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Prevalence of Multi-Drug Resistant Bacterial Isolates in Healthcare Environments

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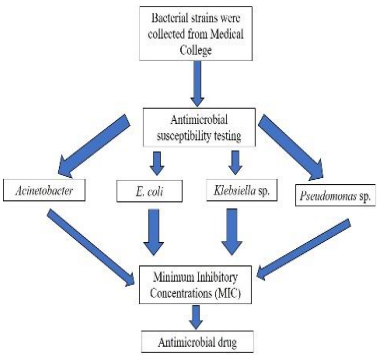
Abstract

To evaluate and measure the frequency of multi-drug resistance bacterial isolates in various healthcare environments. This study employed the disc diffusion susceptibility test to assess the antibiotic resistance of Acinetobacter, E. coli, Klebsiella sp., and Pseudomonas sp. to carbapenem, extended-spectrum β-lactamase (ESBL), and colistin. In the present study, Acinetobacter was generally found resistant to carbapenem at 72% and 71% at 75 µg/ml and minimum for Carbapenem + Colistin at 11% at 150 µg/ml. E. coli counts of the antibiotic-resistant carbapenem samples were 85% and 71% at 150 µg/ml and the lowest in colistin 12% at 225 µg/ml. The Klebsiella isolates obtained were highly sensitive to carbapenem (98%) at 150 µg/ml, followed by 75 µg/ml (96%) and 225 µg/ml (92%). Low colistin resistance was also observed (7%). The evaluation presented in Pseudomonas sp. of the antibiotic-resistant carbapenem samples was high at 47 at 225 µg/ml, and minimum in ESBL + Colistin at 5% at 150 µg/ml. Klebsiella (92%), Acinetobacter (71%), E. coli (64%), and Pseudomonas sp. (47%) were highly sensitive to 75 µg/ml carbapenem. The MICs of carbapenem-resistant Acinetobacter were shown in the 6.45 (OD values) at 75 µg/mL. Carbapenem and ESBL antibiotic-resistant Klebsiella were found at OD values of 9.14, 9.74, 9.61, and 3.45, 3.21 3.67, (OD values) at 75, 150, and 225 µg/mL, respectively, and the highest susceptibility was observed with Carbapenem 9.74 at 150 µg/mL. The ternary colour frequency of the graph indicates that Klebsiella sp. showed good susceptibility to carbapenem, carbapenem + ESBL, carbapenem, and colistin. Carbapenem is a highly effective therapy against infections caused by antibiotic-resistant Klebsiella sp.



Keywords: *Antibiotics, Bacterial strains, MIC, Methods, Plate, Resistance, Telangana*

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Introduction

Antimicrobial resistance has a substantial impact on the treatment, surveillance, and outcomes of a variety of infections and causes, including antibacterial resistance. In the 1990s, researchers predicted that the nineteenth century would signal the start of a new era of epidemiological transformation. This transition was distinguished by the increased influence of individual behaviours and lifestyle choices on human health, in addition to traditional elements such as bacterial infections and their characteristics. The resistance patterns of the identified isolates were consistent with those of the routinely used antibiotics. Almost all isolates exhibited antibiotic resistance, as well as bacterial resistance, which posed a greater public health risk to the population that was indirectly exposed to hospital waste and equipment through disposal routes. The rise of bacteria that produce extended-spectrum β-lactamase (ESBL), particularly in *E. coli* and *Klebsiella*, is now a significant concern for the development of effective treatments against bacterial infections. A study demonstrated that a high level of resistance to antibiotics such as ampicillin, ceftazidime, amoxicillin, piperacillin-tazobactam, cefuroxime, erythromycin, tetracycline, clindamycin, trimethoprim-sulphamethoxazole, and cefepime was observed in the majority of bacterial isolates from diabetic foot patients [1-3].

Antimicrobial resistance poses a significant public health threat in Telangana as it has become increasingly challenging to effectively treat, control, and manage infections caused by treatment-resistant bacterial strains. This resistance can lead to rapid transmission of infections among communities, perhaps resulting in an epidemic [4]. Researchers in the interdisciplinary and departmental fields of health education are increasingly highlighting how modern lifestyles contribute to susceptibility to disease and the proliferation of germs [5,6]. Multiple studies have demonstrated a significant occurrence of antibacterial resistance to frequently used antibiotics, including chloramphenicol, ampicillin, tetracycline, and co-trimoxazole [7]. Despite the widespread use of antibiotic therapy in Telangana, knowledge of the resistance and susceptibility of bacterial strains to antibiotics is lacking. This is mostly because of the absence of monitoring or surveillance in various healthcare facilities. Microorganisms can infect rooms, windows, and high-touch surfaces [8,9]. This can result in ineffective antimicrobial therapies [10,11]. In the present study, the antimicrobial susceptibility pattern in regular analysis was determined using the plate method for a single isolate. In this study, we evaluated antibiotic resistance and minimum inhibitory concentration (MIC) values.

Materials and Methods

Bacterial Strains

Four distinct samples were obtained from the Department of Microbiology at the Chalmeda AnandRao Institute of Medical Sciences in Bommakal Village, Karimnagar District, Telangana, India. These samples were cultured on nutrient agar medium and then incubated at 37°C for 24 h to facilitate bacterial growth. The samples included *Acinetobacter*, *E. coli*, *Klebsiella*, and *Pseudomonas* sp.

Antimicrobial Susceptibility Testing

Confirmed *Acinetobacter* and Mueller-Hinton agar plates were used to assess the antibiotic susceptibility of isolates of *E. coli*, *Klebsiella*, and *Pseudomonas* sp. The modified Kirby-Bauer disc diffusion technique was used, following the Clinical and Laboratory Standards Institute criteria. At 75, 150, and 225 µg/ml per disc, tests were performed for every antibiotic (Carbapenem, ESBL, Colistin, combination of Carbapenem and ESBL, combination of ESBL and Colistin, and combination of Carbapenem and Colistin). A homogeneous solution, similar to the McFarland standard unit (0.5), was obtained by

dissolving the newly produced isolated colonies (16–24 h) in sterile saline. Antibiotic discs were then equally spaced at regular intervals (three discs per plate), and the suspension was grown over the entire surface of the Mueller-Hinton agar. For a duration of 18-24 h, the plates were incubated at 37°C. Then, the size of the zone where the growth stopped was measured. CLSI-established parameters were used to measure the widths of the growth inhibition zones in the antibiograms. The strains were grouped based on their resistance to each treatment [10,12].

Minimum Inhibitory Concentrations MIC

The antibiotics administered were colistin, extended-spectrum β-lactamase (ESBL), carbapenem, colistin plus carbapenem plus ESBL, and carbapenem plus colistin. To combat highly resistant clinical strains of Acinetobacter, *E. coli*, *Klebsiella*, and *Pseudomonas* sp., all possible combinations of these antibiotics were used. Colonies that had grown overnight were collected using a sterile loop and placed in a tube containing 5 ml of Mueller-Hinton broth to measure the minimum inhibitory concentration (MIC) of antibiotics for a particular set of clinical strains. The broth was cultured at 37 °C until it reached a turbidity level similar to that of 0.5 McFarland standards (108 CFU ml^{-1}). The solution was diluted to 1:100 using the broth microdilution method. The strains were subjected to a gradual reduction in drug concentrations, ranging from 225 to 75 µg/mL, following the parameters set by the Clinical Laboratory Standards Institute (CLSI) [13,14].

Statistical Analysis

The data for each assay were analysed using suitable statistical procedures, such as the Student's t-test or analysis of variance (ANOVA). The calculations included the determination of the standard deviations and mean values of the data. A threshold of statistical significance was established at $p < 0.05$ to determine statistical significance.

Results

Antibiotics Resistance Profile

As shown in Figures 1& 2 A, *Acinetobacter* showed resistance to carbapenem at 72% at 75 µg/ml and minimum resistance to Carbapenem + Colistin at 11% at 150 µg/ml. In *E. coli*, the antibiotic-resistant carbapenem samples were 85% and 71% at 150 µg/ml, and the minimum in colistin was 12% at 225 µg/ml (Figure 1& 2 B). *Klebsiella* isolates showed different patterns of antibiotic resistance (Figure 1 & 2 C), and were classified based on these patterns. The *Klebsiella* isolates obtained were highly sensitive to carbapenem (98%) at 150 µg/ml, followed by 75 µg/ml (96%) and 225 µg/ml (92%). Low resistance was observed with Colistin 7%). The lowest amount in ESBL + Colistin was 5% at 150 µg/ml, while the maximum number in *Pseudomonas* sp. of the antibiotic-resistant carbapenem samples was 47 at 225 µg/ml (Figure 1 & 2 D). As shown in Figure 3A, the antibiotic susceptibility profiles of *Pseudomonas* sp. (43%), Acinetobacter (72%), *E. coli* (71%), and *Klebsiella* (96%) were extremely susceptible to 75 µg/ml carbapenem. The antibiotic susceptibility profiles of *Klebsiella* (97%), *E. coli* (85%), *Acinetobacter* (64%), and *Pseudomonas* sp. (34%) were highly sensitive to 75 µg/ml carbapenem, as shown in Figure 3B. The analytical value of the antibiotic susceptibility graph profile of *Klebsiella* (92%), *Acinetobacter* (71%), *E. coli* (64%), and *Pseudomonas* sp. (47%) isolates revealed high sensitivity to 75 µg/ml carbapenem, as shown in Figure 3C.

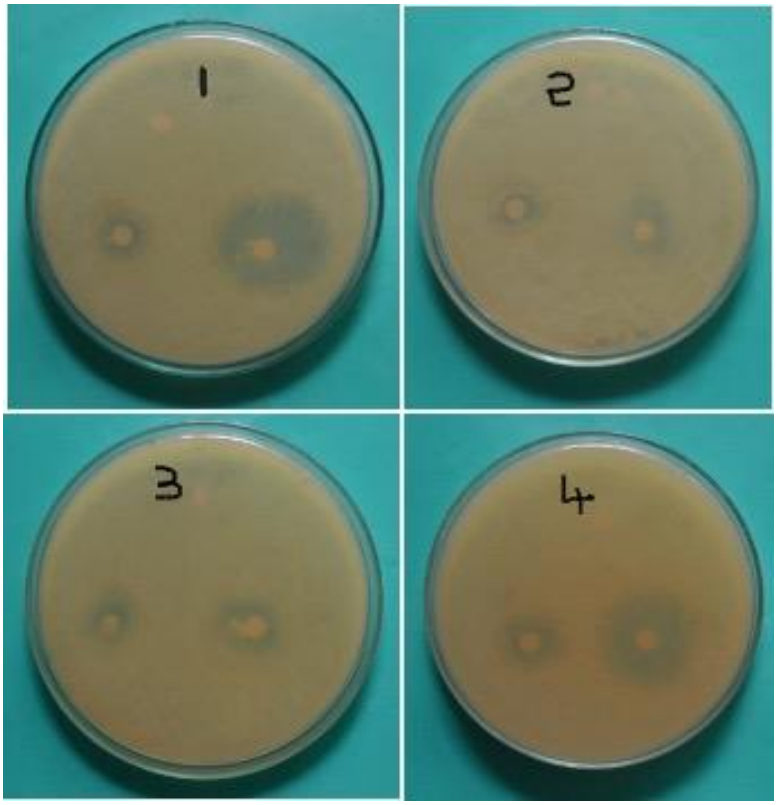

Figure 1. Antibiotic Resistance Patterns of Bacteria (1-*Acinetobacter* sp., 2- *E-coli*, 3- *Klebsiella* sp., 4- *Pseudomonas* sp.)

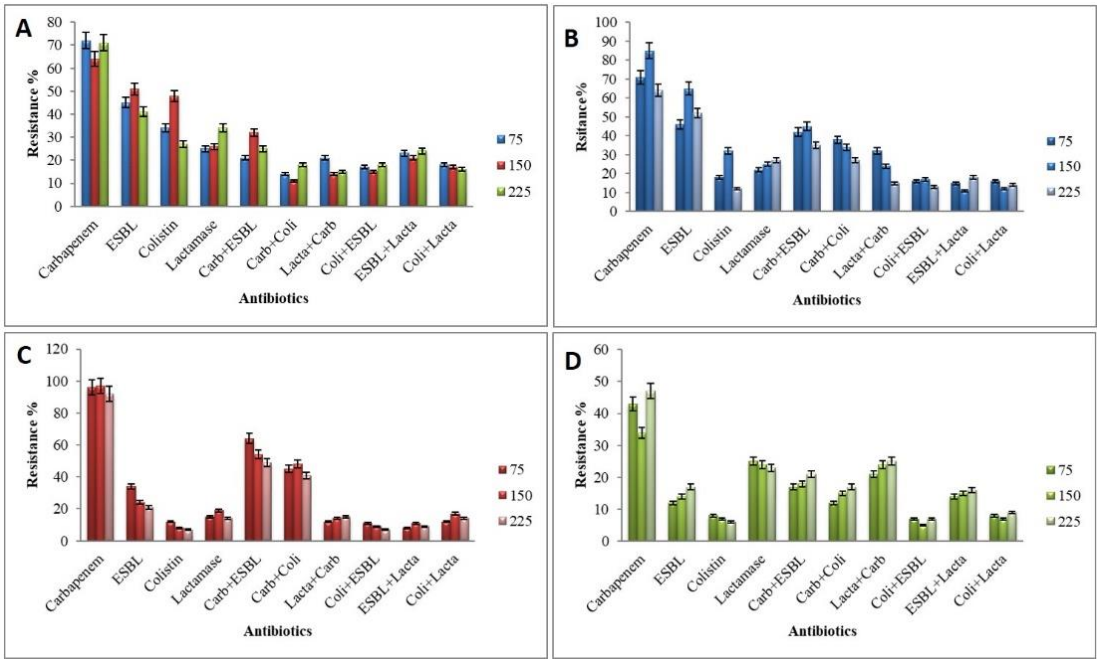

Figure 2. Antibiotic Resistance Pattern of Isolated Bacteria (A-*Acinetobacter* sp., B- *E-coli*, C- *Klebsiella* sp., D- *Pseudomonas* sp.)

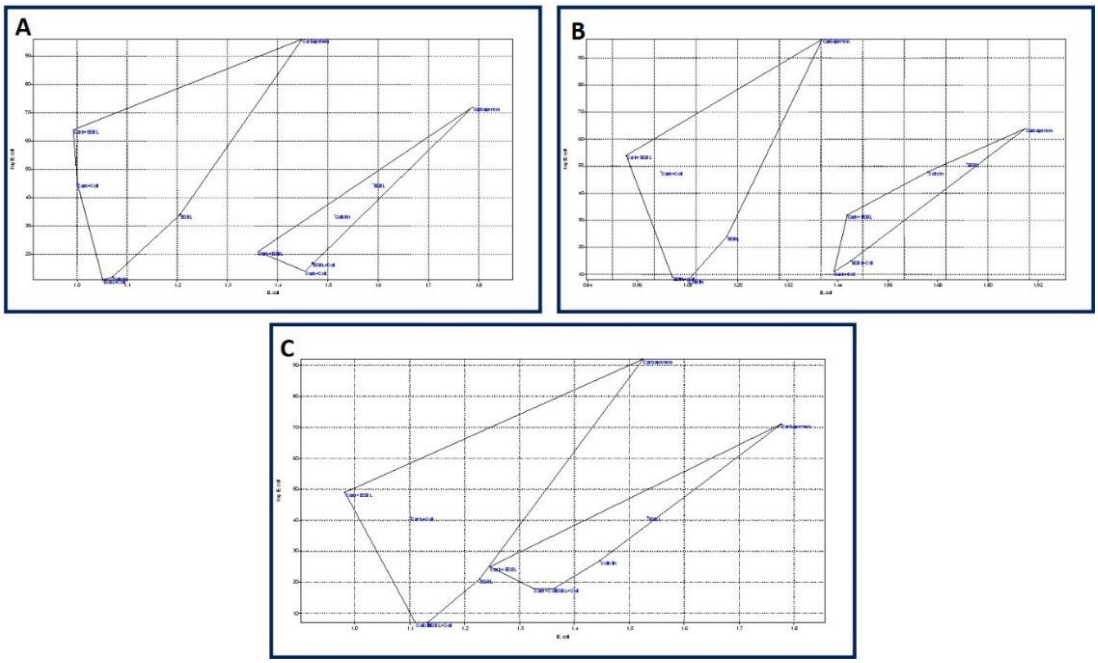

Figure 3. Graph Showing the Antibiotic Resistance at Different Concentrations (A-75 µg/ml, B-150 µg/ml, C-225 µg/ml)

Minimum Inhibitory Concentrations MIC

According to the present investigation, the minimum inhibitory concentrations (MICs) of carbapenem, ESBL, colistin, colistin + extended-spectrum β-lactamase (ESBL), colistin, and colistin against *Acinetobacter*, *E. coli*, *Klebsiella*, and *Pseudomonas* sp. were between 75 and 225 µg/mL. The results of this study indicated that Acinetobacter carbapenem antibiotic resistance was present at 75 µg/mL (6.45 OD values) (Figure 4A). The antibiotic resistance of *E. coli* to carbapenem was observed in the 4.64 at 150 µg/mL (Figure 4B). Moderate susceptibility was noted for carbapenems + ESBL (1.25, 1.26, and 1.21). *Klebsiella* sp. was resistant to carbapenem and ESBL antibiotics at 9.14, 9.74, 9.61, and 3.45, 3.21, 3.67 (OD values) at 75, 150, and 225 µg/mL, respectively (Figure 4C). The highest susceptibility was observed for Carbapenem 9.74 at 150 µg/mL. *Klebsiella* sp. showed good susceptibility to carbapenem antibiotics. For carbapenem antibiotic resistance, *Pseudomonas* sp. was found to be 2.87 at 225 µg/mL (Figure 4D). Low susceptibility to ESBL + Colistin was observed (0.11, 0.18, and 0.17, respectively). A Ternary colour frequency graph of the association between red and blue is shown (Figure 5 A). The colour frequency of carbapenem (75 µg/mL) perfectly indicated a red line of high resistance (6.23, 4.64, 9.74, 2.54) and Carbapenem+ ESBL (moderate level), 3.14, 1.25, 4.67, 1.27at red, blue colour. The colour frequency of carbapenem (150 µg/mL) is indicated by the red line, indicating high resistance (6.23, 4.64, 9.74, and 2.54) and low colistin levels (0.15, 0.24, 1.54, and 0.19, respectively) (Figure 5B).

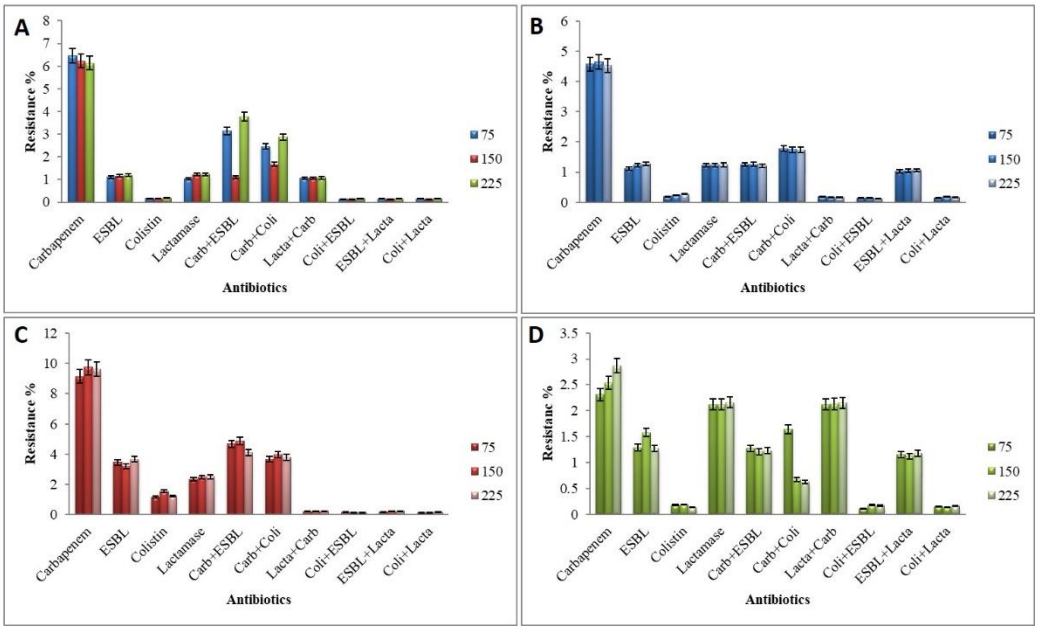

Figure 4. MIC Values of Isolated Bacteria (A- *Acinetobacter* sp., B- *E. coli*, C- *Klebsiella* sp., D- *Pseudomonas* sp.)

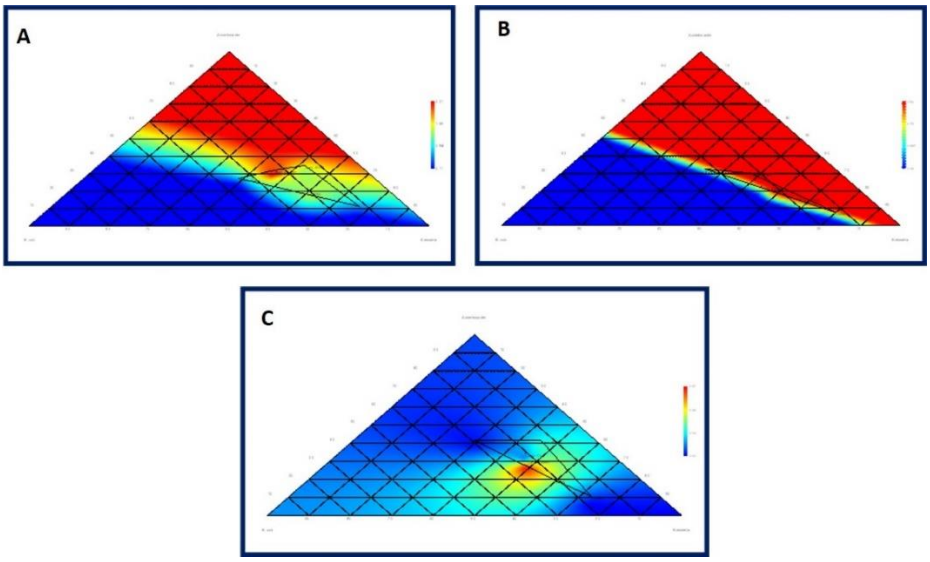

Figure 5. MIC Values of Isolated Bacteria at Different Concentration (A-75 µg/mL, B-150 µg/mL, C- 225 µg/mL)

Discussion

In the present study, the graph line clearly shows that carbapenem antibiotic susceptibility was the highest. According to previous studies, the frequencies of resistance of *S. aureus* to methicillin (MRSA) (84.1%), *A. baumannii* to imipenem (46.3%), *K. pneumoniae* to ceftazidime or ceftriaxone (76.1%), and *P. aeruginosa* to piperacillin (78.0%) have been documented [15]. The degrees of inhibition varied significantly ($P < 0.05$) between different amounts evaluated for all bacteria species, indicating antibiotic resistance. The results obtained using the common superscript are statistically equivalent, while other letters show statistically significant differences in the results.

The health of a patient is significantly affected by trash, personnel, equipment, and assistants; therefore, research should be conducted on the long-term effects of these particles on patient health. Bacterial infections

are becoming more common in Telangana patients owing to unsanitary environmental circumstances. The maximum antibiotic resistance was observed for cefotoxin (77.50%), followed by ampicillin (48.24%), penicillin (43.96%), streptomycin (38.80%), ciprofloxacin (31.50%), cefoperazone (25.60%), erythromycin (23.86%), azithromycin (15.70%), and cephalothin (14.82%) [Suma *et al*. 16]. Vasaikar *et al.* [9] reported high antibiotic resistance rates in the following order: tobramycin, 108 (53.5%) ceftazidime, 124 (61.4%) aztreonam, 126 (62.4%) cefpodoxime, 127 (62.9%) cefuroxime, 129 (63.9%) cefepime, 130 (64.4%) cefazolin, 139 (68.8%) trimeth/sulfa, 143 (70.8%) piperacillin, 160 (79.2%), and amp/sulbactam 167 (82.7%). Minakshi *et al.* [17] reported antibiotic susceptibility results of 16.25% (n ¼ 13) and 71.25% (N ¼ 57) *Pseudomonas* sp., colistin (82.5%), *K. pneumoniae* 29.41% (n ¼ 15) for ampicillin, cefuroxime, ceftriaxone, cefepime, *A. baumannii* colistin (92.59%), and tigecycline (88.89%). Rihab *et al.* [18] reported the lowest rates of resistance to amikacin, piperacillin, tazobactam, ertapenem, meropenem, and tigecycline (22.22%), whereas the highest rates were reported for piperacillin and ampicillin (100%).

Apenteng *et al*. [19] determined that *S. aureus* is highly sensitive to ciprofloxacin (100%), gentamicin (91.23%), and tetracycline (100%), whereas high resistance was observed with cefuroxime (75.44%). The results of antibiotic susceptibility testing showed that the gram-negative bacteria *Acinetobacter, E. coli, Klebsiella, and Pseudomonas* sp. were more resistant to carbapenem (98%), but exhibited moderate resistance to ESBL (65%). Antibiotic-resistant and highly pathogenic *Klebsiella* are rapidly spreading around the state and depend vastly on geographical and population factors. In the present study, periodic assessments of antibiotic usage and monitoring of resistance patterns locally and in detail *in vitro* were compared and investigated. The innovation of this study is that the inhibition of resistant isolates contributes to a healthier lifestyle. In the present study, high levels of resistance to various antibacterial agents were observed during empirical antimicrobial therapy, to improve patient outcomes and reduce treatment costs.

The MIC values of gentamicin and ciprofloxacin revealed that 12 (80%) and 5 (45.4%) isolates and gentamicin had a high level of concentration resistance with 256-512 μg/ml; 256 μg/ml for gentamicin and ciprofloxacin, respectively. MDR was observed with 128 μg/ml ciprofloxacin ($p = 0.0001$) and gentamicin ($p = 0.011$), as previously reported [14]. Zhang *et al*. [20] reported that the MICs of minocycline and trimethoprim/sulfamethoxazole were the most potent against *C. indologenes*, with low resistance ranges of 2.2% (3/135) and 0.7% (1/135), respectively, whereas rifampicin exhibited very high antibacterial activity (23.7%). The MICs of 19 isolates (79.2%) were resistant to aminoglycoside, 23 isolates (95.8%) were resistant to quinolones, eight isolates (33.3%) were resistant to tigecycline and fosfomycin, and 21 (87.5%) were resistant to cotrimoxazole were conducted [21].

Shamsi *et al*. [22] reported that the MICs of doripenem, streptomycin, and cefoxitin ranged from 1024 to 64 g/mL, 4096 to 32 g/mL, and 4096 to 64 g/mL, respectively. The *Klebsiella* obtained in the present study showed high susceptibility to carbapenem, carbapenem + ESBL, and carbapenem + colistin, and high resistance to carbapenem (9.74). Carbapenem is a highly effective therapy against infections caused by antibiotic-resistant *Klebsiella* sp. ESBL on the other offer, to which gram-negative bacteria are most sensitive, is the routine use of antibiotics (3.67), which probably eliminates older antibiotics. Therefore, the present study on bacterial resistance in an exact population can provide an appropriate indication of effective new

medicines for healthcare workers to provide an effective antibiotic schedule to ensure the improved recovery of patient health.

A colour frequency that Carbapenem (225 µg/mL) perfectly indicates medium red colour line of high resistance 6.14, 4.52, 9.61, 2.87 and Carbapenem+ ESBL, Carbapenem + Colistin as moderate level indicates 3.78, 1.21, 4.12, 1.23 and 2.87, 1.73, 3.78, 0.62 at dark blue colour. *Klebsiella* spp. showed good susceptibility to carbapenem + ESBL and carbapenem + colistin (Figure 4C). Previously, Shanahan *et al.* [23] determined the trimethoprim MIC (64 mg/l) and chloramphenicol MIC (256 mg/l) of antimicrobial agents, and ampicillin resistance (128 mg/l) was detected in *S. typhi* ST11.

The highest resistance rate was observed for carbapenems (98%), whereas the weakest resistance was detected for ESBL (5%). In the present study, specific carbapenem + extended-spectrum β-lactamase (ESBL) and carbapenem + colistin appeared to be unique control bacteria when compared to others. The high resistance of *Klebsiella* isolates to carbapenem indicates the production of special enzymes, such as carbapenemase, which provide resistance by deactivating the antibacterial substances of the antibiotic agents, and hence, to the cautious use of these particular antibiotics for the treatment of bacterial infections.

Conclusion

To summarize, the high occurrence of multidrug-resistant bacterial strains in hospital settings necessitates immediate intervention, such as implementing stricter infection control protocols, promoting responsible antibiotic usage, and continuing research endeavours to address this intricate public health issue.

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Authors' Contribution

MKK: Performed the experiments; **CU & SS:** Provided technical assistance; **PS & YM:** Analyzed the data and prepared the manuscript **and PR & PS:** Hypothesized, supervised, and finalized the manuscript.

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Research Content

The research content of This manuscript is original and has not been published elsewhere.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

Ethical Approval

Not applicable.

Data Availability

Data will be available from the corresponding author on request.

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