolate | Nature of isolated sample | Number of samples | % of each CLR isolate |
|---|---|---|---|---|
| *P. Aeruginosa* | 2 | Double lumen | 2 | 45% |
| *P. Aeruginosa* | 1 | Urine | 1 | |
| *P. Aeruginosa* | 1 | Sputum | 1 | |
| *P. Aeruginosa* | 1 | Pus | 1 | |
| *A. Baumani* | 1 | Tissue | 1 | 18% |
| *A. Baumani* | 1 | NBL | 1 | |
| *E. Coli* | 2 | urine | 2 | 18% |
| *K. Pneumoniae* | 1 | NBL | 1 | 9% |
| *K. Oxytoca* | 1 | urine | 1 | 9% |
| **Total CLR isolates** | **11** | | | |

## DISCUSSION

The global challenge of antimicrobial resistance (AMR) is evident in recent studies of Brown et al emphasizing its pervasive impact across healthcare settings and communities [13]. Over the past five years, infections from drug-resistant bacteria have surged posing a considerable threat, especially in intensive care units (ICUs) susceptible to nosocomial infections, as supported by recent research. Most recent results are dominated by gram-negative bacteria, especially those that produce carbapenem-producing Enterobacteriaceae (CPE) and extended-spectrum β-lactamases (ESBL-E) [14].

Our study revealed 11% colistin resistance among XDRs, compared to Qamar et al.'s study in Pakistan showing 15% (using 251 strains, exceeding our sample size [15]. Similarly, Abd El-Baky RM et al. reported 87% colistin-resistant XDR *P. aeruginosa*, aligning with our study, where *P. aeruginosa* also exhibited significant colistin resistance [16]. The study by Abd El Baky et al. found 87% XDR *P. aeruginosa* with 45% colistin resistance. Notably, *P. aeruginosa* emerged as the most common colistin-resistant isolate, aligning with Coșeriu's et al findings, emphasizing the need for heightened vigilance in monitoring urinary tract infections [17].

Bir et al. reported 15% total colistin resistance in Carbapenem-resistant Enterobacterales (CRE) with 16% *E. coli* resistance, comparable to our findings of 11% CLR isolates among 100 XDR, with 18% *E. coli* resistance [18].

Our study's findings of *K. pneumoniae* resistance among 100 mixed isolates are consistent with an Italian investigation that found 43% CLR among 96% of carbamenase-producing *K. pneumoniae* [19]. Our study agrees with Fatima et al., having two resistant *A. baumannii* isolates. However, our small sample size and mixed isolates are study limitations[20].

Matthaiou and Kontopidou rarely stated colistin resistance in *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*, contrary to our study, which included various isolates [21,22]. Ling et al.'s study observed 2.52% resistance in *K. Oxytoa* MDR samples. The collective results indicate a significant increase in colistin resistance over time (2013–2018, 2019–2021), possibly due to increased antibiotic use [23,24].

## LIMITATIONS OF STUDY

Our findings reveal emerging in vitro colistin resistance in extensively drug-resistant (XDR) strains from Pakistan, limiting therapeutic options and underscoring the global health concern of antibiotic resistance. Therapeutic use of broad-spectrum antibiotics should be

reserved for severe infections, emphasizing the need for antimicrobial surveillance and antibiotic stewardship programs. The study suggests further investigation into plasmid-mediated colistin resistance in gram-negative bacteria (GNB) to understand its definitive cause.

## CONCLUSION

Limitations include a single-center study in Karachi, advocating for broader national research to establish a more reliable antibiotic susceptibility pattern against XDR. Genetic studies are crucial for confirming resistant strains, and future research should focus on the clinical significance of acquiring MCR genes and the implications of hetroresistance in colistin susceptibility testing.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

## AUTHORS CONTRIBUTION

**Sadia Talib:** Conception, Analysis
**Abeera Ahmed:** Design, data analysis, interpretation, literature review
**Syeda Hira Abid:** Administrative support, overall supervision of study
**Tahira Assad:** Proofreading, literature review
**Muhammad Nizamuddin and Shaista Sharif:** Critical review

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