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Multidrug Resistance (MDR) Bacteria Causing Nosocomial Infections in Al-Muthanna Province

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ABSTRACT

Background: Hospital-acquired infections (HAIs) are a growing worldwide issue, leading to increase in-hospital mortality, prolonged patient stays, and escalating healthcare expenses. This study investigates the patterns of antibiotic resistance and the variability of harmful microorganisms on surfaces across different departments within Al-Muthanna Hospitals in Iraq. The severity of these infections is influenced by the specific characteristics of the microorganisms present, as well as the prevalence of antibiotic-resistant pathogens in hospital environments.

Methods: A study was reached between October 1, 2024, to December 30, 2024. A collection of samples was made by swabbing surfaces in the hospital, and the isolated bacteria were tested for antibiotic susceptibility using the Kirby-Bauer disk diffusion methods, following the standards set by the CLSI.

Results: %99 of (100) swab samples had positive growth, a total of one hundred twenty microorganisms were isolated. The most frequently identified organisms were as follows: 40 (33.3%) *Staphylococcus aureus* , 29 (24.2%) *Pseudomonas aeruginosa*, 12 (10%) *Escherichia coli* , 11 (9.2%) *Enterococcus* species, and 9 (7.5%) each of *Candida albicans* and *Klebsiella pneumoniae*. Additionally, 6 (5%) of the isolates were *Proteus* and *Shigella*, all of which were multidrug-resistant (MDR).

Conclusions: Antimicrobial resistance is a significant issue in hospitals. Alongside the careful use of antibiotics, we recommend ongoing and thorough monitoring of multidrug-resistant (MDR) bacteria to effectively manage antimicrobial resistance among pathogens.

Keywords: Nosocomial infection, Multidrug resistance, MDR bacteria.

1. INTRODUCTION

Hospital-acquired infections (HAIs) represent a major public health concern due to their high prevalence, severity, and associated complications. According to the WHO, approximately 8.7% of hospitalized patients develop infections during their stay in general hospitals. [1].

Healthcare-associated infections can be classified as internal or external. Internal infections occur when bacteria originate from within the patient's body. External infections happen when pathogens come from hospital personnel, patients elsewhere, or the hospital setting [2].

One of the significant challenges faced by patients and medical staff is microbial contamination in hospitals, particularly in critical locations like emergency areas, intensive care, and surgery rooms. The proliferation of microorganisms in these environments is a major contributor to hospital infections, leading to the development of various illnesses and complications [3].

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Antimicrobial resistance (AMR) develops among microorganisms in intensive care units (ICUs) due to selective pressure from the frequent use of wide-spectrum antibiotics in most ICU patients [4]. As a result, infections caused by MDR pathogens—which include Pseudomonas aeruginosa, *Staphylococcus aureus* , *Enterobacter* sp., and *Klebsiella pneumonia*. are commonly found in the microbiology profiles of healthcare-associated infections (HAIs) within ICUs [5,6]. The presence of HAIs in ICU patients significantly increases hospital stay duration, mortality rates, and financial burdens [7,8].

The objective of our study was to clarify the occurrence of healthcare-associated infections (HAI) in operating rooms, intensive care units, and other departments, along with the microbiological profiles and antibiotic resistance characteristics of the infecting germs, and to determine their impact on patient health related to multi-drug resistant bacteria (MDRB).

2. MATERIAL AND METHODS:

2.1. Obtaining Samples:

A nationwide bacteriological investigation was conducted in hospitals located in Al-Muthanna city, Iraq, from October 1, 2024, to December 30, 2024. One hundred surface samples were collected from the emergency room, operating room, surgical room, and intensive care unit. Using cotton swabs moistened with sterile transport media in accordance with ISO/DIS 14698-1, samples were collected from specific surfaces, including operating tables, surgical lights, hospital beds, medical equipment, floors, walls, and sinks [9].

2.2. Bacteria Isolation and Characterization:

During sample delivery to the laboratory, all the swabs were placed in Brain Heart Infusion broth and incubated for a full day at 37°C. After the initial incubation, the swabs were subcultured onto MacConkey agar, Blood agar, Mannitol salt agar and Eosin Methylene Blue agar and incubated for an additional 24 hours at 37°C. Following the guidelines set by Meunier et al., characteristic colonies have been purified by subculturing in media, which were then incubated for 18 to 24 hours at 37°C to yield pure culture isolates. The bacterial isolates were characterized based on their biochemical, physiological, morphological, and even cultural properties using conventional biochemical techniques [11].

2.3. Antibiotic Testing of Purified Bacteria:

The antibacterial susceptibility test of the isolates was assessed by the disk diffusion methods, as outlined by Bauer et al. In accordance with the McFarland standard, an inoculum that is slightly 1×10^8 CFU/mL was prepared for each isolate and aseptically distributed onto sterile Mueller-Hinton Agar (Oxoid, England). A total of thirteen antibiotic disks were tested, including: 30 µg of amoxicillin-clavulanic acid , 10 µg of imipenem , 30 µg of ceftazidime , 30 µg of ceftriaxone , 100 µg of piperacillin, 10 µg of gentamicin , 15 µg of erythromycin , 30 µg of tetracycline , 5 µg of ciprofloxacin , 1.25/23.75 µg of sulfamethoxazole/trimethoprim. The selection of antibiotics was based on the guidelines from the Clinical and Laboratory Standards Institute (CLSI), relevant literature, and local availability. After the antibiotic disks were placed on the Petri dish, they were incubated for 24 hours at 37°C. The CLSI standards were used to measure and interpret the sizes of the inhibition zones produced by the antibiotics [13].

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3. RESULTS AND DISCUSSION:

Antimicrobial resistance (AMR) is an increasingly concerning issue affecting the majority of healthcare facilities. Multi-drug-resistant healthcare-associated infections are among the leading causes of death and morbidity in hospitalized patients. The intensive care unit (ICU) is often considered a hotspot for HAIs [14]. Therefore, this study was conducted to assess the microbial diversity and resistance patterns of these organisms to commonly prescribed antimicrobial agents in Al-Muthanna hospitals.

A total of 100 samples dispersed as follows and evaluated: 37, Hospital equipment; 28, Floor and wall; 20, Patient beds; 16, Bedside table; 9, Door/window handle.

A total of 120 isolates were discovered from these samples, all of which displayed positive cultures. From a bacteriological perspective, each department had specific criteria for determining positive results 96% (24/25) in emergencies, 92 % (23/25) in surgery, 88% (22/25) in operating rooms and 72% (18/25) in ICU. Table One lists various bacterial pathogens that have been isolated. There is a high prevalence of Gram-negative bacteria. 71 (59.2%) is distributed as follows: 40.8% (29/71) of *Pseudomonas aeruginosa*, 16.9% (12/71) are *E. coli* species, 15.5% (11/71) *Enterobacter* spp., 11.3% of *Klebsiella pneumonia* (8/71), 8.5% (6/71) of *Proteus* spp.and 7% (5/71) of *Shigella* sp. While 40 strains (33.3%) were *Staphylococcus aureus* with occurrence of 7.5% of *Candida albicans*.

According to the survey, 99% of the various services are contaminated with bacteria. At least 88.4% of the country was found to be contaminated, according to research by Chaoui et al. Comparatively, research conducted in Ethiopia and Nigeria documented lower contamination rates of 46.7% and 39.6%, respectively. A study from Taiwan indicated that 63.5% of samples were contaminated. Additionally, a 2018 investigation across seven institutions in Iran revealed a contamination prevalence of 57%. In contrast, a study conducted in a surgical setting in Scotland at the Western General Hospital reported a significantly higher contamination rate of %96. These findings indicate that contamination percentages vary both qualitatively and quantitatively [19-21].

In our study, *S. aureus* (%33.3) was the predominant pathogen, followed by *P. aeruginosa* (%24.2), *E. coli* (10%), which aligns with several previous findings, a cross-sectional descriptive study identified *P. aeruginosa* as the predominant pathogen (%36.17) responsible for HAIs, while *E. coli* ranked fourth (6.38%), with *Enterobacter* and *S. aureus* being less frequently detected. [22].

The rate of surface contamination is influenced by several factors, including the characteristics of microorganisms and their ability to survive in conditions that promote the formation of biofilms. Additionally, the effectiveness of the organic disinfectant being used plays a significant role. Different types of bacteria and various contaminated surfaces can lead to biofilms with distinct properties. When biofilms develop on specific surfaces, their impact on the environment becomes more pronounced. Furthermore, the bacteria present on surfaces are influenced by air quality; larger airborne particles tend to settle on surfaces more quickly. Thus, beyond assessing the effectiveness of bio cleaning, surface samples from a room can also serve as indicators of the performance and potential limitations of the existing air treatment system [23].

The results in Table 1 indicate that the highest level of contamination was found on hospital equipment and the floors of the wards. This was followed by contamination on patient beds and door window handles. The high level of contamination on the floors of the fracture wards can be attributed to the large number of patients and their companions in the inpatient areas, which

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contributes to the transfer and persistence of bacteria on the floors. This finding is consistent with the observations made by [24].

Table (1): Number of surfaces, equipment, and organisms identified during screening.

organisms Observed	site of sampling					
	Patient beds (20)	**Floor and wall (28)**	**Door/ window handle (9)**	**Bedside table (16)**	**Hospital equipment (37)**	**Total = (100)**
Staphylococcus aureus	7	9	8	7	9	40(%33.3)
Escherichia coli	2	3	1	2	4	12(%10)
Klebsiella pneumoniae	2	3	-	1	2	8(%6.6)
Pseudomonas sp.	5	7	6	4	7	29(%24.2)
Proteus sp.	1	2	1	-	2	6(%5)
Shigella sp.	1	1	-	-	3	5(%4.2)
Enterobacter sp.	2	1	1	1	6	11(%9.2)
Candida albicans	2	1	1	1	4	9(%7.5)
Total = 120	22	27	18	16	37	120

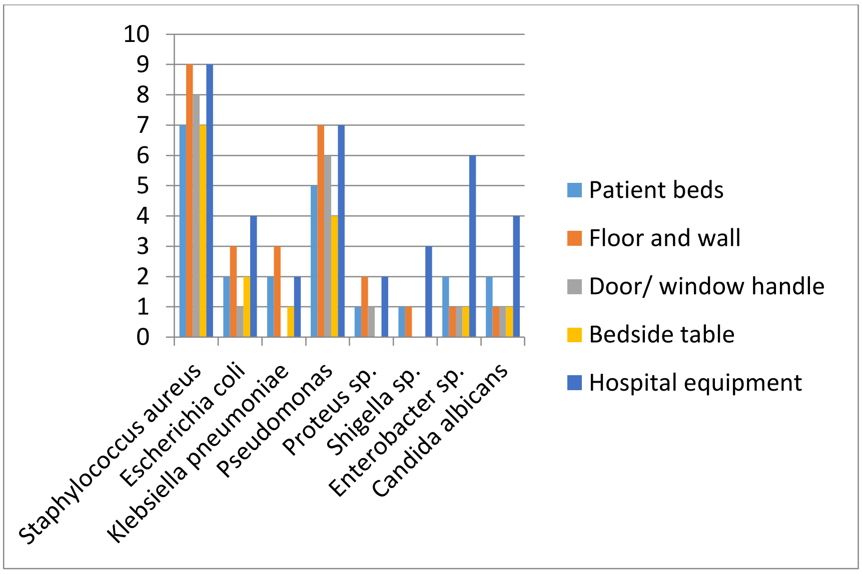

Table Two presents the resistance of Gram-negative bacilli concerning various classes of antibiotics. The isolates of Enterobacteriaceae showed complete sensitivity to Erythromycin, Vancomycin, and Ofloxacin. However, there was significant resistance to amoxicillin-clavulanic acid and Trimethoprim/sulfamethoxazole, with resistance rates of 71.4% and 52.4%, respectively. Furthermore, based on the current definition of MDR, it was found that 82.5% (33 out of 40) of S. aureus strains were resistant to erythromycin, and 51.7% (15 out of 29) of Pseudomonas spp. were resistant to at least two classes of antibiotics.

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The data in Table 1 show the rise of MDRB in hospitals poses a significant threat to public health if measures are not taken to limit their spread. This situation could lead to epidemics that are challenging to control, especially given the transmission of these bacteria between hospitals and the community. Proper management of the cleaning and disinfection procedures for reusable medical equipment is essential to reducing the risk of infections. This practice minimizes the presence of harmful germs, thereby enhancing the effectiveness of sterilization or disinfection processes later on [25]. We recommend expanding the analysis of susceptibility profiles to include all bacteria linked to surface contamination. Additionally, we should develop our understanding of their characteristics to help identify the genes associated with antimicrobial resistance and understand how these genes are transmitted.

Table (2): Antibiotic susceptibility pattern

Antibiotics	**Concentration**	**The percentage of isolates that are resistant to antibiotics**						
		***Staphylococcus aureus* n:40**	***Escherichia coli* n:12**	***Pseudomonas* n:29**	***Klebsiella pneumoniae* n:8**	***Shigella sp* n:5**	***Proteus sp* n:6**	***Enterobacter sp* n:11**
Amoxicillin-clavulanic acid	(30ug)	-	7(%85.3)	-	6(%75)	2(%40)	5(%83.3)	10(%90.9)
Imipenem	(10ug)	-	1(%8.3)	2(%6.9)	00	00	00	00
Ceftriaxone	(30ug)	-	1(%8.3)	4(%13.8)	3(%37.5)	3(%60)	1(%16.7)	6(%54.5)
Ceftazidime	(30ug)	-	2(%16.7)	10(%34.5)	1(%12.5)	2(%40)	1(%16.7)	5(%45.5)
Piperacillin/tazobactum	(100ug/10ug)	-	6(%50)	10(%34.5)	5(%62.5)	2(%40)	00	7(%63.6)
Gentamicin	(10ug)	17(%42.5)	2(%16.7)	15(%51.7)	1(%12.5)	3(%60)	4(%66.7)	7(%63.6)
Erythromycin	(5ug)	33(%82.5)	-	-	-	-	-	-
Vancomycin	(30ug)	11(%27.5)	-	-	-	-	-	-
Ofloxacin	(5ug)	6(%15)	-	-	-	-	-	-
Tetracycline	(30ug)	21(%52.5)	7(%58.3)	-	5(%62.5)	2(%40)	1(%16.7)	8(%73.7)
Ciprofloxacin	(5ug)	22(%55)	1(%8.3)	12(%41.4)	3(%37.5)	3(%60)	5(%83.3)	4(%36.4)
Trimethoprim/sulfamethoxazol	(1.25ug/23.75ug)	18(%45)	8(%66.7)	12(%41.4)	4(%50)	2(%40)	2(%33.3)	6(%54.5)
Chloramphenicol	(30ug)	23(%57.5)	00	-	6(%75)	2(%40)	2(%33.3)	5(%45.5)

CONCLUSION

Our study found that *Pseudomonas aeruginosa* and *Staphylococcus aureus*, as well as *Escherichia coli* were the predominant bacteria responsible for HAIs. The types of drug resistance among these pathogens indicate that antimicrobial resistance continues to be a significant concern. It is crucial for healthcare professionals to be aware of the infection risks posed by MDRB. Based on our findings, to effectively combat antimicrobial resistance in these pathogens, we strongly advocate for responsible antibiotic use alongside rigorous and ongoing monitoring of multidrug-resistant strains.

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Conflict of interests.

There are non-conflicts of interest.

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