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Effects of hydrogen peroxide and ferrous ions on the ability of *Pseudomonas aeruginosa* isolates to adhere to host cells in vitro.

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Abstract :

The current study aimed to demonstrate the effect of oxidative stress generated by free radicals resulting from the exposure of *Pseudomonas aeruginosa* isolates to a sub-minimum inhibitor concentration of hydrogen peroxide (H_2O_2), and ferrous ion (Fe^{+2}) with hydrogen peroxide, on the bacteria's ability to adhere to red blood corpuscles (RBCs). Collecting 237 samples from clinical cases that included: burns, urinary tract infections (UTI), pneumonia, skin infections, keratitis, otitis media, arthritis and cystic fibrosis, and of different ages from Samarra General Hospital and some private clinics in Samarra city in the Salah El-Din governorate for the period from the beginning of December 2021 until the middle of June 2022. The results of the isolates showed a different ability to adhere to RBCs, where the isolates of wounds and burns changed from a positive ability to adhere to the cells before treatment to a loss of that ability when exposed to H_2O_2 and ferrous ion, in contrast to pneumonia isolate, which had high effectiveness of adhesion to RBCs when treated with H_2O_2 only, while, most of the isolates lost their ability to adhere when treated with ferrous ions with hydrogen peroxide, except the two isolates of otitis media and keratitis. It's concluded from the results of the current research that the exposure of *Pseudomonas aeruginosa* to oxidative stress may affect the ability of the bacteria to adhere to host cells and thus affect their severity and pathogenicity.

Key words: *Pseudomonas aeruginosa*, H_2O_2, Free radicals, Oxidative stress.

Introduction:

Oxygen is an essential and important component for energy production through food oxidation. However, the reduction of this element isn´t complete even under normal conditions. Mostly, there are secondary products arise from natural chemicals that are often formed from food transformation processes (metabolism) or from the electron transport chain in mitochondria, which are called free radicals, free radicals attack and destroy the components of cells to cause severe damage to their genetic material and various cellular functions, the accumulation of free radicals may cause many diseases such as cardiovascular diseases, cancer, aging, and others (Dawidowicz *et al*., 2006).

It should be noted that aerobic bacteria use molecular oxygen O_2 for respiration or oxidize nutrients to produce energy, but these processes produce reactive oxygen species (ROS), which are necessary for many physiological functions in cells and the body in general, but an imbalance between their natural levels and antioxidants lead to high levels of ROS, such as H_2O_2, which results in oxidative stress as in humans (Rutherford, 2016). Exposure of bacteria to free radicals (ROS) can damage a variety of macromolecules in the bacterial cell, and lead to mutations or cell death. However, free radicals may at the same time be considered useful compounds as signaling molecules that lead to a coordinated response in the bacterial cell under oxidative stress conditions (Kashmiri and Mankar, 2014). *Pseudomonas aeruginosa* is an opportunistic pathogen that possesses a variety of virulence factors that make it a subject of interest and study by researchers because of causes disorder to human health and is responsible for some of the infections suffered (Filloux and Ramos, 2022).

Pseudomonas aeruginosa is one of the most widespread species of bacteria and its ability to cause infections among patients with wounds and burns, assisted by multiple virulence factors, including the formation of biofilms and the production of toxins that cause extensive damage to tissues and then enter the bloodstream and then spread to body tissues (Liew *et al*., 2019; Filloux and Ramos, 2022). In addition to their production of virulence factors, these bacteria have a high resistance to many antibiotics due to their possession of many self-resistance mechanisms, and they can also develop new resistance when exposed to antibacterial substances, which leads to the emergence of strains with many characteristics, such as multi-resistance to antibiotics (Das, 2021).

Materials and Methods:

Materials:

Hydrogen peroxide (30%) from GmbH & Co., Germany, and Ferrous chloride ($FeCl_2$), as a source of Ferrous ion (Fe^{+2}), supplied by Thomas Baker, India. As for the culture media and materials used in the diagnosis and the study, they were supplied by international companies and different origins such as Oxoid, Himedia, and Difco.

Sample collection:

In the current study, 723 samples were collected from clinically diagnosed patients in the bacteriological laboratory at Samarra General Hospital and some private clinics and centers in Samarra city in Salah al-Din Governorate, for the period from the beginning of December 2021 until the middle of June 2022, as in Table (2).

Diagnosis of bacterial isolates:

The diagnosis was diagnosed initially based on the morphological and cultural characteristics after the growth on culture media, then after that the characteristics of the cells were studied after staining them with a gram stain and examined under a microscope to distinguish the forms and know their shapes and external characteristics, and confirmatory by physiological and biochemical tests (Pitt, 2017 ; Todar, 2011 ; Forbes *et al*., 2007), as in Table (1).

Table 1: Diagnostic morphological and biochemical tests used in the diagnosis of *P. aeruginosa* isolates.

No.	Tests	Results
1	Gram stain	-
2	Cell morphology	Bacilli
3	Pigment production	-/+
4	growth at a temperature of 42 °C	+
5	Growth on a Cetrimide agar	+
6	Catalase	+
7	Oxidase	+
8	Motility	+
9	Hemolysin	+
10	Indole production	-
11	Methyl red	-
12	Voges-proskauer	-
13	Citrate consumption	+
14	Urease	+
15	Gelatin hydrolysis	+
16	CO_2 Gas production	-
17	H_2S Gas production	-
18	Kligler test	K/K
19	Lactose fermentation	-

(-) the negative result of the test. (+) the positive result of the test.
(K/K) the media is alkaline at the bottom and the top surface is slant.

The API-20E system was used to identify and diagnose bacteria and confirm previous tests (Juang and Morgan, 2001). In addition to diagnosing the isolates by using the compact system VITEK 2 to finally diagnosis the isolates, the results were read according to Fritsche *et al*, (2011).

Test for host cell viability and adhesion factors of *Pseudomonas aeruginosa* isolates:

The method presented in Iwahi *et al*, (1983) was followed using the Slide Agglutination test, then the results of agglutination were seen by using a light microscope with the preparation of two slides as a control, on the first slide, putting a

suspension of RBCs was placed with a normal saline only, while the second slide was put on a bacterial suspension with a normal saline for comparison, as in Table (4).

Determination of minimum inhibitory concentration (MIC) and (Sub-MIC) :

MIC and sub-MIC for hydrogen peroxide only and both ferrous ions with hydrogen peroxide were determined according to the dilution method in the medium (Colle *et al*., 1996 ; Vinckx *et al*., 2008), as in Table (3).

The results and discussion:

1- Isolates of *Pseudomonas aeruginosa* bacteria:

The results in Table (2) showed that the total number of samples and the number of isolates of *Pseudomonas aeruginosa* that were collected from different patients and classified and diagnosed in the laboratory and divided according to the type of infection as cases infected with bacteremia, pneumonia, otitis media, keratitis, cystic fibrosis, wounds, bed sores, urinary tract infections, arthritis, and burns.

Table (2): Sources of isolation with the total number of samples, the number of *P. aeruginosa* isolates, and the percentage of studied isolates.

Isolation sources	Total number of samples	Number of *P.aeruginosa* isolates	Studied isolates (%)
Urinary Tract	268	88	32.83
The lung	96	55	57.29
Bed sores	93	40	43.01
Wound	64	20	31.25
Burns	20	9	45
Ear	75	25	33.34
Cornea	77	23	29.87
Blood	23	7	30.43
Bone joints	7	1	14.28
Total	**723**	**268**	

2- Minimum inhibitory concentration (MIC) and sub-MIC for H_2O_2, and H_2O_2 with ferrous ion:

The results in Table (3) showed the minimum inhibitory concentration (MIC) and sub-MIC for hydrogen peroxide, and ferrous ion with hydrogen peroxide for the *P. aeruginosa* isolates.

Table (3): MIC and Sub-MIC of H_2O_2, and H_2O_2 with ferrous ion for the studied *P. aeruginosa* isolates.

Isolation sources	H_2O_2 Conc. (mM)		H_2O_2 with ferrous ion Conc. (mM)	
	Sub-MIC	MIC	Sub-MIC	MIC
Otitis media	60	70	5	6
Keratitis	60	70	5	6

Wound	90	100	5	6
Bed sores	100	110	5	6
Pneumonia	40	50	2.5	3
Bacteremia	30	40	2.5	3
Burns	450	460	3	4
Arthritis	210	220	5	6
Cystic fibrosis	60	70	2.5	3
Urinary tract infections	110	120	2.5	3

3- Viability and adhesion factors with host cells for *Pseudomonas aeruginosa* isolates:

The results in Table (4), showed that all of the studied isolates of *P. aeruginosa* had the ability to adhere to RBCs before exposure to oxidative stress resulting from the studied treatments (H_2O_2, and H_2O_2 with ferrous ion), except for pneumonia and UTI isolates, but after exposure of the isolates to the treatments, their ability differed on the adhesion of RBCs, the otitis media isolate lost its adhesion ability when treated with hydrogen peroxide only, while the keratitis isolate was not affected in its adhesion ability before and after the treatment, while the wound and burn isolates lost its adhesion ability after exposure to the treatments. The bed sore, bacteremia, and cystic fibrosis isolates could adhere to RBCs after exposure to H_2O_2 only, while when treated with ferrous ions with hydrogen peroxide, they lost their ability to adhesion to RBCs, and pneumonia isolate became highly capable of adhesion to RBCs, whereas, isolates of pneumonia and arthritis lost their adhesion ability when exposed to a ferrous ion with H_2O_2.

Table (4) The ability of *P. aeruginosa* isolates to adhesion to RBCs before and after exposure to hydrogen peroxide and ferrous ions.

Isolates of *P .aeruginosa*	**Ability of isolates to adhesion to RBCs**		
	A	**B**	**C**
Otitis media	+	-	+
Keratitis	+	+	+
Wound	+	-	-
Bedsores	+	+	-
Pneumonia	-	+++	-
Bacteremia	+	+	-
Burns	+	-	-
Arthritis	+	+	-
Cystic fibrosis	+	+	-
Urinary tract infection	-	-	-

A: Results of isolates before treatment. B: Results of isolates after treatment with H_2O_2 only.

C: Results of isolates after treatment by H_2O_2 with ferrous ion.

Many reasons may explain the effect of oxidative stress resulting from the exposure of isolates of *P. aeruginosa* to H_2O_2 and ferrous ions on their ability to adhesion to RBCs, including that the treatment may lead to a change in the cell composition of the bacteria and their macromolecules, which may affect at their ability to respond to the bacterial adhesion process, also, may oxidative stress leads to damage of proteins and lipids in the outer membrane of bacteria and change in their structure, or maybe affects on the gene expression of proteins that have a role in bacterial adhesion such as fimbriae (hair-like structures on the surface of bacteria that participate in adhesion), so that it reduces their expression and thus the bacteria lost its adhesion ability (Shinji *et al*., 2018 ; Roy *et al*., 2019). The oxidizing factors that form from oxidative stress which results from exposure to H_2O_2 and ferrous ions may lead to changes in the surface structure of these cells, and this may affect their ability to adhere with bacteria. On the other hand, oxidative stress may affect the external structure of the bacteria, and thus increase the virulence of the bacteria, or the opposite may reduce its virulence (Chakraborti *et al*., 2019).

Conclusions:

The conclusion from the results of the current research is that the exposure of *Pseudomonas aeruginosa* to oxidative stress may affect the ability of the bacteria to adhere to host cells and thus affect their severity and pathogenicity.

Ethical conduct of research:

This research was approved by the ethics committee of healthy ministry in 29/11/2021 code 35/2021036 and the university of Samarra in 1/11/2021 code 1653-07/3.

Conflicts of Interest:

The authors state that the publishing of this paper does not include any conflicts of interest.

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References:

Dawidowicz, A. L., Wianowska, D. and Baraniak, B. (2006). The antioxidant properties of alcoholic extracts from Sambucus nigra L.(antioxidant properties of extracts). *LWT-Food Science and Technology*, *39*(3), 308-315.

Rutherford, M. (2016). Free Radicals & Health. UK ed. Nova Science Publishers Inc, New York, United States. p. 64-97.

Kashmiri, Z. N. and Mankar, S. A. (2014). Free radicals and oxidative stress in bacteria. *Int. J. Curr. Microbiol. Appl. Sci*, 3, 34-40.

Filloux, A. and Ramos, J-L. (2022). Pseudomonas aeruginosa, Biology, Pathogenesis and Control Strategies. 1st ed, Springer Cham, Springer Nature Switzerland. Pp: XVII-445.

Liew, S. M., Rajasekaram, G., Puthucheary, S. A. and Chua, K. H. (2019). Antimicrobial susceptibility and virulence genes of clinical and environmental isolates of Pseudomonas aeruginosa. *PeerJ*, 7, e6217.

Das,T. (2021). Pseudomonas aeruginosa - Biofilm Formation, Infections and Treatments. IntechOpen. Pp:25-102.

Forbes, B.A., Sahm, D.F. and Weissfeld, A.S. (2007) Baily and Scott Diagnostic Microbiology. 12th Edition, Mosby Elsevier, Philadeliphia, 93-107, 187-197, 842-854.

Todar, K. (2011). Diversity of metabolism in procaryotes. Today's Online Textbook of Bacteriology. University of Wisconsin: Madison, WI.

Pitt,S. J. (2017). Clinical Microbiology for Diagnostic Laboratory Scientists. John Wiley & Sons Ltd, p. 1-221.

Juang, D. F. and Morgan, J. M. (2001). The applicability of the API 20E and API Rapid NFT systems for the identification of bacteria from activated sludge. *Electronic Journal of Biotechnology*, 4(1), 1-2.

Fritsche, T. R., Swoboda, S. E., Olson, B. J., Moore, F. M., Meece, J. K. and Novicki, T. J. (2011). Evaluation of the Sensititre ARIS 2X and Vitek 2 automated systems for identification of bacterial pathogens recovered from veterinary specimens. LACROSSE. Univ. Wisconsin.

Iwahi, T., Abe, Y., Nakao, M., Imada, A. and Tsuchiya, K. (1983). Role of type 1 fimbriae in the pathogenesis of ascending urinary tract infection induced by Escherichia coli in mice. *Infection and Immunity*, 39(3), 1307-1315.

Collee,J. G., Marmion,B. D., Fraser,A. G. and Simmons,A. (1996). Mackie & Mccartney practical medical microbiology, 14th ed. Churchill Livingstone, New York, USA.

Vinckx, T., Matthijs, S. and Cornelis, P. (2008). Loss of the oxidative stress regulator OxyR in Pseudomonas aeruginosa PAO1 impairs growth under iron-limited conditions. *FEMS microbiology letters*, 288(2), 258-265.

Shinji, H., Toyofuku, Y. and Matsushita Y. (2018). Effects of hydrogen peroxide on the adherence of Staphylococcus aureus to human red blood cells. *Microbiol Immunol*, 62(2):107-113.

Roy, S., Mukherjee, S., Ghosh, S., Chatterjee, S., Adhikari, A., Biswas, J., Dutta, S. and Karmakar, S.P. (2019). Oxidative stress and antioxidant defense in a bacterial pathogen, Vibrio cholerae. *Genes (Basel)*, Apr 2;10(4):272.

Chakraborti, S., Chakraborti, T., Chattopadhyay, D. and Shaha, C. (2019). Oxidative stress in microbial diseases. 1st ed. Springer Nature, p. 586.

Karupusamy, S., Mustafa, M. A., Jos, B. M., Dahiya, P., Bhardwaj, R., Kanani, P., & Kumar, A. (2023). Torque control-based induction motor speed control using Anticipating Power Impulse Technique. The International Journal of Advanced Manufacturing Technology, 1-9.

Govindarajan, S., Mustafa, M. A., Kiyosov, S., Duong, N. D., Raju, M. N., & Gola, K. K. (2023). An optimization based feature extraction and machine learning techniques for named entity identification. Optik, 272, 170348.

Sudha, I., Mustafa, M. A., Suguna, R., Karupusamy, S., Ammisetty, V., Shavkatovich, S. N., ... & Kanani, P. (2023). Pulse jamming attack detection using swarm intelligence in wireless sensor networks. Optik, 272, 170251.

Hassan, J. A., & Rasheed, M. K. (2022, November). Synthesis and characterization of some benzimidazole derivatives from 4-methyl ortho-phenylene diamine and evaluating their effectiveness against bacteria and fungi. In AIP Conference Proceedings (Vol. 2394, No. 1). AIP Publishing.

Nijris, O. N., Khaleel, Z. I., Hamady, S. Y., & Mustafa, M. A. (2020). The effectiveness of Aqueous Extract of Grape Seeds Vitis vinifera as an antibiotic for some microorganisms and its Protective

Role Histology for Liver, Kidney in Mice. Indian Journal of Forensic Medicine & Toxicology, 14(2), 1838-1845.

Mustafa, H. A., Majid, H. H., Abdulqader, A. T., Mustafa, M. A., & Salih, A. A. (2019). Study On Some Physiological, Biochemical And Hormonal Parameters Of Seminal Fluid Of Infertile Men. Biochem. Cell. Arch, 19(Supplement 1), 1943-1947.

Fadhil, K. B., Majeed, M. A. A., & Mustafa, M. A. (2019). Electronic study of fresh enzyme complexes of antifungal drugs-P450 and Aspergillus kojic acid biosynthesis. W: w saccharose flavus: fructose as a substratum. Annals of Tropical Medicine and Health, 22, 65-72.

Abdulazeez, M., Hussein, A. A., Hamdi, A. Q., & Mustafa, M. A. (2020). Estimate the Complications That Resulting from Delayed Management of Dental Trauma in Tikrit City. Journal of Cardiovascular Disease Research, 11(2), 80-82.

Hama Hasan, T. A., Erzaiq, Z. S., Khalaf, T. M., & Mustafa, M. A. (2020). Effect of Equisetum Arvense Phenolic Extract in Treatment of Entamoeba Histolytica Infection. Systematic Reviews in Pharmacy, 11(11).

Hama Hasan, T. A., Erzaiq, Z. S., Khalaf, T. M., & Mustafa, M. A. (2020). Effect of Equisetum Arvense Phenolic Extract in Treatment of Entamoeba Histolytica Infection. Systematic Reviews in Pharmacy, 11(11).

Nijris, O. N., Khaleel, Z. I., Hamady, S. Y., & Mustafa, M. A. (2020). The effectiveness of Aqueous Extract of Grape Seeds Vitis vinifera as an antibiotic for some microorganisms and its Protective Role Histology for Liver, Kidney in Mice. Indian Journal of Forensic Medicine & Toxicology, 14(2), 1838-1845.

Ali, A., Jassim, A.F., Muhsin, S.N., & Mustafa, M.A. (2020). Study of Lycium Shawii Phenolic Compounds in Treatment of Hyperlipidemia. Journal of cardiovascular disease research, 11, 196-199.

Ibrahim, H. M., Jumaah, L. F., Khalaf, S. A., & Mustafa, M. A. (2021). KNOWLEDGE AND PRACTICE OF BREASTFEEDING AND WEANING IN MOTHERS LIVES SAMARRA CITY, IRAQ. Biochemical & Cellular Archives, 21.

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