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Identification of nosocomial infection bacteria from hospitals, and effect of some antibiotics in Wasit Province

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Abstract--Background: Nosocomial infections are illnesses obtained after medical care. The responsible pathogens are represented by several types of microorganisms and this study aimed to define the causative bacteria and their sensitivity to different antibiotics. Methods: A total of 308 samples have been collected from different places and surfaces prone to bacterial contamination in the three main governmental hospitals in AL-Kut city. Firstly, bacteria were isolated and identified using the biochemical test, then antibiotic sensitivity of isolates was performed using different antibiotics for *Staphylococcus haemolyticus*, *Acinetobacter baumannii*, *Serratia marcescens*, and *Enterobacter cloacae* on Mueller Hinton agar using Kirby-Bauer disk diffusion method. Results: There were 83 (26.9%) bacterial-contaminated samples. The most common type of isolated bacteria was *Staphylococcus haemolyticus* (20.5%) followed by *Acinetobacter baumannii* (12%) of the total bacteria. Culture and sensitivity for *Staphylococcus haemolyticus* bacteria showed that bacteria were resistant to all selected antibiotics reaching 92.9% for Azithromycin and 85.7% for Erythromycin. Results for *A. baumannii* bacteria showed bacterial resistance to all antibiotics and the highest rate (87.5%) was reported for Piperacillin. Results for *Serratia marcescens* bacteria showed bacterial resistance to most antibiotics except for Meropenem with 100% resistance to Augmentin and Nitrofurantoin. The *Enterobacter cloacae* bacteria showed no resistance to Levofloxacin and Nitrofurantoin antibiotics. The highest

resistance rates were 62.5% for Ceftriaxone, Tobramycin, Piperacillin, and Gentamicin. Conclusion: There is an increased rate of bacterial resistance in Wasit province. This may negatively affect Iraqi patients and needs to be considered an alarming health status.

Keywords---bacterial isolation, healthcare-associated infections (HAIs), antimicrobial resistance.

Introduction

The term “nosocomial” applies to any disease contracted by a patient under medical care. Nosocomial infections are also known as healthcare-associated infections (HAI). Both terms are including infections or illnesses that develop while undergoing medical treatment but were absent at the time of admission. They may occur in a variety of healthcare supply unit locations, like hospitals, longstanding care facilities, and outpatient settings, as well as after discharge to home (1, 2). Additionally, work-related infections that may harm healthcare workers personnel are included in healthcare-associated infections (3). The infection results when microorganisms (M.O) infect a patient or other susceptible host. In the current healthcare system, these infections are linked to invasive surgeries, medical devices inserted internally, and prosthetic devices. The etiology of HAIs depends on the source or kind of infection and the M.O that caused it, which could be bacterial, viral, or fungal (1, 2).

HAI is the most frequent adverse event in healthcare that concerns patient safety. They place a heavy financial strain on patients, families, and healthcare systems as well as significantly increased morbidity and mortality (4). The emergence of multi-drug resistant organisms is another complication seen with HAI. HAI affects 3.2% of all hospitalized patients in the United States, 6.5% in the European Union/European Economic Area, and worldwide prevalence is likely much higher (5). Due to a lack of HAI surveillance methods, the burden of HAIs globally is unknown. However, infection prevention and control programs have made a lot of effort to create surveillance systems and infection control techniques (6).

Studies were proved that the most common mean of transmission is through contact, whereby the organisms are spread by direct or indirect contact. Common M.O that may be transmitted through contact is bacteria i.e., *Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter* spp., and *Klebsiella pneumoniae*. Droplet transmissions can happen when large droplets (greater than 5 microns) that leave the respiratory area travel for less than three feet. *Streptococcus pneumonia* and *Neisseria meningitides* are two examples of infectious organisms that are spread by droplets (7). The current study aimed to identify the most common bacteria of nosocomial infection, which possess a strong virulence factor and assess the effect of some selected antibiotics against different pathogenic bacteria.

Material and Method

Study design

For this survey study, the specimens were collected during the period from 9th November 2021 to 15th February 2022. At least 308 samples of pathogenic bacteria spread on the surfaces of different departments in hospitals were collected, including Al-Zahra Teaching Hospital, Al-Karma Hospital, and Al-Kut hospital (Burns, Operating theaters, Birth operations room, Intensive care units (ICUs), preterm, catheter). All samples were taken using sterile disposable cotton and transport swabs. Then they were cultured onto (Cetrimide agar, Mannitol Salt agar, MacConkey agar, MRS agar, and Blood agar) after incubation, samples were identified based on microscopic, colony morphology, Gram stain, and biochemical tests including catalase test, oxidase test, methyl red test, urease test, coagulase test, indole test, then bacteria were identified by API 20 E system, API Staph System, and Vitek2 (8).

Antimicrobial susceptibility test

Antibiotic sensitivity of isolates was identified on Mueller Hinton agar using the Kirby-Bauer disk diffusion method as per CLSI guidelines. The antibiotics that have been tested in the current study included Ciprofloxacin (CIP) 5µg, Levofloxacin (LEV) 5µg, Gentamicin (CN) 10µg, Azithromycin (AZM) 15µg, Amoxicillin-Clavulanate (AUG) 30µg, Ceftriaxone (CRO) 30µg, Amikacin (AK) 30µg, Novobiocin (NO) 30µg, Ceftazidime (CAZ) 30µg, Imipenem (IMI) 10µg, Meropenem (MRP) 10µg, Trimethoprim-sulfamethoxazole (SXT) 25µg, Tobramycin (TOB) 30µg, Piperacillin (PRL) 100 µg, Nalidixic acid (NA) 30µg, Nitrofurantoin 30µg, Erythromycin (E) 15µg, and Rifampicin (RD) 5µg. Bacterial pathogens were considered resistant or sensitive based on the zone of inhibition following the criteria of clinical and laboratory standard institute CLSI (9).

Statistical analysis

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) software program version 26. All categorical variables were presented by frequency and percentages. Association between categorical variables was assessed by the Chi-Square test or Fisher's Exact Test (if > 20% of expected cell counts are less than 5) accordingly. Considering a *P*-value equal to or less than 0.05 as significant.

Results

Sample collection

A total of 308 swab samples have been selected from different places and surfaces for bacterial contamination in the three main governmental hospitals in AL-Kut city. The hospital room floor, walls, and patient's bed were the most frequent places where the samples were selected, which represented 14%, 12.3%, and 12% in the same order. Only one sample was collected from the least contacted subjects like Bathtubs for burn treatment, Kitchen floor, Hospital corridor floor,

Hospital corridor walls, Injector devices in the catheterization unit, and Patient's transport bed (Table1).

Table (1): Frequency distribution of the total number of specimens from the three selected hospitals

Place of sample	Frequency	Percentage
Hospital room floor	43	14.0%
Hospital room wall	38	12.3%
Patient's bed	37	12.0%
Suction machine	33	10.7%
Bandage cart	33	10.7%
Patient medication locker	16	5.2%
Oxygen mask	14	4.5%
Scrubs	13	4.2%
Surgical instruments	13	4.2%
Working medical gloves	12	3.9%
Neonate incubator	11	3.6%
Air conditioner	10	3.2%
Electricity PowerPoint	9	2.9%
Anesthetic devices	9	2.9%
Relative's bed	3	1.0%
Sterilization substances	3	1.0%
Ventilator	2	0.6%
DC shock device	2	0.6%
Bathtubs for burn treatment	1	0.3%
Kitchen floor	1	0.3%
Hospital corridor floor	1	0.3%
Hospital corridor walls	1	0.3%
Injector devices in the catheterization unit	1	0.3%
Patient's transport bed	1	0.3%
Hand washing place in operation theatre	1	0.3%

Isolation of bacteria

Of those 308 isolated swab specimens, only 83 samples (26.9%) were found to be contaminated by bacteria (Table 2). The *Staph. haemolyticus* represented the most frequent isolated bacteria, it accounting for 20.5% of total isolates. Followed 12% for *Acinetobacter baumannii* and then 10.8% for *Enterobacter cloacae*.

Table 2: Frequency distribution of the total isolated bacteria from the whole sample (308) from all 3 hospitals.

Bacterial growth	Frequency	Percentage	
No bacterial growth	225	73.1%	
Bacterial growth	83	26.9%	
Type of isolated bacteria	Frequency	From total samples(n.=308)	From contaminated samples(n.=83)
<i>Staph. haemolyticus</i>	17	5.5%	20.5%

<i>Acinetobacter baumannii</i>	10	3.2%	12.0%
<i>Enterobacter cloacae</i>	9	2.9%	10.8%
<i>bacillus spp</i>	5	1.6%	6.0%
<i>Klebsiella oxytoca</i>	5	1.6%	6.0%
<i>Serratia marcescens</i>	5	1.6%	6.0%
<i>Pseudomonas stutzeri</i>	5	1.6%	6.0%
<i>P. aeruginosa</i>	4	1.3%	4.8%
<i>Listeria spp</i>	4	1.3%	4.8%
<i>Staph. epidermis</i>	4	1.3%	4.8%
<i>Pantoea spp</i>	3	1.0%	3.6%
<i>Proteus penneri</i>	3	1.0%	3.6%
<i>Klebsiella pneumoniae</i>	3	1.0%	3.6%
<i>Leclercia adecarboxylata</i>	2	0.6%	2.4%
<i>Stenotrophomonas</i>	1	0.3%	1.2%
<i>Staph. hominies</i>	1	0.3%	1.2%
<i>Rhizobium Radiobacter</i>	1	0.3%	1.2%
<i>Chromobacterium violaceum</i>	1	0.3%	1.2%

Air conditioners, hospital corridor floor, hospital corridor walls, anesthetic devices, surgical instruments, sterilization substances, neonate incubators, injector devices in the catheterization unit, DC shock devices, patient transport beds, and hand washing places in operation theatres are places where no any bacterial contamination was detected (Figure 1). Fisher's Exact Test found a highly significant association between the places where the samples were derived and the presence of bacterial contamination (P -value <0.001).

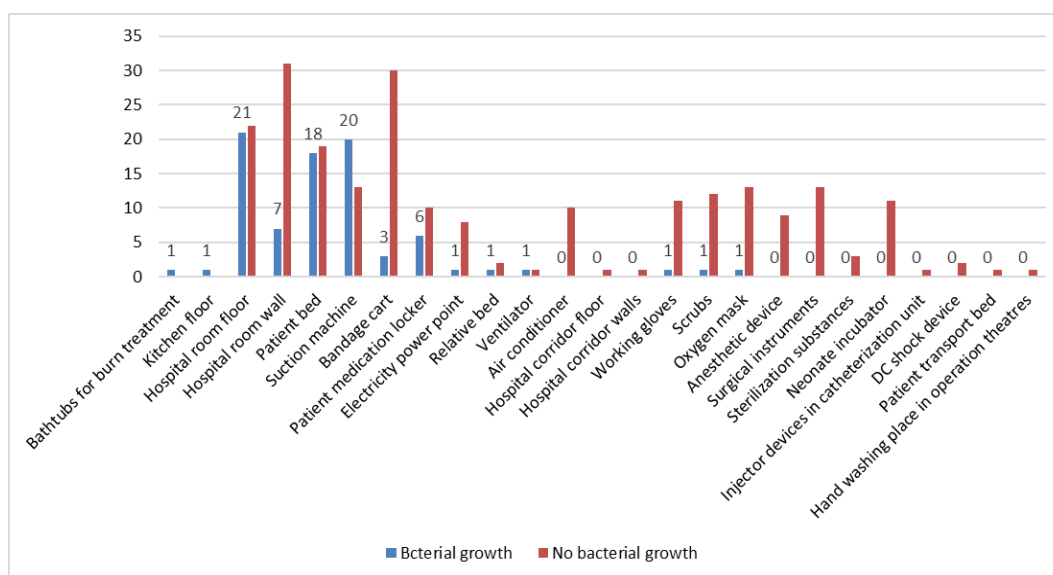


Figure (1): Distribution of bacterial growth in different samples in the three selected hospitals

In Table 3, there was no significant association between the contamination and the hospital where the samples were taken from. In AL-Karama hospital, there were 30.9% of the selected samples found to be contaminated while only 23.5% of samples were contaminated in AL-Kut hospital. The highest rate of contamination was noticed in AL-Zahraa Teaching Hospital, which was divided into two hospitals (Burn hospital and the main one) in percentages of 24.1% and 27% in the same order.

Table 3: Association between the presence of bacterial contamination and the hospital where the samples were derived from

Hospital	Presence of bacteria		P-value
	Bacterial growth	No bacterial growth	
Burn hospital	13(24.1%)	41(75.9%)	0.810 (Chi-square test)
AL-Zahraa Teaching Hospital	41(27%)	111(73%)	
Al-Karama Teaching Hospital	21(30.9%)	47(69.1%)	
Al-Kut Hospital	8(23.5%)	26(76.5%)	

Culture and Sensitivity

Sensitivity test of *S. haemolyticus*

Culture and sensitivity test results for this bacterium showed that these bacteria were resistant to most antibiotics at a high rate, reaching 92.9% for AZM and 85.7% for Erythromycin. The lowest resistant rates were for F and RD antibiotics, in which the resistance rates were 21.4% and 35.7% respectively, as shown in Table 4.

Table (4): Antibiotic sensitivity test of *S. haemolyticus* (n=14)

Antibiotic	Susceptibility		
	S	I	R
LEV	3(21.4)	2(14.3)	9(64.3)
CRO	3(21.4)	0(0)	11(78.6)
AK	5(35.7)	1(7.1)	8(57.1)
TOB	5(35.7)	0(0)	9(64.3)
CIP	2(14.3)	1(7.1)	11(78.6)
AZM	1(7.1)	0(0)	13(92.9)
F	10(71.4)	1(7.1)	3(21.4)
SXT	3(21.4)	1(7.1)	10(71.4)
RD	9(64.3)	0(0)	5(35.7)
E	1(7.1)	1(7.1)	12(85.7)
CN	4(28.6)	1(7.1)	9(64.3)

Fisher's Exact Test (P -value=0.003)

There was a significant association between *S. haemolyticus* bacterial susceptibility responses and the type of antibiotics used ($P=0.003$).

Sensitivity test of *A. baumannii*

Culture and sensitivity test results for this bacterium showed these bacteria were resistant to most antibiotics at a high rate except for LEV, AK, and TOB antibiotics, in which the resistance rate was 37.5% for all of them, as shown in (Table 5). The highest resistance rates were identified for PRL at 87.5%.

Table (5): Antibiotic sensitivity test of *A. baumannii* (n=8)

Antibiotic	Susceptibility No. (%)		
	S	I	R
LEV	4(50)	1(12.5)	3(37.5)
MRP	4(50)	0(0)	4(50)
CRO	1(12.5)	1(12.5)	6(75)
CAZ	2(25)	2(25)	4(50)
AK	4(50)	1(12.5)	3(37.5)
TOB	5(62.5)	0(0)	3(37.5)
IMI	4(50)	0(0)	4(50)
CIP	3(37.5)	1(12.5)	4(50)
PRL	1(12.5)	0(0)	7(87.5)
AUG	3(37.5)	1(12.5)	4(50)
AZM	4(50)	0(0)	4(50)
F	3(37.5)	0(0)	5(62.5)
SXT	2(25)	1(12.5)	5(62.5)
CN	2(25)	1(12.5)	5(62.5)
NA	3(37.5)	0(0)	5(62.5)

Fisher's Exact Test (P-value=0.878)

Sensitivity test of *S. marcescens*

Culture and sensitivity test results for this bacterium showed bacterial resistance to most antibiotics except for MRP. All these bacteria were resistant 100% to F and AUG. While half of them (50%) were resistant to CRO, AK, TOB, CIP, PRL, AZM, and CM as shown in Table 6.

Table (6): Antibiotic sensitivity test of *S. marcescens* (n=4)

Antibiotic	Susceptibility No. (%)		
	S	I	R
LEV	3(75)	0(0)	1(25)
MRP	4(100)	0(0)	0(0)
CRO	0(0)	2(50)	2(50)
CAZ	3(75)	0(0)	1(25)
AK	2(50)	0(0)	2(50)
TOB	2(50)	0(0)	2(50)
IMI	1(25)	2(50)	1(25)
CIP	2(50)	0(0)	2(50)

PRL	2(50)	0(0)	2(50)
AUG	0(0)	0(0)	4(100)
AZM	2(50)	0(0)	2(50)
F	0(0)	0(0)	4(100)
SXT	1(25)	0(0)	3(75)
CN	2(50)	0(0)	2(50)
NA	2(50)	1(25)	1(25)

Fisher's Exact Test (P-value=0.095)

Sensitivity test of *E. cloacae*

Culture and sensitivity test results for this bacterium showed bacteria were not resistant to the LEV and F. The highest rate of resistance was demonstrated for CIO, TOB, PRL, and CN. In which, 5 out of 8 (62.5%) were shown to be resistant to the defined antibiotics (Table 7).

Table (7): Antibiotic sensitivity test of *E. cloacae* (n=8)

Antibiotic	Susceptibility No. (%)		
	S	I	R
LEV	7(87.5)	1(12.5)	0(0)
MRP	5(62.5)	1(12.5)	2(25)
CRO	3(37.5)	0(0)	5(62.5)
CAZ	2(25)	2(25)	4(50)
AK	5(62.5)	1(12.5)	2(25)
TOB	3(37.5)	0(0)	5(62.5)
IMI	3(37.5)	2(25)	3(37.5)
CIP	4(50)	2(25)	2(25)
PRL	3(37.5)	0(0)	5(62.5)
AUG	4(50)	0(0)	4(50)
AZM	5(62.5)	1(12.5)	2(25)
F	6(75)	2(25)	0(0)
SXT	5(62.5)	0(0)	3(37.5)
CN	3(37.5)	0(0)	5(62.5)
NA	6(75)	1(12.5)	1(12.5)

Fisher's Exact Test (P-value=0.095)

No significant association was detected between the susceptibility of *Acinetobacter baumannii*, *Serratia marcescens*, and *Enterobacter* with the selected types of antibiotics.

Discussion

Nosocomial infections (NIs) are linked to several toxins or infectious agents that spread illness to hospitalized patients. During the patient's hospital stay, these illnesses are likely to spread beyond the walls of the hospital (10). Since the treatment of NIs is a protracted process, patients must take antibiotics. However, the misuse of antibiotics created problems because the level of antibiotic

resistance was rising. The use of antibiotics inappropriately, excessively, inconsistently, and insufficiently poses serious risks to patients' health because it exposes bacteria and pathogens to the medications. The severity of the issue led to a reduction in the development of new antimicrobial medications (11). In the current study, culture and sensitivity test results for *S. haemolyticus* bacteria showed that bacteria are resistant to the most antibiotic at a high rate except for F and RD antibiotics which were the lowest rates. Similar to other studies, *S. haemolyticus* displayed considerable resistance to ciprofloxacin (72.3%), gentamicin (72%), and a moderate level of resistance to cotrimoxazole (46.2%), oxacillin (34.6%) and doxycycline (30.7%). No resistance to linezolid, teicoplanin, and vancomycin and sensitive to F and RD in (12). In addition, *S. haemolyticus* showed high resistance was seen to other non- β -lactam antimicrobial agents – ciprofloxacin (58%-72%), gentamicin (26%-72%), and cotrimoxazole (46%-47%). The resistance to ciprofloxacin varied from 84% in *S. haemolyticus* (13, 14). While *S. haemolyticus* isolates showed resistance to fluoroquinolones, macrolides, and aminoglycosides, three antibiotic classes are known as vitally important for humans. Fluoroquinolones were previously used heavily in Lithuania to treat domestic animals (15). Culture and sensitivity test results for *A. baumannii* bacteria showed bacteria were resistant to the most antibiotic at a high rate except for LEV, AK, and TOB antibiotics. The same results were shown in Tewari *et al.* (16) study as imipenem sensitivity was (53.3%), amikacin sensitivity (33.3%), and ciprofloxacin sensitivity was (20%), while the frequency of resistance was high in all other antibiotics used. Similar reports of an increase in bacterial trends have been made from various regions of the world. Vakili *et al.* (17) reported that 95% of *A. baumannii* were isolates from Iran, while Nazmul *et al.* (18) reported 85% of *Acinetobacter* isolates from Malaysia. Similar to this investigation, Lahiri *et al.* (19) found that the percentage of isolates exhibiting was the highest. While in Malaysia, it was discovered that *Acinetobacter spp.* were up to 92.5% resistant to imipenem (20). Additionally, it was noted that *Acinetobacter spp.* had a high resistance rate to carbapenems (90%) (21), which disagreed with our data.

In the present study, it was recorded that *S. marcescens* was resistant to the most antibiotic at a high rate except for levofloxacin (LEV), meropenem (MRP), Ceftazidime (CAZ), IMI, and nalidixic acid (NA) antibiotics, in which resistance rate was (25), (0), (25), (25) and (25) respectively. Current data were agreed with a study conducted by Tıraş *et al.* (22), who found that all 10 strains of *S. marcescens* were susceptible to imipenem, meropenem, amikacin, and ciprofloxacin at the same rates. However, *S. marcescens* is frequently resistant to third-generation cephalosporins and new fluoroquinolones, which are both commonly used to treat infections (23). While *S. marcescens* isolates indicated high resistance rates to ceftriaxone and ceftazidime 22.7% and 19.6%, respectively. The study found that clinical isolates of *Serratia spp.* showed the highest resistance to ceftriaxone, ceftazidime, and piperacillin/tazobactam. However, the rate of resistance detected to cefotaxime and gentamicin (0.6%) was very low. The findings suggested that cefotaxime and gentamicin are the most effective antibiotics for treatment. According to Çiftçi Türetken *et al.* (3), *Serratia* strain isolates were highly susceptible to the antibiotics amikacin (95.7%), ciprofloxacin (91.5%), imipenem (88.9%), cefotetan (85.3%), gentamicin (82.2%), ampicillin/sulbactam (5.9%), ceftazolin (6.8%), tetracycline (9.1%), and ampicillin

(9.1%) (24). *E.cloacae* in the current study was resistant to the most antibiotic at a high rate except for LEV, MRP, AK, IMI, CIP, AZM, F, SXT, and NA antibiotics, in which resistance rate was (0), (25), (25), (37.5), (25), (25), (0), (37.5) and (12.5) percentages respectively. Similar findings were found in a study conducted in Turkey by Ozcan *et al.* (25) and Kaleli *et al.* (26) who evaluated 109 *Enterobacter* spp. for antimicrobial resistance; 15% of the strains tested positive for amikacin; however, resistance to ceftazidime, ceftriaxone, cefaclor, and cephalothin was found to be 47%, 50%, 72%, and 83%, respectively. While it was reported resistant strains among 213 *E. cloacae* isolates. These disagreed with our data. A summary of what was written in the discussion above that *S. haemolyticus* showed that the bacteria are highly resistant to most antibiotics and non- β -lactam antibiotics while they are sensitive to F and RD, and the *A. baumannii* showed resistance to the most antibiotic at a high rate except for LEV, AK and TOB. The *S. marcescens* was resistant to the most antibiotic at a high rate except for LEV, MRP, CAZ, IMI, and NA and the best antibiotics for treatment are cefotaxime and gentamicin. The *E.cloacae* was resistant to the most antibiotic at a high rate except for LEV, MRP, AK, IMI, CIP, AZM, F, SXT, and NA antibiotics.

Conclusions

In the present study, outcomes indicated that *S. haemolyticus*, *A. baumannii*, *S. marcescens*, and *E.cloacae* were the most prevalent bacterial nosocomial infections in main Wasit hospitals. In addition, there was a high rate of bacterial resistance to the most used antibiotics; particularly *S. haemolyticus* which was the most resistant bacteria to all antibiotics. This increase in antibiotic resistance must be alarming to the health status of patients and the Iraqi government to treat or limit nosocomial infections.

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