

Isolation and Identification of Gram-positive and Gram-negative Bacteremia in patients admitted to Intensive Care Units in Mosul City Hospitals

Mahmood Y. Younis^{1*} and Hiyam Adel Altaii²

¹Nineveh Health Department, Mosul Iraq.

²Department of Biology, College of Science, University of Mosul, Mosul Iraq.

*Email: mahmoodyaseen1981@yahoo.com

Abstract

The present study aimed to identify bacteremia in ICU patients, determine the antibiotic resistance profiles and minimum inhibitory concentrations (MICs) of all isolates by using the Vitek-2 compact system. 90 blood samples from both sexes and from different age groups ranging from (21-90) years were collected from different hospitals in Mosul city, for the period between the first of July and December of 2022. Samples were subjected to bacterial diagnosis, antibiotic sensitivity test by using Vitek2. The results of the current study indicated that 66(73.33%) blood samples revealed negative cultures and 24(26.67%) blood sample revealed positive cultures consisting of 14(58.34%) Gram-positive bacteremia were predominant, and 10(41.66%) Gram-negative bacteremia. Antimicrobial susceptibility test (AST) revealed that Vancomycin, linezolid, Imipenem and Rifampicin provided the best antibacterial effect against most Gram-positive bacteremia while Meropenem, Imipenem and Colistin were the best against the most of gram negative bacterial isolates.

Keywords: ICU patients, Bacteremia, Antibiotics, Sensitivity, Resistance

1. Introduction

The main objective of the respiratory system is to facilitate the process of air exchange, carrying oxygen and transporting it through the respiratory system, and removing carbon dioxide (Hsia et al., 2016). The respiratory system was described as one of the organs most susceptible to infection with microbes, being in direct contact with the atmospheric air and the external environment, as it begins with the nostrils and ends downward with the lung alveoli. It is divided into two parts, the upper respiratory tract (URT) and the lower respiratory tract (LRT), (Pawlina, W., & Ross, M. H. 2018). In addition to a role in air transport, each of these areas also plays an important role in defending the respiratory tract against infection, the mucociliary blanket of the sinuses, middle ear, and tracheobronchial tree clears particulate matter and contains immunoglobulin and other antimicrobial substances, if particles reach the alveoli, resident macrophages ingest organisms; polymorphonuclear leukocytes and monocytes are recruited once the lung becomes inflamed (Mahon et al., 2015). Hospitalization-acquired pneumonia is one of the most frequent infections in critical patients. It is primarily associated with mechanical ventilation leading to severe illness and high mortality (Perez et al., 2021). Community-acquired pneumonia by *Streptococcus pneumoniae* and other pathogens is a focus of infection in patients with community-acquired bacterial meningitis, invasive disease by these pathogens is preceded by nasopharyngeal colonization, after which the bacteria are able to pass the mucosal barrier, invade the bloodstream and eventually cross the blood-brain barrier to

cause meningitis (Figueired et al., 2020).

The heart, like every other organ in the body, is susceptible to infection. Infective endocarditis (IE) has a high mortality rate, the majority of organisms causing IE include *S.aureus*, *S.epidermidis* can enter through the skin or intravenous/central lines and causes a highly toxic febrile illness, particularly associated with prosthetic valves. Other organisms like *S.viridans*, *S.bovis*, *Enterococci*, *Pseudomonas aeruginosa*, *Brucella*, *Coxiella burnetii* and HACEK-group (Vaswani et al., 2022).

Blood stream infections (BSIs) refer to presence of microorganisms (bacteria, viruses, fungi and parasites) in blood, which are a threat to every organ in the body; however as bacteria account for the majority of BSIs and it is called bacteremia (Apurba, S.S., & Sandhya, B.K. 2019a).

Worldwide, bloodstream infections (BSIs) with Gram-Positive and Gram-Negative bacteremia which are a major health problem, constitute an important cause of morbidity and mortality in clinical settings (Habyarimana et al 2021). However, BSIs remain undiagnosed due to limited diagnostic laboratory facilities resulting in poor clinical outcomes and the increasing risk of antimicrobial resistance (Leal, et al 2019). Identify risk factors of BSIs lead to describe epidemiological patterns for early onset bacteremia (EOB) and late onset bacteremia (LOB), (Lee, et al 2020).

2. Materials and Methods

Collection of blood samples

90 blood samples were collected from patients admitted to intensive care unit in Mosul city hospitals (Ibn Sina teaching hospital, Mosul general

hospital and AL-Salam teaching hospital) in Nineveh Governorate/Iraq after their diagnosis by specialist doctor, both of sexes and for different age groups ranging from (21-90) years. Approximately 10ml of venous blood was withdrawn by researcher himself using plastic medical syringes.

Methodology procedure and techniques used in our study

Figure(1) below illustrates methodology of procedure assay of our study and techniques used in the diagnosis of microorganisms and measurement of immunological parameters.:

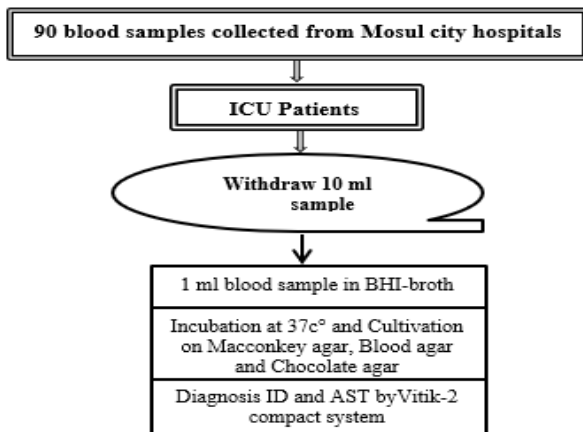


Figure 1: methodology of procedure assay

Isolation and Identification of Gram-Positive and Gram-Negative Bacteremia

All the appeared bacterial colonies on the specific culture media were identified by gram stain, biochemical tests(Catalase and Oxidase test) (Granato *et al.*, 2019), VITEK 2compact system was also used utilizing ID-GNB and ID-GPB cards (BioMerieux, France) (Bagudo *et al.*, 2020).

3. Results and Discussion

The demographic characteristic of the study

According to Figure(2) below, the results of our study may differ with local and international previous studies, it is difficult for the results of our study to agreed for several reasons, including the difference in the size of the clinical sample, geographic and climatic differences, the immune response of population groups, microbiologic techniques in pathogen isolation, in addition to the application of primary health care programmes, medical staff working in intensive care units and infection control programs. The current study included 90 blood sample taken from ICU patients, patients group including (40 male and 50 female patients), this is demographic characteristic of the study group as shown in Figure (2) below.

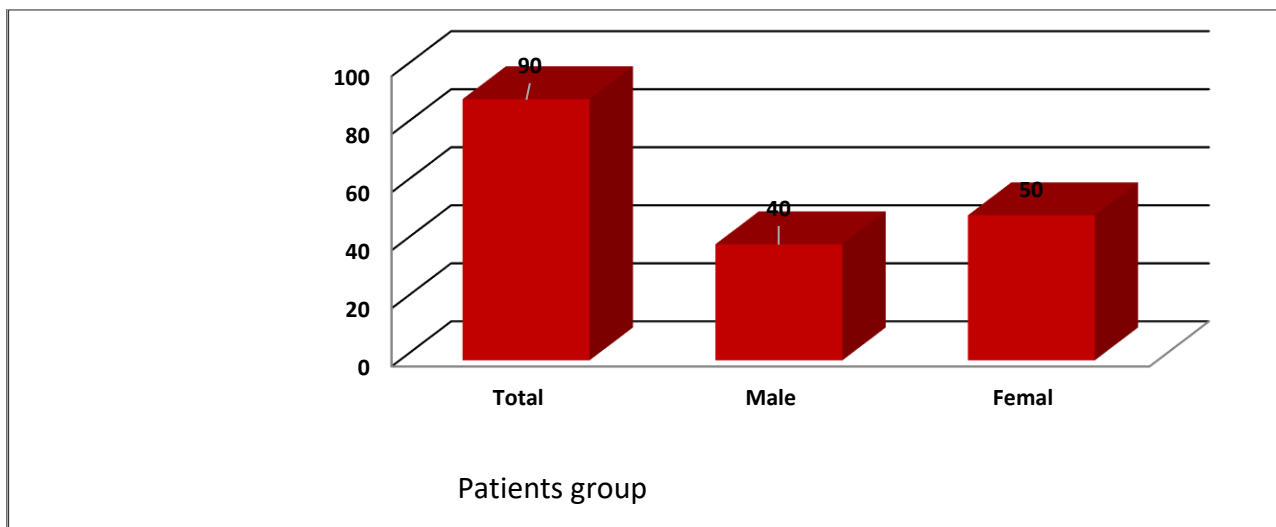


Figure 2: The demographic characteristic of the study.

Distribution of the patients group according to sex

Figure (3) illustrates 90 blood samples were collected from patients confirmed to be infected with the Gram-positive and Gram-negative Bacteremia and those hospitalized in ICU in Mosul city hospitals in Nineveh Governorate/Iraq after their diagnosis by specialist doctor. It is found that 24 blood samples are positive culture (male=9, female =15), and 66 blood samples revealed negative cultures without growth (male= 31, female= 35), for different age groups ranging from (21-90) years, The result of current study showed that the incidence of bacteremia for both sexes of

the study group was 24(26.67%) while the percentage of non-bacteremia of the study group was 66(73.33%). The results of our study may agree with previous studies in close proportions such as local and international studies(Al-Saadi, 2011; Akbar, 2000). The result also showed that the incidence of bacteremia for females(62.5%) higher than the incidence of bacteremia in males(37.5%). Our results indicated the presence of bacteremia which were also obtained by other investigators(Komori *et al.*, 2020). Distribution of the patients group with bacteremia and non-bacteremia according to sex as shown in Figure (3) below.

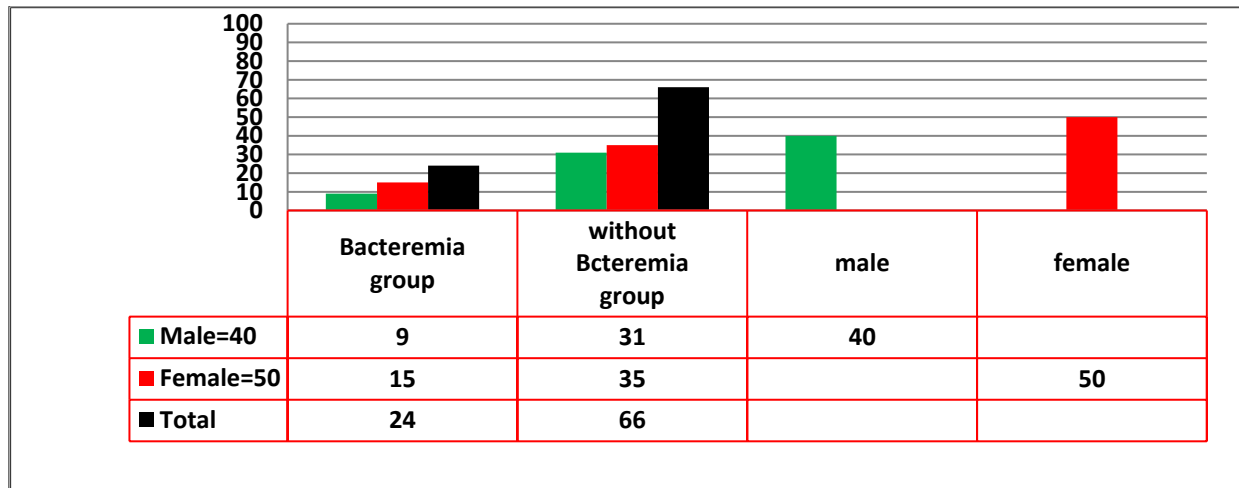


Figure 3: Distribution of the patients group according to sex

Percentage of Gram-positive and Gram-negative bacterial species

The bacterial isolates are distributed into 24 isolates, 14(58.34%) belonging to Gram-positive bacteremia were predominant, and 10(41.66%) belonging to Gram-negative bacteremia

Percentage of Gram-positive bacterial species

Figure (4) below showing that Gram-positive

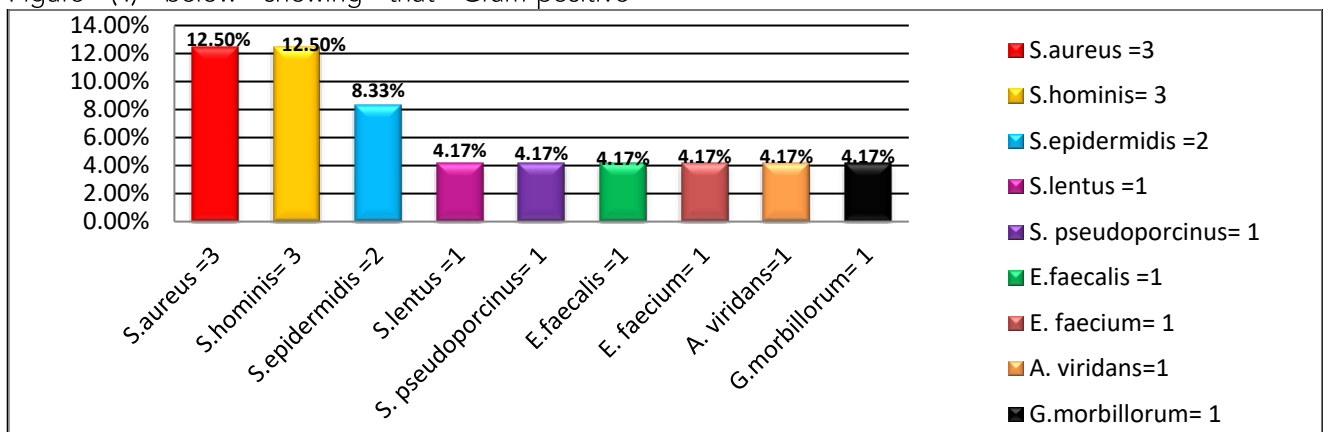


Figure 4: Percentage of gram- positive bacterial species

Percentage of Gram-negative bacterial species

Figure(5) below showing that Gram-negative bacterial isolate were represented by *K.pneumonia*

2(8.33%), *S.marcescens* 2(8.33%), *E.coli* 2(8.33%), *A.baumannii* 2(8.33%), *P.aeruginosa* 1(4.17%). and *B.cepacia* 1(4.17%), these percentage of negative bacterial species as shown in Figure(7) below:

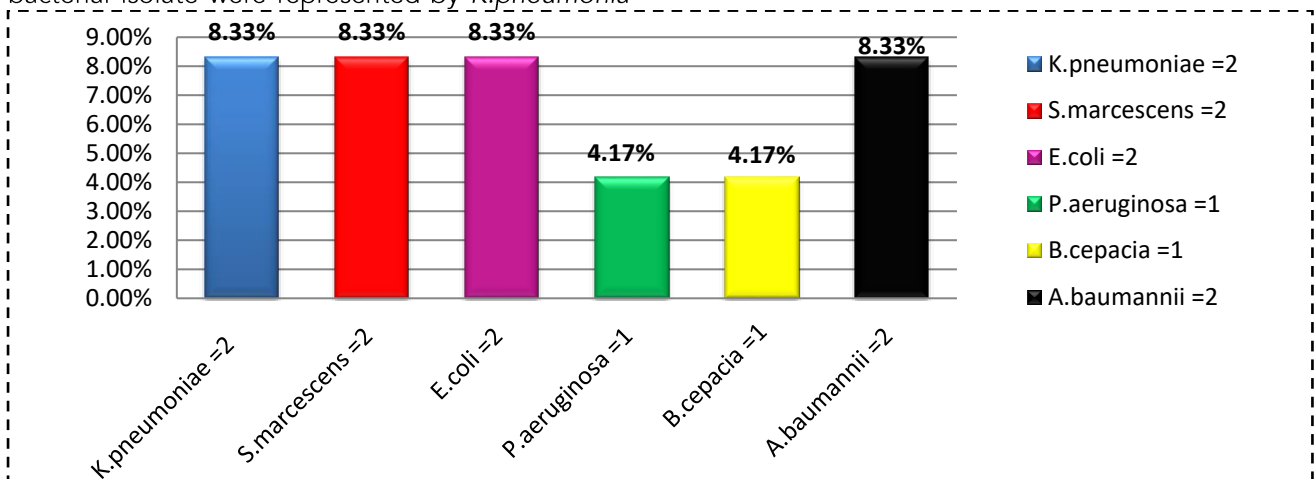


Figure 5: Percentage of gram-negative bacterial species

AST and MICs Profile for Gram positive and Gram negative bacteremia

All 24 isolates under study were subjected to an antibiotic susceptibility test and minimum inhibitory concentrations(MICs) in order to find out the prevalence of antibiotic resistance among these isolates. In this test, different groups of antibiotics were used such as the group of penicillins, B-lactam-B-lactamase inhibitor, carbapenems, cephalosporins, glycopeptides, sulfonamides, fluoroquinolone, and other groups by using VITEK 2compact system for infection control to diagnose, report BSIs etiology and restrict

therapeutic failures that may be triggered by the traditional methods.(Ahmed *et.al.*,2019). The etiology of bacteria that cause Urinary tract infection(UTIs) and BSIs, as well as their resistance to antimicrobials, have changed over time and differs between countries. (Livermore and Pearson,2007; Magliano *et al.*2012).

AST-Profile for Gram positive bacteremia

Table(1) revealed that the results of antibiotic susceptibility tests, showing effects of Vancomycin, linezolid, Imipenem, Rifampicin, Tetracycline and Tigecycline best antibacterial effect against most Gram-positive bacteremia.

Table 1: Antibiotics susceptibility tests in AST card for Gram-positive bacteria.

No	O	AB	G+ bacterial isolates																	
			S. aureus (3)		S. hominis (3)		S. epidermidis (2)		S. lentus (1)		E. faecalis (1)		E. faecium (1)		S. Pseudoporcinu(1)		A.iridans (1)		G.morbillorum (1)	
			S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R
1	P	2 (66.7)	1 (33.3)	1 (33.3)	2 (66.7)	2 (100)	0(0)	0(0)	1(100)	0(0)	1 (100)	1 (100)	0(0)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)	1 (100)
2	CIP	1 (33.3)	2 (66.7)	2 (66.7)	1 (33.3)	1(50)	1(50)	0(0)	1(100)	1 (100)	0(0)	0(0)	1 (100)	1 (100)	0(0)	1 (100)	0(0)	0(0)	0(0)	1 (100)
3	CM	1 (33.3)	2 (66.7)	3 (100)	0(0)	1(50)	1(50)	1 (100)	0(0)	0(0)	1 (100)	0(0)	1 (100)	1 (100)	0(0)	0(0)	1 (100)	1 (100)	0(0)	0(0)
4	E	1 (33.3)	2(66.7)	3 (100)	0(0)	2 (100)	0(0)	0(0)	1(100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)
5	FA	0(0)	3 (100)	0(0)	3 (100)	1(50)	1(50)	0(0)	1(100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)
6	GM	3 (100)	0(0)	1 (33.3)	2 (66.7)	0(0)	2 (100)	0(0)	1(100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)
7	IPM	3 (100)	0(0)	3 (100)	0(0)	2 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)
8	LEV	2 (66.7)	1 (33.3)	1 (33.3)	2 (66.7)	2 (100)	0(0)	1 (100)	0(0)	0(0)	1 (100)	0(0)	1 (100)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)
9	LNZ	3 (100)	0(0)	3 (100)	0(0)	2 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)
10	OXI	1 (33.3)	2 (66.7)	3 (100)	0(0)	2 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)	1 (100)
11	RA	3 (100)	0(0)	2 (66.7)	1 (33.3)	2 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)	0(0)	1 (100)
12	TEC	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7)	0(0)	2 (100)	1 (100)	0(0)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)	1 (100)
13	TE	3 (100)	0(0)	3 (100)	0(0)	2 (100)	0(0)	1(100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)
14	TIC	3 (100)	0(0)	3 (100)	0(0)	2 (100)	0(0)	1(100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)
15	SXT	1 (33.3)	2 (66.7)	2 (66.7)	1 (33.3)	0(0)	2 (100)	0(0)	1 (100)	0(0)	1 (100)	1 (100)	0(0)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)
16	VA	3 (100)	0(0)	3 (100)	0(0)	2 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)

Abbreviations: P=Benzylpenicillin, CIP=Ciprofloxacin, CM=Clindamycin, E=Erythromycin, FA=FusidicAcid, GM=Gentamicin, IPM=Imipenem LEV=Levofloxacin, LNZ=Linezolid, OXI=Oxacillin, RA=Rifampicin,TEC=Teicoplanin, TE=Tetracycline,TIC= Tigecycline, SXT= Trimethoprim/Sulfamethoxazole, VA= Vancomycin. R=Resistance, S=Sensitive, %= Percentage, AB= Antibiotics, G+ = gram positive

AST - Profile for Gram negative bacteremia:

Table(2) revealed that the results of antibiotic susceptibility tests, showing effects of

Ceftazidime/Avibactm, Ceftolozane/Tazobactam, Meropenem, Imipenem and Colistin were the best antibacterial effect against the most of gram negative bacterial isolates.

Table 2: Antibiotics susceptibility tests in AST card for Gram-negative bacteria.

NO	AB.	G- bacterial isolates											
		E. coli (2)		K. Pneumonia (2)		S. marcescens (2)		P. aeruginosa (1)		A. baumannii (2)		B. cepacia (1)	
		S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
1	PEF	1(50)	1(50)	0(0)	2(100)	1(50)	1(50)	0(0)	1(100)	0(0)	2(100)	0(0)	1(100)
2	AK	1(50)	1(50)	1(50)	1(50)	2(100)	0(0)	1(100)	0(0)	1(50)	1(50)	0(0)	1(100)
3	FED	0(0)	2(100)	0(0)	2(100)	1(50)	0(0)	1(100)	0(0)	1(50)	1(50)	0(0)	1(100)
4	CFT	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	1(100)	0(0)	2(100)	0(0)	1(100)
5	CAZ	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	1(100)	0(0)	2(100)	1(100)	0(0)
6	AVC	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	1(100)	0(0)	0(0)	2(100)	1(100)	0(0)
7	TZC	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	1(100)	0(0)	0(0)	2(100)	1(100)	0(0)
8	CIP	0(0)	2(100)	2(100)	0(0)	2(100)	0(0)	1(100)	0(0)	0(0)	2(100)	0(0)	1(100)
9	CS	2(100)	0(0)	1(50)	1(50)	2(100)	0(0)	1(100)	0(0)	1(50)	1(50)	1(100)	0(0)
10	GM	0(0)	2(100)	1(50)	1(50)	0(0)	2(100)	1(100)	0(0)	0(0)	2(100)	0(0)	1(100)
11	IPM	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	1(100)	0(0)	1(50)	1(50)	0(0)	1(100)
12	MEM	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	1(100)	0(0)	1(50)	1(50)	1(100)	0(0)
13	TZP	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	0(0)	1(100)	0(0)	2(100)	0(0)	1(100)
14	TIC	2(100)	0(0)	1(50)	1(50)	2(100)	0(0)	1(100)	0(0)	0(0)	2(100)	0(0)	1(100)
15	SXT	1(50)	1(50)	0(0)	2(100)	1(50)	1(50)	0(0)	1(100)	0(0)	2(100)	0(0)	1(100)

Abbreviations:PEF=Ampicillin/Sulbactam,AK=Amikacin,FED=Cefepime,CFT=Cefotaxime,CAZ=Ceftazidime,AVC=Ceftazidime/Avibactam,TZC=Ceftolozane/Tazobactam,CIP=Ciprofloxacin,CS=Colistin,GM=Gentamicin,IPM=Imipenem,MEM=Meropenem,TZP= Piperacillin/Tazobactam,TIC=Tigecycline,SXT=Trimethoprim/Sulfamethoxazol,R=Resistance,S=Sensitive,%=Percentage,AB=Antibiotics,G= gram negative.

MICs - Profile for Gram positive and Gram negative bacteremia

The minimum inhibitory concentrations(MICs) were determined also by a VITEK 2compact device based on a dilute series for the concentrations of the antibiotics prepared in the AST card for bacterial isolates. Susceptible when the calculated

MICs value is less than breakpoint (CLSI, 2022).

MICs - Profile for Gram positive bacteremia

Table(3) showing the results of our study to determine MICs of the antibiotics in AST card which used against Gram-positive bacterial isolates.

Table 3: Antibiotics in AST card with MICs for Gram-positive bacteria

Antibiotics (G+ve)			
Class		Type	Symbolcode
1	Penicillins	Benzylpenicillin	P
		Oxacillin	OXI
2	Carbapenems	Imipenem	IPM
3	Glycopeptide	Teicoplanin	TEC
		Vancomycin	VA
4	Glycylcycline	Tigecycline	TIC
5	Oxazolidinones	Linezolid	LNZ
6	lincomycin	Clindamycin	CM
7	Aminoglycosides	Gentamicin	GM
8	Fluoroquinolones	Ciprofloxacin	CIP
9	Tetracycline	Tetracycline	TE
10	Sulfonamides	Trimethoprim/Sulfamethoxazole	SXT
11	Macrolide	Erythromycin	E
12	Fusidic Acid	Fusidic Acid	FA
13	Quinolones	Levofloxacin	LEV
14	Rifamycins	Rifampicin	RA

MICs - Profile for Gram negative bacteremia

determine MICs of the antibiotics in AST card which used against Gram- negative bacterial isolates

Table(4) showing the results of our study to

Antibiotics (G-ve)		Symbolcode	MICs \leq , \geq	
Class	Type			
1	Penicillins	Ampicillin/Sulbactam	PEF	$\geq 16\mu\text{g}$
2	B-lactam-B-lactamase inhibitor	Piperacillin/Tazobactam	TZP	$\leq 4\mu\text{g}$
		Ceftolozane/Tazobactam	TZC	$\leq 0.25\mu\text{g}$
3	Cephalosporines	Ceftazidime	CAZ	$2\mu\text{g}$
		Cefepime	FED	$2\mu\text{g}$
		Cefotaxime	CFT	$32\mu\text{g}$
		Ceftazidime/Avibactm	AVC	$0.12\mu\text{g}$
4	Fluoroquinolones	Ciprofloxacin	CIP	$0.06\mu\text{g}$
5	Aminoglycosides	Gentamicin	GM	$1\mu\text{g}$
		Amikacin	AK	$\leq 1\mu\text{g}$
6	Polypeptide	Colistin	CS	$\leq 4\mu\text{g}$
7	Glycylcycline	Tigecycline	TGC	$0.25\mu\text{g}$
8	Sulfonamides	Trimethoprim/Sulfamethoxazole	SXT	$\geq 40\mu\text{g}$
9	Carbapenems	Imipenem	IPM	$\leq 0.25\mu\text{g}$
		Meropenem	MEM	$\leq 0.25\mu\text{g}$

4. Conclusions

We found that Gram-positive bacteremia were predominant more than Gram-negative bacteremia especially *S. aureus* are still the classical causative agents of BSIs in Mosul city hospitals. Vancomycin, linezolid, Imipenem, Rifampicin, Tetracycline and Tigecycline are the best antibiotics against gram positive bacterial strains associated with BSIs whereas Ceftazidime/Avibactm, Ceftolozane/Tazobactam, Meropenem, Imipenem and Colistin were the best antibacterial effect against the most of gram negative bacterial isolates. Therefore, it is important to control the use of these antibiotics in the hospital setting to prevent the emergence of aminoglycosides and fluoroquinolone-resistant strains, and to restrict the use of antimicrobials when these resistant strains are detected. The present study cited the diagnosis of drug-resistant Gram-negative *Acinetobacter baumannii* isolates.

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