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Synergistic effect of siderophores extract with antibiotics against drug resistant pathogenic bacteria

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Abstract
Bacterial resistance to antibiotics is a global threat that drives the continuous development of new treatment strategies due to the rapid and increasing emergence of this resistance. The current study aimed to find the synergistic effect of siderophore extract with antibiotics to overcome antibiotic resistance.
The results showed the ability of (*K. pneumonia*, *E. coli* 1, *E. coli* 2, *E. coli* 3 *S. aureus* 1, *S. aureus* 2) isolates to produce siderophore, also results showed that different siderophore extracts had a different inhibitory effect on the growth of the studied isolates. The results of synergistic effect showed that all the isolates have different responses depending on type of studied isolates as for *P. mirabilis* isolate, the results of all extracts showed clear synergy with the Trimethoprim-Sulphamethoxazol (SXT), ranging between (11-21 mm), while the synergistic results on *S. aureus* 3 was noted that Penicillin (P) showed synergy with all extracts, with inhibition zone between (10-15 mm). *E. coli* 4 showed less synergistic activity ranging between (1-8 mm).
Our results indicates the possibility of using siderophore extracts as delivering agents for antibiotic and future additional studies are required to complete extraction and purification of siderophore and direct study of their activity.

Keywords: Siderophore, antibiotic resistance, synergistic effect, *E. coli*, *S. aureus*

Introduction
The World Health Organization describes antimicrobial resistance as a natural process that happens when pathogen organism stop responding to medications that were formerly effective in treating them. Drug resistance makes infections more difficult to cure, increasing the chance of the spread of fatal infectious illnesses (Mancuso *et al*., 2021) [18]. It is unsatisfactory to define the spread of antimicrobial resistance AMR as a phenomenon brought on by the misuse of antibiotics because it is well known that AMR develops spontaneously over time through diverse pathways (Saleem *et al*., 2019; Cepas and Soto, 2020) [23, 8].
Therapeutic Synergy is defined as the phenomenon in which two compounds are combined to increase their individual efficacy. Or, it increases the inhibitory effect of antibiotics when used together with other substances (Al-Taee, 2013) [5]. And that the synergistic effect may be due to the formation of certain compounds that may become more effective in inhibiting or killing a certain type of microorganism (Ahmed *et al*., 2010) [2].
Siderophores are organic chelators with extremely low molecular weights that are highly selective for Fe (III). Organisms produce siderophores to absorb the required iron from the environment with low iron content (Cavas and Kirkiz, 2022) [7]. Siderophores are particularly helpful for avoiding drug resistance related to membrane by leveraging their iron moving capacity to deliver pharmaceuticals into cells by combining the siderophores and antibiotic employing the Trojan horse method: an antibiotic that is unable to cross the bacterial membrane barrier is coupled to a siderophore. When the siderophore-Fe (III) complex is recognized by the homologous receptor, the complex and medicine are both transported through the membrane. When the medication-siderophore complex accesses the cytoplasmic material, the pathogen may be eliminated in several ways, including drug release, overall antibacterial agent action, and blocking iron absorption (Ribeiro and Simões, 2019) [21].
The current study aimed to investigate the possibility of overcoming antibiotic resistance through the synergistic effect between bacterial siderophore extract and antibiotics against bacteria resistant to these antibiotics.

Materials and Methods
Bacterial isolates
All bacterial isolates (*E. coli* ***1***, *E. coli* 2, *E. coli* 3, *E. coli* 4, *S. aureus* 1, *S. aureus* 2, *S. aureus* 3, *K. pneumonia, P. mirabilis, E. faecalis*) which used in current study were obtained from Biology department / College of science / University of the Mosul, Iraq***.***

Extraction of siderophore
The isolates were grown in Tris-Minimal Succinate medium (TSM) prepared according to (Sebulsky *et al*., 2000) [25], Then after growth has occurred, the tubes are centrifuged by a super cooled centrifuge at a speed of 11,000 revolutions per 5 minutes, The centrifugation was repeated twice, then the filtrate is sterilized by filtering to get rid of bacterial cell residues. And the $FeCl_3$ test was performed according to (Jalal and Helm, 2017) [12] to determine the presence or absence of siderophores in the filtrate. Siderophores were extracted from the filtrate free of bacterial cells according to the (Rogers, 1973) [22].

Inhibitory effect of siderophore against the studied isolates
The method of disc diffusion on Mueller-Hinton agar medium was used in this study by preparing filter paper discs saturated with siderophore extract in concentration (100 µg / ml.) .Then the discs were fixed by sterile forceps on the surface of medium inoculated with studied isolates, the plates were incubated at 37 °C for 24 hours, then the

diameter of the inhibition zone was measured in millimeters (Khelkal *et al*., 2021) [15]. The letter in table and figure represented siderophore extract in Which A represents: Siderophore extracts from *E. coli* ***2***, B: Siderophore extracts from *E. coli* 3, C: Siderophore extracts from *S. aureus* 2, D: Siderophore extracts from *K. pneumonia*, E: Siderophore extracts from *S. aureus* 1 and F: Siderophore extracts from *E. coli* 1.

Antibiotic susceptibility test

The fallowing antibiotic was used against studied enterc bacteria (Azithromycin (AZM) 15µg/disc, Amoxicillin clavnlanatn (AMC)10/20µg/disc, Cefotaxime (CTX) 30µg/disc, Gentamicin (CN)10µg/disc, Levofloxacine (LEV) 5µg/disc, Trimethoprim-Sulphamethoxazol (SXT)1.25/23.75µg/disc, Tobramycin (TOB)10µg/disc, Cefixime (CFM)5µg/disc, Aztreonam (ATM)30µg/disc, Meropenem (MEM)10µg/disc, Ofloxacin (OFX)5µg/disc, Imipenem (IPM) 10µg/disc). while the fallowing antibiotic was used against *S. aureus* (Amickacin(AK) 10µg/disc, Bacitracin (B)10µg/disc, Clindamycin (DA)10µg/disc, Chloramphenicol (C) 10µg/disc, Erythromycin (E)10µg/disc, Gentamicin (CN)10µg/disc, Levofloxacine (LEV) 5µg/disc, Nitrofurantion (F)100µg/disc, Ofloxacin (OFX)5µg/disc, Penicillin (P)10µg/disc, Trimethoprim-Sulphamethoxazol (SXT)1.25/23.75µg/disc***)***.

Disc diffusion modified Kirby-Bauer method on Mueller-Hinton agar medium was used in this study. The Clinical and Laboratory Standards Institute (CLSI) guidelines used for interpretative the results (Neel, 2012) [19].

Synergetic test

A synergetic test was carried out for siderophores extracts with antibiotics according to (Alani and Al Nuaimi, 2020) [3]. Antibiotics discs that were resisted by the isolates were fixed by sterile forceps on the surface of medium inoculated with studied isolates, then by using a micropipette, 10 microliters of each of the siderophore extracts were added to each disc, then the plates were incubated, and the inhibition diameters were measured. The results were calculated based on that the synergistic effect is the increase in the diameter of inhibition for each of the antibiotics and siderophore extract together compared to the diameter of inhibition for each of them separately (Al-Taee, 2013) [5].

Results

The $FeCl_3$ test was used to investigate the presence of siderophore in bacteria, as the results showed the capability of (*K. pneumonia, E. coli* 1**,** *E. coli* 2, *E. coli* 3, *S. aureus* 1, *S. aureus* 2) isolates, which was inferred by the appearance of a reddish-orange color (Figure 1) by the addition of aqueous ferric chloride to the cell-free filtrate, as a result of the reaction of iron and siderophore present in the cell-free filtrate.

Fig 1: Results of $FeCl_3$ test

The inhibitory effect of siderophore

the Siderophores were extracted from 6 bacterial isolates that showed their ability to produce siderophore. After extracting, the inhibitory effect of these extracts was test against various types of bacteria, the effectiveness of siderophore extracts was evaluated by measuring the diameter of the inhibition zone, and the bacteria were considered sensitive when the diameter of the inhibition zone was greater than 8 mm (Montagna *et al*., 2019). Table (1) and figure (2) shows the results of this test. The results showed a clear variation in the sensitivity of the studied isolates towards siderophore extracts, where the different siderophore extracts had a different inhibitory effect on the growth of the isolates.

Table 1: The sensitivity of the studied bacteria to different siderophore extracts

Isolates	**siderophore extracts at a concentration of 100 mg/mL**						**Sensitivity %**
	A	**B**	**C**	**D**	**E**	**F**	
E. coli 1	S	S	S	S	R	-	66.6
E. coli 2	S	S	R	R	R	R	33.3
E. coli 3	R	-	S	R	S	S	50
E. coli 4	R	S	R	R	S	S	50
P. mirabilis	S	S	S	S	R	R	66.6
K. pneumonia	S	R	R	-	R	R	16.6
E. faecalis	S	S	S	S	R	R	66.6
S. aureus 1	S	S	R	R	-	S	50
S. aureus 2	R	R	-	R	R	S	16,6
S. aureus 3	R	R	R	R	-	R	0
for sensitivity **%**	60	60	40	30	20	40	

(-): No test was conducted, Resistant: R, Sensitive: S

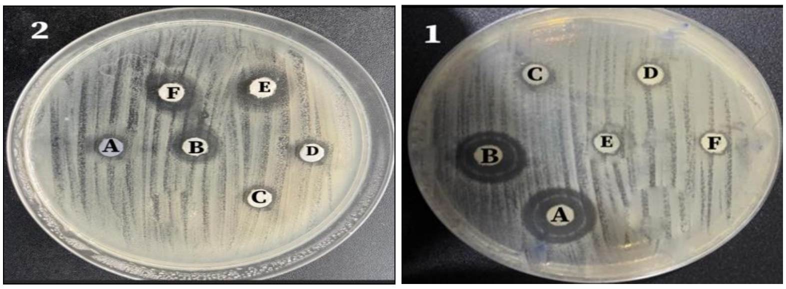

Fig 2: Inhibitory effect of siderophore extract on (1): *E. coli* 2 and (2) *E. coli* 4 in Which A represents: Siderophore extracts from *E. coli* 2, B: Siderophore extracts from *E. coli* 3, C: Siderophore extracts from *S. aureus* 2, D: Siderophore extracts from *K. pneumonia*, E: Siderophore extracts from *S. aureus* 1 and F: Siderophore extracts from *E. coli* 1.

Antibiotic sensitivity results

The sensitivity test was conducted for all the isolates under study in order to find out the prevalence of antibiotic resistance among these isolates. Tables (2), (3), and Figure (3) shows the results of this test. It was found that all intestinal bacteria had multiple resistances (MDR), except for *E.coli* 3, which was sensitive to all types of antibiotics used.

Table (2): Antibiotics sensitivity results of intestinal bacteria.

Isolates	Antibiotics												The multiple resistance%
	IPM	**AZM**	**CN**	**SXT**	**OFX**	**CTX**	**TOB**	**ATM**	**LEV**	**CFM**	**AMC**	**MEM**	
E.coli 1	S	S	R	R	R	R	R	S	R	R	S	S	58
E.coli 2	S	S	S	I	I	R	R	S	R	R	R	S	42
E.coli 3	S	S	S	S	S	I	S	S	S	S	I	S	0
E.coli 4	S	R	S	S	R	R	S	R	I	R	I	S	42
P. mirabilis	R	R	I	R	I	R	I	S	I	R	I	S	42
K. pneumonia	S	S	R	S	S	R	R	S	R	R	S	S	42
E. faecalis	S	R	I	S	R	R	R	R	R	R	R	S	67
The single resistance %	14.2	42.8	28.5	28.5	42.8	85.7	57.1	28.5	57.1	85.7	28.5	0	

Resistant: R ‹Intermediate: I ‹Sensitive: S

As for the results of the sensitivity of *S. aureus* isolates shown in Table (3), figure (3) they show that all isolates were resistant to one type of antibiotic, except for *S. aureus* 3, which was resistant to two types of antibiotics.

Table 3: Antibiotics sensitivity results of *S. aureus*

Isolates	Antibiotics												The multiple resistance%
	AK	**E**	**DA**	**F**	**B**	**C**	**CN**	**RA**	**LEV**	**OFX**	**SXT**	**P**	
S. aureus 1	I	S	S	I	S	S	S	S	S	S	S	R	8
S. aureus 2	I	S	S	I	I	S	S	S	S	S	S	R	8
S. aureus 3	I	R	S	I	S	I	S	S	S	S	S	R	17
The single resistance%	0	*33.3*	0	0	0	0	0	0	0	0	0	100	

Resistant: R ‹Intermediate: I ‹Sensitive: S

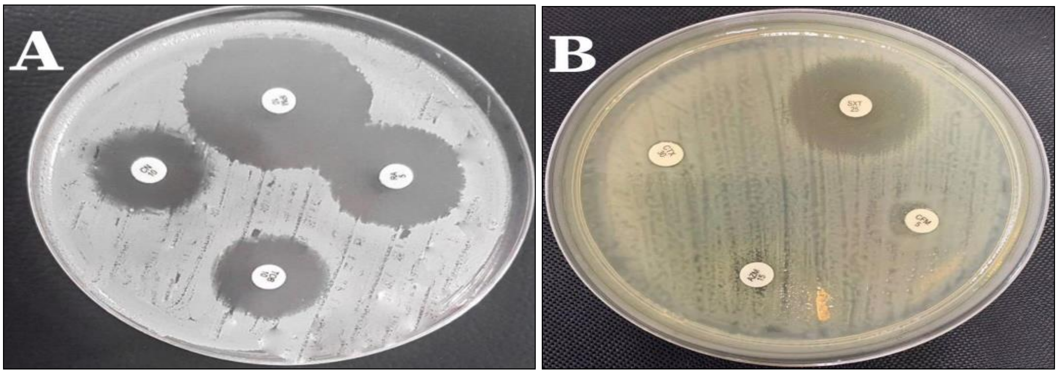

Fig 3: Models of the sensitivity of some isolates towards the studied antibiotic as (A) represent *S. aureus* 1 and (B) *E. faecalis.*

Synergistic results

The study was carrying out to find the synergistic effect between extracts of siderophore and antibiotics to which bacteria showed resistance to it. Some studies indicate that the synergistic effectiveness is important when it is equal to 5 mm or more (Adwan and Mhanna, 2008 [1]; Stefanovic and Comic, 2012) [26], and the result of this test are shown in the table (4), (5) and (6), also figure (4). The numbers fixed in the results tables of these test represents the difference in the diameter of inhibition between the synergistic result and the highest inhibition result of the extracts of siderophore or antibiotics separately.

Table 4: Synergistic effect (diameter of inhibition in mm) between extracts of siderophore and studied antibiotics in *P. mirabilis*.

Antibiotics	Siderophore extracts at a concentration of 100 mg/ml					
	A	**B**	**C**	**D**	**E**	**F**
SXT	11	21	17	*16*	*17*	*14*
CTX	-	-	-	-	-	-
AZM	3	2	8	*7*	*12*	*7*
IPM	-	-	-	-	-	-
CFM	-	-	-	-	-	-

(-): no synergistic

Table 5: Synergistic effect (diameter of inhibition in mm) between extracts of siderophore and studied antibiotics in *S. aureus* 3.

Antibiotics	Siderophore extracts at a concentration of 100 mg/ml					
	A	B	C	D	E	F
P	10	14	15	13	11	11
E	12	14	-	5	1	10

(-): no synergistic

Table 6: Synergistic effect (diameter of inhibition in mm) between extracts of siderophore and studied antibiotics in *E. coli* 2.

Antibiotics	Siderophore extracts at a concentration of 100 mg/ml					
	A	B	C	D	E	F
ATM	1	3	1	4	-	2
CFM	1	-	8	1	-	-
OFX	-	-	-	-	-	-
CTX	-	-	-	-	-	-
AZM	1	-	-	1	-	-

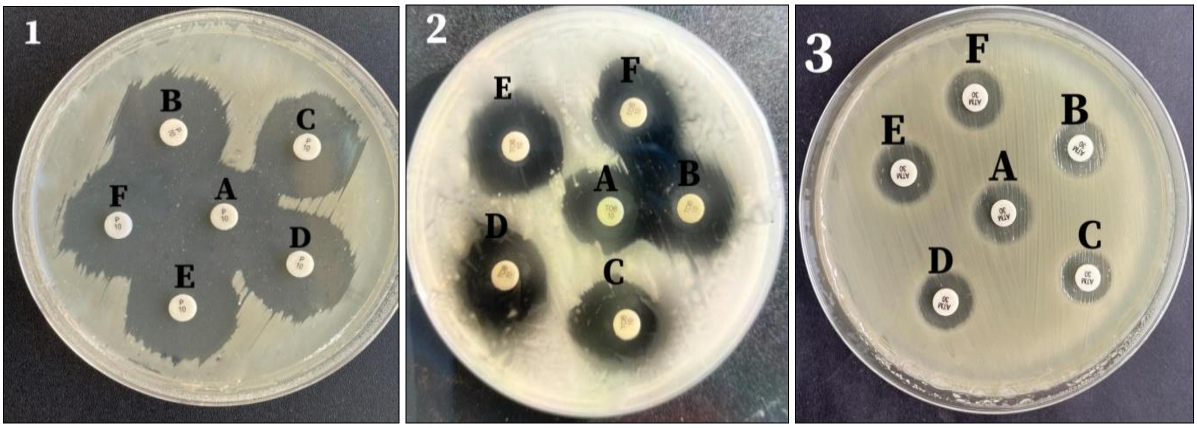

Fig 4: Synergism of siderophore extracts with antibiotic. (1): *S. aureus* 3 bacteria and penicillin antibiotic, (2): *P. mirabilis* bacteria and Tobramycin antibiotic, (3): *E. coli 4* bacteria and Aztreonam antibiotic

Discussion

We found that the isolates (*K. pneumonia, E. coli* ***1***, *S. aureus* 1, *S. aureus* 2, *E. coli* 2, *E. coli* 3) were able to thrive in TSM containing succinate which gave us first glow that it produces siderophore, which was later verified by a $FeCl_3$ test. When $FeCl_3$ was added to the culture supernatant, the color changes from yellow to orange, indicating the existence of a siderophore (Figure 1). Our findings also agree with a study by Kumar *et al*., 2021 [17], which discovered that the majority of *E. coli* strains had the ability to manufacture siderophore.

It is noted through the results shown in Table (1) that all the isolates under study were highly sensitive to more than one extract of the studied siderophore extracts, except for the two isolates *S. aureus* 2 and which was sensitive to only one types of siderophore extracts and *S. aureus* 3 which was resistance to all siderophore extracts. The results also showed a clear variation in the percentage of the inhibitory activity of the different siderophore extracts, and it was the highest in the case of each extract *E. coli* 3 and *E. coli* 2 extract with rates of (60%), while the effectiveness of the siderophore extract from *E. coli* 1 and *S. aureus* were (40%), while the rest of the extracts had lower inhibitory activity rates against the studied bacteria.

There are two hypotheses to explain the inhibitory activity of siderophore: the first it assumes that depriving bacteria of iron resulting from the binding it with siderophore leading to make the iron unavailable to bacteria and thus starving and killing bacteria, also unavailable of iron cause disruption of proteins and enzymes involved in vital functions (Gokarn and Pal, 2018; Kramer *et al*,. 2020) [11, 16].

The second hypotheses it is related to the mechanism of resistance of G^- bacteria, which includes the production of Metallo-β-lactamase (MBL) enzymes, which are a variety of enzymes and need zinc for their activity. It was found that the use of EDTA to withdraw Zn^{2+} leads to the inactivation of MBLs, which makes pathogens susceptible to beta-lactam antibiotic. Thus, it was hypothesized that a similar mechanism might be responsible for the inhibitory activity. siderophore have the ability to bind to Fe^{3+} with a high affinity and convert it to Fe^{2+}, which has a lower affinity for siderophore. Zn^{2+} has a high affinity for binding with siderophore and thus make Zn^{2+}unavailable and will inactivate MBL enzymes (Gokarn and Pal, 2018) [11].

The excessive use of antibiotics led to the spread of resistance among bacteria due to the phenomenon of natural selection (Bottery *et al*., 2021) [6]. The results in table (2) showed a clear variation in the rates of resistance of the isolates to antibiotics, and the highest rates were in the case of CFM and CTX (85.7), while the rest of antibiotic have fewer resistance rates and all isolates were sensitive to IPM.

The results in table (3) also showed a difference in the resistance ratios, as the highest was in the case of P with a rate of (100%), and for the rest of the antibiotics, the bacteria showed complete sensitivity to it.

The resistance of bacteria to antibiotics can occur through three main mechanisms, 1- changing and modifying the target site in the bacteria so antibiotics cannot bind, 2- preventing the entry of antibiotics into the cell by reducing the permeability of the membrane and also By expelling the antibiotic to the outside through pumps efflux and 3- the ability to produce enzymes that analyze antibiotics and make them ineffective (Sawa *et al*., 2020; Kakoullis *et al*., 2021) [14, 24]. Resistance genes may be transmitted vertically or horizontally. There are three basic methods of horizontal genetic transmission, which are Transduction, Conjugation and Transformation (Oladeinde *et al*., 2022) [20]. The results of this study were consistent with the results of many other studies regarding the presence of multiple antibiotic resistances in bacteria, including the study of Jalil and Al Atbee, 2022 [13], the study of Al-Nuaimi *et al*., 2022 on intestinal bacteria. As well as the study of Zhang *et al.*, 2022 [37] and the study of AL-Bakry, 2022 [4] on staphylococci.

The synergistic mechanism between different substances and antibiotics is one of the promising methods for addressing the problem of resistance. Synergistic effect is the phenomenon in which two compounds are combined to increase the individual effectiveness of each (Al- Taee, 2013) [5]. The results of this study showed, in general, that all the isolates showed different responses. In some cases, there was a synergistic effect reached (21mm).

Regarding the *P. mirabilis* isolate, the results of synergistic activity were somewhat high Table (4) and Figure (4), as all extracts showed clear synergy with the SXT antibiotic, ranging between (11-21 mm), and the majority of extracts gave Clear synergistic activity with AZM antibiotic, while no synergistic activity was observed between CTX, IPM and CFM with all siderophore extracts used. In general, bacteria need iron for a large number of vital activities, and during infection they need additional amounts of iron, so the bacteria developed several mechanisms to acquire iron, including the production of siderophore. The use of iron transport systems is a promising strategy to deliver antibiotics into bacteria, which called a Trojan horse strategy in which the bacteria are effectively tricked into killing themselves. (Dassonville-Klimpt and Sonnet, 2020) [10].

From the synergistic results of the S. aureus 3, it was found that there is a synergistic activity between most of the extracts with the antibiotics used and with high values Table (5) Figure (4), most of extracted gave Clear synergistic activity with both P and E antibiotic, while the antibiotic P showed synergy with all extracts, with diameters ranged between (10-15 mm). Many researchers studied the possibility of binding antibiotics, especially β-lactams, macrolides with siderophore, as antibiotic P belongs to the group of β-lactams. Mostly, β-lactams + siderophore were used because of their ability to cross the outer membrane of Gram-negative bacteria (Dassonville-Klimpt and Sonnet, 2020) [10]. Our results were consistent with the results of Gokarn and Pal (2018) [11], which showed that siderophore associated with antibiotics have the ability to inhibit and prevent the growth of S. aureus.

The results of the synergistic activity on E. coli 4 are shown in Table (***6***) and Figure (4), where the highest synergistic activity was between the Siderophore extracts from S. aureus 2 and CFM (8 mm). The results of this study show that some isolates showed high resistance to the effect of sidrophore+antibiotic, for example, isolate E. coli 4 showed resistance to the effect of Siderophore extracts from S. aureus 1 with all the antibiotics used, and the reason may be due to the synergistic activity may be Negatively affected by the secondary metabolites secreted by bacteria, as some enzymes secreted by bacteria may lead to the occurrence of the process of deformation of siderophore and thus affect its composition or affect its association with the antibiotic (Khelkal *et al.*, 2021) [15].

Reference

1. Adwan G, Mhanna M. Synergisticy effects of plant extracts and antibiotic on Staphylococcus aureus strains Isolated from clinical specimens. Middle–East Journals of Scientific Research,2008:3(3):134-139.
2. Ahmed Z, khan SS, khan M, Tanveer A, Lone ZA. Synergistic effect of *Salvadora Persica* extracts, tetracycline and penicillin against Staphylococcus aureus. African Journal of Basic and Applied Sciences,2010:2(1-2):25-29.
3. Alani MS, Al Nuaimi LM. Synergistic Effect of Some Natural Substances in Combination with Antibiotics on MDR Klebsiella isolates. Medico Legal Update,2020:20(3):1112-1118.
4. AL-Bakry, Amina Yarub Hameed Abdullah. Inhibition of virulence enzymes produced by *Staphylococcus aureus* isolated from different sources. Master Thesis. University of Al Mosul, Mosul, Iraq, 2022.
5. Al-Taee, Hala Zaid Najem. Bacteriological and molecular study on some Enterococcus spp. which resistant to some antibiotics isolated from different environmental sources. Master Thesis, College of Science, University of Mosul, Iraq, 2013.
6. Bottery MJ, Pitchford JW, Friman VP. Ecology and evolution of antimicrobial resistance in bacterial communities. The ISME Journal,2021:15(4):939-948. https://doi.org/10.1038/s41396-020-00832-7
7. Cavas L, Kirkiz I. Characterization of siderophores from Escherichia coli strains through genome mining tools: an antiSMASH study. AMB Express,2022:12(1):74-74. https://doi.org/10.1186/s13568-022-01421-x
8. Cepas V, Soto SM. Relationship between virulence and resistance among gram-negative bacteria. Antibiotics,2020:9(10):719. https://doi.org/10.3390/antibiotics9100719
9. Clinical, Laboratory Standards Institute. performance Standards For Antimicrobial Susceptibility Testing. M100, 32th ed. CLSI, Wayne, PA, 2022.
10. Dassonville-Klimpt A, Sonnet P. Advances in 'Trojan horse'strategies in antibiotic delivery systems. Future medicinal chemistry,2020:12(11):983-986. https://doi.org/10.4155/fmc-2020-0065
11. Gokarn K, Pal RB. Activity of siderophores against drug-resistant Gram-positive and Gram-negative bacteria. Infection and drug resistance,2018:11:61. doi: 10.2147/IDR.S148602
12. Jalal MA, van der Helm D. Isolation and spectroscopic identification of fungal siderophores. In CRC Handbook of microbial iron chelates, 2017, 235-270. CRC press. DOIhttps://doi.org/10.1201/9780203712368
13. Jalil MB, Al Atbee MYN. The prevalence of multiple drug resistance *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with urinary tract infections. *Journal of Clinical Laboratory Analysis*,2022:36(9):e24619.
14. Kakoullis L, Papachristodoulou E, Chra P, Panos G. Mechanisms of antibiotic resistance in important gram-positive and gram-negative pathogens and novel antibiotic solutions. *Antibiotics*,2021:10(4):415. https://doi.org/10.3390/antibiotics10040415
15. Khelkal IN, Risan FA, Abdol Razzaq FN, Hashim KA. Increased Antibacterial Activity of Ciprofloxacin by Combination with Staphylococcus aureus Siderophores. International Journal of Drug Delivery Technology,2021:11(2):483-487.
16. Kramer J, Özkaya Ö, Kümmerli R. Bacterial siderophores in community and host interactions. *Nature Reviews Microbiology*,2020:18(3):152-163. doi: https://doi.org/10.1038/s41579-019-0284-4

17. Kumar G, Kumar Y, Kumar G, Tahlan AK. Selection and characterization of siderophores of pathogenic *Escherichia coli* intestinal and extraintestinal isolates. Open Agriculture,2021:6(1):456-465. https://doi.org/10.1515/opag-2020-0104
18. Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: The most critical pathogens. Pathogens,2021:10(10):1310. https://doi.org/10.3390/pathogens10101310
19. Neel R. Isolation of pathogenic microorganisms from contaminated paper currency notes in circulation from different market places in Korogwe and Mombo towns in Tanzania. Journal of Microbiology and Biotechnology Research,2012:2(3):470-474.
20. Oladeinde A, Abdo Z, Zwirzitz B, Woyda R, Lakin SM, Press MO, *et al*. Litter commensal bacteria can limit the horizontal gene transfer of antimicrobial resistance to Salmonella in chickens. Applied and Environmental Microbiology,2022:88(9):e02517-21. https://doi.org/10.1128/aem.02517-21
21. Ribeiro M, Simões M. Advances in the antimicrobial and therapeutic potential of siderophores. Environmental Chemistry Letters,2019:17:1485-1494. https://doi.org/10.1007/s10311-019-00887-9
22. Rogers HJ. Iron-binding catechols and virulence in Escherichia coli. Infection and immunity,1973:7(3):445-456. DOI: https://doi.org/10.1128/iai.7.3.445-456.1973
23. Saleem M, Deters B, de la Bastide A, Korzen M. Antibiotics overuse and bacterial resistance. Annals of Microbiology and Research,2019:3(1):93.
24. Sawa T, Kooguchi K, Moriyama K. Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. Journal of intensive care,2020:8:1-13. https://doi.org/10.1186/s40560-020-0429-6
25. Sebulsky MT, Hohnstein D, Hunter MD, Heinrichs DE. Identification and characterization of a membrane permease involved in iron-hydroxamate transport in Staphylococcus aureus. Journal of bacteriology,2000:182(16):4394-4400. DOI: 10.1128/JB.182.16.4394-4400.2000
26. Stefanovic O, Comic L. Synergistic antibacterial interaction between Melissa officinalis extracts and antibiotic. Journal of Applied Pharmaceutical Science,2012:2(1):1-5.
27. Zhang H, Qin L, Jin C, Ju H, Jiang R, Li L, Lv G. Molecular Characteristics and Antibiotic Resistance of Staphylococcus aureus Isolated from Patient and Food Samples in Shijiazhuang, China. Pathogens,2022:11(11):1333. https://doi.org/10.3390/pathogens11111333