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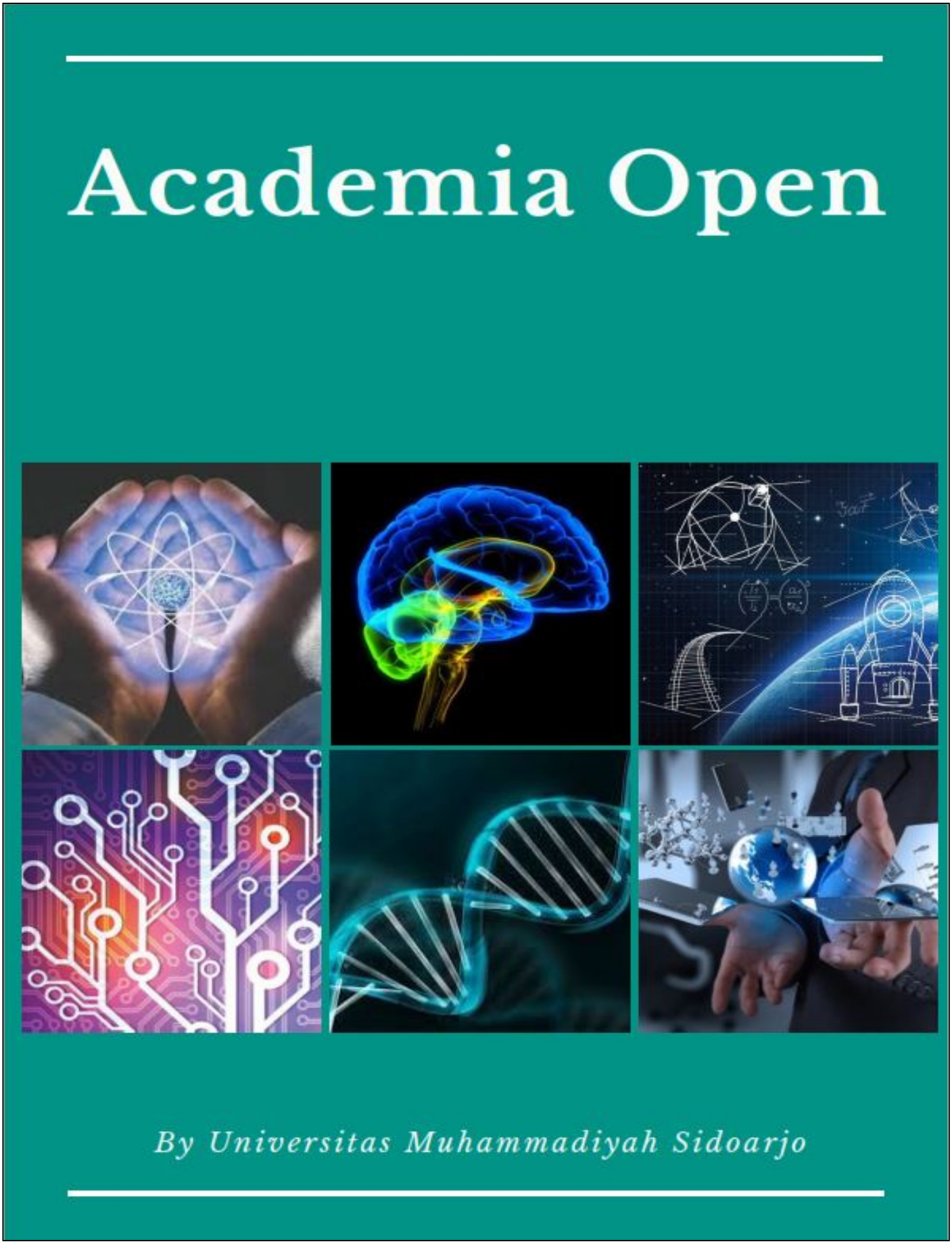

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Optimizing Conditions to Combat Antibiotic Resistance in Pathogenic Bacteria

Mengoptimalkan Kondisi untuk Memerangi Resistensi Antibiotik pada Bakteri Patogen

Adawia Fadhel Abbas, Adwa_a2000@yahoo.com, (1)
Department of Biology- College of Education of Pure Science - Diyala University- Iraq, Iraq

(1) Corresponding author

Abstract

Background: Antimicrobial resistance (AMR) is a significant adaptive trait that allows pathogenic bacterial subpopulations to out-compete and out-survive their microbial neighbors and overcome host defenses. **Specific Background:** Despite extensive research, the influence of various environmental parameters on antibiotic sensitivity in pathogenic bacteria remains underexplored. **Knowledge Gap:** There is limited understanding of how temperature, pH, bacterial inoculum volume, and culture medium amount affect the antibiotic resistance of both gram-negative and gram-positive bacteria. **Aims:** To investigate the effects of these parameters on the antibiotic sensitivity of four standard laboratory strains: Escherichia coli, Proteus spp., Klebsiella spp., and Staphylococcus aureus. **Results:** Our findings indicate imipenem exhibited the highest sensitivity, with percentages varying significantly based on temperature (92% at 35-39°C), pH (83% at pH 6-8), inoculum volume (42% at 0.1-1.0 µL), and medium volume (67% at 15-35 ml). Conversely, antibiotics such as Piperacillin, Amoxicillin, Erythromycin, Tetracycline 30, and Cephalexin showed high resistance, with Tetracycline 10 being the most resistant. **Novelty:** This study highlights the significant impact of environmental conditions on bacterial antibiotic resistance, emphasizing the need for tailored antibiotic use based on specific bacterial characteristics and growth conditions. **Implications:** The results suggest that optimizing environmental parameters can enhance antibiotic efficacy and inform better clinical practices to combat AMR, thus improving treatment outcomes for bacterial infections.

Highlights:

1. Parameter Influence: Temperature, pH, inoculum, medium amount affect antibiotic sensitivity.
2. Highest Sensitivity: Imipenem most effective across conditions.
3. Tailored Use: Optimize conditions for better antibiotic efficacy.

Keywords: Antimicrobial resistance, bacterial sensitivity, environmental parameters, Imipenem, pathogenic bacteria

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Introduction

The ability of a microbe to endure and proliferate in the presence of an antimicrobial agent that would typically suppress or kill this specific type of organism is known as antimicrobial resistance [1]. Stability bacterial subpopulations may exhibit or acquire a variety of adaptive features, including antimicrobial resistance, that allow them to outcompete and outlive their microbial neighbors and outwit host tactics that are intended to harm them [2, 3]. [5,6] The rate at which antibiotic resistance frequently arises, as well as how quickly it travels throughout the world and among various bacterial species, are concerning nowadays [7, 8]. More bacterial infections with multiple drug resistance are being reported globally as a consequence of the sequential, cumulative development of resistance characteristics against several medicines. Many bacterial organisms have developed resistance to previously highly effective antibiotics as a result, including important human and animal diseases like Salmonella species and Mycobacterium [10]. Bacterial organisms need to be able to obstruct one or more of the necessary processes for the antimicrobial agent's effective action in order to survive in the presence of an antibiotic. Bacterial species can counteract the intended mechanisms of action of antibiotics through many means [11]. This could entail blocking the antibiotic from entering the bacterial cell or even causing the antimicrobial agent's active ingredient to break down. It is believed that multiple mechanisms of resistance contribute to the resistance shown in bacterial organisms [12].

Methods

Bacterial strains: Three well-characterized standard laboratory strains were used in this study gram-negative (Escherichia coli, Proteus spp, Klebsiella spp, Staphylococcus aureus)

Antibacterial Susceptibility Testing.

The study was conducted in the lab. Science College used standard bacterial strains (Escherichia coli Staphylococcus aureus, Proteus spp Klebsiella spp) obtained from this agar plate, and a bacterial isolate was tested for resistance to each of twelve different antibiotics [13].

Disk Diffusion Method

The disk diffusion method is arguably the most used technique for identifying antibiotic resistance in private veterinary clinics due to its affordability, effectiveness, and ease of use. The isolate of interest is first uniformly seeded over the plate using a growth medium, often Mueller-Hinton agar, and diluted to a standard concentration (about 1 to 2 × 108 colony-forming units per ml).

Next, uniformly distributed and gently pressed disks that have been commercially manufactured and pre-impregnated with a standard concentration of a specific antibiotic are placed onto the agar surface [14,15,16,]. The test antibiotic starts to spread outward from the disks right quickly, forming a gradient in the agar's antibiotic concentration where the concentration is highest near the disk and lowest farther out. Following an overnight incubation period, the bacterial growth surrounding every disc is examined. [17]. A distinct region of "no growth" will be seen surrounding that specific disk if the test isolate is responsive to that particular antibiotic [15, 18]. Since this roughly corresponds to the lowest antibiotic dose necessary to stop the test isolate from growing, the area surrounding an antibiotic disk that is devoid of growth is known as the zone of inhibition. The isolate is then classified as susceptible, intermediately susceptible, or resistant based on the measurement of this zone in millimeters and comparison with a standard interpretation chart. [19, 20].

Antimicrobial Effect on Bacterial Isolates Under Different Conditions:

To study the effect of the different parameters on antibiotic bacterial resistance in the culture medium, the following steps were taken:

1.Purecoloniesweretakenfroma24-hourfarmforisolationtotesttubes containing 5 ml of heart and brain broth. The tubing was incubated at 37 ° C for 4-8. hours until the center was exposed. The growth curve formed by the standard tube muffler (McFarland Tube)

2.for parameters that affect studying:

a.Temperature effect: To study the temperature effect on bacterial growth, the following steps have been taken:

Muller Hinton was attended and distributed in three Petri dishes containing the culture medium (45-50) ° C after the completion of the sterilization process for the bacterium under study

b.PH effect: Attended the Muller Hinton and distributed in three test tubes, and adjusted the pH to (6.0, 7.0, and 8.0), respectively. Using NaOH and HCl one Normality and then sterilized by Autoclave (heat 121 ° C for 15

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minutes under a pressure of 15 lb / kg 2).

c.Bacterial inoculum value effect studying: Muller Hinton was attended and distributed in three Petri dishes containing the culture medium (45-50) ° C after the completion of the sterilization process for the bacterium under study. Bacterial dishes were injected with different amounts of bacterial vaccine (0.1 ml, 0.5 ml, 1.0 ml).

d.Effect of amount of plant medium: attended the Muller Hinton center and distributed in three Petri dishes containing the culture medium in different amounts (15 ml, 25 ml, 35 ml) and then injected with bacterial isolates under study.

3.Bacterial dishes were incubated at three different temperatures (35, 37, 39).

4.Then read the results by measuring the area of inhibition after incubation and as explained in (Appendix 1). PH Effect: To study the effect of pH on bacterial growth, the following steps have been taken:

1.Pure colonies were taken from a farm with a life of 24 hours per isolation to test tubes containing 5 ml of heart and brain broth. The tubes were incubated at 37 ° C for 4-8 hours until the middle of the tube was exposed. McFarland Tube)

2.Attended the Muller Hinton and distributed in three glass bottles, and adjusted the pH to (6.0, 7.0, 8.0), respectively by using NaOH and HCl

3.Return the culture medium (45-50) ºC after the sterilization process is completed and pour into the dishes and incubate the bacterial dishes 24 ° C for 24-48 hours.

Result and Discussion

Tables (1, 2) show the effect of temperature on the sensitivity of bacteria to antibiotics for Four Bacteria gram-negative (Escherichia coli, Proteus spp, Klebsiella spp) and gram-positive (Staphylococcus aureus) in different temperatures (35ºC, 37ºC, 39ºC). The results showed, as shown in table (1.2) Effect of temperature the standard isolates' sensitivity of the bacteria when the temperature was 35º m, where the standard four isolates showed the sensitivity of each of the antibiotics CL, IMP, E. The increased sensitivity and low resistance for anti-CL, IMP, E, and TE30 at temperature 37 o C differently from their sensitivity to temperature 35º m, and then the drop in the sensitivity got off the case of medium sensitivity in all life antibiotics except TE10, where he observed stiff resistance in all thermal grades 35º, 37, 39° C

° C, respectively.

Temp.	Bacteria	Antibiotic						
		AMX	CL	E	IMP	PRL	TE10	TE30
35ºC	E.coli	I	R	R	S	R	R	R
	Klb	R	R	S	S	R	R	I
	Pro	R	R	S	S	R	R	R
	Staph	R	S	S	S	R	R	R
37ºC	E.coli	R	R	R	S	R	R	S
	Klb	R	R	I	I	R	R	R
	Pro	R	S	S	S	R	R	R
	Staph	R	I	I	S	R	R	R
39ºC	E.coli	R	R	I	S	I	R	R
	Klb	R	R	R	S	I	R	I
	Pro	R	I	R	S	S	R	R
	Staph	I	S	I	S	I	R	R

Table 1. *Sensitivity test results for Four Bacteria with antibiotics and temperatures (35ºC , 37ºC , 39ºC).*

Antibiotic	Sensitivity value	Statistics	Temp. with C.			Total	C. S.(*)
			35	37	39		
AMX	R	Freq	3	4	3	10	Likelihood Ratio test P.value= 0.403 NS
		% of Total	25.0%	33.3%	25.0%	83.3%	
	I	Freq	1	0	1	2	
		% of Total	8.3%	0.0%	8.3%	16.7%	
	S	Freq	0	0	0	0	

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		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
CL	R	Freq	3	2	2	7	Likelihood Ratio test P.value= 0.755 NS
		% of Total	25.0%	16.7%	16.7%	58.3%	
	I	Freq	0	1	1	2	
		% of Total	0.0%	8.3%	8.3%	16.7%	
	S	Freq	1	1	1	3	
		% of Total	8.3%	8.3%	8.3%	25.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
E1	R	Freq	1	1	2	4	Likelihood Ratio test P.value = 0.091 NS
		% of Total	8.3%	8.3%	16.7%	33.3%	
	I	Freq	0	2	2	4	
		% of Total	0.0%	16.7%	16.7%	33.3%	
	S	Freq	3	1	0	4	
		% of Total	25.0%	8.3%	0.0%	33.3%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
IMP	R	Freq	0	0	0	0	Likelihood Ratio test P.value = 0.303 NS
		% of Total	0.0%	0.0%	0.0%	0.0%	
	I	Freq	0	1	0	1	
		% of Total	0.0%	8.3%	0.0%	8.3%	
	S	Freq	4	3	4	11	
		% of Total	33.3%	25.0%	33.3%	91.7%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
PRL1	R	Freq	4	4	0	8	Likelihood Ratio test P.value = 0.004 HS
		% of Total	33.3%	33.3%	0.0%	66.7%	
	I	Freq	0	0	3	3	
		% of Total	0.0%	0.0%	25.0%	25.0%	
	S	Freq	0	0	1	1	
		% of Total	0.0%	0.0%	8.3%	8.3%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
TE10.1	R	Freq	4	4	4	12	---
		% of Total	33.3%	33.3%	33.3%	100.0%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
TE30.1	R	Freq	3	3	3	9	Likelihood Ratio test P.value = 0.431 NS
		% of Total	25.0%	25.0%	25.0%	75.0%	
	I	Freq	1	0	1	2	
		% of Total	8.3%	0.0%	8.3%	16.7%	
	S	Freq	0	1	0	1	
		% of Total	0.0%	8.3%	0.0%	8.3%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	

Table 2. *Crosstabs between antibiotics with sensitivity values (Resistance, Intermediate , Sensitive) and*

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temperature for Four Bacteria (Staph., Pro., klb., E.coli)

(*) Note : (HS) High significant at P<0.01 ; (S) Significant at P<0.05 ; (NS) Non Significant at P>0.05

The tables (3,4) shows the effect of pH on Sensitivity of bacteria to antibiotics for Four Bacteria (Escherichia coli, Proteus spp, Klebsiella spp, Staphylococcus aureus) indifferent pH (6,7, 8).

The results showed that, sensitivity of bacterial isolates was more with antibiotics IMP at pH= 6. And showed the sensitivity to the same antibiotic at pH 7 and pH 8,

but in lower than PH 6. To some extent its effect was 83% sensitive and the Intermediate sensitivity 17%. Bacterial isolates showed the highest resistance to antibiotics AMX, CL, PRL, TE10 and TE30 with 6 pH and increasing this resistance when acidic value decreasing and with pH 7 and pH 8.

Temp.	Bacteria	Antibiotics						
		AMX	CL	E	IMP	PRL	TE10	TE30
6	E.coli	I	R	I	S	I	R	R
	Klb.	R	I	S	S	R	R	R
	Pro.	R	R	I	S	R	R	R
	Staph.	R	R	S	S	R	R	R
7	E.coli	R	R	I	S	R	R	R
	Klb	R	R	I	I	R	R	I
	Pro	R	S	S	S	R	R	R
	Staph.	R	R	R	S	R	R	R
8	E.coli	R	R	R	S	R	R	R
	Klb.	R	R	R	S	R	R	R
	Pro.	R	R	I	S	R	R	R
	Staph.	R	I	R	I	R	R	R

Table 3. *Sensitivity test results for Four Bacteria with antibiotics and pH (6 , 7 , 8)*

Antibiotics :) Amoxicillin , Cephalexin , Erythromucin,Imipenem ,Piperacillin , Tetracycline)

Strains : (Staphylococcus aureus ,Escherichia coli, Proteus spp , Klebsiella spp)

Antibiotic	Sensitivity value	Statistics	PH			Total	C. S.(*)
			35	37	39		
AMX	R	Freq	3	4	4	11	Likelihood Ratio test P.value= 0.303 NS
		% of Total	25.0%	33.3%	33.3%	91.7%	
	I	Freq	1	0	0	1	
		% of Total	8.3%	0.0%	0.0%	8.3%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
CL	R	Freq	3	3	3	9	Likelihood Ratio test P.value= 0.431 NS
		% of Total	25.0%	25.0%	25.0%	75.0%	
	I	Freq	1	0	1	2	
		% of Total	8.3%	0.0%	8.3%	16.7%	
	S	Freq	0	1	0	1	
		% of Total	0.0%	8.3%	0.0%	8.3%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
E1	R	Freq	0	1	3	4	Likelihood Ratio test P.value = 0.112 NS
		% of Total	0.0%	8.3%	25.0%	33.3%	
	I	Freq	2	2	1	5	

		% of Total	16.7%	16.7%	8.3%	41.7%	
	S	Freq	2	1	0	3	
		% of Total	16.7%	8.3%	0.0%	25.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
IMP	R	Freq	0	0	0	0	Likelihood Ratio test P.value = 0.403 NS
		% of Total	0.0%	0.0%	0.0%	0.0%	
	I	Freq	0	1	1	2	
		% of Total	0.0%	8.3%	8.3%	16.7%	
	S	Freq	4	3	3	10	
		% of Total	33.3%	25.0%	25.0%	83.3%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
PRL	R	Freq	3	4	4	11	Likelihood Ratio test P.value = 0.303 NS
		% of Total	25.0%	33.3%	33.3%	91.7%	
	I	Freq	1	0	0	1	
		% of Total	8.3%	0.0%	0.0%	8.3%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
TE10	R	Freq	4	4	4	12	---
		% of Total	33.3%	33.3%	33.3%	100.0%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
TE30	R	Freq	4	3	4	11	Likelihood Ratio test P.value = 0.303 NS
		% of Total	33.3%	25.0%	33.3%	91.7%	
	I	Freq	0	1	0	1	
		% of Total	0.0%	8.3%	0.0%	8.3%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	

Table 4. *Crosstabs between antibiotics with sensitivity values (Resistance , Intermediate , Sensitive) and pH for Four Bacteria (Staph., Pro., klb., E.coli)*

(*) Note : (HS) High significant at P<0.01 ; (S) Significant at P<0.05 ; (NS) Non-Significant at P>0.05

Tables (5, and 6) show the effect of temperature on the Sensitivity of bacteria to antibiotics for Four Bacteria gram-negative (Escherichia coli, Proteus spp, Klebsiella spp, Staphylococcus aureus) indifferent inoculums values (0.1, 0.5, 1)

2.As shown in the tables the effect of inoculum values on the sensitivity of bacterial isolates showed that when inoculums' values were 0.1, Lμ, sensitivity and moderate sensitivity included IMP antagonists. IMP was the most potent antimicrobial antagonist; Thus, IMP antibiotic had a major effect on all isolates by 91.7% while the Intermediate sensitivity was 58.3%.

However, bacterial isolates showed the highest resistance to antibiotics, TE10 and TE30 in three difference inoculum values 0.1, 0.5, 1.0 Lμ, Thus, TE10 and TE30 antibiotics was the major resistance of all isolates by 100.0 %

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inoculums' values	Bacteria	Antibiotics						
		AMX	CL	E	IMP	PRL	TE10	TE30
0.1	E.coli	R	I	R	S	R	R	R
	Klb	R	R	R	I	R	R	R
	Pro	R	S	R	S	R	R	R
	Staph	R	I	R	S	R	R	R
0.5	E.coli	R	R	R	I	R	R	R
	Klb	I	R	R	S	I	R	R
	Pro	R	I	R	I	R	R	R
	Staph	R	R	R	I	R	R	R
1	E.coli	R	I	R	I	R	R	R
	Klb	R	S	R	S	R	R	R
	Pro	R	R	R	I	R	R	R
	Staph	R	R	R	I	R	R	R

Table 5. *Sensitivity test results for Four Bacteria with antibiotics and inoculums' values (0.1 , 0.5, 1) Lμ*

Antibiotic	Sensitivity value	Statistics	inoculums' values			Total	C. S.(*)
			0.1	0.5	1		
AMX	R	Freq	4	4	3	11	Likelihood Ratio test P.value= 0.303 NS
		% of Total	33.3%	33.3%	25.0%	91.7%	
	I	Freq	0	1	0	1	
		% of Total	0.0%	8.3%	0.0%	8.3%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
CL	R	Freq	1	3	2	6	Likelihood Ratio test P.value= 0.535 NS
		% of Total	8.3%	25.0%	16.7%	50.0%	
	I	Freq	2	1	1	4	
		% of Total	16.7%	8.3%	8.3%	33.3%	
	S	Freq	1	0	1	2	
		% of Total	8.3%	0.0%	8.3%	16.7%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
E1	R	Freq	4	4	4	12	Likelihood Ratio test P.value = 0.099 NS
		% of Total	33.3%	33.3%	33.3%	100.0%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
IMP	R	Freq	0	0	1	1	Likelihood Ratio test P.value = 0.303 NS
		% of Total	0.0%	0.0%	8.3%	8.3%	
	I	Freq	3	3	1	7	
		% of Total	25.0%	25.0%	8.3%	58.3%	
	S	Freq	4	4	3	11	
		% of Total	33.3%	33.3%	25.0%	91.7%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
PRL	R	Freq	4	3	4	11	Likelihood Ratio test P.value = 0.303 NS
		% of Total	33.3%	25.0%	33.3%	91.7%	
	I	Freq	0	1	0	1	

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		% of Total	0.0%	8.3%	0.0%	8.3%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
TE10	R	Freq	4	4	4	12	---
		% of Total	33.3%	33.3%	33.3%	100.0%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
TE30	R	Freq	4	4	4	12	---
		% of Total	33.3%	33.3%	33.3%	100.0%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	

Table 6. *Crosstabs between antibiotics with sensitivity values (Resistance , Intermediate , Sensitive) and inoculums values for Four Bacteria (Staph., Pro., klb., E.coli)*

(*) Note : (HS) High significant at P<0.01 ; (S) Significant at P<0.05 ; (NS) Non Significant at P>0.05

The tables (7, 8) shows the effect of temperature on Sensitivity of bacteria to antibiotics for Four Bacteria Escherichia coli, Proteus spp, Klebsiella spp, Staphylococcus aureus) indifferent culture media amount (21, 22) (ml)

As shown in Table (7.8), the effect of media amount on the sensitivity of bacterial isolates showed that when the media amount was 15 ml, sensitivity and moderate sensitivity included CL, E, and IMP. Medium sensitivity was found to be more sensitive than other isolates. On the other hand, increasing the media amount up to 35 mL increased the resistance isolates by a large difference from the 15 ml medium and found that Pro isolation was also the most sensitive of the other isolates. The IMP antagonist was the major effect of all isolates with 67% and 33% sensitivity. Where the change in the amount of the medium controls the provision of the amount of food needed for the growth of bacteria in the center of the plant and therefore affects the intensity of growth.

Media size (ml)	Bacteria	Antibiotics						
		AMX	CL	E	IMP	PRL	TE10	TE30
15	E.coli	R	R	R	S	R	R	R
	Klb	R	R	R	I	R	R	R
	Pro	R	S	S	S	R	R	R
	Staph	R	S	I	S	R	R	R
25	E.coli	R	R	R	S	R	R	R
	Klb	I	R	I	I	R	R	R
	Pro	R	S	S	S	R	R	R
	Staph	R	R	R	S	R	R	R
35	E.coli	I	R	R	S	R	R	R
	Klb	R	R	I	I	R	R	R
	Pro	R	S	R	S	R	R	R
	Staph	R	R	R	I	R	R	R

Table 7. *Sensitivity test results for Four Bacteria with antibiotics and culture media amount (ml) (15 , 25 , 35)*

Antibiotic	Sensitivity value	Statistics	media amount (ml)			Total	C. S.(*)
			15	25	35		
AMX	R	Freq	4	3	3	10	Likelihood

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		% of Total	33.3%	25.0%	25.0%	83.3%	Ratio test P.value= 0.403 NS
	I	Freq	0	1	1	2	
		% of Total	0.0%	8.3%	8.3%	16.7%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
CL	R	Freq	2	3	3	8	Likelihood Ratio test P.value= 0.693 NS
		% of Total	16.7%	25.0%	25.0%	66.7%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	2	1	1	4	
		% of Total	16.7%	8.3%	8.3%	33.3%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
E1	R	Freq	2	2	3	7	Likelihood Ratio test P.value = 0.755 NS
		% of Total	16.7%	16.7%	25.0%	58.3%	
	I	Freq	1	1	1	3	
		% of Total	8.3%	8.3%	8.3%	25.0%	
	S	Freq	1	1	0	2	
		% of Total	8.3%	8.3%	0.0%	16.7%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
IMP	R	Freq	0	0	0	0	Likelihood Ratio test P.value = 0.693 NS
		% of Total	0.0%	0.0%	0.0%	0.0%	
	I	Freq	1	1	2	4	
		% of Total	8.3%	8.3%	16.7%	33.3%	
	S	Freq	3	3	2	8	
		% of Total	25.0%	25.0%	16.7%	66.7%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
PRL	R	Freq	4	4	4	12	---
		% of Total	33.3%	33.3%	33.3%	100.0%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
TE10	R	Freq	4	4	4	12	---
		% of Total	33.3%	33.3%	33.3%	100.0%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	Total	Freq	4	4	4	12	
		% of Total	33.3%	33.3%	33.3%	100.0%	
TE30	R	Freq	4	4	4	12	---
		% of Total	33.3%	33.3%	33.3%	100.0%	
	I	Freq	0	0	0	0	
		% of Total	0.0%	0.0%	0.0%	0.0%	
	S	Freq	0	0	0	0	

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	Total	Freq	4	4	4	12
		% of Total	33.3%	33.3%	33.3%	100.0%

Table 8. *Crosstabs between antibiotics with sensitivity values (Resistance, Intermediate, Sensitive) and culture media amount (ml) for Four Bacteria (Staph. Pro., klb., E.coli)*

(*) Note : (HS) High significant at P<0.01 ; (S) Significant at P<0.05 ; (NS) Not Significant at P>0.05

Discussion

In general, bacterial isolates were not significantly affected by their susceptibility or resistance to antibiotics that were selected in the way of dispersion in pests. The increase in bacterial growth in the middle increases the bacterial growth in the medium, increasing the bacterial competition for sufficient and necessary food to grow [8, 23].The increase in the vaccine leads to a lack of food, shortening the life of the bacterial farm and the early killing of the bacterial plant. This gives a false idea of the cause of the bacteria dying due to the lack of food and not the pharmacological sensitivity of the selected antibiotics in the research. [24, 12] The IMP antibiotic was the major effect of all isolates with 67% and 33% sensitivity. Where the change in the amount of the medium controls the provision of the amount of food needed for the growth of bacteria in the center of the plant and therefore affects the intensity of growth [25, 23]. Imipenem acts as an antimicrobial agent by inhibiting cell wall synthesis of various Gram-positive and Gram-negative bacteria [10, 26]. It remains very stable in the presence of β- lactamase (both penicillin and cephalosporinase) produced by some bacteria, a potent inhibitor of β-lactamases of some Gram-negative bacteria that resist most β-lactam antibiotics have developed resistance to Imipenem in varying degrees. There are not many types of resistance to imipenem except Pseudomonas aeruginosa [27,28]

Conclusion

When studying the effect of some factors on antibiotic sensitivity (Amoxicillin, Cephalexin, Erythromycin, Imipenem, Piperacillin, and Tetracycline) performed on the isolates of bacterial pathological (Escherichia coli, Staphylococcus aureus, Proteus mellitus, and Klebsiella spp.) Showed the following:

1.The effect of temperature (35, 37, 39) ° C on the antibiotic sensitivity was have slight effect of the sensitivity of the bacteria at a temperature of 39 ° C, but a non-significant effect, and the highest effect of antibiotic sensitivity towards the antibiotic Imipenem and Erythromycin

2.For the effect of pH (6, 7, 8) on antibiotic sensitivity, was highest sensitivity forward imipenem was found at pH 6. This increase in bacterial killing may be due to increased acidity of the culture medium not only to the effect of Antibiotics on bacterial growth.

3.The effect of the inoculums' values on the antibiotic sensitivity showed that there was an inverse condition between the inoculums' values and the antibiotic sensitivity. Inhibition zone decreased when the inoculums' values increased. This indicates that increasing of inoculums' values leads to an increase in the density of the bacteria growth on the culture media. The result was Increasing bacterial killing in the inhibition zone and this is used when using 0.1

ML volume. Bacterial isolates showed the highest sensitivity of the Imipenem antibiotic at0. l ML inoculums' values

4.As for the media amount, increase amount of the media caused grow more bacteria on the surface of the culture media this is the result of increased nutrients which helped to grow bacteria, the highest sensitivity to Imipenem when the media amount was 15 ml and 25 ml with IMP antibiotic.

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