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VARIABLE BACTERIAL RESPONSES TO OXIDATIVE STRESS IN DIFFERENT BACTERIAL SPECIES

By

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ABSTRACT

Background: Living organisms are exposed to oxidative stress due to internal or external stimuli. It results from the imbalance between the production and elimination of reactive oxygen species. This leads to loss of homeostasis. **Objective**: To test the effect of oxidative stress on the level of the production of reduced glutathione (GSH) as an antioxidant, malondialdehyde (MDA) as a measure of lipid peroxidation, and of the siderophore enterobactin as an oxidative stress response, in different bacterial species.

Materials and Methods: H_2O_2 minimum inhibitory concentration (MIC) was determined in *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603, using broth-macrodilution method. The levels of GSH and MDA were measured in *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 and in clinical isolates of *E. coli*, *K. pneumoniae* and *Staphylococcus aureus* after exposure to lethal H_2O_2 concentration, using Glutathione Reduced Kit and Lipid Peroxide-Malondialdehyde Kit, respectively. The level of expression of *ent*C gene, involved in enterobactin biosynthesis, in presence of 0.25 and 0.5 MIC of H_2O_2 was determined using quantitative reverse transcription-polymerase chain reaction.

Results: H_2O_2 MIC for both *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 was 1.5 mM. Exposure of *E. coli* to H_2O_2 resulted in a significant increase in GSH (p=0.0001) and MDA (p=0.0001) levels. However, in *K. pneumoniae*, a significant decrease in the GSH (p=0.0001) and MDA levels (p=0.0001) was recorded upon H_2O_2 exposure. No change in MDA and GSH levels was detected in *S. aureus* isolates exposed to H_2O_2. The expression of *ent*C gene in both *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 was reduced in presence of 0.25 and 0.5 H_2O_2 MIC.

Conclusion: Bacteria responded differently to oxidative stress, with *S. aureus* bacteria as the least affected by oxidative stress. Enterobactin role in oxidative stress needs reevaluation.

Keywords: Oxidative stress, Malondialdehyde, Reduced glutathione, enterobactin, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus.*

INTRODUCTION

Oxygen is essential for the growth of living organisms. Under normal physiological conditions, free radicals are generated due to the escape of electrons from the electron transport chain, leading to formation of reactive oxygen species (ROS) as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical *($OH^{\cdot}$; McBee et al., 2017)*. If the generated ROS exceeds the elimination capacity of the organism, this leads to loss of homeostasis, and the organism encounters a state of oxidative stress *(Su et al., 2019)*.

Excess ROS, generated during the oxidative stress, results in adverse modifications of cell components as lipids, proteins and DNA. Polyunsaturated fatty acids of the cell membranes are highly susceptible to ROS damage; a process called lipid peroxidation. Lipid peroxidation is a chain reaction that results in the production of breakdown products such as malondialdehyde (MDA) and 4-hydroxynonenal; these products may cause disruption of the bacterial cell membrane *(Ayala et al., 2014)*.

The attack of ROS may also affect the protein activity through nitrosylation, carbonylation, disulphide bond formation and glutathionylation. In addition, the breakdown products of lipid peroxidation may form conjugates with the protein *(Repetto et al., 2012)*. Oxidative attack on DNA results in deoxyribose oxidation, removal of nucleotides, strand breakage, base modification, and DNA-protein crosslinks. This may lead to malfunctions or complete inactivation of the encoded protein and finally can lead to mutations *(Sharma et al., 2012* and *Su et al., 2019)*.

Microorganisms produce antioxidants to alleviate the oxidative stress. Antioxidants can be enzymes as superoxide dismutase (SOD) which catalyzes the conversion of superoxide anion into H_2O_2, catalase and peroxidase enzymes which decompose H_2O_2 into H_2O. Antioxidants can also be non-enzymatic molecules as reduced glutathione (GSH), ascorbic acid, and α-tocopherol *(Staerck et al., 2017)*.

Reduced glutathione (the tripeptide γ glutamylcysteinylglycine) is one of the most abundant non-protein thiols. GSH reacts with free radicals preventing the damage of cellular components; it is also involved in the formation and maintenance of proteins' disulphide bonds *(Ribas et al., 2014)*.

The role of the enterobactin siderophore in alleviation of oxidative stress produced by hydrogen peroxide and paraquat was reported in *Escherichia coli (Adler et al., 2014* and *Peralta et al., 2016)*. Also, *Staphylococcus aureus* siderophore transporter SirABC was found to be induced by oxidative stress *(Nobre and Saraiva, 2014)*. In this study, the effect of the classical stressor; hydrogen peroxide on different bacterial species was determined. The level of the two antioxidant molecules; GSH, and enterobactin as well as MDA as a measure of lipid peroxidation, were determined.

The present study aimed to study the effect of H_2O_2 on different bacterial strains regarding GSH, MDA and enterobactin.

MATERIALS AND METHODS

Bacterial strains:

E. coli ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used in the study. Clinical isolates of *E. coli* (n=59), *K. pneumoniae* (n=11) and *S. aureus* (n=9) were obtained from the Faculty of Medicine, Cairo University and Faculty of Medicine, Ain Shams University.

Determination of H_2O_2 minimum inhibitory concentration (MIC):

The MIC of H_2O_2 was measured using broth-macrodilution method, according to the *Clinical and Laboratory Standards Institute (2016)*. Briefly, the overnight culture of *E. coli* ATCC 25922 and *K.*

pneumonia ATCC 700603 were diluted to have an optical density equivalent to that of 0.5 McFarland standard (contains approximately $2x10^8$ CFU/mL). This was diluted 1:150 to contain $5x10^5$ CFU/mL. Then, one mL of different H_2O_2 concentrations prepared by two-fold serial dilutions (6mM to 0.0117 mM) was inoculated with one ml of the prepared inoculum. The culture of the organism without addition of H_2O_2 was used as a positive control while un-inoculated broth was used as a negative control. Tubes were incubated overnight at 37 ºC for 20 hours, and the MIC was determined as the lowest concentration of H_2O_2 which completely inhibited the growth of the organism in the tubes.

Determination of GSH and MDA levels:

The effect of H_2O_2 on the levels of MDA and GSH was tested at lethal H_2O_2 (10xMIC level; *Jenkins et al., 1988*). Cell extracts were obtained by the method of *Daily et al. (1978)*. Briefly, the tested strains were grown aerobically for 24 hours in brain heart infusion broth at 37 ºC in a rotary shaker at 250 rpm. The culture was divided into two portions; one portion was treated with 15 mM H_2O_2 (50% w/v) and the other was kept without treatment (control). The flasks were incubated in the shaking incubator for 90 minutes at 37 ºC. Cells were harvested by centrifugation for 10 minutes at 7000xg and washed with 0.05 M potassium phosphate (pH 7.8) containing 1mM ethylenediaminetetraacetic acid (EDTA; potassium phosphate EDTA buffer). Washed cells were resuspended in 10 mL potassium phosphate EDTA buffer and centrifuged for 3 minutes at 7000xg. The supernatant was discarded and 5mL potassium phosphate EDTA buffer was added. Cells were disrupted for 3 minutes with a sonicator (Branson sonifier, USA). Cell debris was removed by centrifugation at 10000 xg for 5 minutes and the cell extracts were stored at -70 ºC until used. The level of reduced glutathione was assayed in the cell extracts using Glutathione Reduced kit (Biodiagnostic, Egypt) according to manufacturer's protocol. The level of GSH was determined by measuring absorbance of the yellow color produced after the reaction with 5,5` dithiobis (2-nitrobenzoic acid) at 405 nm. The concentration of GSH was calculated using the following equation:

GSH concentration (mmol/L) = Sample absorbance x 2.22

MDA level in the extracts was measured using Lipid Peroxide-Malondialdehyde kit (Biodiagnostic, Egypt) according to manufacturer's instructions, where MDA reacts with thiobarbituric acid (TBA) in acidic medium forming thiobarbituric acid reactive product of pink color. The absorbance of the produced color was measured at 534 nm; the concentration of MDA was calculated using the following formula:

MDA concentration (nmol/mL) = (Sample absorbance/Standard absorbance) x 10

Determination of *ent*C gene expression:

The effect of the oxidative stress exerted by H_2O_2 on the level of *ent*C gene expression in *E. coli* and *K. pneumoniae* was determined using quantitative reverse transcription-polymerase chain reaction (RT-PCR). *ent*C gene encodes the

isochorismate synthase enzyme responsible for the conversion of chorismate to isochorismate which is converted finally to enterobactin, through different enzyme-catalyzed steps. The sequence of the primers used for *ent*C gene quantitation in *E. coli* was as follows:

F'ACCTCCTCTCCACAATTGATTAC and R'AGCAGACAAGCCAAAGTCA, while the sequence of those used in *K pneumoniae* was as follows **F'TGGCTGAGGATGTACAGAAAC and R'GCAGCCTGAGGTGCTAAA**. *rpo*S and *rpo*B were used as housekeeping genes for *E. coli* and *K. pneumoniae,* respectively. The following primers were used for quantification of *rpo*S and *rpo*B, respectively:

F'ACGGCCGAAGAAGAAGTTTAT and R'TTACCACCAGACGCAAGTTAC and F'CGAAATCGAAGGTTCCGGTAT and R'ATCGTCCACTTCGCCTTTAC.

Tested species were incubated in luria bertani broth overnight at 37 ºC. Overnight cultures were diluted to reach an optical density of 0.05 at 600 nm. H_2O_2 was added to 20 mL culture at a concentration of 0.25 of MIC (0.375 mM) and 0.5 of MIC (0.75 mM). Culture without H_2O_2 addition was used as a control. The cultures were incubated at 37 ºC in a shaking incubator at 180 rpm until the exponential phase of growth (OD 600 =0.2). The synthesis and degradation of RNA were blocked by adding 1/5 volume of stop solution (90% ethanol /10% phenol). The RNA was purified using RNeasy mini kit (QIAGEN, Germany) according to manufacturer's protocol. RNA was quantified by measuring the absorbance at 260nm. cDNA synthesis and the RT-PCR were carried out using KAPA SYBR® FAST One-Step qRT-PCR Master Mix (2X) Kit (Sigma-Aldrich, U.S.A) as per manufacturer's recommendations.

Statistical analysis: SPSS version 18.0 was used for statistical analysis. Comparisons of the results of the H_2O_2 treated cultures with the untreated ones were accomplished using the Mann Whitney U rank test.

RESULTS

H_2O_2 MIC:

The H_2O_2 MIC of both *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 was found to be 1.5 mM.

Level of MDA and GSH under oxidative stress:

The levels of MDA and GSH were determined in response to oxidative stress exerted by lethal H_2O_2 concentration. *E. coli* strains (*E. coli* ATCC 25922 and the clinical isolates) recorded a significant increase in the level of GSH in presence of a lethal concentration of H_2O_2 (p=0.0001). However, *K. pneumoniae* strains (*K. pneumoniae* ATCC 700603 and the clinical isolates) treated with lethal concentration of H_2O_2 recorded a slight but a significant decrease in GSH level (p=0.0001). In *S. aureus* strains, no difference in the level of GSH by H_2O_2 treatment was detectable (p=0.9; Table 1). The level of MDA increased significantly in *E. coli* cells treated with lethal H_2O_2 concentration (p=0.0001), while in *K. pneumoniae*, a significant decrease in MDA level (p=0.0001) by H_2O_2 treatment was recorded. In *S. aureus*, MDA level was not affected by H_2O_2 treatment (p=0.97; **Table 1**).

Table (1): Level of malondialdehyde and reduced glutathione in untreated and H_2O_2-treated bacterial cells

Test / Organism	Number of tested clinical isolates	MDA (nmol/mL)			GSH (mmol/L)		
		Mean ± SD		*p* Value	Mean ± SD		*p* Value
		Control	H_2O_2 treated		Control	H_2O_2 treated	
E. coli	59	2.15±1.8	3.97±1.5	0.0001	9±1.95	15±7.8	0.0001
K. pneumoniae	11	2.9±0.048	2.6±0.07	0.0001	7±0.8	4.6±1.3	0.0001
S. aureus	9	7±0.05	7±0.07	1.0	10.7±0.05	10.7±0.07	1.0

GSH: Reduced Glutathione; MDA: Malondialdehyde

Expression of *ent*C gene under sublethal concentrations of H_2O_2:

The expression of *ent*C gene in both *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 was reduced to approximately 20% and 1% of its original level in untreated cells in presence of 0.25 and 0.5 MIC of H_2O_2, respectively.

DISCUSSION

Oxidative stress is a universal phenomenon to which all organisms are exposed; however, the oxidative stress response differs from one organism to another and differs in the same organism according to the applied stressor. The mechanisms of homeostasis in bacteria are becoming a very attractive target for the development of new anti-infective agents and are a promising strategy to circumvent antimicrobial resistance *(Mourenza et al., 2020)*.

H_2O_2 is considered one of the reactive oxygen species that results from normal cell metabolism and can cause damage to various cell components if exceeded a certain level. ROS, including hydrogen peroxide can play an important role in redox signaling *(Phaniendra et al., 2015)*, where they are needed for the normal physiological functioning of cells. Redox signaling includes reversible modification

either oxidation or covalent adduct formation with specific target proteins, allowing further translations of a signal. Cysteine residues of a target protein are the most susceptible to oxidation. Hydrogen peroxide is considered a critical signaling molecule involved in redox signaling *(DiMarzo et al., 2018)*. It has a very good stability and is able to pass through transmembrane water channels (aquaporins), where specific isoforms (peroxiporins) are present for hydrogen peroxide transport *(Bienert & Chaumont, 2014* and *Prata et al., 2019)*. In this way, H_2O_2 acts as messenger to carry a redox signal from its generation site to the target site *(Rani et al., 2015)*. In Gram negative bacteria, H_2O_2 modulates the activity of the transcription factor Oxy R; the main peroxide sensor that regulates the transcription of genes responsible for defense against cellular H_2O_2 *(Jo et al., 2015)*. Similarly, PerR, a functional equivalent of OxyR, is used by many Gram positive bacteria for defense against oxidative stress *(Ji et al., 2015)*.

In this study, the effect of H_2O_2 as a stressor was studied. H_2O_2 is a classical stressor and is one of the ROS produced normally in living cells under normal physiological conditions *(Phaniendra et al., 2015)*. The response of the tested species to H_2O_2 was different. *E. coli* cells treated with H_2O_2 showed a significant increase in the MDA level compared to the untreated cells. MDA is a biomarker of oxidative stress; it's one of the byproducts of lipid peroxidation *(Hong et al., 2012)*. Elevation of the level of MDA under oxidative stress results from the attack of the ROS on the unsaturated fatty acids of the bacterial-cell membrane, where polyunsaturated fatty acids are the major substrates for lipid peroxidation in cell membrane *(Ayala et al., 2014)*. Similar increase in MDA level by oxidative stress was reported previously in *E. coli (Arenas et al., 2011*; *Joshi et al., 2011* and *Hong et al., 2012)*.

In contrast to *E. coli*, *K. pneumoniae* strains subjected to H_2O_2 stress showed a slight but a significant decrease in the MDA level, although there was a reported significant increase in MDA level in *K. pneumoniae* exposed to the oxidative stress caused by lavender oil *(Yang et al., 2020)*. This difference in the level of produced MDA by oxidative stress may be due to the difference in the used stressor. The lower level of MDA in H_2O_2 treated *K. pneumoniae* cultures compared to the control groups may also have resulted from a shift towards a higher level of saturation of membrane lipids to confer protection against oxidative stress and ROS attack, as suggested by *Prione et al. (2016)*. However, similar to our results, a significant decrease in MDA level of *Pantoea ananatis* by oxidative stress was reported.

In *S. aureus*, no variation in the level of MDA by H_2O_2 treatment was detectable. This may be due to the presence of phophatidylethanolamine (PE) as a minor component of the phospholipids of *S. aureus* cell membrane *(Onyango and Alreshidi, 2018)*, while PE is a major component of the phospholipids of the *E. coli* outer membrane *(Bogdanov et al., 2020)*. *Poyton et al. (2016)* revealed that the rate of oxidation increases linearly with the increase in PE content in the membrane. The difference in response to oxidative stress between *S. aureus* and *E. coli* can also be attributed to the presence

of a thicker peptidoglycan wall in *S. aureus* as suggested by *Dakal et al. (2016)* that may hinder the penetration of H_2O_2 inside *S. aureus* cells.

H_2O_2 has similar chemical properties to that of water and can be transported by the aquaporins *(Bienert and Chaumont, 2014)*. Therefore, *S. aureus* can have a limited transport of H_2O_2, which can also explain their lack of response towards H_2O_2. However, some reports recorded a significant increase in the MDA level in *S. aureus* exposed to oxidative stress by blue light, which is an oxidative stress inducer *(Wu et al., 2018)*.

The level of GSH was also measured as a defense mechanism used by many species against oxidative stress. There was a significant increase in GSH level in H_2O_2-treated *E. coli* cells. Similar increase in the GSH level was reported when different *E. coli* strains were exposed to different oxidative stressors *(Arenas et al., 2011* and *Smirnova et al., 2012)*. On the other hand, *Korshed et al. (2016)* reported a significant decrease in the GSH level in *E. coli* JM109 strain by silver nanoparticles-induced oxidative stress. It is unclear why H_2O_2-treated *K. pneumoniae* strains recorded a significant decrease in the level of GSH compared to untreated cells. *K. pneumoniae* are able to overcome the oxidative stress by increasing the level of various biomarkers as glutathione –S-transferase *(Kulkarni et al., 2014)*.

In the present study, H_2O_2-treated *S. aureus* strains showed no significant difference in GSH levels. This may also be accounted for by the lack of canonical aquaporins in Gram-positive bacteria and consequently the limited H_2O_2 transport. On the contrary, the ROS produced due to treatment of *S. aureus* with silver nanoparticles which crossed the cell wall and the cell membrane reported a reduced GSH level *(Dakal et al., 2016*; *Yuan et al., 2017* and *Hamida et al., 2020)*.

Although, it was reported previously that enterobactin production increases with oxidative stress *(Peralta et al., 2016)*, we reported a reduction in the level of enterobactin in both *E. coli* and *K. pneumoniae* strains tested under the effect of different peroxide concentrations. According to *Achard et al. (2013)*, catechols not only function in iron uptake by the cells but they have the ability to scavenge the reactive oxygen species that enhance oxidative stress through Fenton reaction. This variation in enterobactin level on exposure to oxidative stress was suggested to arise from the effect of different regulators that predominate in low cell densities situation *(Adler et al., 2014)*. According to *Faulkner and Helmann (2011)*, peroxide stress may increase the expression of the *E. coli* Fur protein in a dose dependent manner; Fur protein is a negative regulator of the *ent*C gene and this may be involved in modulating the effect of oxidative stress on enterobactin production. The role of catechol siderophores in alleviating the oxidative stress was reported previously in different organisms as *Bacillus anthracis (Lee et al., 2011), Acinetobacter oleiovorans (Kim et al., 2015), Salmonella enterica* ser Typhimurium *(Achard et al., 2013)*, and *E. coli (Adler et al., 2014)*. In addition, catechol siderophores were reported to protect the bacteria from the oxidative stress caused by antimicrobial agents *(Zhang et al., 2017)*.

CONCLUSION

Different bacterial species respond differently to oxidative stress. The used stressor may also affect the response of different organisms. The role of enterobactin in oxidative stress needs further evaluation.

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الإستجابات المتنوعة لأنواع مختلفة من البكتيريا تجاه ضغط الأكسدة

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خلفيـــة البحـــث: تتعـــرض الكائنـــات الحيـــة لضـــغط أكســـده نـــاتج مـــن كـــل مـــن مـــؤثرات داخليـــة أو خارجيـــة وذلـــك نتيجـــة عـــدم التـــوازن بـــين إنتـــاج والـــتخلص مـــن جزيئـــات الأكسجين النشطة مما ينتج عنه فقدان الإتزان الداخلى للخلية.

الهـــدف مـــن البحـــث: يهـــدف هـــذا البحـــث الـــى دراســـة تـــأثير مســـتوى ضـــغط الأكســـدة علـــى مســـتويات إنتـــاج الجلوتـــاثيون المُختـــزَل (GSH) كمضـــاد للأكســـدة و ثنـــائى المـــالون الديهايـــد (MDA) كنـــاتج مـــن نـــواتج أكســـدة الليبيـــدات بالإضـــافة الـــى اســـتجابة الإنتيروباكتين وذلك فى أنواع مختلفة من البكتيريا.

مـــواد وطـــرق البحـــث: تـــم قيـــاس التركيـــز المثـــبط الأدنـــى (MIC) لفـــوق أكســـيد الهيـــدروجين (H_2O_2) لكـــل مـــن بكتيريـــا الإشـــيريكية القولونيـــة (*Escherichia coli* ATCC 25922) و بكتيريـــا الكلبســـيلة الرئويـــة (*Klebsiella pneumoniae* ATCC 700603) باســـتخدام طريقـــة التخفيـــف الماكرويـــة الســـائلة كمـــا تـــم قيـــاس مســـتوى كـــل مـــن الجلوتـــاثيون المُختـــزَل و ثنـــائى المـــالون الديهايـــد فـــى مجمـــوعتين مـــن البكتيريـــا مجموعـــة لـــم تتعـــرض لفـــوق أكســـيد الهيـــدروجين والمجموعـــة الأخـــرى تعرضـــت لتـــأثير فـــوق أكســـيد الهيـــدروجين المثـــبط وذلـــك فـــى كـــل مـــن بكتيريـــا الإشـــيريكية القولونيـــة و بكتيريـــا الكلبســـيلة الرئويـــة وبكتيريـــا المكـــورة العنقوديـــة (*Staphylococcus aureus*) والمـــأخوذه مـــن حـــالات مرضـــية. كمـــا تـــم قيـــاس مســـتوى التعبيـــر الجينـــي لجـــين الإنتيروبـــاكتين *entC* والمســـؤول عـــن تخليـــق الإنـــزيم المحفـــز لإنتـــاج الإنتيروبـــاكتين الـــذى يشـــارك فـــى عمليـــات الـــتحكم فـــى ضـــغط الأكســـدة الخلـــوى وذلـــك باســـتخدام طريقـــة تفاعـــل البلمـــرة المتسلســـل الكمـــى وقـــد تـــم ذلـــك القيـــاس تحت تأثير 0.25 و 0.5 وحدة MIC لفوق أكسيد الهيدروجين.

نتـــائج البحـــث: وقـــد وجـــد أن التركيـــز المثـــبط الأدنـــى (MIC) لفـــوق أكســـيد الهيـــدروجين يســـاوى 1.5مـــل مـــول لكـــل مـــن بكتيريـــا الإشـــيريكية القولونيـــة (*Escherichia coli* ATCC 25922) و بكتيريـــا الكلبســـيلة الرئويـــة (*Klebsiella pneumoniae* ATCC 700603). وقـــد أدى تعـــرض باكتيريـــا الإشـــيريكية القولونيـــة لفـــوق أكســـيد الهيـــدروجين الـــى زيـــادة معنويـــة فـــى كـــل مـــن تركيزالجلوتـــاثيون المُختـــزَل وثنـــائى المـــالون الديهايـــد. أمـــا فـــى البكتيريـــا الكلبســـيلة الرئويـــة فتعرضـــها لفـــوق أكســـيد الهيـــدروجين أدى الـــى إنخفـــاض معنـــوى فـــى كـــلا مـــن تركيزالجلوتـــاثيون المُختـــزَل وثنـــائى المـــالون الديهايـــد كمـــا لـــم تبـــد بكتيريـــا المكرويـــة العنقوديـــة أى تغيـــر فـــى تركيـــزات كـــل مـــن الجلوتـــاثيون المختـــزَل وثنـــائى المـــالون الديهايـــد أمـــا مســـتوى التعبيـــر الجينـــي للجـــين *ent*C فقـــد أبـــدى إنخفاضـــا فـــى كـــل مـــن بكتيريـــا الإشـــيريكية القولونيـــة (*Escherichia coli* ATCC 25922) و بكتيريـــا الكلبســـيلة الرئويـــة (*Klebsiella pneumoniae* ATCC 700603) المعرضـــة لتـــأثير0.25 و 0.5 وحدة (MIC) من فوق أكسيد الهيدروجين.

الإســـتنتاج: نســـتنتج مـــن هـــذه الدراســـة أن البكتيريـــا تســـتجيب بطـــرق مختلفـــة لضـــغط الأكســـدة وأن البكتيريـــا المكـــورة العنقوديـــة هـــى أقـــل الأنـــواع تـــأثرا بضـــغط الأكســـدة فـــى هـــذه الدراســـة كمـــا أن دور الإنتيروبـــاكتين فـــى الإســـتجابة لضـــغط الأكســـدة يحتـــاج لمزيـــد من التقييم.

الكلمـــات الدالـــة: ضـــغط الأكســـدة، وثنـــائى المـــالون الديهايـــد، الجلوتـــاثيون المُختـــزَل، إنتيروباكتين، الإشيريكية القولونية، الكلبسيلة الرئوية، المكروية العنقودية.