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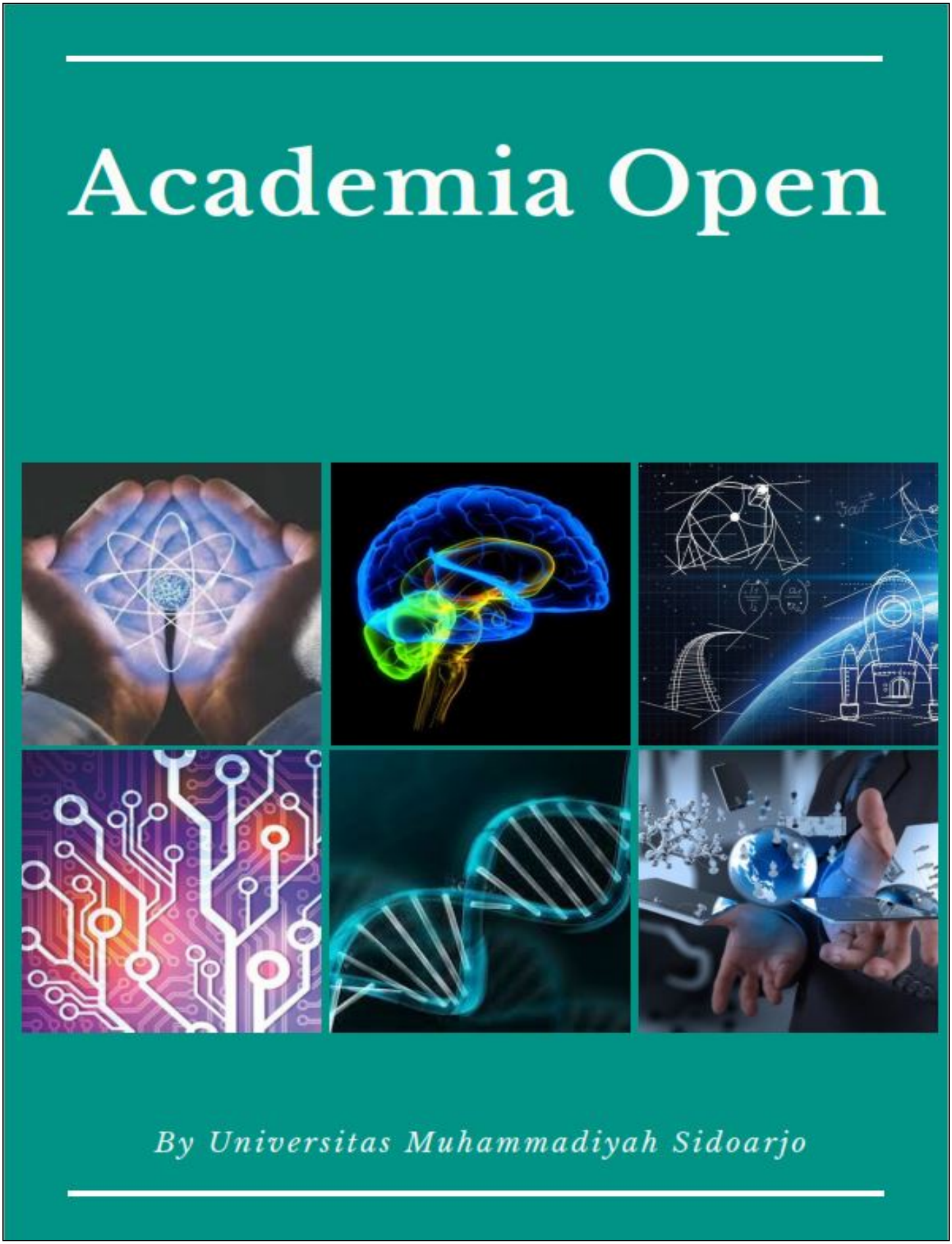

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Identification of Carbapenem-Resistant Bacterial Species Clinically Isolated From Patients

Identifikasi Spesies Bakteri yang Resisten terhadap Karbapenem yang Diisolasi Secara Klinis dari Pasien

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Abstract

Background: The global rise of antibiotic-resistant Gram-positive and Gram-negative bacteria, particularly Enterobacterales, has become a significant health concern, particularly carbapenem resistance due to carbapenemase production. **Knowledge Gap:** While the global threat of carbapenem-resistant bacteria is well-recognized, there remains a need for localized studies that provide detailed insights into the prevalence and characteristics of these resistant strains, particularly in developing regions. **Aims:** This study aims to identify carbapenem-resistant isolates in various clinical samples and explore phenotypic methods for carbapenemase production. **Methods:** 100 bacterial isolates from Batool Teaching Hospital were collected for identification, antibiotic susceptibility, colony morphology, Gram staining, biochemical reactions, and carbapenemase production, following CLSI guidelines. **Results:** The most frequent isolate was *Escherichia coli* (36%), with 24.5% showing carbapenem resistance. Other significant isolates included *Staphylococcus aureus* (17%), *Pseudomonas aeruginosa* (11.5%), and *Proteus mirabilis* (7%). Carbapenem resistance was higher in females (53%) compared to males (47%), with the age group 21-45 showing the highest resistance rates. **Novelty:** This study provides one of the first region-specific profiles of carbapenem-resistant strains in this area, highlighting significant gender and age-related trends. **Implications:** The findings underscore the urgent need for stricter antibiotic stewardship and infection control measures to prevent the further spread of carbapenem-resistant organisms, which pose a critical risk to both patients and the wider community.

Highlights:

1. *Escherichia coli* had the highest carbapenem resistance at 24.5%.
2. Females showed higher resistance (53%) than males (47%).
3. Highest resistance observed in ages 21-45.

Keywords: Carbapenem resistance, antibiotic-resistant bacteria, clinical isolates, *Escherichia coli*, carbapenemase production

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Introduction

Many microbes can cause hospital infections, and the causative microbes vary according to patients, departments, institutions, health facilities, and countries. (1) Viruses, fungi, and even parasites can also cause hospital infections, However, the most frequent cause of these diseases is bacteria. Bacteria are considered the most common microbes that cause hospital infections, and they are divided into bacteria residing naturally in the body (Normal flora) and pathogenic bacteria (Pathogenic bacteria (2), the type of bacteria causing hospital infections has changed over the years. In the period before antibiotics, most infections were caused by bacteria that were positive for the gram stain, especially Streptococcus pyogenes and Staphylococcus aureus. After using antibiotics, gram-negative bacteria emerged, such as P. aeruginosa and Escherichia coli. (3)

Recently, the proportion of antibiotic-resistant Gram-positive bacteria, such as coagulase-negative Staphylococci and methicillin-resistant S. aureus and Enterococci, has increased due to the use of broad-spectrum antibiotics and medical instruments that penetrate the body. (4) In addition to raising the germ count. Both external and self-infection are possible ways for the germs that cause hospital illnesses to spread (5) .The most important bacteria causing nosocomial infections are MRSA, vancomycin-resistant Staphylococcus aureus (VRSA), Pseudomonas aeruginosa, carbapenem-resistant Enterobacteriaceae, vancomycin-resistant enterococci (VRE), coagulase-negative Staphylococci, Acinetobacter baumannii, and the list continues. (6) It is becoming more complex as we talk about strains resistant to all types of antibiotics available. For this reason, preventing infection with these microbes has become very difficult (7).

1.1 Endogenous Infection Microflora

Bacteria within the Normal Flora can cause infection when they reach outside their natural habitat, for example when these bacteria reach the urinary system or damaged tissues (wounds and burns), or if there is inappropriate treatment with antibiotics that allows the growth of many other microbes. (7) Or when Gram-negative bacteria from the digestive system reach wound sites in abdominal surgeries or through tubes installed for urine extraction in patients with urinary system problems (8).

Exogenous Interaction

Patients can contract bacteria from one another by direct hand-to-hand contact, bodily fluids such as saliva, or other secretions. or in the air via dust or spray that has the patient's microorganisms in it.(9) Or through health facility workers while they care for the patient through direct contact with them or their contaminated clothing. Some workers become temporary or permanent carriers of hospital infection bacteria (Transient or Permanent Carriers) while carrying out their work. Infection can occur due to tools contaminated by the patient or contaminated by workers, visitors, or other environmental sources such as water, food, etc. (10)

Bacterial epidemiology

Many types of bacteria can survive in the hospital environment in humid places, and sometimes they may even be present in sterile and disinfected tools (Pseudomonas, Acinetobacter, Mycobacterium) used in patient care. (11) If cleaning processes are not good, the microbes need moisture or appropriate temperature and nutrition to live, survive, and cause infection. One of the diseases may also come from bacteria from food served to the patient or through dust or droplets generated when coughing. Bacteria that are smaller than 10 micrometers remain in the air for several hours and can be inhaled by others. (8)

Methods

Collection of Specimens

The clinical samples were obtained from various sources, including blood cultures, cerebrospinal fluid, burns, pleural fluid, wounds, pus, sputum, urine, and vaginal swabs. The study period ran from March 2024 to June 2024, and the participants were recruited for the laboratory of Microbiology at Batool Teaching Hospital. Patients of all ages and genders submitted these clinical samples. The bacteria were identified by using the Vitek 2 compact system, colony morphology, Gram stain, and biochemical reaction—standard laboratory techniques for microbiological culture. Throughout the study, (100) isolates were obtained from diverse samples.

Antibiotic Sensitivity Pattern Test

The Vitek 2 compact system was used to test these isolates for antibiotic resistance. If the isolates passed the test, the Kirby Bauer Disk Diffusion Test was used to confirm the production of Carbapenemases, by guidelines (CLSI, 2021). (14)

Statistical Analysis

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All statistical analysis was conducted using the R 4.2.2 Statistical Computing for Windows in R Studio 2022.07.0 user interface. The results' crucial levels of significance, or P 0.05 for statistical significance, were taken into consideration. The Institutional Ethics Committee of the Medicine College of Diyala University gave its approval for this prospective investigation.

Result and Discussion

Result

Among the 100 isolates, the maximum number of isolates belonging to Escherichia coli at 36 (36%) followed by, Staphylococcus aureus 17(17%), Pseudomonas aeruginosa. 15 (15 %), Proteus mirabilis 11 (11%), Klebsiella pneumoniae 9 (9%), Streptococcus pyogenes 7 (7%), Acinetobacter baumannii and 5 (5%). The total number of female patients at 57(57%) outnumbered male patients at 43(43%).

Isolates	No. %	Male	Female
Escherichia coli	36 (36%)	11(11%)	16 (16%)
Staphylococcus aureus	17(17%)	9(9%)	11(11%)
Pseudomonas aeruginosa	15 (15 %)	6(6%)	7 (7%)
Proteus mirabilis	11 (11%)	5(5%)	8 (8%)
Klebsiella pneumoniae	9 (9%)	5(5%)	6(6%)
Streptococcus pyogenes	7 (7%)	4(4%)	5(5%)
Acinetobacter baumannii	5 (5%)	3(3%)	4(4%)
Total	100	43(43%)	57(57%)

Table 1. *Gender-wise distribution of Specimens.*

Of the 100 isolates, 70 (70%) were Carbapenem-resistant isolates. The age group of 21–45 years had the highest resistance at 26 (26%), followed by 46–60 years at 18 (18%), 1–20 years at 15 (15%), and then in the age group over 60, the least amount of resistance was observed at 11 (11%). as shown in (Table 2).

Age group (In years)	AgAge group (In years)	Total of isolates Carbapenem-resistant
1 - 20	22	15 (15%)
21 - 45	37	26 (26%)
46 - 60	23	18 (18%)
≥ 60	18	11 (11%)
Total	100	70 (70%)

Table 2. *Distribution of carbapenem resistance by age group*

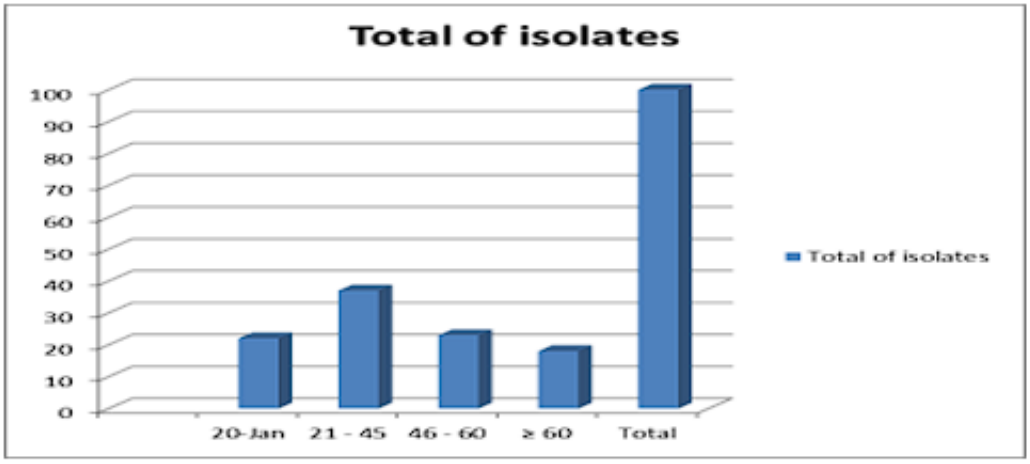

Figure 1. *Carbapenem resistance distribution by age group*

Carbapenem resistance isolates were higher in females at 37 (53%) than in males at 33 (47 %) Maximum number of Carbapenem-Sensitive isolates was at 17(56%(in females, followed by Males at 13 (44 %) as shown in (Table 3).

Gender	Carbapenem-resistant isolates (%)	Carbapenem-sensitive isolates (%)	Total isolates (%)
Male (%)	33 (47 %)	17 (56% (	50%
Female (%)	37(53%)	13 (44%)	50%
Total	70%	30%	100 %

Table 3. *Distribution of carbapenem resistance by gender*

Escherichia coli accounted for the greatest number of carbapenem-resistant isolates from the 70 total specimens. 17 /22 (24.5 %) followed by Staphylococcus aureus 11/14 (15.5 %), Pseudomonas aeruginosa 8/10 (11.5 %), Proteus mirabilis 5/8 (7%), Klebsiella pneumoniae 6/7 (8.5 %), Streptococcus pyogenes 3/5 (4.5 %), and lastly Acinetobacter baumannii 2 /4 (2.25 %). While The maximum number of carbapenem-sensitive isolates were Escherichia coli 5 /22 (7 %) followed by Staphylococcus aureus 3/14 (4.5 %), Pseudomonas aeruginosa 2/10 (3%), Proteus mirabilis 3/8 (4.5 %), Klebsiella pneumoniae 1/7 (1.5%), Streptococcus pyogenes 2/5 (2. 5 %), and lastly Acinetobacter baumannii 2 /4 (2.25 %). (Table 5).

Isolates	No. %	Carbapenem- resistant isolates (%)	Carbapenem-sensitive isolates (%)
Escherichia coli	22 (31.5%)	17 (24.5 %)	5 (7%)
Staphylococcus aureus	14 (20%)	11 (15.5 %)	3 (4.5 %)
Pseudomonas aeruginosa	10 (14.5 %)	8 (11.5 %)	2(3 %)
Proteus mirabilis	8 (11.5 %)	5 (7%)	3 (4.5 %)
Klebsiella pneumoniae	7 (10%)	6 (8.5 %)	1 (1.5 %)
Streptococcus pyogenes	5 (7 %)	3 (4.5%)	2(2.5%)
Acinetobacter baumannii	4 (5.5%)	2 (2.25 %)	2(2.25 %)
Total	70 (100%)	54 (77 %)	16(23%)

Table 4. *Distribution of Carbapenem-resistant isolates.*

Our study includes a representation of the male and female patient demographics in the area, across a wide range of age groups, from those who are infants to those who are over 60 years old, based on the data gathered from the various clinical samples used in the study. The data also includes a wide variety of patient biological sample types as well as the numerous medical departments from which the samples were obtained.

Discussion

Based on data generated from the various clinical samples included in the study, our analysis represents patient demographics in the region, both male and female, across a wide range of age groups, from individuals in the infant age group to those over 60. The data also shows a broad range of the kinds of biological samples that patients have, as well as the different hospital departments that provided the samples. The distribution of specimens was the maximum number the Escherichia coli 36/100 (36%), followed by Staphylococcus aureus 17(17%), Pseudomonas aeruginosa 15(15 %), Proteus mirabilis 11(11%), Klebsiella pneumoniae 9(9%), Streptococcus pyogenes 7 (7%), the maximum number was Acinetobacter baumannii 5 (5%). this result agreement with a study conducted in 2019, Carbapenem-resistant (CRE) infections are becoming more common throughout Europe, according to the European Centre for Disease Prevention and Control (ECDC), with 43% of nations reporting regional or interregional CRE spread (4). In 2017, European countries reported 137,728 cases of carbapenem-resistant E. Coli and 32,461 cases of carbapenem-resistant K. pneumoniae invasive isolates. (15)

Escherichia coli had the highest number of isolates that were resistant to carbapenem, followed by Staphylococcus aureus. This is comparable to what Kumar et al. (16) found. Similar to the study by Astha et al. (9), where the predominant age group for carbapenem resistance was 21 to 40 years (36.25%) and where carbapenem resistance was higher in females at 37 (53%) than in males at 33 (47%), our study found that the maximum carbapenem resistance was observed in the age group of 21 to 45 years at 26% (26/70). which resembles the research done by Gunasekaran et al. (2010). Revealed the Maximum carbapenem resistance was observed in our study among admitted patients, with a maximum of 42 isolates from the Klebsiella species, or 24.41% (42/172). Additionally, Saseedharan et al. (11)

In this study, the most common isolate was Escherichia coli n=36 (36%). The prevalence of Carbapenem resistance

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was 17 (24.5 %) which is similar to Saseedharan et.al. (11), and then Staphylococcus aureus 17(17%), The most common Carbapenem resistance was 11 (15.5 %),

Pseudomonas aeruginosa 8 (11.5 %) Proteus mirabilis 5 (7%), Klebsiella pneumoniae 6 (8.5 %) Streptococcus pyogenes 3 (4.5%) Acinetobacter baumannii 2 (2.25 %). This is similar to the study by Ramasubramanian et.al. (12). Both of which reported the same trend antibiotic stewardship policies are clearly needed to address the problem of antibiotic resistance, and they must be developed and strictly followed. Globally, the World Health Organization expresses concern over this issue. (17) Additionally, numerous research on this subject, including those by Carlet (17) and Vadala (18), additionally highlight it.

Conclusion

Numerous studies have shown a direct correlation between the overuse of antibiotics and the development and dissemination of antimicrobial resistance in pathogenic bacteria. While some bacteria are resistant to certain antibiotics by nature (a phenomenon known as intrinsic resistance), improper antibiotic use (such as using an antibiotic for a viral infection) can cause non-pathogenic bacteria to become resistant to antibiotics or allow resistant pathogens to proliferate and eventually replace non-pathogenic ones.

In summary, it was discovered that 70% of the isolates at the hospital had an overall prevalence of carbapenem resistance. Escherichia coli was the isolate type with the highest number.

1. The age range of 21 to 45 years old exhibited the highest level of carbapenem resistance.

2. The prevalence of carbapenem resistance was higher in women than in males.

3. Carbapenemases-producing organisms have been found to exhibit multi-drug resistance. This presents a significant risk to both the community and other admitted patients, as these organisms have the potential to spread and cause outbreaks.

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