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A Bacteriological study of patients with bacteremia in Erbil city

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ABSTRACT

In this study, we investigate bacteria that cause sepsis and determine infection rates by gender. Aerobic bacteria were isolated and identified by a series of biochemical tests, and the results were confirmed using the Vitek2 system, which detected resistance to 13 antibiotic discs. The most common isolated bacteria were Staphylococcus aureus (23.42%), followed by Escherichia coli (18.35%), Staphylococcus epidermidis (10.76%), Micrococcus luteus (7.59%), and *Klebsiella pneumoniae* (6.96%). The infection rate among patients with bacteremia was higher in females (57.59%) than in males (42%). 41% respectively.

Keywords: bacteremia, *Staphylococcus aureus*, gender factor, multidrug resistance

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دراسة بكتريولوجية للمرضى المصابين بتجرثم الدم في مدينة اربيل

بيمان علي كريم

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الملخص

في هذه الدراسة، قمنا بدراسة أنواع البكتيريا المسببة لعدوى الإنتان، وتحديد نسبة الإصابة بناءً على حسب الجنس. تم عزل البكتيريا الهوائية والكشف عنها باستخدام التشخيص المجهري والمظهري، بالإضافة إلى سلسلة من الفحوصات الكيميائية الحيوية. وقد تم تأكيد النتائج باستخدام تقنية نظام Vitek2 للكشف عن أنواع البكتيريا المقاومة لـ 13 نوعًا من المضادات الحيوية. كانت البكتيريا المعزولة الأكثر شيوعًا هي المكورات العنقودية الذهبية (23.42٪)، تليها الإشريكية القولونية (18.35٪)، والمكورات العنقودية البشروية (10.76٪)، والمكورات الصفراء (7.59٪)

والكلبسيلة الرئوية (6.96٪). كانت نسبة الإصابات بين الاناث والذكور أعلى لدى المرضى الإناث المصابات بتجرثم الدم من الذكور بنسبة 57.59% و 42.41% على التوالي.

INTRODUCTION

The majority of cases with occult bacteremia resolve on their own, and significant complications are becoming less prevalent. However, significant bacterial infections occur, including pneumonia, septic arthritis, osteomyelitis, cellulitis, meningitis, brain abscesses, and sepsis, which can result in death [(1)]. It can arise spontaneously, with specific tissue infections, and with the use of indwelling genitourinary or intravenous catheters, or after dental, gastrointestinal, genitourinary, wound care, or other procedures. Bacteremia may cause metastatic infections, including endocarditis, especially in patients with valvular heart abnormalities. Although it can cause fever, transient bacteremia is often asymptomatic. When additional symptoms appear, they typically indicate a more serious illness, like sepsis or septic shock [(2)].

The identification of S. aureus in at least one blood culture was referred to as SAB. The time between the first SABU sampling date and the first SAB reported during hospitalization was known as the interval between Staphylococcus aureus bacteriuria (SABU) and S. aureus bacteremia (SAB). Effective antimicrobial treatments were found to be cefazolin or flucloxacillin for methicillin-susceptible S. aureus and vancomycin, linezolid, or daptomycin for methicillin-resistant S. aureus (MRSA)[(3)]. Staphylococcus aureus bacteriuria (SABU) is linked to Staphylococcus aureus bacteremia (SAB) in a small percentage of cases (0.8–4.3%).

This association is attributed to asymptomatic catheter-associated urinary tract infections, which may cause bacteremia [(4, 5).]

According to several studies, concurrent SABU may be a sign of complex SAB and unfavorable clinical outcomes, such as ICU hospitalization or death[(6, 7)]. Several studies evaluated risk factors and the proportion of SAB in SABU; however, they either used different inclusion/exclusion criteria or covered only specific groups. Kouijzer et al. discovered a 6.9% prevalence of SAB in SABU in a recent population-based study (Canada). They also identified risk factors for SAB, such as male sex, urinary procedures, inpatient status, and pure S. aureus culture[(8)]. Building on this discovery, we investigated the percentage and risk factors of SAB in patients with SABU at a hospital.

Within the medical community, antibiotic resistance has been recognized as a significant factor influencing both patient outcomes and overall hospital resource use [(9-11)]. Antibiotic-resistant germs are emerging and spreading faster than ever before, posing a threat to hospitals worldwide. Hospital-acquired illnesses have been linked to Antibiotic-resistant gram-positive and gram-negative bacteria [(12, 13)]. Few antimicrobial drugs remain available for successful treatment in many cases, especially against gram-negative bacteria that produce extended-spectrum β-lactamases conferring resistance to numerous antibiotics, and methicillin- and vancomycin-tolerant Staphylococcus aureus[(14, 15)]. To lessen the emergence and spread of infections caused by antibiotic-resistant bacteria, several recent editorials and studies have recommended additional efforts to intensify current infection control procedures[(9, 16, 17)]. Additionally, the discovery of new antimicrobial treatments targeting these emerging diseases has recently accelerated, driven by the clinical evaluation of promising therapies [(18)]. These relative differences in some research findings can be attributed to both the patient's characteristics (such as age, psychological stress, and immunological status) and certain environmental factors [(19)].

Increased awareness of inadequate antibiotic therapy for illnesses in both community and hospital settings is one effect of rising antimicrobial resistance [(20, 21)]. The issue of insufficient antibiotic therapy for infections in hospitalized patients, especially in specialized areas of the hospital, has emerged as a key contributor to the rise in illnesses caused by antibiotic-resistant bacteria. Some key areas with insufficient antimicrobial therapy include intensive care units, oncology/bone marrow transplantation wards, and dialysis units. [12, 13, 22, 23].

Our study's objectives were to determine the prevalence of bacteremia in patients in Erbil city using blood sample analysis and to examine antibiotic susceptibility testing and multidrug resistance.

MATERIALS AND METHODS

Samples Collection:

A total of 1124 blood samples were collected from bacteremia patients hospitalized in Erbil city from January 2021 to January 2022. Following the collection, each bacterial isolate was subjected to a battery of verification assays. Palpation helps select the vein for puncture. Cleansing the region surrounding the planned puncture site with a prepared alcohol swab or a gauze pad soaked in 70% isopropanol is recommended. It is best to wait to touch the cleaned skin until after the venipuncture is finished. A tourniquet is worn 4–6 inches (10–15 cm) above the planned puncture site after the skin has been cleaned to widen the veins and prevent venous blood from returning to the heart. The phlebotomist should select the appropriate tubes and estimate the volume of blood to be drawn before performing a venipuncture. Also, the proper needle needs to be chosen for the desired serum or plasma tests. Gauges 19 to 22 (1.06–0.71 mm outer diameter) are the most widely used sizes [24].

The samples were directly injected into culture media to isolate bacteria; MacConkey agar plates and blood culture were then incubated aerobically at 37°C for 24 to 48 hours. Species identification, biochemical testing, morphological analysis, and pathogen antibiograms were used to isolate pure colonies of microorganisms using the Vitek 2 system [25].

Vitek2 system:

The first step in identifying isolates with VITEK 2 (BioMérieux, Inc., France) was performing a Gram stain to determine which card to use. Each isolate's pure colony was removed, recultured on nutrient agar, and incubated for 24 hours at 37˚C. 2.5 milliliters of 0.45% saline were used to suspend three to five colonies. According to the system's instructions, the suspended bacteria were employed after being adjusted to a McFarland turbidity level of 0.50 to 0.63. Before being loaded into the carousel incubator, inoculated cards were passed via a mechanism that closes the card and cuts off the transfer tube. Up to 30 or 60 cards can fit inside the carousel incubator. At 35.5 ± 1.0 °C, all card types were incubated online. Every 15 minutes, each card was removed from the carousel incubator, sent to the optical system for reading, and then returned to the incubator until the next read time. Over the incubation period, data were collected every 15 minutes [26].

Antimicrobial susceptibility test by Vitek 2 compact system:

An advanced expert system (AES) built into the system examines minimum inhibitory concentration (MIC) trends to identify phenotypes in the majority of tested species. For lean laboratory management, this maximizes laboratory efficiency. Quick results enable doctors to switch from empiric therapy to targeted therapy, improving patient outcomes and strengthening antibiotic stewardship[27] Crucial to Vitek2 technology is the AES, which allows exact "fingerprint" detection of bacterial resistance mechanisms and phenotypes. This Vitek2 card has 64 micro-wells. Antimicrobials or identification substrates are present in every well. For the discovery and testing of organisms' susceptibility to antibiotics, Vitek2 provides a full menu [28]. Due to its sealing, the Vitek2 test card reduces spills, aerosols, and personal contamination. More than 80% less disposable waste is produced when using micro titration techniques.

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RESULTS AND DISCUSSION

Distribution of Gram-positive and Gram-negative bacteria isolated according to gender

Out of 1124 isolates only 158 were positive from patients as in Table (1) and Figure (1) Results showed that Staphylococcus aureus isolates were higher in females (23, 62.16%) than in males (14, 37.84%), Escherichia coli isolates were 17 (58.62%) in females and 12 (41.38%) in males, Klebsiella pneumonia isolates were 8 (72.73%) in females and 3 (27.27%) in males, Enterococcus isolates were 5 (55.55%) in females and 4 (44.44%) in males, Staphylococcus saprophyte isolates were 2 (66.66%) in females and 1 (33.34%) in males, and Enterobacter isolates were higher in males than females (5 (71.43%) in males). While Kocuria rhizophila was present in 1 (100%) of the females and 0 (0%) of the males, Staphylococcus hominis was present in 4 (40%) of the females and 6 (60%) of the males. And for *Streptococcus mutans* was 1(50%) in female and 1(50%) in male for *Micrococcus luteus* was 7(58.33%) in female and 5(41.67%) in male for *Staphylococcus epidermidis* 12(70.59%) in female and 5(29.41%) in male, *Salmonella typhi* 0(0%) in female and 1(100%) in male, while for *Aerococcus viridans* was higher in females than males 1 (100 %) in female 0 (0%) in male. *Dermacoccus nishinomiyaensis, Serratia marcescens* and *Kocuria Kristina* in females 0(0%) and in male 1 (100%) but for *Streptococcus anginosus* was 0 (0%) in female and 2(100%) in male, *Kocuria rosea* and *Pseudomonas erogenous* were 1(50%) in female and 1(50%) in male for *Acetobacter* was 4(57.14%) in female and 3(42.86%) in male for Streptococcus *constellatus* and *Streptococcus agalactiae* 1(100%) in female and 0(0%) in male.

Table 1: Distribution of Gram-positive and Gram-negative bacteria isolates according to gender.

Bacteria	Gender					
	Female		Male		Total	
	No.	%	No.	%	No.	%
Staphylococcus aureus	23	62.16	14	37.84	37	23.42
Escherichia coli	17	58.62	12	41.38	29	18.35
Klebsiella pneumoniae	8	72.73	3	27.27	11	6.96
Enterococcus	5	55.55	4	44.44	9	5.70
Staphylococcus saprophyticus	2	66.66	1	33.34	3	1.89
Enterobacter	2	28.57	5	71.43	7	4.43
Staphylococcus hominis	4	40.00	6	60.00	10	6.33
Kocuria rhizophila	1	100.00	0	0.00	1	0.63
Streptococcus mutans	1	50.00	1	50.00	2	1.27
Micrococcus luteus	7	58.33	5	41.67	12	7.59
Staphylococcus epidermidis	12	70.59	5	29.41	17	10.76
Salmonella typhi	0	0.00	1	100.00	1	0.63
Aerococcus viridans	1	100.00	0	0.00	1	0.63
Dermacoccus nishinomiyaensis	0	0.00	1	100.00	1	0.63
Serratia marcescens	0	0.00	1	100.00	1	0.63
Streptococcus anginosus	0	0.00	2	100.00	2	1.27
Kocuria rosea	1	50.00	1	50.00	2	1.27
Acetobacter	4	57.14	3	42.86	7	4.43
Pseudomonas aeruginosa	1	50	1	50	2	1.27
Kocuria Kristina	0	0	1	100	1	0.63
Streptococcus constellatus	1	100	0	0	1	0.63
Streptococcus agalactiae	1	100	0	0	1	0.63
Total	91	57.59	67	42.41	158	100

No Number of bacteria, %=Percentage

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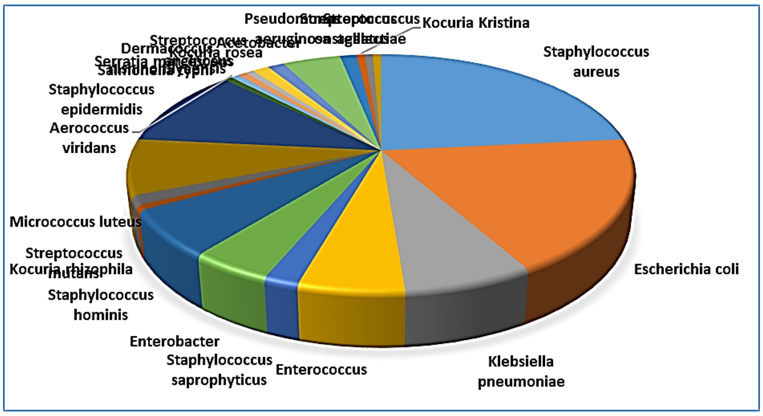

Fig. 1. Distribution of bacteremia according to gender

The most common cause of death and morbidity among healthcare-associated diseases is bacteremia, defined as the presence of bacteria in the circulation. Although diagnostic methods have advanced rapidly, blood culture remains the gold standard for diagnosis [29].

The results of the current study showed that the incidence of bacteremia was higher in females than in males; 91 (57.59%) samples were female and 67 (42.41%) were male. Our study's findings closely matched those of earlier research [30]. The findings indicated that the incidence of bacteremia was 15 (62.5%) in females and 9 (37.5%) in males. Our findings, along with those of other researchers, showed the presence of bacteremia [31, 32]. These relative differences in some research findings can be attributed to both the patient's conditions (such as age, psychological stress, and immune status) and certain environmental factors.

Gram-positive bacterial antibiotic susceptibility pattern testing in bacteremia patients:

158 of the 1124 cases tested positive, and 100 (63.29%) of the 13 antibiotics tested for resistance in Gram-positive bacterial isolates. Clinical and laboratory standards for antimicrobial sensitivity testing were used to evaluate the data. Isolates of Gram-positive bacteria exhibited high resistance to erythromycin (100%) and tetracycline (81.82%). However, as shown in Table 2, the lowest resistance was to Vancomycin (13.63%), Rifampin (22.73%), Gentamycin (36.36%), and both Clindamycin and Amoxicillin (31.82%).

Table 2: Gram-positive bacterial antibiotic susceptibility pattern testing in bacteremia patients

Antibiotic	Resistance		Intermediate		Susceptible	
	No.	%	No.	%	No.	%
Ampicillin	—	—	9	40.90	13	59.09
Ciprofloxacin	14	63.64	5	22.73	3	13.63
Clindamycin	7	31.82	4	18.18	11	50.00
Ceftriaxone	11	50.00	8	36.36	3	13.63
Erythromycin	22	100.0	—	—	—	—
Gentamycin	8	36.36	—	—	14	63.64
Levofloxacin	11	50.00	5	22.73	6	27.27
Amoxicillin	7	31.82	—	—	15	68.18
Rifampin	5	22.73	3	13.63	14	63.64
Sulfamethoxazole	9	40.90	—	—	13	59.09
Tetracycline	18	81.82	—	—	4	18.18
Tigecycline	6	27.27	4	18.18	12	54.55
Vancomycin	3	13.63	3	13.63	16	72.73

No Number of bacteria, %=Percentage

Our result differs from the previous study [33]. Showed high resistance to Tetracycline and Oxacillin 100%.

Gram-negative bacterial antibiotic susceptibility pattern testing in bacteremia patients:

58 (36.71%) of the 158 positive Gram-negative bacterial isolates from the 1124 cases were tested for resistance to 13 common antibiotics. Clinical and laboratory standards for antimicrobial sensitivity testing were used to evaluate the data. Our results in Table 3 show that Gram-negative bacterial isolates exhibited the lowest levels of resistance to ciprofloxacin and levofloxacin (20%), 26.67% to Gentamycin, 33.33% to Rifampin, and 100% to Vancomycin (80%) against Tetracycline.

Table 3: Gram-negative bacterial antibiotic susceptibility pattern testing in bacteremia patients

Antibiotic	Resistance		Intermediate		Susceptible	
	No.	%	No.	%	No.	%
Ampicillin	7	46.67	3	20	5	33.33
Ciprofloxacin	6	40	—	—	9	60
Clindamycin	8	53.33	7	46.67	—	—
Ceftriaxone	7	46.66	4	26.67	4	26.67
Erythromycin	9	60	5	33.33	1	6.67
Gentamycin	4	26.67	3	20	8	53.33
Levofloxacin	6	40	4	26.67	5	33.33
Amoxicillin	8	53.33	7	46.67	—	—
Rifampin	5	33.33	10	66.67	—	—
Sulfamethoxazole	11	73.33	—	—	4	26.67
Tetracycline	12	80	3	20	—	—
Tigecycline	3	20	3	20	9	60
Vancomycin	15	100	—	—	—	—

No = Number of bacteria, % = Percentage

According to some researchers, the severity of the underlying disease, the patient's initial condition, and the type of antibiotic treatment employed all have a greater impact on the probability of death than the degree of antibiotic resistance. The primary cause of a high mortality rate appears to be improper antibiotic therapy [34].

Resistance rate of Gram-positive bacteria isolates from patients with bacteremia:

Our results in Table 4 and Figure 2 showed that *Staphylococcus aureus*. Resistance to Erythromycin followed by Aztreonam, Ciprofloxacin, levofloxacin, Cefaxime, Rifampin and Ampicillin percentages of resistance being (92.86%, 50%, 42.86%, 35.71%, 28.57%, 14.29%), respectively. *Enterococcus, resistance to Erythromycin, Ciprofloxacin, Gentamycin, Rifampin, and Cefexim was* 100% and 66.67%, respectively. *Staphylococcus hominis* with resistance to Aztreonam, Ceftazidime, Ciprofloxacin, and Erythromycin was observed; all antibiotics showed the same level of resistance (50%). Streptococcus mutans is not resistant to any antibiotics. *Staphylococcus epidermidis* showed resistance to Cefaxime, Aztreonam, Piperacillin and Erythromycin, with a percentage of resistance (100%), followed by Ampicillin and Gentamycin, with a percentage of resistance (50%). If the organism being highlighted is a Gram-negative bacterium, such as Streptococcus pneumoniae or Enterococcus, antibiotic therapy is more appropriate[35].

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Table 4: Gram-positive bacterial isolates' resistance rate in bacteremia patients

Antibiotic	*S. aureus* (14)		*Enterococcus* (3)		*S. hominis* (2)		*Strep. mutans* (1)		*S. epidermidis* (2)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	2	14.29	—	—	—	—	—	—	1	50
Cefazolin	1	7.14	—	—	—	—	—	—	—	—
Ceftriaxone	2	14.29	—	—	—	—	—	—	—	—
Cefaxime	5	35.71	2	66.67	—	—	—	—	2	100
Aztreonam	7	50	—	—	1	50	—	—	2	100
Piperacillin	—	—	—	—	—	—	—	—	2	100
Ceftazidime	—	—	—	—	1	50	—	—	—	—
Imipenem	—	—	—	—	—	—	—	—	—	—
Gentamycin	2	14.29	2	66.67	—	—	—	—	1	50
Ciprofloxacin	6	42.86	3	100	1	50	—	—	—	—
Levofloxacin	5	35.71	—	—	—	—	—	—	—	—
Erythromycin	13	92.86	3	100	1	50	—	—	2	100
Rifampin	4	28.57	2	66.67	—	—	—	—	—	—

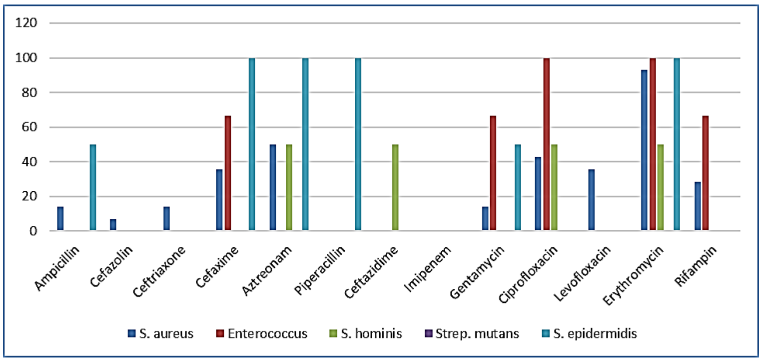

Fig. 2: Gram-positive bacterial isolates' resistance rate in bacteremia patients

Gram-negative bacterial isolates from bacteremia patients' resistance rate:

The results shown in Figure 3 and Table 5 suggested that Acetobacter baumannii. Resistance to Piperacillin, then Ceftriaxone, Imipenem and Ampicillin, with percentages of resistance which were (66.67%, 50%, 33.33%, 16.67%) respectively. *Escherichia coli* showed resistance to Aztreonam and Piperacillin, with a percentage of resistance of 100%, followed by erythromycin, Ciprofloxacin and Cefazolin, with percentages of resistance which were (75%, 50%, 25%, respectively. Klebsiella pneumonia showed resistance to Piperacillin and Ceftazidime, with a percentage of resistance of 100%, followed by Ampicillin and Levofloxacin, with percentages of resistance of 66.67%, 33.38%) respectively. Pseudomonas aeruginosa showed 100% resistance to Aztreonam, Piperacillin, Gentamycin, and Imipenem. It showed that the endpoints of 90-day mortality, persistent bacteremia, relapse, or treatment failure were not improved by adding an anti-staphylococcal beta-lactam to vancomycin or daptomycin [36].

Table 5: Gram-negative bacterial isolates from bacteremia patients' resistance rate

Antibiotic	Number of Resistance % of Gram-negative							
	A. baumanni (6)		*E. coli* (4)		*K. pneumoniae* (3)		*P. aeruginosa* (2)	
	No.	%	No.	%	No.	%	No.	%
Ampicillin	1	16.67	1	25.00	2	66.67	—	—
Cefazolin	1	16.67	1	25.00	2	66.67	—	—
Ceftriaxone	3	50.00	1	25.00	1	33.33	—	—
Cefaxime	2	33.33	—	—	—	—	—	—
Aztreonam	—	—	4	100.0	1	50.00	2	100.0
Piperacillin	4	66.67	4	100.0	3	100.0	2	100.0
Ceftazidime	3	50.00	—	—	3	100.0	—	—
Imipenem	2	33.33	—	—	1	33.33	2	100.0
Gentamycin	3	50.00	—	—	—	—	2	100.0
Ciprofloxacin	2	33.33	2	50.00	1	33.33	2	100.0
Levofloxacin	2	33.33	—	—	1	33.33	—	—
Erythromycin	3	50.00	3	75.00	—	—	—	—
Rifampin	—	—	—	—	—	—	—	—

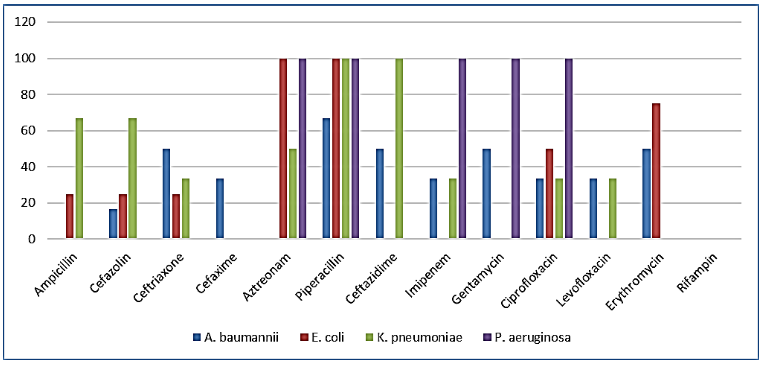

Fig. 3: Resistance rate of Gram-negative bacterial isolates from patients with bacteremia

Numerous factors contributing to the emergence of antibiotic-resistant bacteria in these units are likely linked to the increase in illnesses caused by these germs. This pressure is caused by several causes, including the extensive use of broad-spectrum antibiotics, the concentration of patients with high disease acuity in relatively small, specialized hospital regions, the reduction of nursing and support staff as a result of financial strains, which raises the risk of antibiotic-resistant bacteria spreading from person to person; and the rise in the number of acutely and chronically ill patients who need lengthy hospital stays and frequently carry antibiotic-resistant bacteria [37].

CONCLUSION

According to the study, the bacteremia rate in Erbil has risen in recent years. Strong resistance to currently used antibiotics is a defining feature of their prognosis. This would monitor antimicrobial resistance and necessitate strict control of antibiotic prescriptions. Additionally, there is a strong push to rationalize antibiotic use. To prevent infections caused by drug-resistant organisms, such as bacteremia, better antibiotic use regulation and improved infection prevention are required.

Ethical Approval and Consent: The patients provided consent, and the bacterial strains used in this investigation were isolated from standard clinical collections. The Scientific and Research

Ethics Committee of the University of Salahaddin, College of Agricultural Engineering Sciences, gave its approval to this work.

Conflict of interests: The authors declared no conflicts of interest.

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