Microbes and Infectious Diseases 2025; 6(3): 5064-5071

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Effect of different carbon and nitrogen sources on biofilm formation of *Acinetobacter baumannii*

Farah J. Abdullrazaq, Estabraq Ali Maklef *, Amal Aziz Kareem

1- College of Health and Medical Techniques, Middle Technical University (MTU), Baghdad, Iraq

ARTICLE INFO

Article history:
Received 21 April 2025
Received in revised form 1 June 2025
Accepted 4 June 2025

Keywords:
A. baumannii
biofilm formation
carbon
nitrogen sources

ABSTRACT

Background: Biofilm is the major problem in patients suffering from antibiotics resistance because it protects the bacteria from many environment effectors. The hospital infections are one of the most important pathogens which increase by A.baumannii Aim: Detection of biofilm and factors affecting on biofilm formation in A. baumannii: **Methods:** One -hundred of A. baumannii isolates were collected from patients suffering from different complaints such as surgical wound infections, burns infection, urinary tract infection and pulmonary infections.Biofilm formation detection and the nitrogen and carbon sources affecting on biofilm production done by microliter plate method. **Results:** The results of the current study observed there were (34%) of A. baumannii bacterial isolates produced strong biofilm out of 100 cases, high production of biofilm was with Trypton and yeast extract sources than the other nitrogen sources, while; the carbon sources including fructose, sucrose and lactose were the good sources for biofilm formation for the 9 isolates. **Conclusion:** The highest percentage of isolation (42%) was of sputum and the lowest percentage (3%) was from urine. A. baumannii produced biofilm with three scores (34%) strong biofilm, (13%) moderate biofilm, and (53%) weak biofilm, quantitatively the carbon sources were more good than nitrogen for biofilm formation.

Introduction

A baumannii is a gram-negative coccobacillus firstly considered to be an opportunistic pathogen, which acting a vital role as a most important cause of healthcare-associated infections. In current years, *A. baumannii* has become resistant to greatest current antimicrobial agents and producing a high incidence percentage of morbidity and mortality specially in the intensive care unit (ICU) in several countries, *A. baumannii*, a significant emerging pathogen of nosocomial infections, is recognized for its capability to form biofilms on both biotic and abiotic surfaces, promoting survival on indwelling medical devices, hospital surfaces, or in then unfavorable environments [1]. Biofilm formation is a multistage process, initiation with the primary attachment, continuing to strong adhesion and aggregation of cells into micro colonies, followed through biofilm growth and maturation, before cell diffusion into the environment [2]. The first stage is attachment to the bacteria in a planktonic phase, contact with a surface, either of human matrix or foreign body material, and attempt to adhere to it [3]. In the second stage, collective cells bond on the surface then split into daughter cells, multiply outward and upward from the point of bond to form cell clusters, the separating cells produce exo polysaccharides (EPS) and quorum sensing molecules, so accumulating cells in Micro colonies and biofilms stick to surface on which it is made [4]. Aggregate

DOI: 10.21608/MID.2025.377700.2727
* *Corresponding author:* Estabraq Ali Maklef
E-mail address: *astabraqals124@gmail.com*

number of organisms cause micro colonies which come to be bigger and rise of quantity of EPS produced likewise increasing of signaling molecules [5]. The completely mature biofilm structure consists of the polymer matrix, bacterial cells and interstitial water channels that simplify the exchange of nutrients and wastes in and out of the biofilm into the periphery environment [6]. *A. baumannii* may causes bacteremia, pneumonia, meningitis, urinary tract infection and wound infection[1]. It is responsible for wound infections as well as infections of the lung in addition to skin/soft tissue and bloodstream and urinary tract, it is almost considered novel pathogen, several researchers suggested the primary appearance of this pathogen was in Iraq specially in military treatment facilities through the Iraq war and was called "Iraqibacter [2]. One of the main factor involved in bacterial resistance to antimicrobial, chronic infections or survival in varying environments is the capability to form biofilms[3].Factors affecting of *A. baumannii* biofilm production is greatly influenced by media components ,especially carbon and nitrogen sources [4]. It was documented that the growing of bacterial cells were better when increasing the concentration of peptone in the media, but the amount of biofilm were less [5].In one study observed the biofilm formation was reduced when growing with nitrogen sources, while the carbon and nitrogen sources were increase bacterial cell growth [5].Therefore, the aim of this study represented the effect of different carbon and nitrogen sources on *A. baumannii* biofilm formation.

Nomenclature			
C F U	Colony forming unit	ICU	Inensive care unit
CLSI	Clinical and Laboratory Standards Institutes	C : N : P	Carbon: nitrogen: phosphate
ESKAPE.	*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa,* and *Enterobacter spp.*	F.E.P	Fisher 's exact probability test
EPS.	Exopolysaccharides	BHIB	Brain Heart Infusion Broth
O.D	Optical density	DW	Distilled water

Materials and methods

Specimen collection

One hundred clinical samples were isolated from different sources of infections (sputum, burns, Bed sore, blood, wound swabs and urine).

Bacterial identification

Samples were streaked on Blood agar, MacConkey agar and Drigalski Lactose agar.

VITEK-2 compact system (BioMerieux \DensiCHEK plus (France) used to confirm the diagnoses of isolates.

Nitrogen and carbohydrate sources preparation

Effect of nitrogen sources production

For testing the effect of nitrogen sources six different type of material were selected including Trypton, extract of yeast, ammonium chloride (NH_4Cl), sodium nitrate ($NaNO_3$), casein and peptone, all the nitrogen sources were added to the media in a concentration of 1 g/100ml of the most efficient source of nitrogen [6].

Effect of carbon source

Five types of sugar were used as a carbone sources including fructose, lactose, sucrose, starch and for testing the amount of biofilm formation by *A.baumannii* . These sugar materials were added to broth medium at a concentration of 0.1g/100ml [7, 8].

Biofilm detection methods was adapted from[9],overnight cultures of the bacteria in Brain Heart Infusion Broth (BHIB) ,the bacteria cultured after diluted and adjusted by McFarland tube 0.5, then 200 µl of each bacterial dilution deposited in three wells of a sterile 96-well polystyrene microliter plate and incubated under constant conditions at 37ºC for 24 h. [10].Biofilm production measured the optical density (O.D) by ELISA reader then calculate the results as follows: OD_{630} (bacteria) divided on 3 then Biofilm = OD_{630} (bacteria) – OD_{630} (control).Biofilm producing strains were scored as strong ,moderate, and weak as mentioned[11].The results was read as follows: Weak: ODc <OD ≤2x ODc, Moderate: 2xODc < OD ≤ 4xODc, Strong : OD > 4xODc [11].

Minimal media preparation

This medium is composed of K_2HPO_4 0.3g,KH_2PO40.15g,$MgSO_4 7H_2O$0.15g, $FeSO4.7H_2O$0.03g, $ZnSO_4.7H_2O$ 0.003g,$CaCO_3$ 0.6g, all these components were liquefied in 300ml of distilled water (DW), pH was adjusted to (7.0) and then in autoclaved for (15) min [12].

Statistical analysis

Statistical Analysis System version (2012) program was utilized to determine the impact of various factors on the study's parameters. To significantly compare between means, the T-test and

Least Significant Difference (LSD) test (ANOVA) were utilized. The Chi-square test was utilized in this study to significantly compare percentages and 0.05 and 0.01 as level of significant [13].

Results and Discussion:

One hundered *A. baumannii* isolated from different specimens type there were (42) isolates from sputum (42 %), burns with percentage 29(29%), from the bed sore 16(16%) , from the wound 4(4%) , from blood 6 (6%) ,and the lower percent was from urine 3(3%), significant difference was noticed at p-value = 0.0001 (H.S), the percent of *A. baumannii* distribution according to the specimen. as illustrated in Table 1.

Because of the rise in hospital-acquired infections and the growth of treatment resistance, doctors face a huge problem due to infection by *A. baumannii* (12).Results declared that (86) isolates were diagnosed as *A. baumannii* by growth on blood agar they seemed opaque creamy and non-hemolytic colonies, convex, gray or white color, While MacConkey agar media their colonies seemed small, pale yellow and lactose non fermenter,who mention that isolates obtained from MacConkey agar media were identified according to the Gram stain, *A. baumannii* showed gram negative coccobacilli and arranged in diplococcic as illustrated in Figure-1 and Figure-2 .

The similarities and differences in sites and distribution of infection of bacteria show difference from country to another due to patients condition number of patients examined ,health practices, personal hygiene environment of condition and laboratory procedures[14].

The results show the highest percentage of isolation (42%) was of sputum this result agreement with two Iraqi and international studies results shown highly respiratory tract infection by[15]. And the lowest percentage of isolation (3%) was of the urine this result agreement with Iraqi study[16]. The results revealed (34%) strong biofilm formation, (13%) moderate biofilm and (53%) isolates were weak biofilm formation, the results were illustrated in figure 3-A.

Biofilm-encased cells have narrow metabolic activity and are shielded through the extracellular formed matrix, production them more resistant to antibiotics and innate immune components of the host[17].The *A. baumannii* bacteria are non-motile have organisms and form a biofilm at the air-liquid interface[18].

Testing the factors affecting of A. baumannii biofilm production

Nine *A.baumannii* isolates from different biofilm stages were selected to detect some factors affecting on biofilm formation.

Effect of nitrogen sources

To determine the effect of nitrogen sources on the biofilm formation, media were supplemented with different nitrogen sources then cultured with the bacteria. The results indicated high production of biofilm with Trypton and yeast extract than the other nitrogen sources, by measuring the means concentrations of biofilm [19]. shows that (mean+S.D) of Trypton as follow :(0.423 ±0.03, 05 0.316 ±0.02), as strong and moderate biofilm induction by Trypton while yeast extract (0.334 ±0.05, 0.236 ±0.07) as strong and moderate biofilm induction by yeast extract. This effect of carbon sources were significant (P-value $\leq$ 0.05) for Trypton, Yeast extract and NH4CL.Table 2 and figure4 Other researcher found decrease in biofilm formation when the bacteria were cultured with nitrogen sources in minimal medium such as peptone, Cell growth was encouraged by both carbon and nitrogen sources, but only nitrogen supplies prevented the formation of biofilms.

Effect of carbon source

The results revealed that the fructose,sucrose and lactose were the good sources for biofilm formation for the 9 isolates which indicated by measuring the means concentrations of biofilm, the results shown (mean+ S.D) like follow: : (0.423 ±0.04, 0.374 ±0.07, 0.009 ±0.006), for strong, moderate and weak biofilm formation by fructose, the best biofilm carbon source for biofilm formation, while glucose were the least source for biofilm formation for the 9 isolates (3 strong,3 moderate and 3 weak biofilm producing strains, these differences in the scores of biofilm formation under the effect of carbon sources were significant (P-value $\leq$ 0.05) as arranged in Table .3.

Five carbon sources (starch, lactose, fructose, glucose, sucrose) were used as a sole source of carbon and energy to determine the best one for *A.baumannii* These carbon sources were added to the production medium (broth medium) 0.1g/100ml minimal medium [8]. Microorganisms differ in their needs to nitrogen sources according to their nature and growth requirements, in general organic nitrogen sources support growth and metabolism more than inorganic [20].

Exo polysaccharide production through *Aureobasidium pullulans* cells increased below conditions where growth was extended through the high carbohydrates content in the medium, such for example glucose, mannose, fructose, ribose, arabinose, , xylose, , maltose sucrose and lactose [21]. Additional maltose, lactose, and sucrose acted as good a carbon source as glucose. Figure.5. [22] However, no previous studies focused on study the effects of different carbon and nitrogen sources on the biofilm formation of *A. baumannii* bacterial isolates. According to reports, *A. baumannii's* biofilm development is influenced by the environment's stressors and growth circumstances [23].

Both veterinary and clinical sources have noted that A. baumannii exhibits significant inter-strain variability and growing medium requirement for biofilm development [24]. It was revealed high formation of biofilm by *P. aeruginosa* in the present of yeast extract and peptone (organic sources) compared with inorganic sources NH_4Cl and $NaNO_3$ [25].In many studies focused on the effects of Physicochemical factors including Type and nutrient availability which conculded the, concentration-dependent effect on biofilm accumulation,Higher nutrient concentrations decrease biofilm accumulation due to detachment and reduced competition among bacterial isolates, reduced media nutrients lead to decreasing in the amount of biofilm formation.

Extreme nutritional deficiency causes a drop in exopolysaccharide synthesis, which in turn causes a decrease in biofilm production.The presence of sucrose, calcium, and phosphate promotes the formation of biofilms [26,27,28,29,30].

Table 1. Numbers and percentage for *A. baumannii* isolated from various clinical samples

Samples	(No and %) of bacteria isolates	(No and %) *A. baumannii*
Sputum	80(26.66%)	42(42.0%)
Burn	64(21.33%)	29(29.0%)
Bed sore	52(17.33%)	16(16.0%)
Blood	20(6.66%)	6(6.12%)
Wound	50(16.66%)	4(4.0%)
Urine	34(11.33%)	3(3.0%)
Total N. (%)	300(100%)	100(100%)
P-value	0.0063**	0.0001**

** ($P \leq 0.01$).

Table 2. Nitrogen sources affecting on biofilm formation stages (strong, moderate and weak) in *A.baumannii*.

Type of nitrogen source	Mean ± SE			LSD value
	Strong (n=3)	Moderate (n=3)	Weak (n=3)	
Trypton	0.432 ±0.03	0.316 ±0.02	0.140 ±0.06	0.114 *
Yeast extract	0.334 ±0.05	0.236 ±0.07	0.164 ±0.12	0.098 *
NH4CL	0.145 ±0.03	0.106 ±0.01	0.035 ±0.02	0.089 *
NaNo3	0.115 ±0.03	0.081 ±0.02	0.071 ±0.01	0.051
Casein	0.104 ±0.02	0.072 ±0.03	0.080 ±0.06	0.088 *
Peptone	0.046 ±0.01	0.050 ±0.03	0.038 ±0.02	0.0298
LSD value	0.094 *	0.122 *	0.157 *	---

* ($P \leq 0.05$).

Table 3. The effect of carbon sources on *A.baumannii* biofilm formation stages.

Type of sugar	Mean ± SE			LSD value
	Strong (n=3)	Moderate (n=3)	Weak (n=3)	
Fructose	0.423 ±0.04	0.374 ±0.07	0.009 ±0.006	0.104 *
Lactose	0.342 ±0.02	0.361 ±0.01	0.028 ±0.01	0.102 *
Sucrose	0.331 ±0.04	0.254 ±0.05	0.018 ±0.01	0.096 *
Starch	0.161 ±0.01	0.113 ±0.01	0.022 ±0.002	0.083 *
Glucose	0.110 ±0.01	0.056 ±0.02	0.122 ±0.07	0.0693*
LSD value	0.094 *	0.122 *	0.157 *	---

* ($P \leq 0.05$).

Figure 1. Colony morpholgy of *A. baumannii* on MacConkey agar at 37 °C for 24hr.

Figure 2. Colony morpholgy of *A. baumannii* on Drigalski Lactose agar at 37 °C for 24hr.

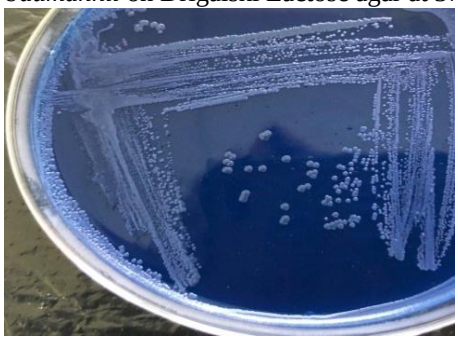


Figure 3. A- Type of biofilm producing strain of *A. baumannii*, B- biofilm formation in Microtiter plate stained by Crystal Violate.

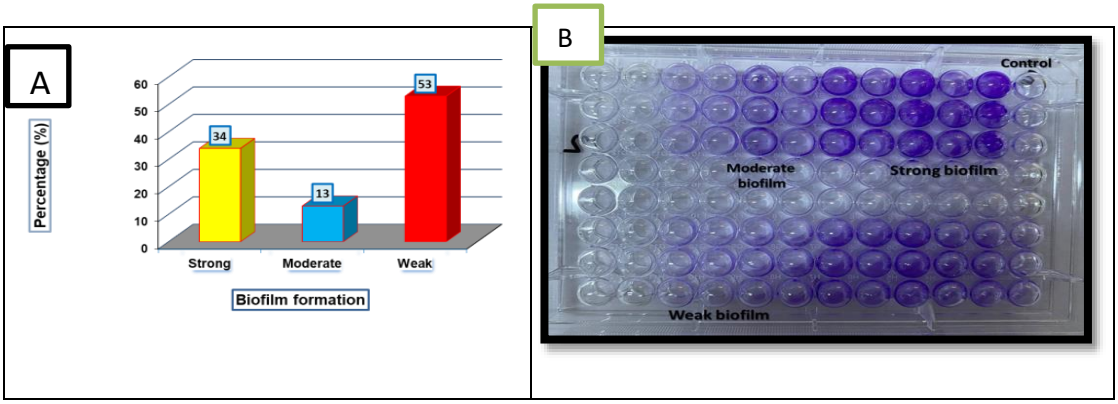


Figure 4. Nitrogen sources affecting on biofilm formation stages (strong, moderate and weak) in *A.baumannii*.

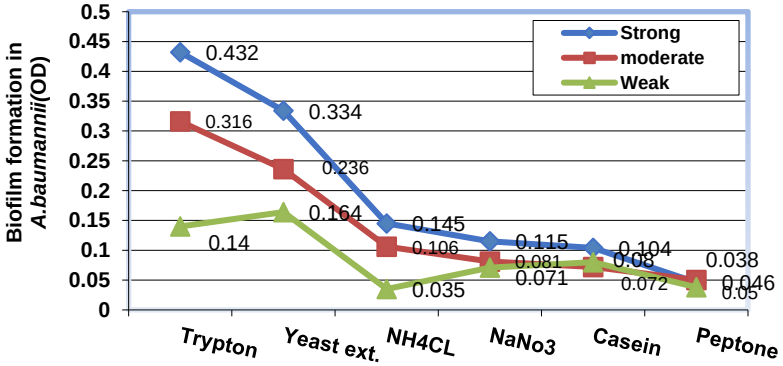
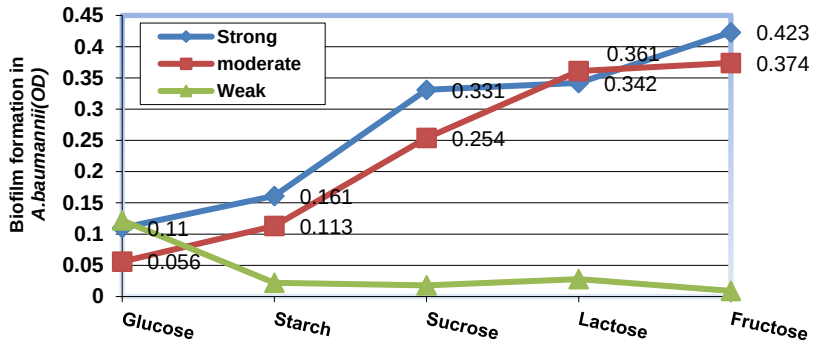


Figure 5. Effect of carbon sources on *A.baumannii biofilm* formation stages.



Conclusions

The highest percentage of isolation (42%) was of sputum and the lowest percentage (3%) was from urine. *A. baumannii* produced biofilm with three stages, (34%) strong biofilm (13%) moderate biofilm and (53%) weak biofilm, the biofilm increased significantly in media contain (Trypton and Yeast extract) as nitrogen sources and carbon sources like (fructose, sucrose and lactose).

Acknowledgement

The authors are thankful to all the staff members in the Al-Hussein Hospital for Burns in Karbala.

Ethical Approval

Ethical approval for this study was granted from the ethical committee of the Iraqi Ministry of Health Karbala. Directorate of Health (1866/3 2021/12/2).

Conflict of interest

None declared.

Financial disclosure

None declared.

References

1- Gedefie A., Demsis W., Ashagrie M., Kassa Y., Tesfaye M., Tilahun M., et al. Acinetobacter baumannii biofilm formation and its role in disease pathogenesis: a review. Infection and Drug Resistance 2021; 14: 3711.

2- Jalali Y., Jalali M., and Payer J., Acinetobacter baumannii Emergence of a Superbug, Past, Present, and Future 2022. DOI: 10.5772/intechopen.104124

3- Eze E. C., Chenia H. Y., and El Zowalaty M. E., Acinetobacter baumannii biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments," Infection and drug resistance, 2018; 11:2277-2299. doi: 10.2147/IDR.S169894.

4- Kashif, A.; Rehman, R.; Fuwad, A.; Shahid, M.K.; Dayarathne, H.N.P.; Jamal, A.et al. Current advances in the classification, production, properties and applications of microbial biosurfactants—A critical review. Adv. Colloid Interface Sci 2022; 306: 102718

5- Ham Y. and Kim T.-J., "Conditions for preparing Glycyrrhiza uralensis extract for inhibiting biofilm formation of Streptococcus mutans, Journal of the Korean Wood Science and Technology 2019; 47: 178-188.

6- Balderas-Ruíz K. A., Bustos P., Santamaria R. I., González V., Cristiano-Fajardo S. A. et.al. Bacillus velezensis 83 a bacterial strain from mango phyllosphere, useful for biological control and plant growth promotion, Amb Express 2020; 10: 1-19.

7- Prusiner S., "Regulation of glutaminase levels in Escherichia coli," Journal of bacteriology1975; 123(3) : 992-999. https://doi.org/10.1128/jb.123.3.992-999.1975

8- Dang Y., Zhao F., Liu X., Fan X., Huang R., Gao W., Wang S.and Yang.C, "Enhanced production of antifungal lipopeptide iturin A by Bacillus amyloliquefaciens LL3 through metabolic engineering and culture conditions optimization," Microbial cell factories 2019;18:1-14.
9- O'Toole G. A. Microtiter dish biofilm formation assay . J Vis Exp 2011 ; 30:(47):2437.doi: 10.3791/2437.
10- Atmanto Y. K. A. A., Paramita K., and Handayani I., "CULTURE MEDIA." International Journal of Current Research in Science Engineering & Technology 2022; 4(04): 2213-2225.
11- Mazraeh F. N., Hasani A., Sadeghi J., Kafil H. S., Barhaghi M. H. S., Sefidan F. and Rezaee, M., High frequency of blaPER-1 gene in clinical strains of Acinetobacter baumannii and its association with quorum sensing and virulence factors," Gene Reports 2021;24: 101232.
12- Aina O., Atuanya E., Oshoma C., Omotayo A., and Olaleye O., Biodegradation potential of rhizospheric microorganisms of rhizophora racemosa in crude oil contaminated mangrove swamp in the niger delt mallery a," African Journal of Health, Safety and Environment 2021; 2: 91-102.
13- D. George and P., IBM SPSS statistics 26 step by step: A simple guide and reference: Routledge, 16th Edition, 2019: 402
14- AL-Sudani E. A. and S. A., Prevalence of Urinary Tract Infections in Adult and Child Patients, Indian Journal of Public Health 2019;10: 1861.
15- Nasser S. J. and Abdulrazaq R. A., Effect of Purified Bacteriocin from Acinetobacter baumannii on some Pathogenic and Environmental Isolates and Its Inhibitory Effect on Hemolysin Production from S. aureu," Journal of Chemical Health Risks.JCHR 2022;12(4):649-658
16- Radhi S. H. and Al-Charrakh A. H., Occurrence of MBLs and Carbapenemases among MDR and XDR Acinetobacter baumannii Isolated from Hospitals in Iraq," SCOPUS IJPHRD CITATION SCORE 2019; 10: 668.
17- Arciola C. R., Campoccia D., and Montanaro L., Implant infections: adhesion, biofilm formation and immune evasion, Nature reviews microbiology 2018;16: 397-409.
18- Primo E. D., Cossovich S., and Giordano W., "A simple method to evaluate biofilm formation in exopolysaccharide-producing strains of Sinorhizobium meliloti," Journal of Biological Education 2020; 54 :355-362.
19- Thompson L., V. Gray, Lindsay D., and Von Holy A., "Carbon: nitrogen: phosphorus ratios influence biofilm formation by Enterobacter cloacae and Citrobacter freundii," Journal of applied microbiology 2006; 101:1105-1113.
20- N. S. Zbar, A. W. B. Al-Shaibani, and H. M. Jasim, "Optimum Conditions for Glutaminase Production by Serratia marcescens N1, Al-Nahrain Journal of Science 2014; 17: 147-153.
21- Zou M. and Liu D., Effects of carbon sources and temperature on the formation and structural characteristics of food-related Staphylococcus epidermidis biofilms," Food Science and Human Wellness 2020; 9: 370-376.
22- Ham Y. and Kim T.-J., Nitrogen sources inhibit biofilm formation by Xanthomonas oryzae pv. oryzae. .Journal of Microbiology and Biotechnology 2018; 28: 2071-2078.
23- Sharma S., A. Das, Banerjee T. Barman, H., Yadav G., and Kumar A., Adaptations of carbapenem resistant Acinetobacter baumannii

(CRAB) in the hospital environment causing sustained outbreak, Journal of Medical Microbiology 2021 ;70: 001345.

24- Nocera F. P., Attili A.-R., and Martino L. De, "Acinetobacter baumannii: its clinical significance in human and veterinary medicine, Pathogens 2021; 10:127.

25- Al-Sa′adi A. A. The relationship between collagenase production and biofilm formation by Pseudomonas aeuroginosa .Thesis 2014:76.

26- Dolma, K. G., Khati, R., Paul, A. K., Rahmatullah, M., de Lourdes Pereira, M.Virulence Characteristics and Emerging Therapies for Biofilm-Forming Acinetobacter baumannii: A Review. Biology 2022; 11(9):1343.

27- Al-Wrafy, F. A., Al-Gheethi, A. A., Ponnusamy, S. K., Noman, E. A., & Fattah, S. A. Nanoparticles approach to eradicate bacterial biofilm-related infections: A critical review. Chemosphere 2022; 288: 132603.

28- Mahmood, A., Liu, X., Grice, J., Medley, G., & Roberts, M. The Efficacy of Quinolones and Fluoroquinolones in the Medicine: a review study. Iraqi Journal of Medical and Health Sciences2024;1(1): 27–35. https://doi.org/10.51173/ijmhs.v1i1.17.

29- Suhad Hassan Aubaid, Ekhlas Saddam Falih, & Suhaib khalid Ibrahim. Biofilm Formation of Staphylococcus aureus in Multiple Sclerosis Patients and its Essential Role in the Pathogenicity of the Disease. Journal of Techniques 2022; 4(3): 14–18. https://doi.org/10.51173/jt.v4i3.511.

30- Zidan, M. E., Samanje, J., & Nasir, H. M. Identification of Antibiotic Resistance of Acinetobacter baumannii in Iraqi Patients. Journal of Techniques 2022; 4: 28–32. https://doi.org/10.51173/jt.v4i33.589.