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DNA Fingerprinting of the Local Pathogenic Bacteria Which Treated with Copper

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Abstract
Copper (Cu) is an essential trace element for all aerobic organisms. It functions as a cofactor in enzymes that catalyze a wide variety of redox reactions due to its ability to cycle between two oxidation states, Cu(I) and Cu(II). This same redox property of copper has the potential to cause toxicity if copper homeostasis is not maintained. Studies suggest that the toxic properties of copper are harnessed by the innate immune system of the host to kill bacteria. The aim of this research is show the DNA fingerprinting of two local pathogenic bacteria(*Staphlococcus aureus* isolated from body fluid and *E.coli* isolated from urine) which treated with Copper to clarify the role of copper's antibacterial activity against bacterial species could be utilized in health care facilities and in food processing plants.
Keywords: *Staphlococcus aureus*, *E.coli*, Copper, copper's antibacterial.

I- Introduction

Copper is the 26th most abundant in the earth's crust and exists as 2 stable and 9 radioactive isotopes. A transition metal, copper primarily exists as one of two stable oxidation states: Cu^{2+} in the oxidized cupric form, and Cu^{+} in the reduced cuprous form. Cu^{+} is a closed shell $3d^{10}$ transition metal ion with diamagnetic properties (Frausto da Silva and Williams, 1993). A soft Lewis acid, it favors tetrahedral coordination with soft bases such as hydrides, alkyl groups, cyanide, phosphines, and thiols from cysteine and thioether bonds with methionine (Crichton and Pierre, 2001). Cu^{2+} has a $3d^{9}$ configuration, is paramagnetic, and is an intermediate Lewis acid. In addition to ligands bound by Cu^{+}, Cu^{2+} forms square planar complexes with sulphates, nitrates, nitrogen donors such as histidine, and oxygen donors like glutamate and aspartate (Bertini *et al.*, 2007). Different ligand combinations, oxygenation levels, pH, organic matter, sulfates and carbonates, generate differential metal speciation and distinct metal coordination environments. Copper's value as a bioelement lies mainly in its unique electrochemical properties. The Cu^{+}/Cu^{2+} couple has a high redox potential, which allows it to act as an electron donor/acceptor in redox reactions (Crichton and Pierre, 2001).

Copper (Cu) is a critical component of proteins involved in a variety of cellular processes. As a redox-active metal ion, Cu exists in the reduced ["Cu(I)" or "Cu^{+}"] or oxidized state ["Cu(II)" or "Cu^{2+}"](Ladomersky and Petris 2015), thereby providing a rich chemical environment for diverse biological ligands that are partners for its many structural and catalytic roles. Enzymes and proteins such as Cu, Zn superoxide dismutase, cytochrome oxidase, methane mono-oxidase, dopamine β-hydroxylase and the ethylene receptor all bind Cu as an essential ligand for their activity. Computational genome analysis for proteins with potential Cu-binding domains estimates bacterial proteomes are ~0.3% cuproproteins (Andreini et al., 2008). Furthermore, analysis of 450 bacterial genomes found 72% encode at least one putative Cu-dependent protein (Ridge et al., 2008).Despite the critical role of Cu in a wide array of biological processes, too much Cu is toxic. The antimicrobial benefits of Cu have been known for thousands of years and Cu has been used in healthcare and agriculture by many cultures. One of the earliest testimonies of Cu dates as far back as 2400 B.C. in an ancient Egyptian medical text known as the Smith Papyrus, where Cu was reported for its water and wound sterilization properties. The benefits of Cu to human health were also reported during the cholera epidemics in Paris in the 1800's, when Cu workers were found to be less susceptible to the disease (Samanovic et al. 2012).

Today, Cu continues to be used for its antimicrobial properties in plumbing (Borkow and Gabbay, 2005, Russell, P. E. (2005), and trials are underway to determine if Cu-containing surfaces can significantly reduce nosocomial infections (Casey et al., 2010; Marais et al., 2010; Mikolay et al., 2010). Human and animal studies now suggest a parallel between ancient medicinal copper use and antibacterial immune function (Chaturvedi and Henderson, 2014).

II- Material and methods

1- Bacteria:

The bacterial strains which used were :

A. *Staphlococcus aureus* isolated from body fluid.
B. *E.coli* isolated from urine.

The bacteria were from Teaching laboratory / Medical city of Baghdad. All bacteria were grown on

brain heart infusion agar and Blood agar and BHI broth for for 24h in 37c° with shaking at 180 rev min^{-1}.
$CUSO_4$. $5H_2O$ was used in all experiment in 0.0392 ppm .

2- Evaluation of Copper

Liquid culture method for Copper tests were conducted and four bacteria (*Staphlococcus aureus* and *E.coli*) were used. After stationary phase, bacterial cell density was adjusted to an optical density (O.D.=600) of 0.3 cell density was measured using a spectrophotometer and samples were diluted ten-folds. After (24h) of incubation. The cell density was measured using spectrophotometer. The concentration of Copper (0.02, 0.2, 2, 20& 200ppm) were used and growth curves of the bacteria were obtained.

3- Extraction of DNA:

The process of extraction was according to kit (**Geneaid. USA**). Measurement of the concentration and purity of DNA by using Nanodrop was done. The concentration was (1.84-1.98) mg/ml and the purity of samples were in between (1.56-1.84). (The dependable range of purity of Nanodrop is 1.8-2 the ratio of 260/280).

4- Agarose gel electrophoresis:

DNA extraction was confirmed by agarose gel electrophoresis **[**Sambrook *et al*.,1989**]**. Agarose gel was prepared by dissolving 1.5gm of agarose powder in 100 ml of TBE buffer (PH=8) in boiling water bath, allowed to cool to 50°C and ethidium bromide in the concentration of 0.5µg/ml was added. The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was poured gently into the tray, allowed to solidify at room temperature for 30 min. The comb was then removed gently from the tray. The tray was fixed in an electrophoresis chamber filled with TBE buffer that covered the surface of the gel. 3µg of loading buffer and 10µg of DNA sample was transferred into the wells in agarose gel, and to one well there is 10µg of DNA ladder. The electric current was allowed at 70 volt for 50min.The gel was removed from the tank, and the excess liquid was drained .The gel was placed in the dark room, and visualized at UV beam at 480 nanometer**.** The product band differentiated and was compared it with ladder band as a control. Image for the gel was captured by digital camera connected to imaging system.

III- Results

Fig-1 shown the growth curves and the concentration of Copper in two local pathogenes bacteria(*Staphlococcus aureus & E.coli*) .In Fig-2 the DNA fingerprinting of the two local pathogenic bacteria which treated with Copper.

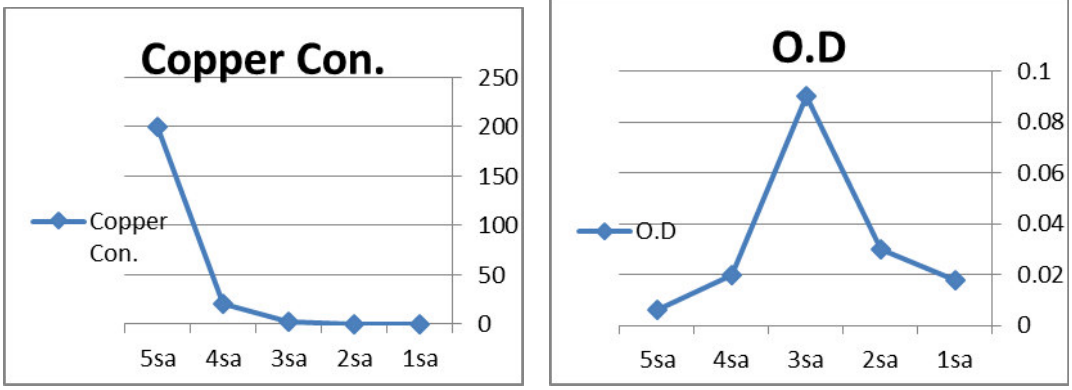

Fig-1-a) the growth curve and the concentration of Copper in *Staphlococcus aureus* isolated from body fluid.

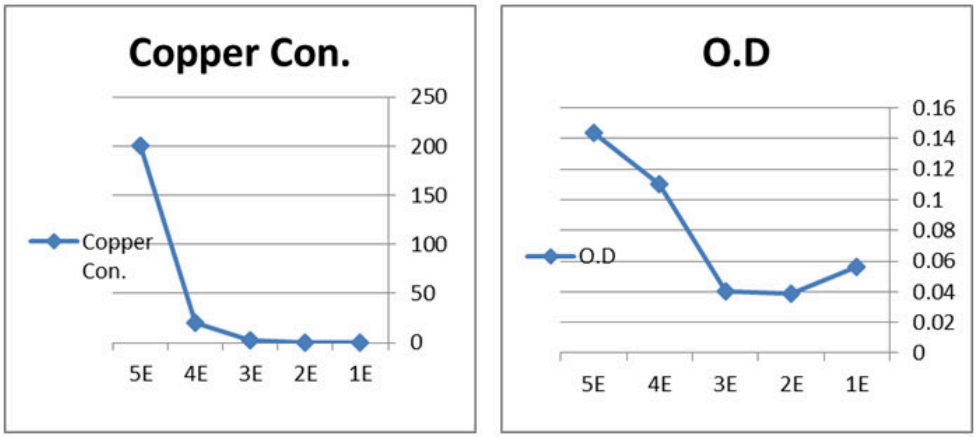

Fig-1-b) the growth curve and the concentration of Copper in *E.coli* isolated from urine.

Fig-1 (a,b,) the growth curve and the concentration of Copper of *Staphlococcus aureus(Sa)* and *E.coli*(E).

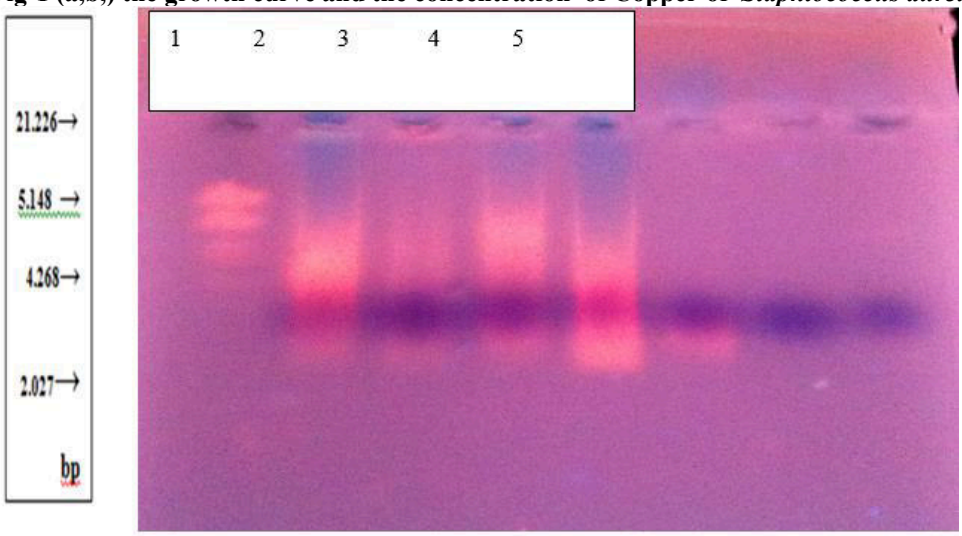

Fig-2 the DNA fingerprinting of the two local pathogenic bacteria which treated with Copper.
Lane- 1- DNA marker (Lambda DNA\EcoR+Hind 111),
Lane-2- *Staphlococcus aureus(Sa)* .
Lane-3- *Staphlococcus aureus(Sa)* treated with Copper.
Lane-4- *E.coli*(E).
Lane-5- *E.coli*(E*)* treated with Copper.

V- Discussion

The results shown when increase the concentration of Copper (0.02, 0.2, 2, 20& 200ppm) the growth of *Staphlococcus aureus (* which was local strains pathogenic bacteria) decrease and then increase to reach the highest pick in (3Sa),then decrease when increase the concentration of Copper,(Fig-1 a), but in *E. coli* ,(Fig-1 b) when increase the concentration of Copper (0.02, 0.2, 2, 20& 200ppm) the growth of *E. coli* (which also was local strains pathogenic bacteria) this may be the strain became resistance for the Copper or may be not enough to kill it. In Fig-2- the DNA fingerprinting of the two local pathogenic bacteria which treated with Copper, in *Staphlococcus aureus (Sa)* there was two bands one of them about (5.148bp) and an ather was about (4.268bp), but in *Staphlococcus aureus(Sa)* which treated with Copper the bands were disappered this because of treated with Copper . In *E.coli*(E) there was three bands were from (5.148bp) to (4.268bp), but in *E.coli*(E*)* which treated with Copper the bands were very light this may be of treated with Copper.

Copper carries out an essential role as an electron donor/ acceptor in many enzymes, but copper can also take part in Fenton-like reactions leading to the generation of hydroxyl radicals, hydrogen peroxide and superoxide, which can cause cellular damage (reviewed by Grass *et al.*, 2011). This has been generally accepted as the major mechanism for copper toxicity. However, recent experimental evidence from experiments in liquid culture shows that coppermediated ROS generation occurs largely in the periplasm of *E. coli*, so the importance of ROS generation by copper as a cellular toxicity mechanism has been under debate (Macomber *et al.*, 2007& Tina *et al.*, 2012).

The importance of ROS generation by copper as a cellular toxicity mechanism has been under debate (Macomber et al., 2007). Gram-positive bacteria lack a periplasm, and although many are tolerant to hydrogen peroxide (Solioz *et al.,* 2010), recent evidence from *Staphylococcus aureus* shows oxidative stress resistance and protein misfolding repair transcriptional responses, and hydrogen peroxide scavenging defence (Baker *et al.,* 2010). According to the Irving–Williams series, copper has a higher affinity than other first-row transition metals for ligands, and displacement of iron from iron–sulphur clusters by copper in liquid culture experiments has been reported to be an important mechanism of copper toxicity (Macomber & Imlay, 2009). There is also a role for copper and ROS in phagosome killing of bacteria (reviewed by German *et al.*, 2013). The rapid killing of bacteria on solid copper surfaces is thought to be due to cellular damage caused by very high local concentrations of copper dissolving from the surface, which causes membrane rupture, coupled with ROS generation leading to further cellular destruction, including degradation of plasmid and chromosomal DNA (Grass *et al.,* 2011).Various laboratory and clinical studies have confirmed that solid copper/copper alloy surfaces promote rapid killing of Gram-negative and Gram-positive bacteria(Hobman& Crossman , 2014).

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