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Synthesis and Evaluation the Activity of 1,3,4-Thiadiazole Derivatives as Antibacterial Agent Against Uncommon Bacteria Causes of Urinary Tract Infections

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ABSTRACT

Schiff base compound 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol was prepared from condensation reaction of 2-amino-5-mercapto-1,3,4-thiadiazole with benzaldehyde. New Schiff bases react with copper (II), Ferric (III), Cobalt (II) and Zinc (II) to form four complexes. The Schiff base complexes were identification by using FTIR and UV-VIS. The antibacterial activity of complexes (Copper (II) LM_1, Ferric (III) LM_2, Cobalt (II) LM_3 and Zinc (II) LM_4 complexes) were studied against *Kocuria kristinae, Alloiococcus otiti* and *Aerococcus urinae* as a model of Gram positive, *Pseudomonas stutzeri, Ochrobactrum anthropic and Pantoea agglomerans* as a model of Gram negative to determine the activity of synthesized complexes. Identification of these uncommon bacteria that isolated from urinary tract infection confirmed by using VITEK2 compact system. Several antibiotics have been chosen to investigate the ability of these isolates to resist the conventional antibiotic. The results showed higher activity of the new compounds relative to the chosen antibiotics.

Keywords: Synthesis, 1,3,4-thiadiazole, uncommon bacteria, antibiotic, antibacterial agent.

تصنيع وتقييم فعالية مشتقات 1,3,4-Thiadiazole كعامل مضاد للبكتريا غير الشائعة المسببة لالتهابات المسالك البولية

الملخص

تم تحضير مركب قاعدة شيف 5-بينزيلادين امينو-1,3,4 ثاديازول-2-ثايول (5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol) من تفاعل تكثيف 2- امينو-5 ميركابتو-1,3,4 ثاديازول 2-amino-5-mercapto-1,3,4-thiadiazole مع بينزالديهايد benzaldehyde. ولتكوين اربع معقدات جديدة تم تفاعل قواعد شيف الجديدة مع النحاس (II)، الحديد (III)، الكوبلت (II) و الزنك (II). شخصت معقدات قاعدة شيف باستعمال جهاز محول الاشعة تحت الحمراء Fourier Transform-Infrared Spectroscopy (FTIR) و جهاز المطياف الضوئي (UV-VIS) V-visible spectroscopy. ولتحديد فعالية المعقدات المصنعة تم دراسة الفعالية البيولوجية للمعقدات (النحاس (II)LM_1، الحديد (III) LM_2، الكوبلت (II) و الزنك (II)LM_4 ضد بكتريا *Kocuria kristinae*، *Alloiococcus otit* و *Aerococcus urinae* كنموذج للبكتريا الموجبة لصبغة غرام وبكتريا *Pseudomonas stutzeri*، *Ochrobactrum anthropic* و *Pantoea agglomerans* كنموذج للبكتريا السالبة لصبغة غرام. تم تأكيد تشخيص البكتريا الغير شائعة والمعزولة من التهابات المسالك البولية باستعمال LM_3

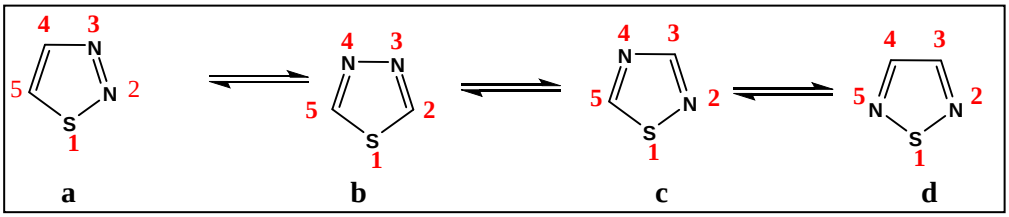
جهاز الفايتك 2. اختيرت العديد من المضادات الحيوية للتحري عن قابلية تلك العزلات على مقاومة المضادات الحيوية التقليدية. وقد اظهرت النتائج فعالية عالية للمركبات الجديدة مقارنة بالمضادات الحيوية المنتخبة.

الكلمات الدالة: تصنيع، 1,3,4-thiadiazole، بكتريا غير شائعة، مضادات حيوية، عامل مضاد بكتيري.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common and frequent medical infections and affects all ages (Hooton, 2000). Accurate diagnosis and successful treatment of infection in most cases has helped minimize secondary outcomes. UTI may occur in up to 50% of all women in their lifetimes and frequently require medication (Zafenello *et al.,* 2010). There are some bacterial species that are uncommon or infrequently exist in clinical sample such as *Kocuria kristinae* that was first described in 1974 (Kloos *et al.,* 1974). There are some reports of *K. kristinae* associated infections in patients, especially those with malignancies or other immunosuppressed states (Cheung *et al.,* 2011). *Alloiococcus otitis* has been quite difficult to isolate using conventional culture methods. On the other hand, studies have indicated that *A. otitis* has the ability to stimulate the immune system and to induce a local immune response in the middle ear cavity (Leskinen *et al.,* 2002) *Aerococcus urinae* is a newcomer in clinical and microbiological practice, first reported in 1989 and designated in 1992 (Aguirre and Collinis 1992). *A. urinae* has also been isolated from blood from patients suffering from urogenic bacterimea or septicemia with or without endocarditis (Skov *et al.,* 1995). *Pseudomonas stutzeri* that causes local and systematic infections (Grimaldi *et al.,* 2009) and *Ochrobactrum anthropi* that become increasingly recognized as a potentially problematic, opportunistic, and nosocomial pathogen (Chain *et al.,* 2011). Clinical strains of *O. anthropi* are multiresistant to common antibiotics, in particular they are usually resistant to all β-lactams except imipenem. (Nadjar *et al.,* 2001). *Pantoea agglomerans* formerly known as *Enterobacter agglomerans*, is most commonly isolated in hospitals (De Baere *et al.,* 2004).

Heterocyclic compounds possessing 1,3,4-thiadiazol ring system show antifungal, bacteriostatic and containing compounds represent an important class of heterocyclic nitrogen compounds and their derivatives are characterized with a broad spectrum of biological activity in both agrochemical (Ana *et al*., 2010) as well as anthelmintic effects. Compounds containing the above ring also exhibit anti-inflammatory, antimicrobial (Laura *et al*., 2010) properties and the depression effect on the central nervous system. In the field of archaeological conservation, amino-mercapto-1, 3, 4-thiadiazole is the most widely used corrosion inhibitor in the treatment of bronze artifacts. Schiff bases are characterized by the -N=CH-(imine) group which is important in elucidating the mechanism of transformation in biological systems. Due to great flexibility and diverse structural aspects a wide range of Schiff bases have been synthesized and their complexation behavior studied .Furthermore Schiff bases are reported to show a variety of interesting biological activities including antibacterial, antifungal, anti-mouse hepatitis virus (MHV), anticancer and herbicidal activities. It is also known that the presence of an azo moiety in different types of Schiff bases can lead them to exhibit pesticidal activities (Yasser *et al*., 2010). Both Schiff bases and azo compounds are important structures in the medicinal and pharmaceutical fields and it has been suggested that the azomethine linkage might be responsible for biological activities displayed by Schiff bases (Ispir *et al*., 2005). Furthermore, Schiff bases have five membered heterocyclic compounds show various types of biological activity among them 2,5-disubstituted 1,3,4-thiadiazoles are associated with diverse biological activities probably, due to –N=C-S- grouping (21). Thiadiazoles are five membered aromatic ring compounds with three hetero atoms .One sulfur atom and two nitrogen atoms (Georgeta *et al*., 2010). There are four isomeric types of thiadiazoles (Vasiliy *et al*., 2017): (a) 1,2,3-thiadiazole; (b) 1,3,4-thiadiazole; (c) 1,2,4-thiadiazole; and (d) 1,2,5-thiadiazole as shown scheme (1):

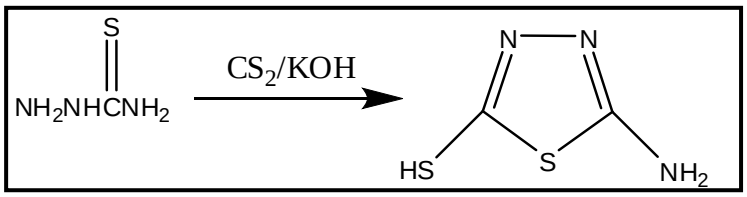


Scheme 1: Structures of Thiadiazoles isomer

MATERIAL AND METHODS

Preparation of 2-amino-5-mercapto-1,3,4-thiadiazole (Salih, 2005).

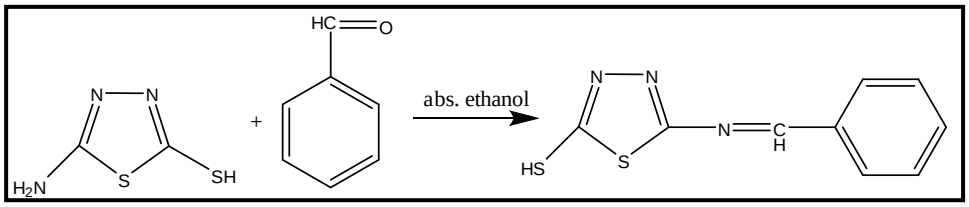
A mixture of (13.5 g , 0.1 mole) of thiosemicarbazide and (0.56 g, 0.1mole) of KOH in 70 ml absolute ethanol, to this solution (18.3 g, 0.24mole) of carbon disulfide was added drop wise. The resulting mixture was heated under reflux for 6 hours, and then was allowed to cool down to room temperature. Carefully acidified with concentration HCl to give pale yellow precipitate. The crude product was filtered and washed with cold water, re-crystallized from ethanol to give the desired product as yellow needles, melting point was reported (180-185)ºC, shown in scheme (2).



Scheme 2: preparation of 2-amino-5-mercapto-1,3,4-thiadiazole

Preparation of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol (Abdul-Jabar *et al*., 2014).

A mixture of 2-amino-5-mercapto-1,3,4-thiadiazole (1.33g,0.01mole) and benzaldehyde (0.01 mole) was dissolved in 20 ml absolute ethanol and 1-2 drops of glacial acetic acid were refluxed for (5-6) hrs. After cooling to room temperature the precipitate was filtered and dried. The product was re-crystallized from ethanol yield white crystal precipitate, melting point was reported (140-145)ºC, shown in scheme (3).



Scheme 3: Preparation of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol.

Preparation of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol complexes

All the complexes were prepared according to (Kadhim *et al*., 2014; Singh *et al*., 2015).

Copper (II) Complexes (LM_1)

Add (0.219g, 0.001mole) of 5-(benzylideneamino)-1,3,4 thiadiazole-2-thiol to alcoholic solution [$CuCl_2.2H_2O$ (0.341g, 0.002 mole) in 10 ml of absolute ethanol], then the mixture was refluxed for 3 hrs. The gray precipitate was product and then filtered and washed with absolute ethanol and dried it in room temperature.

Ferric (III) Complexes (LM_2)

Add (0.219g, 0.001mole) of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol to alcoholic solution [$FeCl_3.6H_2O$ (0.273g,0.001 mole) in 10 ml of absolute ethanol], then the mixture was refluxed for 3 hrs. The gelatinous olive precipitate was product and then filtered and washed with absolute ethanol and dried it in vacuum.

Cobalt (II) Complexes (LM_3)

Add (0.219g, 0.001 mole) of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol to alcoholic solution [$CoCl_2.6H_2O$ (0.23 g, 0.002 mole) in 10 ml of absolute ethanol], the mixture was refluxed for 3hrs. The brown precipitate was product and then filtered and washed it with absolute ethanol and dried it in room temperature.

Zinc (II) Complexes (LM_4)

Add (0.219 g, 0.001 mole) of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol to alcoholic solution [$ZnCl_2.2H_2O$ (0.237g, 0.002 mole) in 10 ml of absolute ethanol], the mixture was refluxed for 3hrs. The yellow gelatinous precipitate was product and then filtered and washed it with absolute ethanol and dried it in room temperature.

Instrument Chemical Analyzes

Infrared Spectra

The Infrared Spectra of Schiff bases compounds and complexes were recorded by using FTIR spectroscopy (Bruker, ALPHA), this analysis was carried out in the Chemistry Department of Al-Nahrain University.

UV -VIS Spectra

The UV -VIS spectra of Schiff bases compounds and complexes were recorded by using Shimadzu UV- 1650PC –Visible recoding spectro photometer, this analysis was carried out in the Chemistry Department of Al-Nahrain University.

Collection of Uncommon Pathogenic Bacterial Isolates

All uncommon pathogenic bacteria were obtained from the (Laboratory of the Department of Biology/ College of Education for Pure Sciences/ University of Samarra). The identification of bacteria was performed using VITEK2 compact system. The pathogenic bacterial isolates were cultivated on selective media in the laboratory and stained by Gram stain and some biochemical tests were done as confirmation diagnostic tests (Mahon *et al*., 2015). All the collected isolates were isolated from urinary tract infections.

Antibiotic Susceptibility Test

The disc diffusion method was used to determine antibiotic sensitivity of the isolates using the method described by Kirby-Bauer cited by (Vandepitte *et al*., 2003). The results were compared with the standard diameter of inhibition zones for each antibiotic according to (CLSI, 2007). The twenty four antibiotic discs used in this research were Meropenem, Dorepenm, Azteronam, Penciilin G, Pipracillin, Ampicillin, Amoxiclave, Cefotacxime, Ceftriaxon, Cefazolin, Cephalothin, Cefadroxil, Cefexime, Cefepem, Ceftazidime, Ciprofloxacin, Levofloxacin, Amikacin, Netilmicin, Tobramycin, Nitrofurantion, Doxicycline, Tetracycline and Trimethoprime. (Bioanalyse/ India).

Antibacterial Activity of Complexes

The antibacterial activities of synthesized complexes were evaluated against some uncommon (*Kocuria kristinae*, *Alloiococcus otiti* and *Aerococcus urinae* as gram positive) and (*Pseudomonas stutzeri, Ochrobactrum anthropic and Pantoea agglomerans* as model of gram negative) using well

diffusion method. 0.2 ml of fresh cultures of each organism was inoculated into 5 ml of sterile nutrient broth (Himedia/ India) and incubated for 3–5 h to standardize the culture to McFarland standard (1.5×10^8 CFU/ml). 0.1 ml of each culture of microorganism was spreading on Mueller Hinton Agar (Himedia/ India). Wells were made using gel puncture (6mm) according to (Egorove, 1985), then 0.1 mL of different dilutions (0.1, 0.03. 0.05 M) in case of Copper (II) complexes : (Dissolved Copper (II) complexes (0.1 g, 0.2 g and 0.3 g) in 2ml Dimethyl sulfoxide (DMSO), while the dilutions (0.1, 0.2, 0,3 M) in case of Ferric (III) complexes: (Dissolved (0.1 g, 0.2 g and 0.3 g) of Ferric (III) complexes in 2ml abs. ethanol) , Cobalt (II) complexes : (0.1 g,0.3 g and 0.5 g of Cobalt (II) complexes and dissolved in 2ml DMSO), and Zinc (II) complexes: (Add 2ml ethanol to each of (0.1 g,0.2 g and 0.3 g) Zinc (II) complexes) . The petri plates were incubated at 37 $^{\circ}$C for 24 hours in incubator during which activity was evidenced by the presence of a zone of inhibition (mm) surrounding the well.

RESULTS AND DISCUSSION

Characterization of Complexes Synthesis

5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol Schiff bases compound was used to evaluate biological activity. These complexes were analyzed using Infrared and UV-vis spectra. The FTIR spectrum for Schiff bases, showed appearance of N-H_{str}, peak at (3407) cm^{-1}, S-H_{str} band at (2594)cm^{-1}. The absorption bands at (1592)cm^{-1} due to C=N_{str}, (1367) cm^{-1} due to C=S and showed the C-S vibrational band at (944.1) cm^{-1}this property made it easily coordinating with most metals forming clonal complexes with nitrogen and sulfur atoms shown in scheme (3). the complexes showed weak vibrational bands of N-H_{str} ,S-H_{str} at same region (3379.1-3147.6) cm^{-1} respectively because of tautomerism (S–H) with (N–H) groups, while appears shifting strong sharp double band for C=N_{str} from (1477.4)cm^{-1} to (1398.3) cm^{-1}. The absorption bands at (1271-1245.9) cm^{-1} and at (944.1-696.3) cm^{-1} due to C-S_{str}, this indicated coordination of metals with C=N and S-H of Schiff bases compound, shown (Table 1).

UV-visible spectrum of Schiff bases compound (C=N) and (C=S) showed intense at (224nm) and (313nm) which refer to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transition respectively, however its complexes demonstrates shifting the absorption band at (307nm), (292nm), (272nm), (320nm) due to LM_1, LM_2, LM_3, LM_4 respectively that refer to electronic transition at $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ shown (Table 2).

Table 1: Infrared Spectroscopy (FTIR) wave number cm^{-1} for Schiff bases and complexes compound

Groups	Schiff bases compounds	LM_1	LM_2	LM_3	LM_3
S-H_{str}	2594	3379.1	3412.1	3147.6	3450.4
N-H_{str}	3407	3379.1	3412.1	3147.6	3450.4
C=N_{str}	1592.1	1477.4	1413.7	1398.3	1425.3
C=S_{str}	1367	1271	1263	1245.9	1265.2
C-S_{str}	944.1	995.2	940.3	873.7	696.3

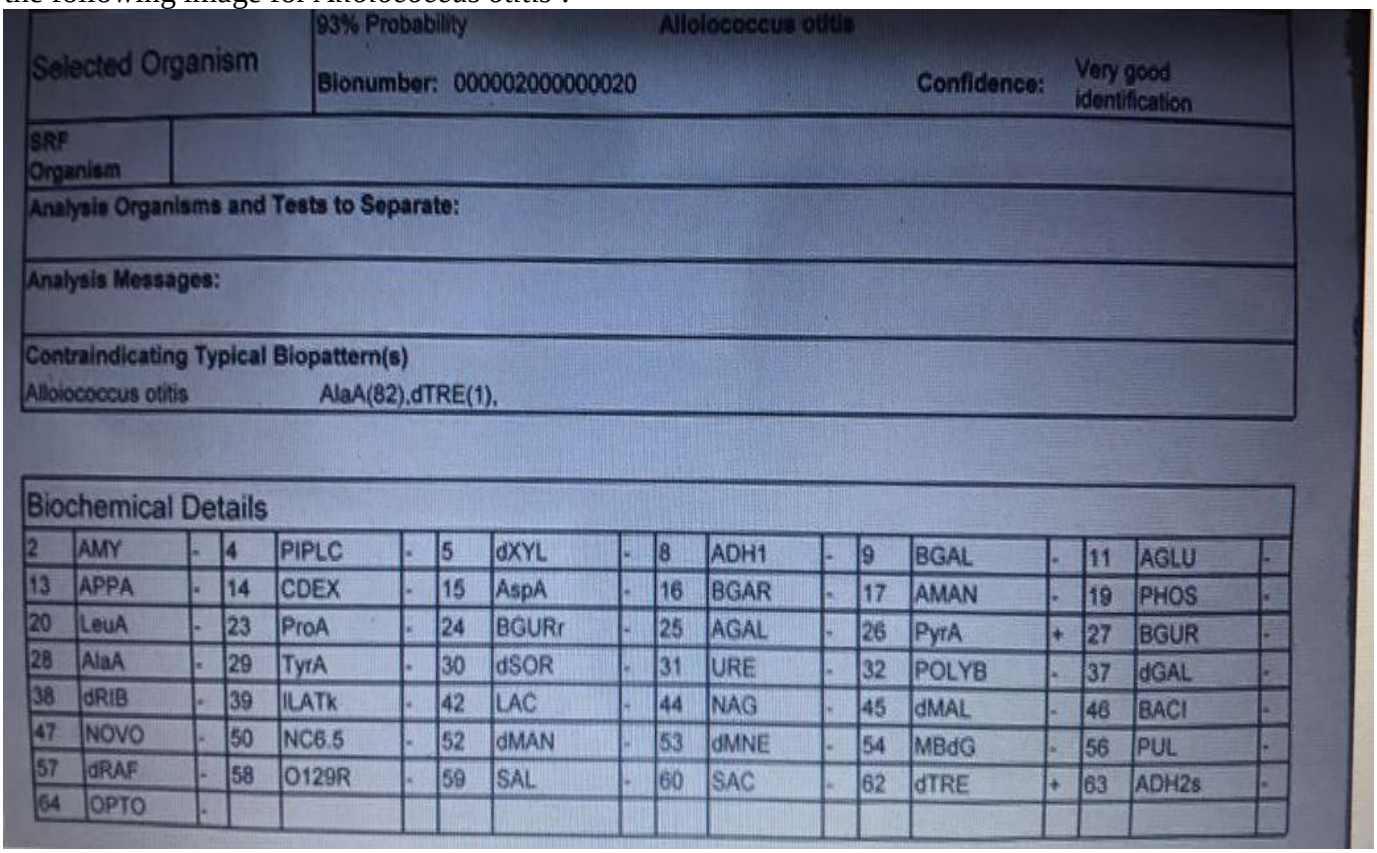
Table 2: Ultraviolet spectroscopy (UV-VIS) wave length λ nm for Schiff bases and complexes compound

Compound	wave length λ (nm)
Schiff bases compound	313
LM_1	307
LM_2	292

LM_3	272
LM_4	320

Bacterial Identification

The identification of bacteria was performed using VITEK2 compact system, as described in the following image for *Alloiococcus otitis* :



Selected Organism: 93% Probability — Alloiococcus otitis
Bionumber: 000002000000020 — Confidence: Very good identification

SRF Organism

Analysis Organisms and Tests to Separate:

Analysis Messages:

Contraindicating Typical Biopattern(s)
Alloiococcus otitis AlaA(82),dTRE(1),

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-		
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-		
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-		
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-		
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-		
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-		
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	+	63	ADH2s	-		
64	OPTO	-																	

The confirmation tests of diagnostic bacterial isolates that depends on biochemical and phenotypic properties were described in (Table 3).

Table 3: Routine identification tests and characteristics of gram negative and positive uncommon isolates

Bacterial species	Gram stain	Lactose fermenting	Pigment production	Oxidase	Catalase	Indole	Methyl red	VP	Citrate
Kocuria kristinae	G^+ ve	+	+	+	-	ND	ND	ND	ND
Alloiococcus otitis	G^+ ve	_	+	-	+	ND	ND	ND	ND
Aerococcus urinae	G^+ ve	_	-	-	-	ND	ND	ND	ND
Pseudomonas stutzeri	G^- ve	-	-	+		-	-	-	-
Ochrobactrum anthropi	G^- ve	-	-	+	+	-	-	-	-

Pantoea agglomerans	G⁻ ve	-	+	-	+	-	-	+	+

G⁻ ve: Gram negatine , G⁺ ve: Gram positive, ND: not done

Susceptibility of Uncommon Bacteria to Antibiotics

Antibiotic resistance is now generally accepted as a major public health issue. *Kocuria kristinae* was resistant to 18 (75%) antibiotics but sensitive to meropenem, doripenem, ciprofloxacin, tetilmycin, Doxicycline and tetracycline as shown in (Table 4). Also *Alloiococcus otitis* was resistant to 22(91.6%) antibiotics but it was sensitive for nitrofurantoin and netilmicin. *Aerococcus urinae* was resistant to 23(95.8%) antibiotics expect nitrofurantoin. *Pseudomonas stutzeri* was resistant to 17 (70.84%) antibiotics and it was inhibited by carbapenem and fluoroquinolones in addition to tobramycin, tetilmycin and amikacin that are aminoglycosides which prevent translation of mRNA during binding to the 30S ribosomal subunit (Kiser *et al.*, 2011). *Ochrobactrum anthropic* was resistant to 20 (83.3%) antibiotics and it was sensitive for carbapenem (meropenem, doripenem) and fluoroquinolones (levofloxacin, ciprofloxacin). Resistance to carbapenems occurs and mediated by mechanisms like the loss of outer membrane proteins and production of β-lactamase that is capable of hydrolyzing carbapenems. Resistance to all β-lactams, except imipenem, is mediated through the production of an AmpC β-lactamase, OCH-1 (membrane bound mannosyltransferase) (Romano *et al.,* 2009). On the other hand the reason for the resistance to the fluoroquinolones antibiotics is that these bacteria have a gene responsible for the resistance as well as mutations that lead to alterations in the DNA gyrase or may be due to mutations leading to the production of efficient efflux systems (Wang *et al.*,2004), while *Pantoea agglomerans* was resistant to all the antibiotics except sensitive to tetilmycin. Tetracycline group are bacteriostatic that inhibit the binding of aminoacyl-tRNA to the 30S ribosomal subunit of the bacterial ribosome. Bacteria resistance to tetracycline resistance results from the loss of bacterial outer membrane proteins, which reduces the permeability of the antibiotic into the bacteria (Brooks *et al*., 2007). All bacterial isolates were 100% resistant to the cephalosporin antibiotics. The bacterial sensitivity to the beta-lactam group is due to the ability of these antibiotic to bind to the PBPs on the bacterial cell wall, thus inhibiting the manufacture of the bacterial cell wall. The bacterial resistance to these antibiotic is due to the ability to change the target sites of the antibiotic, and the production of the beta lactamase enzymes which break the ring of Beta-lalctam, change in the permeability barrier or weaken the affinity between the antibiotic and target sites PBPs (Talaro, 2010). Results of the present study disagree completely with (Bosley *et al.,* 1995) who demonstrated intermediate levels of resistance to β-lactams, including expanded spectrum cephalosporins, and were resistant to trimethoprim sulfamethoxazole and erythromycin. Also Humphries and Hindler (2014) conducted no standardized susceptibility test methods or interpretive criteria have been proposed for *Aerococcus urinae.*

Results of the present study is correlate to the study in Erbil city by Abdullah and Barzani (2016) showed the susceptibility of some gram positive to 13 antibiotics, they found that most of the isolates were highly resistant to amoxicillin/ clavulanic acid with the percentage 81.53% while, the most effective antibiotics were imipenem with the percentage 96% for both antibiotics and also showed variable sensitivity to other antibiotics.

Table 4: resistance of bacterial isolates to antibiotics

Bacteria species / Antibiotic	Abbreviation	*K. kristinae*	*A. otitis*	*A. urinae*	*P. stutzeri*	*O. anthropi*	*P. agglomerans*
Meropenem	MEM	S	R	R	S	S	R
Dorepenm	DOR	S	R	R	S	S	R
Azteronam	ATM	R	R	R	R	R	R
Amoxiclave	AMC	R	R	R	R	R	R
Pipracillin	PRL	R	R	R	R	R	R
Penciilin G	PG	R	R	R	R	R	R
Cefadroxil	CER	R	R	R	R	R	R
Cephalothin	KF	R	R	R	R	R	R
Ampicillin	AMP	R	R	R	R	R	R
Cefazolin,	KZ	R	R	R	R	R	R
Cefotacxime	CTX	R	R	R	R	R	R
Ceftazidime	CAZ	R	R	R	R	R	R
Ceftriaxon	CRO	R	R	R	R	R	R
Cefexime	CFM	R	R	R	R	R	R
Cefepem,	CFP	R	R	R	R	R	R
Ciprofloxacin	CIP	S	R	R	S	S	R
Levofloxacin	LEV	R	R	R	S	S	R
Amikacin	AK	R	R	R	S	R	R
Tobramycin	TOB	R	R	R	S	R	R
Netilmicin	NET	S	S	R	S	R	S
Doxicycline	DO	S	R	R	R	R	R
Tetracycline	TE	S	R	R	R	R	R
Nitrofuranton	F	R	S	S	R	R	S
Trimethoprime	W	R	R	R	R	R	R

Antibacterial Activity of Complexes

The growing numbers of antimicrobial-resistant pathogens, which are increasingly associated with nosocomial infection, place a significant burden on healthcare systems and have important global economic costs (Bush and Jacoby, 2010). The complexes Cobalt (II) complexes, Ferric (III) complexes, Copper (II) complexes and Zinc (II) complexes were screened in vitro for their ability to inhibit the growth of some uncommon pathogenic bacteria. It can be observed from (Table 5) that the Cobalt (II) complexes exhibit high activity of inhibition at concentration (0.1M) against the bacterium *Kocuria kristinae* with clear zone of inhibition about 34 mm. *Alloiococcus otitis, Aerococcus urinae, Pseudomonas stutzeri* and *Ochrobactrum anthropic* with 30, 25, 20, 24 mm respectively. The zone of inhibition represent the activity of this complex on *P. agglomerans* with less value 16 mm. Ferric (III) complexes exhibit close results with Cobalt (II) complexes with the zone of inhibition (30 mm) for *K. kristinae, A. otitis, A. Urinae, O. anthropi* and *P. agglomerans.*

Whereas *P aeruginosa.* exhibit less sensitivity about 22 mm in concentration 0.3 M. Copper (II) complexes exhibited a greater activity against all the studied pathogenic bacteria compared with Cobalt (II) complexes and Ferric (III) complexes as showed in (Table 5). The highest antimicrobial activity observed against *A. urinae* with zone of inhibition about 34 mm at 0.3 M concentration compared with (30,28, 20,30, 28) for *K. kristinae, A. otitis, P. stutzeri, O. Anthropi* and *P. agglomerans* respectively at the same concentration . The results of antibacterial activity of Zinc (II) complexes in concentration 0.3 M revealed a broad spectrum of activity against all uncommom pathogens compared with other complexes as showed in (Table 5). The higher value was 40 mm of Gram positive uncommon bacteria *K. kristinae, A. otitis* and *A. urinae,* followed by 35, 36, 30 mm of *P. stutzeri, O. anthropi* and *P. agglomerans.* Fig. (1, 2, 3 and 4) showed the zone of inhibition against some pathogenic uncommon bacteria).

Several studies were investigate the effectiveness of Thiadiazoles and its derivatives on varies pathogenic microbes as a new treatment, so this results was similar to (Rehab and Eiman, 2014) who demonstrated that the [3-dicyclohexyl amino methyl -2- mercaptobenzothiazole] [E] and its derivatives have high activity against *E.coli and S.aureus.* Also our results are in consistence with (Seelam *et al.*, 2013) who synthesized N-benzylidene- 5-ptolyl-1,3,4-thiadiazole derivatives.

Table 5: Antibacterial activities of complexes

Bacteria	**Complexes**							
	con./M	**E1**	**con./M**	**E2**	**con./M**	**E3**	**con./M**	**E4**
K. kristinae	0.03	25	0.1	25	0.1	20	0.1	30
	0.05	30	0.2	28	0.2	24	0.2	35
	0.1	34	0.3	30	0.3	30	0.3	40
A. otitis	0.03	18	0.1	25	0.1	22	0.1	28
	0.05	24	0.2	28	0.2	25	0.2	30
	0.1	30	0.3	30	0.3	28	0.3	40
A. urinae	0.03	12	0.1	22	0.1	15	0.1	30
	0.05	18	0.2	28	0.2	28	0.2	36
	0.1	25	0.3	30	0.3	34	0.3	40
P. stutzeri	0.03	10	0.1	15	0.1	12	0.1	22
	0.05	15	0.2	20	0.2	15	0.2	30
	0.1	20	0.3	22	0.3	20	0.3	35
O. anthropi	0.03	16	0.1	22	0.1	12	0.1	28
	0.05	20	0.2	25	0.2	20	0.2	30
	0.1	24	0.3	30	0.3	30	0.3	36
P. agglomerans	0.03	8	0.1	22	0.1	18	0.1	22
	0.05	12	0.2	26	0.2	24	0.2	28
	0.1	16	0.3	30	0.3	28	0.3	30

E1: inhibition zone (mm) of (Cobalt (II) complexes)
E2: inhibition zone (mm) of (Ferric (III) complexes)
E3: inhibition zone (mm) of (copper (II) complexes)
E4: inhibition zone (mm) of (Zinc (II) complexes)

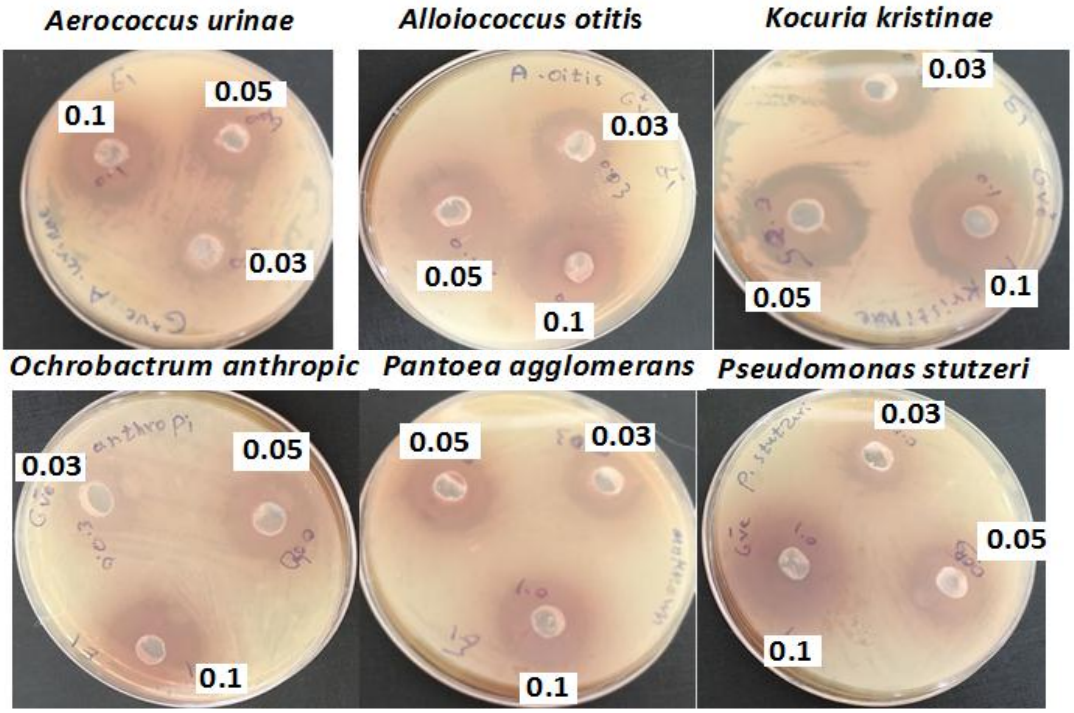


Fig. 1: The activity of Cobalt (II) **complexes on some uncommon bacteria**

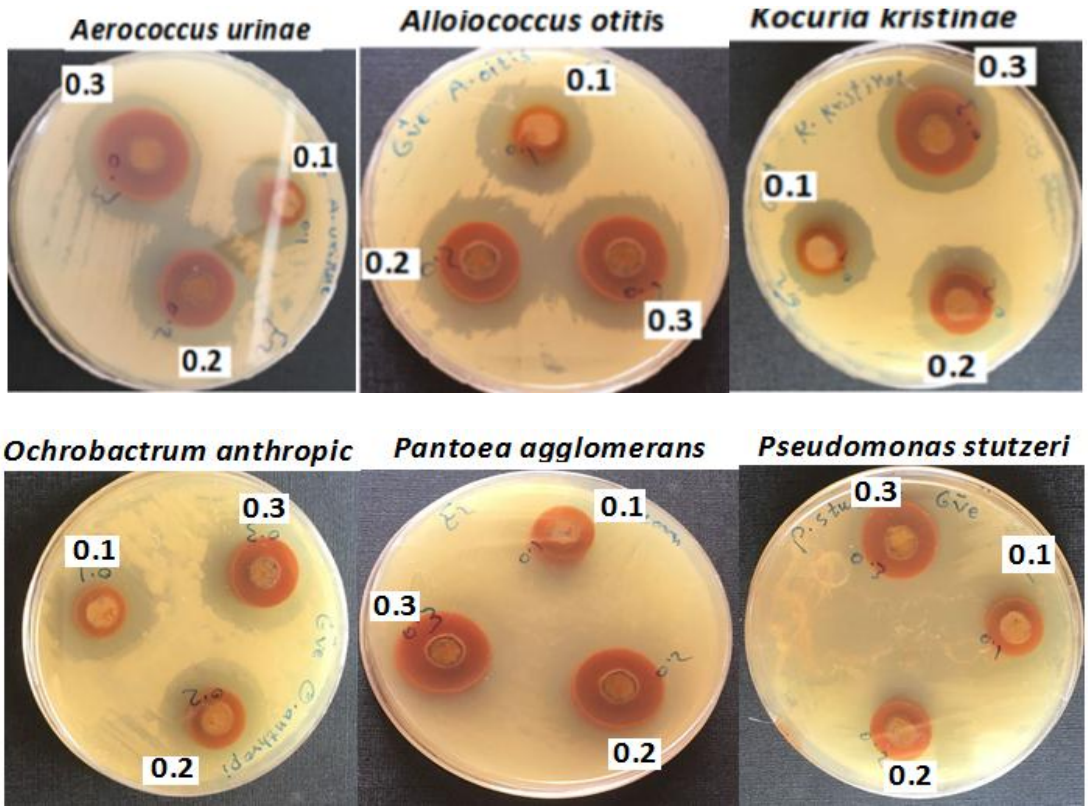


Fig. 2: The activity of Ferric (III) complexes on some uncommon bacteria

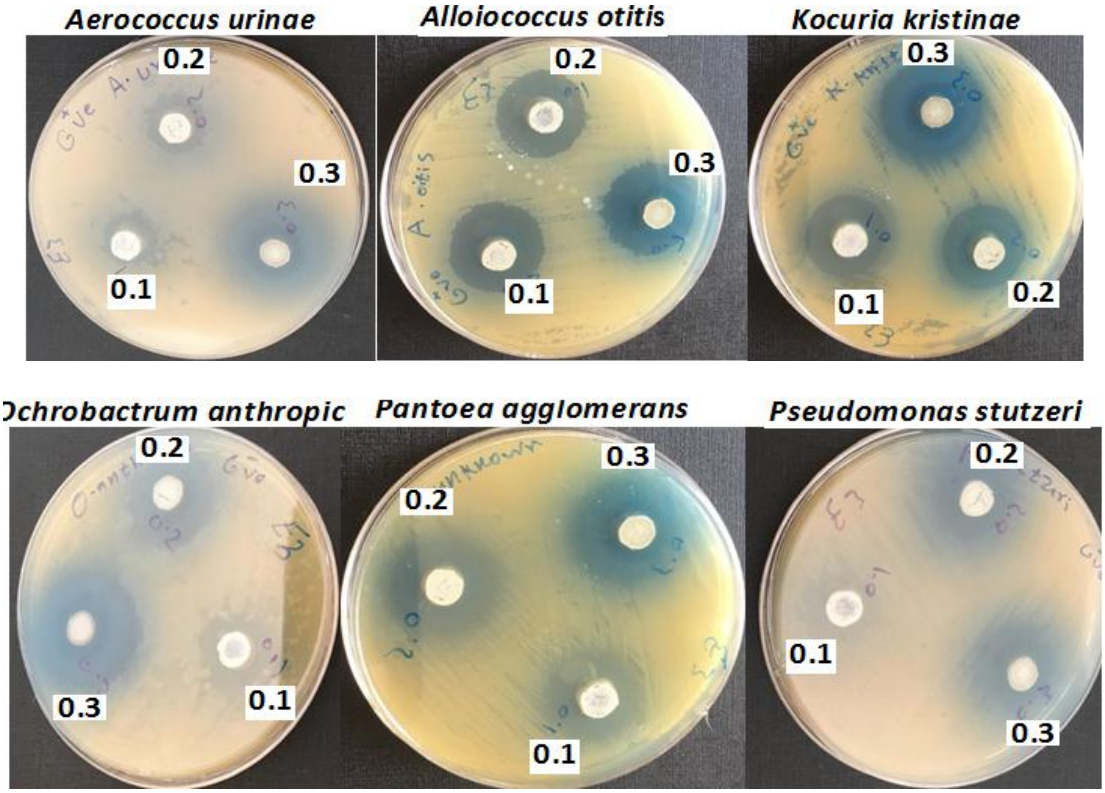


Fig. 3: The activity of Copper (II) complexes on some uncommon bacteria

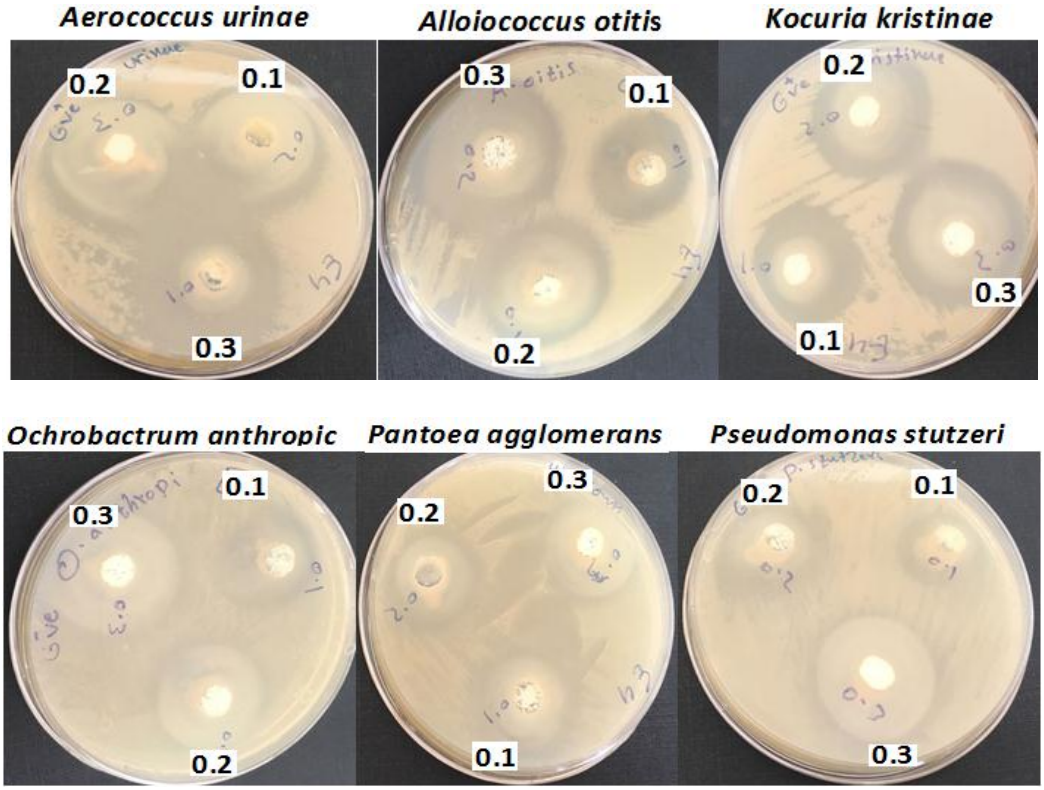


Fig. 4: The activity of Zinc (II) complexes on some uncommon bacteria

CONCLUSION

Zinc (II) complexes have the greater activity against uncommon pathogenic bacteria compared with other complexes and traditional antibiotic, followed by Copper (II) complexes which exhibit a

higher effectiveness toward Gram positive compared with Gram negative bacteria. Cobalt (II) complexes revealed moderate activity when compared with other complexes (Zinc (II) complexes and Copper (II) complexes) Ferric (III) complexes was exhibit less activity compared with other complexes, but when compared with other antibiotic this complex showed a great activity toward all the uncommon pathogenic bacteria.

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