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RESEARCH ARTICLE

Silver Nanoparticles Produced by Some Enteric Bacteria from Chronic Rhinosinusitis

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Abstract

Thirty five sinus samples obtained from Endoscopic patients who were suffering from chronic sinusitis in Mosul Republic hospital in Mosul city from March 2013 to March 2014 were conducted .Samples were cultured and pure isolates were identified to species level using morphological, biochemical and physiological tests. The results indicate that *Klebsiella pneumoniae* which was isolate at (13.3 %) ,one isolate for each of *Citrobacter freundii*, *Moraxella* spp.,*Serratia marcescens*, *Staphylococcus capitis*,*Staph. lugdunensis*,*Staph. saprophyticus* and *Staph. sciuri* at (6.6%) all from males samples . *Escherichia coli* at (15%) ,*Acinetobacter lwoffii*, *Proteus mirabilis* ,*Enterobacter aerogenes*,*Enterobacter cloacae*, *Serratia marcescens*, *Staph.epidermidis*, *Staph.hominis*, *Staph.warneri* and *Staph.xylosus* at (5%) all from females sampels. All isolates were completely resistant to $Ampicillin_{25}$, Augmantin(Amoxicillin + Clavulanic acid)$_{30}$,Ampiclox (Ampicillin+Cloxacillin)$_{30}$, $Amoxicillin_{25}$, $Erythromycin_{15}$, $Meropenem_{10}$, and $Carpencillin_{25}$.Isoletes give high resistance to $Cefotaxime_{10}$ and $Cephalothin_{30}$ at (95.24%), they were slightly resistant to $Amikacin_{10}$, $Gentamicin_{10}$ and $Imipenem_{10}$ at (9.52%). Finally all isolates were sensitive to $Ciprofloxacin_{10}$ and $Norfloxacin_{10}$ which were resistant to high stable active silver colloidal nanoparticle(NP) 10 ppm.Detection of some virulence factors such as urease, hemolysin, ,protease,gelatinase and lipase. Biosynthesis of silver nanoparticles was investigated in two isolates *E.coli* and *Kleb. pneumoniae* , the extracellular synthesis of AgNPS was initially detected by visual inspection of culture flask for a change in the color of cultural medium from clear to brown/green .

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Introduction

Chronic RhinoSinusitis(CRS) defined as an inflammation of the nasal cavities mucosa and the paranasal sinuses for more than 12 week(1).

CRS prompts an estimated (18 - 22) million annual office visit and 545,000 annual emergency room visit in the United States(2).The factors that may associated with CRS are:-Allergic rhinitis, Asthma, Ciliary and Immune dysfunction, defective mucociliary clearance, Host ostia patency, cystic fibrosis, anatomic abnormalities, air pollution, indoor dampness , mold exposure, Active & second hand cigarette smoking, genetic factor, gastroesophageal reflux (laryngopharyngeal reflux) and biofilm.(3). Functional endoscopic sinus surgery (FESS) has become an increasingly popular treatment for CRS (4).Advances in technology with the development of small fiber optic endoscopy computerized tomography(CT) scanning of the paranasal sinuses have allowed more direct and accurate study of sinus disease that was impossible previously(4,5).The microbiology of chronic maxillary sinusitis is polymicrobial, consisting of aerobic and anaerobic bacteria(6).Aerobic bacteria include:-

Gram positive bacteria:-*Corynebacterium* spp., *Staph. aureus*, *Staph.* coagulase-negative, *Staph. epidermidis* and *Streptococcus viridans*.

Gram negative bacteria:-*Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Haemophilus influenzae*, *Morganella morganii*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Serratia marcescens*(1).The field of nanotechnology is one of the most active areas of research in modern material science.Biological methods of synthesis have paved way for greener synthesis of nanoparticles and thus have proved to be better methods due to slower kinetics. They offer better manipulation and control over crystal growth and their stabilization(7).Bacterial synthesis of silver nanoparticles(AgNPs) is particularly attractive due to existence of well-known silver resistance machinery in few silver resistant bacterial species, thus making their study significantly important for biomedical applications (8). Moreover, silver nanoparticles have remained an attractive choice of nanomaterial because of their ability of encompassing broad application area from electronics to medicine and food technology. Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problem and can revolutionize the field of health and medicine. The new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer and fight human pathogens like bacteria. The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health therefore, there is a strong incentive to develop new bactericides (9).

Aim:-We try to detect the ability of Enteric bacteria isolates from CRS patient to produce silver nanoparticles and some of their virulence factors.

Material and Methods

Thirty five sinus samples obtained from endoscopic patients who were suffering from chronic sinusitis in Mosul Republic Hospital, Al-Rabeeh and Al-Zahrawi Hospitals in Mosul city from March 2013 to March 2014 were included. Nasal and sinus swab were collected and transferred by aims transport medium and cultured on Macconkey, blood and chocolate media then incubated at (37°C) for (24-48h.).

The isolates were identified to species level depended on morphological, biochemical and physiological tests and confirmed by Remel RapID^{TM} ONE system.

The effect of different antibiotics on the isolates in this study was studied by using disc diffusion method. the Plates were inoculated with suspensions of studied isolates of (1.5×10^{18}) cfu/ml 0.5 McFarland standard concentration, then these antibiotic discs AK_{10}, AM_{25}, AMC_{30}, APX_{30}, AX_{25}, AZM_{15}, C_{10}, CAZ_{10}, CFM_5 , CIP_{10} ,CN_{10} , CRO_{10},CTX_{10},CX_{10},DA_{10}, $DO_{10,}E_{15}$, F_{100}, IPM_{10},KF_{30}, L_{10}, ME_{10}, MEM_{10}, NA_{30}, NOR_{10}, NV_{30}, OX_{10}, P_{10U}, PRL_{30}, PY_{25}, RA_5, S_{25}, SXT_{25}(1.125/23.75), T_{30}, TE_{10}, TMP_{10}, TOB_{10}, VA_{10} were placed on the streaked plates within 15 minutes 7 antibiotics/plate. The cultures were incubated at 37°C for 24h. The results were recorded at the next day . The inhibition zone diameter around each antibiotic disc was measured by electronic digital caliper in millimeter and compared with standard inhibition zone diameter(10).The effect of colloidal silver (10 ppm) on the species in this study was studied too.

Biosynthesis of silver nanoparticles by *Escherichia coli* and *Proteus mirabilis* :-

1-Strains of *Escherichia coli* and *Proteus mirabilis* were initially grown at (37°C) for (24h.) in (500ml) without added NaCl in a shaker incubator .

2-Following bacterial growth, all the culture suspensions were incubated with aqueous 3Mm/ml and 5Mm/ml solutions of AgNO3 at (37°C) in a shaker incubator in the dark, the reactions were carried out for up to 120 h. (5 days).

3-The extracellular AgNPs were separated from bacterial cells by centrifuging aliquots of culture supernatants(1.5ml) at (3000) rpm for (6min.) at (25°C) and then analyzed by FTIR.

Fourier Transform Infrared Spectroscopy (FTIR) analysis:-

1. **The Source:** Infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample and ultimately to the detector.

2. The Interferometer: The beam enters the interferometer where the "spectral encoding" takes place. The resulting interferogram signal then exits the interferometer.

3. **The Sample:** The beam enters the sample compartment where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.

4. **The Detector:** The beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.

5. The Computer: The measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation.

Result and Discussion

The samples were collected from endoscopic patients from both sexes. The results in Table (1) showed the percentage and numbers of gram positive and negative bacteria in both sexes in different age, which indicate that the high incidence of chronic rhinosinusitis are between (10-25) years and (40-55) years of gram positive and (10-25) years of gram negative .The main pathogens in chronic rhinosinusitis include *Enterobacteriaceae* spp. The role of bacteria in chronic rhinosinusitis has been difficult to understand but it refers to resistant to many antibiotics and the formation of biofilm by many of bacterial species (11).Impact of gender on the clinical presentation of chronic rhinosinusitis indicate that several of the presenting symptoms predisposing factors and co-morbidities of chronic rhinosinusitis and paranasal sinus disease is more common among women and this agree with our results (12).Table (2) showed the percentage and number of bacterial species under study , *Klebsiella pneumoniae* were isolated at (13.3 %) , *Citrobacter freundii*, *Moraxella* spp.,*Serratia marcescens*, *Staph. capitis*,*Staph. lugdunensis*,*Staph. Saprophyticus* and *Staph. Sciuri* were at (6.6%) all from males sampels . Three showed positive for *Escherichia coli* (15%),one isolate for each of *Acinetobacter lwoffii*, ,*Enterobacter aerogenes*,*Enterobacter cloacae*, *Proteus mirabilis* ,*Serratia marcescens*, *Staph.epidermidis*, *Staph.hominis*, *Staph.warneri*, *Staph.xylosus* (5%) all from female sampels.The total percentage was similar to that obtained of (13) who stated that the percentage of *E.coli* (6.3%), *Entero. aerogenes*(1.2%), *Entero. cloacae*(2.4%), *Kleb. pneumoniae* and *Citro. freundii* (2.1%), *Proteus mirabilis*(5.1%) and *Serratia marcescens*(1.8%).The results in Table (3) showed that all bacterial species were completely resistant to AM_{25} , AMC_{30},APX_{30}, AX_{25}, E_{15}, MEM_{10}, and PY_{25}, because most of these antibiotics from penicillin group antibiotics and these bacteria producing β-lactamase enzyme which inactivate certain β-lactam antimicrobial agent .

The other antibiotics CTX_{10}, KF_{30}, CX_{10}, CAZ_{10},and CFM_5 affect against bacterial species in different ratio.

Finally all bacterial species were sensitive to CIP_{10} and NOR_{10} , Ciprofloxacin from aminoglycosides which bind to (30s) of ribosome in prokaryotes (70s) and affect on protein synthesis.

The synthesis of silver nanoparticles in the medium showed In figure (1) it was characterized by the change in color of reaction mixture from light yellow to light brown after (5) days of incubation the color intensity increased with period of incubation due to the reduction in AgO as stated in previous studies (14).

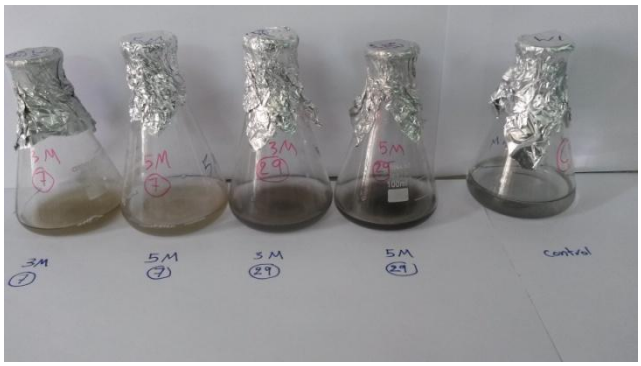
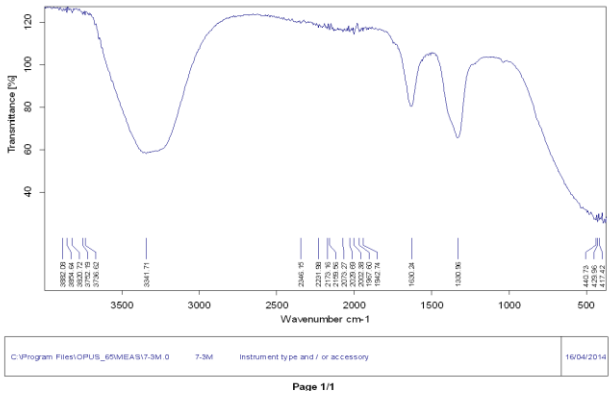


Figure 1:Medium with AgNO3(5,3Mm) and controlled sets

FTIR analysis:-

The characterization of the nanoparticles and resulting silver nanoparticles was analyzed by FTIR absorption spectra of the silver nanoparticles solution was shown in figure(2).

The absorbance bands analysis in bioreduction and absorbed in the regions in (fig.1:A &B) are 3854.64 cm-1,3736.62cm-1 and 3341.71 and the (fig.1:C &D) absorbance bands analysis in bioreduction and absorbed in the regions 3854.73cm-1, 3712.50cm-1 ,2216.44cm-1 and 2058.42cm-1assigned to O-H stretching and aldehydic C-H stretching respectively.The peak 2346.15cm-1, 2029.69cm-1 corresponds to C-N stretching of amine analysis.


Transmittance [%]
Wavenumber cm-1
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7-3M
Instrument type and / or accessory
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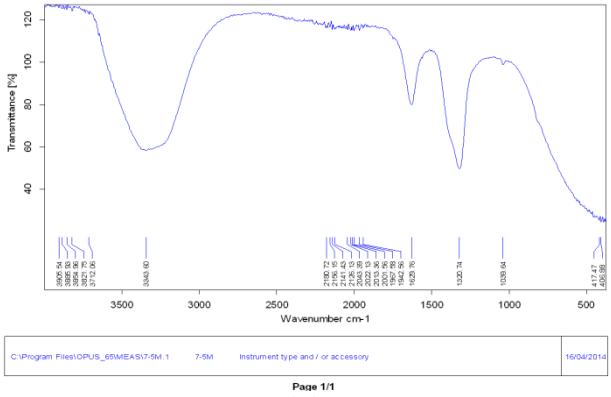
(A)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Instrument type and / or accessory
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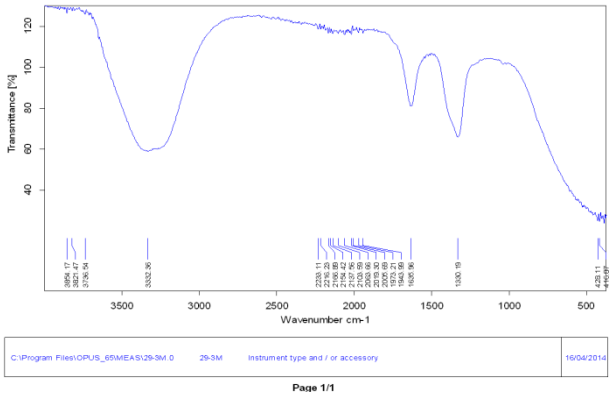
(B)


Transmittance [%]
Wavenumber cm-1
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29-3M
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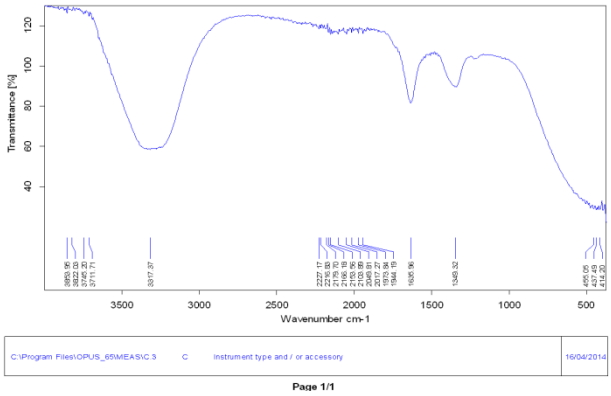
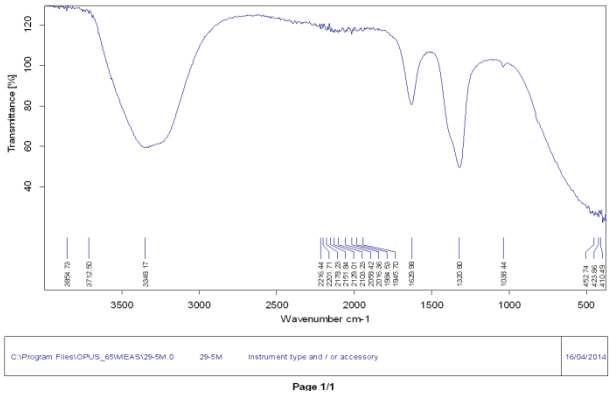
(C)



(D)



(E)

Figure2:The FTIR results (A) sample 7-3mM, (B) sample 7-5mM, (C)sample 29control. -3Mm, (D) sample 29-5mM, (E)

This suggest that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aquous medium (15).

Table(1) Percentage and numbers of gram positive and negative bacteria in both sexes in different age, under study.

Age / Sex	Gram Positive bacteria				Gram Negative bacteria				No Growth			
	10-25	25-40	40-55	55-70	10-25	25-40	40-55	55-70	10-25	25-40	40-55	55-70
Male 15(42.9)	2(13.3)	1(6.7)	1(6.7)	0(0)	4(26.7)	0(0)	2(13.3)	0(0)	1(6.7)	4(26.6)	0(0)	0(0)
Female 20(57.1)	1(5)	1(5)	2(10)	0(0)	1(5)	4(20)	2(10)	0(0)	2(10)	5(25)	1(5)	1(5)

Table(2) Percentage and number of bacterial species under study.

Bacteria isolates	Male No.(%)	Female No.(%)	Total No.(%)
Acinetobacter lwoffii	0(0)	1(5)	1(2.8)
Citrobacter freundii	1(6.6)	0(0)	1(2.8)
Enterobacter aerogenes	0(0)	1(5)	1(2.8)
Enterobacter cloacae	0(0)	1(5)	1(2.8)
Escherichia coli	0(0)	3(15)	3(8.57)
Klebsiella pneumoniae	2(13.3)	0(0)	2(5.7)
Moraxella spp.	1(6.6)	0(0)	1(2.8)
Proteus mirabilis	0(0)	1(5)	1(2.8)
Serratia marcesens	1(6.7)	1(5)	2(5.7)
Staphylococcus capitis	1(6.7)	0(0)	1(2.8)
Staph.epidermidis	0(0)	1(5)	1(2.8)
Staph.hominis	0(0)	1(5)	1(2.8)
Staph.lugdunensis	1(6.7)	0(0)	1(2.8)
Staph.saprophyticus	1(6.7)	0(0)	1(2.8)
Staph.sciuri	1(6.7)	0(0)	1(2.8)
Staph.warneri	0(0)	1(5)	1(2.8)
Staph.xylosus	0(0)	1(5)	1(2.8)
No growth	6(40)	8(40)	14(40)
Total	15(42.86)	20(57.14)	35(100)

Table(3): Percentage of sensitivity of bacterial isolates to different antibiotics .

Antibiotics / Species	AK	AM	AMC	APX	AX	AZM	C	CAZ	CFM	CIP	CN	CRO	CTX
Acinetobacter lwoffii	0	100	100	100	100	0	0	100	100	0	0	100	100
Citrobacter freundii	0	100	100	100	100	MS	0	100	100	0	0	100	100
Enterobacter aerogenes	100	100	100	100	100	MS	0	100	100	0	0	100	100
Enterobacter cloacae	0	100	100	100	100	MS	S(66.7) R(33.3)	100	MS	0	0	MS	100
Escherichia coli	0	100	100	100	100	MS (66.7) S(33.3)	S(66.7) R(33.3)	R(66.7) MS (33.3)	S,MS,R (33.33)	0	S(66.7) R(33.3)	MS (66.7) R(33.3)	100
Klebsiella pneumoniae	0	100	100	100	100	MS	0	MS	MS	0	0	MS	MS (50) R(50)
Moraxella spp.	0	100	100	100	100	MS	100	100	100	0	0	100	100
Proteus mirabilis	0	100	100	100	100	MS (50) R(50)	100	MS	0	0	0	0	100
Serratia marcesens	50	100	100	100	100	MS	MS(50) R(50)	100	S(50) R(50)	0	0	MS	100
Staphylococcus capitis	MS	100	100	100	100	100	0	100	100	0	100	100	100
Staph.epidermidis	0	100	100	100	100	100	100	100	100	0	0	100	100
Staph.hominis	MS	100	100	100	100	0	MS	100	100	0	0	100	100
Staph.lugdunensis	0	100	100	100	100	0	100	100	100	0	0	100	100
Staph.saprophyticus	MS	100	100	100	100	100	MS	100	100	0	0	100	100
Staph.sciuri	MS	100	100	100	100	100	MS	100	100	0	0	100	100
Staph.warneri	MS	100	100	100	100	100	MS	100	100	0	0	100	100
Staph.xylosus	MS	100	100	100	100	MS	MS	100	100	0	0	100	100

R:Resistant, MS:Moderate Sensitive, S: Sensitive

CX	DA	DO	E	F	IPM	KF	L	ME	MEM	NA	NOR	NV
100	100	0	100	100	0	100	100	100	100	0	0	100
100	100	0	100	100	0	100	100	100	100	100	0	100
100	100	MS	100	100	100	100	100	100	100	MS	0	100
100	100	100	100	100	MS	100	100	100	100	0	0	100
100	100	MS (33.3) R(66.7)	100	S(33.3) R(66.7)	0	100	100	MS (33.3) R(66.7)	100	S(33.3) R(66.7)	0	100
100	100	S(50) I(50)	100	100	S(50) MS (50)	100	100	100	100	0	0	100
100	100	0	100	100	0	100	100	100	100	0	0	100
100	100	100	100	100	0	100	100	100	100	MS	0	MS
100	100	100	100	100	S(50) R(50)	100	100	S(50) R(50)	100	0	0	100
0	0	0	100	0	0	100	0	MS (33.3) R(66.7)	100	100	0	0
100	100	0	100	100	0	100	100	100	100	MS	0	100
100	0	100	100	100	0	100	MS	100	100	100	0	0
100	100	0	100	100	0	100	100	100	100	100	0	100
100	0	0	100	0	0	0	0	0	100	100	0	0
0	0	0	100	MS	0	100	0	MS	100	100	0	0
0	0	0	100	100	0	100	0	0	100	100	0	0
100	0	0	100	MS	0	100	0	MS	100	100	MS	0

OX	P	PY	PRL	RA	S	SXT	T	TE	TMP	TOB	VA	Nanosilver solution
100	100	100	0	100	0	MS	100	100	100	0	100	100
100	100	100	100	100	0	0	MS	100	0	MS	100	100
100	100	100	MS	100	MS	0	MS	100	0	0	100	100
100	100	100	100	100	0	0	100	100	0	100	100	100
MS(33.3) R(66.7)	100	100	MS(33.3) R(66.7)	100	MS(33.3) R(66.7)	S(33.3) R(66.7)	100	100	S(33.3) R(66.7)	S(33.3) MS(33.3) R(33.3)	100	100
100	100	100	S(50) R(50)	100	S(50) MS(50)	0	100	100	0	MS(50) R(50)	100	100
100	100	100	0	100	0	100	100	100	100	0	100	100
100	100	100	0	100	0	MS	100	100	100	0	100	100
100	100	100	S(50) MS(50)	100	MS(50) R(50)	0	100	100	0	100	100	100
0	100	100	100	100	0	0	0	MS	MS	0	0	100
100	100	100	100	0	0	0	0	0	0	MS	0	100
100	100	100	MS	0	0	0	0	0	0	0	0	100
100	100	100	100	100	0	100	0	MS	0	0	0	100
0	100	100	100	0	0	0	0	0	0	0	0	100
0	100	100	100	0	0	0	0	0	0	0	0	100
0	100	100	100	0	MS	0	0	0	0	0	0	100
0	100	100	100	0	MS	0	0	0	0	0	0	100

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