

The Ever-Worsening Antimicrobial Resistance in Enterobacterales; A wakeup call

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ABSTRACT

Objective: To determine the comprehensive antibiotic susceptibility profile of carbapenem-resistant *Enterobacterales* among clinical samples received at the Armed Forces Institute of Pathology (AFIP), Rawalpindi.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, from Apr to Sep 2020

Methodology: Over six months, 150 carbapenem-resistant *Enterobacterales* were isolated at the Armed Forces Institute of Pathology, Rawalpindi Pakistan. The antimicrobial susceptibility testing was performed using the Kirby-Bauer Disk-Diffusion technique, and their susceptibility was interpreted according to the CLSI 2020 and EUCAST 2020 guidelines.

Results: Out of 150 clinical isolates resistant to Carbapenems, 99(66%) were identified as *Klebsiella Pneumoniae*, followed by *Escherichia Coli* 38(25.7%), *Proteus Mirabilis* 7(4.6%), *Enterobacter Cloacae*, *Serratia Marcescens*, *Citrobacter Freundii*, *Providencia retgerii* 1(0.6%) each. The isolates were highly resistant to the following categories of antibiotics tested. For Penicillins /Cephalosporins and their combinations, the resistance range was between 94 to 100%, and it was and >79% for Aminoglycosides, >97% for Fluoroquinolones and >48% for Tetracyclines. Isolates showed the highest susceptibility to Colistin, 94(92%) out of 102 then to Tigecycline 81(81%) out of 100, followed by Fosfomycin 60(78.9%) out of 76, Minocycline 41(52%) out of 79 and Doxycycline 33(50%) out of 66.

Conclusion: Extensively drug-resistant *Enterobacterales* 'Superbugs' with ever-worsening antimicrobial resistance threaten the human race back to the pre-antibiotic era. This real menace cautions against the lack of antimicrobials for treating lethal and hazardous infections caused by such difficult-to-treat bacteria in times to come.

Keywords: *Enterobacterales*, Carbapenems, *Klebsiella pneumoniae*.

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INTRODUCTION

Gram-negative bacilli belonging to the family *Enterobacterales* are implicated in causing various community-acquired infections.¹ However, they also cause difficult-to-treat nosocomial infections with higher morbidity and mortality rates. Initially, these pathogens expressed resistance against the most important and widely used group of antibiotics called β -lactams, such as all Penicillins, Cephalosporins and Monobactams, by producing enzymes called Extended Spectrum β -Lactamases (ESBLs).² Thus, the utility of the Carbapenem group of antibiotics increased, which possesses the greatest potency and the broadest spectrum of activity against Gram-negative and Gram-positive bacteria.³ Widespread and injudicious use of these antibiotics resulted in a broadened spectrum of activity of β -lactam hydrolyzing enzymes, encompassing carbapenems too.⁴ This resulted in the emergence of carbapenem-resistant Gram-negative bacilli.⁵

Carbapenem resistance should be considered a 'Ticking time bomb'. Infections caused by such bacteria have limited treatment options. Such pathogens can drag us back into the Preantibiotic era and are rightly called 'Super-bugs'.⁶ They often carry genes that confer resistance to multiple other antimicrobial agents, resulting in the emergence of Multi-Drug Resistant (MDR), Exten-sively-Drug Resistant (XDR) and even Pan-Drug Resis-tant (PDR) bacteria.⁷ According to European Center for disease control (ECDC) and the Center for disease control and Prevention (CDC), an MDR isolate shows acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR, isolate remains susceptible to only one or two categories and a PDR pathogen is non-susceptible to all agents from all antimicrobial categories.^{8,9}

Thus, it is the need of the hour to be thoroughly knowledgeable about the comprehensive antibiotic susceptibility pattern of pathogenic bacteria of a specific geographical area.¹⁰ It will go a long way in helping clinicians to decide about the most appropriate

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and targeted empirical therapy and the best antimicrobials available to treat infections caused by bacteria that are real threats to global public health and to emphasize the importance of knowledge and practice of antimicrobial stewardship. In addition, the results of our study highlight the importance of developing novel antimicrobial agents to combat this menace of growing antimicrobial resistance.

METHODOLOGY

The cross-sectional study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan. AFIP is a reference laboratory that receives samples from all tertiary care hospitals of Rawalpindi, Islamabad, and other areas of Pakistan. The Ethical approval was taken from the Ethical Review Board of AFIP, Rawalpindi, (MP-MIC19-3/READ-IRB/21/120)

Inclusion Criteria: Non-repetitive clinical samples which yielded growth of carbapenem-resistant *Enterobacterales* were included in the study.

Exclusion Criteria: Clinical samples which yielded growth of carbapenem-sensitive *Enterobacterales* were amongst the exclusion criteria.

All the samples received in the microbiology department during the study period were included. Furthermore, 150 carbapenem-resistant *Enterobacterales* isolated were received in the laboratory over six months from April-September 2020. Specimens processed in the laboratory included sputum, endobronchial washings, non-directed bronchial lavage, pus, blood, tissue, and various body fluids, including urine, pleural fluid and ascitic fluid. Specimen processing was done according to the American Society of Microbiology guidelines.¹¹ The specimens were then cultured on blood agar, chocolate agar, MacConkey agar and CLED agar as per protocol for specific samples. Bacterial isolates were identified according to the colony morphology and Gram stain characteristics and various rapid biochemical tests such as catalase, oxidase, and other biochemical reactions as determined using API-20E. Antimicrobial susceptibility testing was performed on Gram-negative bacilli by the Kirby-Bauer method using the Disk-Diffusion technique.¹² Antimicrobials were tested according to CLSI 2020 guidelines for *Enterobacterales*.¹³ The antibiotics used in the panel included Ampicillin (10µg), Gentamicin (10µg), Amoxicillin/Clavulanic Acid (20/10µg), Piperacillin/Tazobactam (100/10µg), Ceftriaxone (30µg), Cefepime (30µg), Imipenem (10µg), Merope-nem (10µg), Amikacin (30µg), Trimethoprim/

Sulfamethoxazole (1.25/23.75µg), Ciprofloxacin (5µg), Doxycycline (30µg), Minocycline (30µg). For urinary iso-lates, nitrofurantoin (100µg) was also applied. The Colistin agar dilution method was used to test the susceptibility of isolates to Colistin. Interpretation of results was made according to CLSI 2020 guidelines. EUCAST 2020 guidelines for Tigecycline (15µg) and Fosfomycin (200/50µg) susceptibility testing were followed.¹⁴

Only those isolates showing resistance or intermediate susceptibility to one or both of the Carbapenems used were considered the Test isolates. According to Centers for Disease Control and Prevention (CDC), Carbapenem-resistant *Enterobacterales* are *Enterobacterales* that are non-susceptible, i.e., intermediate or resistant to Carbapenem, MIC >4µgm/ml for Doripenem, Imipenem, Mero-penem or >2µgm/ml for Ertapenem or documented to produce a Carbapenemase.¹⁵

Data were analyzed using Statistical Package for the Social Sciences version 26 and the results were expressed as frequency and percentages.

RESULTS

In this study, 113(75.3%) specimens were collected from male patients and 37(24.6%) from female patients. The patients were mostly hospitalized in various hospital wards, medical, surgical and neonatal intensive care units and high dependency units, with only 6(4%)specimens from patients from the Outpatient Department. The graphical representation of the type and number of isolates yielded from various clinical specimens is shown in Figure-1.

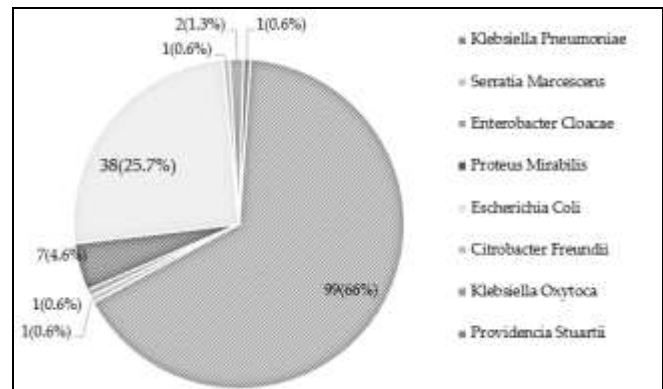


Figure-1: Carbapenem Resistant *Enterobacterales* yielded from Various Clinical Specimens (n=150)

The graphic representation of the resistance pattern to the antibiotics belonging to the carbapenem group is shown in Figure-2. Of the 150 carbapenem-

resistant *Enterobacteriales*, 142(94.6%) were resistant to both Carbapenems. Only 1(0.6%) isolate was resistant to Meropenem but sensitive to Imipenem; One *Escherichia Coli* was imipenem sensitive but Meropenem resistant. The comprehensive antimicrobial profile of carbapenem-resistant *Enterobacteriales*, according to the recommendations of CLSI and EUCAST 2020 guidelines, is shown in Figure-3.

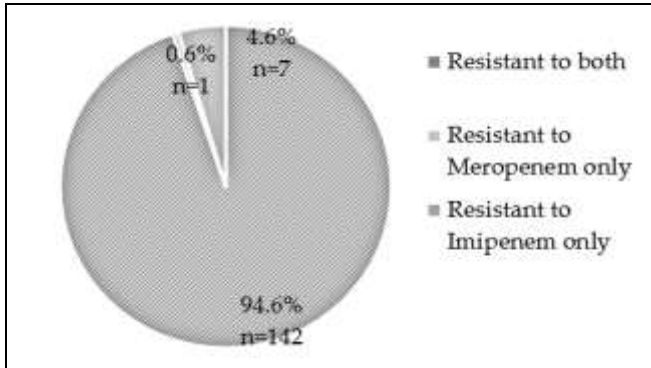


Figure-2: Carbapenem Resistance in *Enterobacteriales* (n=150)

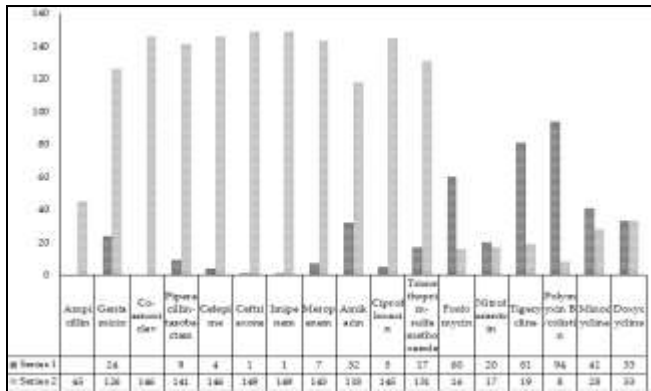


Figure-III: Antibiotic Susceptibility Profile (n= number of Isolates in which Susceptibility of the Respective Antibiotic was Carried out)

Ampicillin was tested against only those isolates which are not intrinsically resistant to the antibiotic. Resistance of 44 out of 44 for ampicillin and 146 out of 146 for Amoxicillin Clavulanate 100% was observed. While a resistance rate of 146(97.3%) out of 150 and 141(94%) out of 150 were observed among test isolates for the third-generation Cephalosporin (Ceftriaxone) and fourth-generation Cephalosporin (Cefepime) respectively.

In our study, the antibiotic which showed the highest in vitro efficacy against the carbapenem-resistant *Enterobacteriales* was Colistin, with 94(92.1%) out of 102 sensitivity. Urinary isolates showed 20(54%) out of 37 sensitivity against Nitrofurantoin.

Out of 150 isolates, only 1(0.6%) isolate was resistant to Penicillins and Imipenem. However, the rest fall into the categories of Multi-drug Resistant (MDR), Extensively-drug Resistant (XDR) and Pan-drug Resistant (PDR), as shown in the Table.

Table: Antimicrobial resistance pattern (n=150)

Multi-drug Resistant (MDR)	Extensively-drug Resistant (XDR)	Pan-drug Resistant (PDR)
n(%) 0%	n(%)	n(%)
87(58%)	60(40%)	2(1.3%)

DISCUSSION

In our study, *Klebsiella Pneumoniae* (66%) was the most abducted pathogen isolated among the carbapenem-resistant isolates, followed by *Escherichia Coli* (25%). The most common specimen to yield carbapenem-resistant isolates was blood culture (28%), followed closely by urine specimens (26.6%) & pus (24.6%).

The most important reason for Carbapenem resistance in our isolates must be the production of Carbapenemases. As described by Nordmann *et al.* Carbapenemase production is the main mechanism for Carbapenem resistance in *Enterobacteriales*, followed by carbapenem impermeability.⁹ The main class of Carbapenemase produced must be metallo-β-lactamase as the main reservoir of New Delhi metallo-β-lactamase (NDM) producers is thought to be the subcontinent (Pakistan, India, and Sri Lanka). One *E. coli* isolated from ascitic fluid was resistant to all β-lactams, including meropenem but was sensitive to imipenem only. Harino *et al.* from Japan was the first to describe an Imipenem-susceptible Meropenem-resistant *Klebsiella Pneumoniae* strain and was designated as ISMRK.¹⁰ Later, a study demonstrated that the reason might be the co-production of IMP-6 and ESBL (CTX-M-2) by *Klebsiella pneumoniae*.¹¹

In our study, almost all β-lactam drugs are ineffective against our test isolates. It is quite evident from our study that 100% resistance was observed for ampicillin. The isolates were also highly resistant to the effects of β-lactam-β-lactamase inhibitor combinations such as Amoxicillin-Clavulanate (100%) and Piperacillin-Tazobactam (93.3%). Likewise, a study showed 100% resistance to Ampicillin and 99.4% resistance to β-lactam-β-lactamase inhibitor combinations.¹²

In a study conducted in China by Chen *et al.* in 2020, all CRE isolates were completely resistant to ceftriaxone and Cefepime. Whereas in our study, it was found that 97.2 % and 94% of isolates showed resistance to both drugs.¹³

According to CLSI 2020, gentamicin is included in primary, routine testing and reporting panels, but our isolates showed 80% resistance, which was higher than Amikacin, which was observed to be 75.4%. Whereas, in another study the rate of resistance to Amikacin was 72.2%, and for Gentamicin, it was 67.3%.¹⁴ In our study, isolates showed 97.4% resistance to ciprofloxacin. According to Zhao *et al.* 53.3% of CRE were resistant to Ciprofloxacin, and 37.3% were resistant to Levofloxacin.¹⁵ The results of a study indicated the possibility of co-occurrence of quinolone resistance genes alongside Carbapenemase genes on a single large conjugative plasmid.¹⁶

Our study showed that 7.9% of isolates showed resistance to Colistin, which is thought to be the last-line antimicrobial to combat infections caused by extensively drug-resistant bacteria. Due to the rise in resistance to β -lactam antibiotics, our dependence on non- β -lactam antibiotics increased. It resulted in the emergence of resistance to Polymyxin B and Colistin due to the spread of the plasmid-encoded *mcr* gene, as described in a previous study.¹⁷

In our study, the test isolates showed the highest sensitivity of 92.1% to Colistin. According to Morrill *et al.* 79.8% of Carbapenem-resistant *Enterobacteriales* from the Arabian Peninsula were susceptible to Colistin.¹⁸ Although nephrotoxicity due to Colistin, at doses required to treat infections caused by CRE, is an important factor that limits its use, as indicated by Sangal *et al.*¹⁹ In our study, the isolates showed 81% and 92.1% sensitivity to tigecycline and colistin, respectively. In our study, among the carbapenem-resistant *Enterobacteriales*, there were 58% MDR, 40 % XDR and 1.3% PDR isolates.

CONCLUSION

Antibiotic resistance among bacterial pathogens is on the rise. Colistin, Fosfomycin and Tigecycline are last-resort antibiotics used to treat life-threatening infections caused by extensively drug-resistant bacteria. Nevertheless, these drugs have limitations because of sub-optimal pharmacokinetics and higher toxicity levels. Knowledge of the antimicrobial susceptibility profile of Carbapenem-resistant isolates is paramount in advocating antimicrobial stewardship, infection control and prevention and sensitization to the need to develop newer antimicrobials.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

FA & IAM: Conception, study design, drafting the manuscript, critical review, approval of the final version to be published.

HW & HZ: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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