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Biological Balance Role of Oxidative Status for Some Bacterial Species

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Abstract:
It has been chosen three types of bacterial species consisting gram positive and gram negative types, in order to evaluate the oxidative status of culture broth. *E. coli* is normal flora with pathogenic bacteria species such as *Staphylococcus aurous* and *Klebsiella pneumonia*. The results shows that *E. coli* has different criteria than others, of them reduced glutathione appear to be higher in the *Klebsiella pneumonia* in association with increased amount in Glutathione-S-transfrease activity to about 3.3 µU/L which gives indication about its defense system against free radicals peroxynitrate to reduced it to the a mount of 50 pM, however the indication of lipid peroxidation malondialdhyde (MDA) calculated to be 0.138 M. This new biological balance role of such status has been proved.
Keywords: Bacteria, Oxidative chess, lipid peroxidation, Glutathione-S-transfrease(GST), Peroxynitrate

1. Introduction:

The oxidative status composed of the formation of free radicals and removal by antioxidant . However, this study try the evaluated such status in new pathway of biological system by using cell free broth of different types of bacteria which include *Escherichia coli*, *Staphylococcus aurous* and *Klebsiella pneumonia*. *E. coli* is one of the most important of enterobactereacae species; it is gram negative rod, usually motile, produce polysaccharide capsule, positive test for indole and shows typical morphology with iridescent sheen on differential media such as EMB grow on and may grow on non-selective media (Brooks *et al*., 2007). The other type of bacteria, Staphylococci, are gram positive cocci occurring in grape-like clusters (Garrity *et al*., 2005). There are aerobic or facultative an aerobic can grow well on normal culture medium. However, it is pathogenicity of staphylococci contributes to hemolysis of the blood, coagulation of plasma and production of extracellular enzymes (Mims *et al*., 2004). It expresses many potential virulence factors such as: surface proteins that promote colonization of host tissues, factors that probably inhibit pathogenesis, toxins that damage host tissues and cause disease symptoms (Archer, 1998). The third type involved in this study is gram negative, *Klebsiella pneumonia*, non-motile, encapsulated, found as normal flora in the mouth, skin and in the intestine (Ryan and Ray, 2004). It is clinically the most important number of the *Klebsiella* genus of enterobactereacae. *Klebsiella* is one of the major pathogens responsible for nosocomial infection (Green wood *et al*., 2002). Free radicals is a molecule or molecular fragment that contains one or more unpaired electrons in its other orbital (Vasudevan and Sreekumaric, 2001). Several powerful oxidants are produced during the course of metabolism; those include superoxide (O_2^-), hydrogen peroxide (H_2O_2), peroxy radicals (ROO^-) and hydroxyl radicals (OH^-), peroxynitrate ($ONOO^-$) (Murry *et al*., 2003). Reactive oxygen species (ROS) damage to lipids and proteins is addressed largely by degradation and synthesis in such bacterial species or even in mammals (Baynes and Dommiczak, 2004). The defenses against free radical fall into the categories of antioxidant defense enzymatic and non-enzymatic type (Marks *et al*., 2005). Bacterial glutathione transfrease play a crucial role in cellular detoxification (Oakley, 2005). The primary role is to catalyze the conjugation of glutathione (GSH) with the electrophoric centers of a wide variety of molecules (Oakley, 2011). And in bacteria GST, is involved in a variety of distinct process such as biotransformation of toxic components, production against several stress and antibacterial drug resistance (Laroche and Leisinger, 1990; Rigsby *et al*., 2007).

Aims of study: The aim of the present study is to evaluate the oxidative stress of some bacterial species, as this is the first time to our knowledge to screen such parameters in broth of bacteria.

2. **Material and methods:** All chemicals used in this study with highly purified material and no farther purification done.

2.1 Collection of specimens:
The specimens were generally collected from different sites of infection. Mid-stream urine samples were collected from patients with urinary tract infections. Blood samples were taken from normal person to isolate normal microflora. All samples had been inoculated on the culture media (MacConky, blood and nutrient agar medium) and incubated aerobically at 37^{o}C for 24-48 hrs. The bacterial isolates were identified after staining with gram stain, specific biochemical tests were done to reach the final identification according to McFadden, (2000) .

2.2 Determination of Glutathione-S-transfrase:
The measurement has been done according to method illustrated by (Habig *et al.,* 1974).

2.3 Determination of reduced glutathione:
The measurement has been done according to method illustrated by (Buritis and Ashood, 1999).

2.4 Determination of Malondialdhyde
The measurement has been done according to method illustrated by (Lunec J. ,1990)

2.5 Determination of peroxynitrate:
According to method illustrated by (BecKman *et al*., 1992).

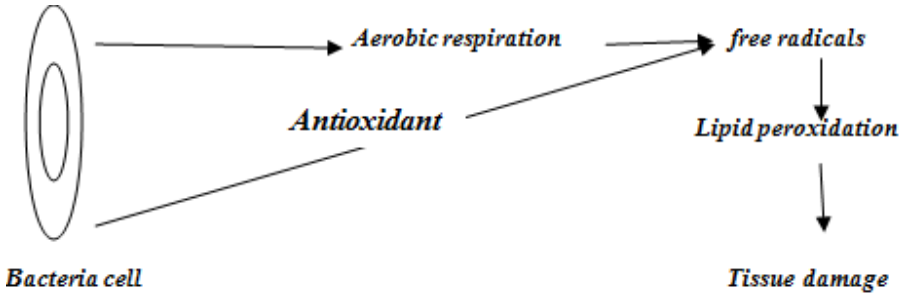
3. Results:
It is shown in Table (1) the bacteria that isolated from different sources, urine, blood and stool. These types of bacteria used in this study to show the effect of oxidative stress.Fig. (1) shows the different levels of peroxynitrate produced in such bacteria group. Since peroxynitrate is an oxidant and nitrating agent, it can damage a wide array of molecules in cells including DNA and protein (Pacher *et al*., 2007),while Fig. (2) shows the concentration of MDA produced in this matter,while Fig.(3) shows the levels and correlation between (GSH) and (GST).

4. Discussion:
The results indicated that bacteria group selected in this study can produce negative oxygen species which lead to lipid peroxidation which in general agreed with previous study (Bouhafs, 1999).

As shown in Fig. (1) the increment in peroxynitrate fellow the order of *Klebsiella pneumonia* > *Staphylococcus aurous*> *E. coli* which are agree with conciliation that pathogenic bacteria produce more oxidant species to be more violent, therefore it is decided to have measurement of MDA to prove such lipid peroxidation as MDA indicator for lipid peroxidation as showed in Fig. (2).

The MDA levels shows increment in its amount as large as peroxynitrate produced accordingly. This results lead to suggest that the decline in levels of free radicals revealed a coupled with increased antioxidant enzyme activities and the reverse is true, but also, critical balances between the generations of oxygen free radicals and antioxidant defense enzymes during development (Kohen and Nyska, 2002). This critical balance in generation of free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity as shown in Fig.(3), (Sies, 1991).

The increase in the oxidative stress may be a reason for such defense system, as a results of increased endogenous production of the free radicals, thus this study hypothesized that the formation of antioxidant enzymes during development is related to the change in the levels of free radicals and since increased oxidative stress displays a strong correlation with activation of the immune system, the antioxidant effects seen to be mediated through direct quenching of reactive oxygen species by the gene expression of major antioxidant enzymes (O. Grundmann *et al.,* 2010).



References:

Arsher,G.L.(1998).Staphylococcus aureus; a well- armed pathogen.Clin.Infect.Dis. 26:1179-1181

Brooks,G.S.,Butel,J.S.and Morse,S.A.(2007).Medical Microbiology,23ed.Large Medical Books, Mc.Graw-Hill

Baynes, J.W. and Dominiczak, H.M. (2004). Medical biochemistry. 2nd ed. Elsevier Mosby. P: 499-505.

Burtis, C.A and Ashwood, E.R. (1999). Tietz text book of clinical chemistry, 3rd, B. sounders company, Tokyo, pp:1034-1054.

Beckman, T.S., Lschiopoulos, H., Zhu, L. and Tsai, M. (1992). Arch. Biochem. Biophys. 298: 498-445.

Bouhafs, R.K. and Jarstrand, C. (1999). Lipid peroxidation of lung surfactant by bacteria. Lung. 19(1): 21-3.

Green wood,D.,Slack,R.C.,Pentherer,J.F.(2002).Medical microbiology, a guide to microbial infections, pathogenesis ,immunity, Laboratory diagnosis and control 16 th ed. Churchill vingstone.

Garrity,G.M.,Don J.B.,Noel R.k.,Staley J.T.(2005),Bergeys manual of systemic bacteriology, the proteobacteria,part A. Introducing assays.Springer-verlag,New York.2nd.Ed. (2).pp(35).

Habig, W.H., Past, M. J. and Jakoby, W.B. (1974). Biochemistry. 249:7130-7139.

Kohen, R., and Nyska, A. (2002). Oxidation of biological system: oxidative stress phenomena, antioxidant, nedox reactions and methods for their quantification. Toxical pathol. 30: 620-650.

Laroche, S.D. and Leisinger, T. (1990). Sequence analysis and expression of bacterial dichoromethane dehalogenase structural gene. J. Bacteriol. 72(1): 164-71.

Lunec J. (1990), Free radicals: their involvement in disease processes, *Ann.Clinic Biochem*.,27:173-182.

Mims,C.,Docknell,H.M.,Goering,R.V. and Roitt,I.(2004).Medical microbiology.3rd ed.,Elsevier limited.

McFadden, J. F. (2000). Biochemical tests for identification of medical bacteria 3rd edition lippincott Williams and Williams, USA.

Murry, R.K., Granner, D.K. and Mayes, P.A. (2003). Haper's illustrated biochemistry. 26th ed. Lange medical books/McGraw-Hill. P: 87-91.

Marks, D.B., Marks, A.D. and Smith, C.M. (2005). Basic medical biochemistry: clinical approach. 2nd ed. William and Wilkins. P: 339-454.

Oakley A.D. (2005). Glutathione transfrases; new functions. Current opinion in structural biology 5(6): 716-23.

Oicley, A.J. (2011). Glutathione transferases a structural perspective. Dtug. Metab. Rev. 43(2): 138-51.

O. Grundmann, O. Kleber, and V. Butterweek. (2010). Mechanism of St. John's wort extract (ST W_3-VI) during chronic restraint stress in mediated by the inter relationship of the immune, oxidative defense, and neuroendocrine system. Neuropharmacology. 658: 767-773.

Pacher, P., Bechman, J. S. and Liaudel, L. (2007). Nitric oxide and peroxynitrate: in health and disease "physiological Reviews". 87(1): 315-424.

Ryan KJ, Ray CG (2004). Sherris Medical Microbiology, 4th ed., McGraw Hill, pp. 551–552

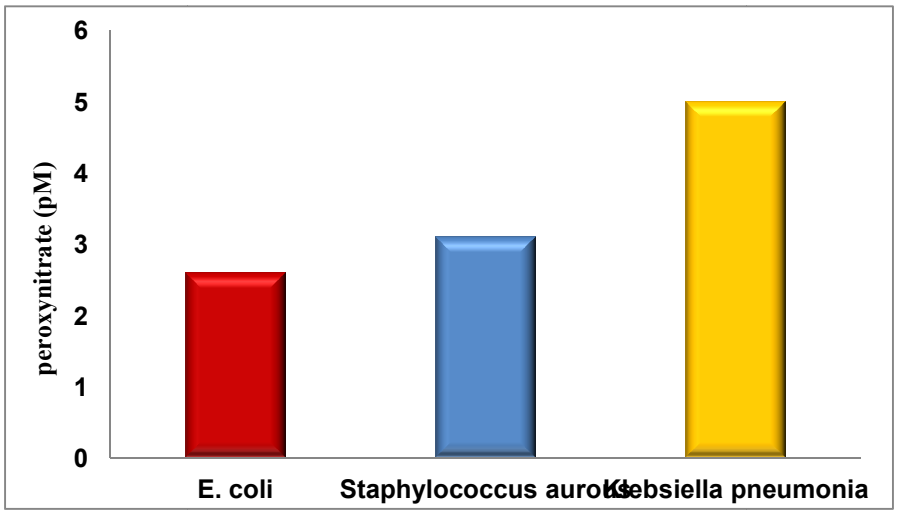
Rigsby, R.E., Brown, D.D., Dawson, E., Lybrand, T.B. and Armstrong, R.N. (2007). A model for glutathione binding and activation of biochemistry and biophysics. 464(2): 277-83.

Sies, H. (1991). Oxidative stress:oxidants and antioxidant. New York. Academic Press.

Vasudevan, D.M. and Sreekumaric, S. (2001). Test book of biochemistry for medical students 3rd ed. Japee Brother Medical Publisher LTD. P: 212-215.

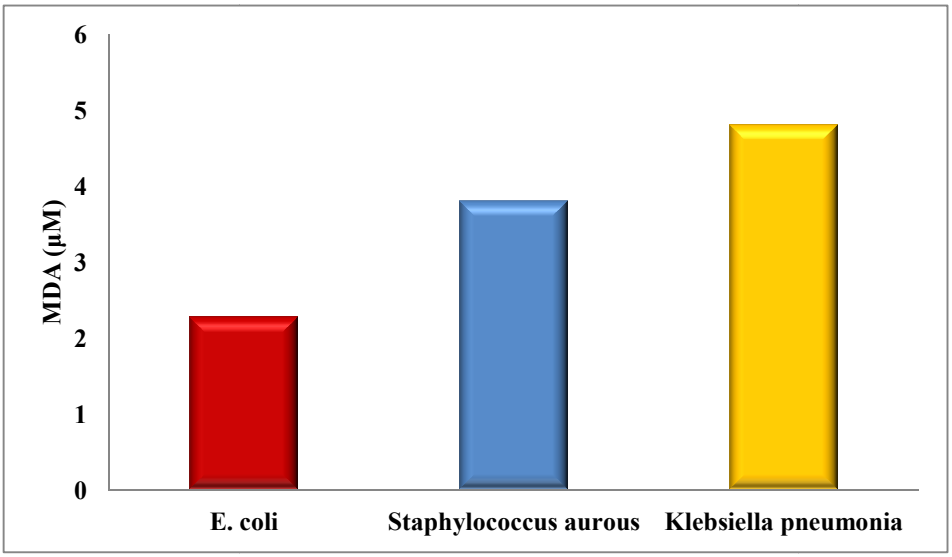
Table (1) Showed the bacterial isolated from different sources

Samples	Type of bacteria
Urine	*Klebsiella pneumonia*
Blood	*Staphylococcus aurous*
Stool	*E. coli*



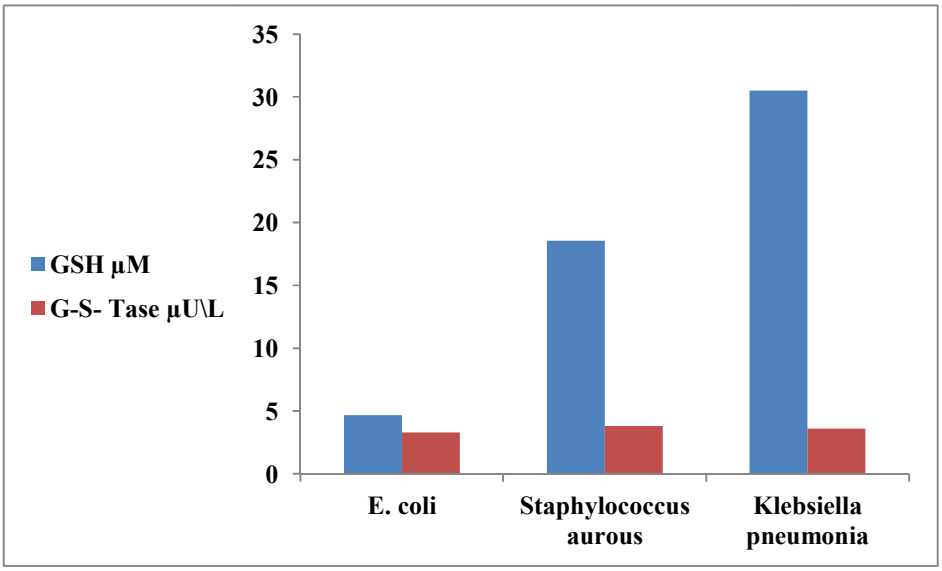
E. coli	26 pM
Staphylococcus aurous	31 pM
Klebsiella pneumonia	50 pM

Fig. (1) peroxynitrate levels in broth of bacteria



E. coli	2.27
Staphylococcus aurous	3.8
Klebsiella pneumonia	4.7

Fig. (2) MDA level in broth of bacteria (µM)



Bacteria	GSH µM	G-S- Tase µU\L
E. coli	4.65	3.3
Staphylococcus aurous	18.55	3.8
Klebsiella pneumonia	30.5	3.6

Fig. (3) GSH level and G-S-transferase activity

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