In vitro effects of deferoxamine on antibiotic susceptibility in Gram-negative bacteria

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Abstract

Background. Iron is a vital element for the growth of bacteria. Bacteria use several strategies to scavenge iron, such as siderophores, which are thought to be important virulence components. The mammalian host uses various iron-binding substances to make iron unavailable for bacterial uptake. Deferoxamine (DFO) is a semi-synthetic iron chelator that has been licensed for medical use. Iron chelators like DFO may provide an alternative therapeutic technique for treating Gram-negative bacteria infections, which frequently display multidrug resistance.

Objectives. We assumed that iron deprivation or interactions with the cell membrane caused by DFO or increased siderophore synthesis may cause the inhibition or inactivation of proteins and enzymes necessary for critical processes in bacteria. Additionally, we proposed that these bacterial alterations might be the origin of synergistic interactions between DFO and several antibiotics.

Materials and methods. To test this hypothesis, we used disc diffusion, broth microdilution and checkerboard synergy testing methods on combinations of DFO with ceftriaxone, cefepime, meropenem, amikacin, levofloxacin, and tigecycline, respectively, in a total of 55 isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Proteus mirabilis* strains – 11 isolates for each genus).

Results. No synergistic or antagonistic interactions were observed between DFO and the tested antibiotics in the *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* isolates, while the addition of DFO significantly increased the inhibition zone diameters of cefepime, amikacin, meropenem, tigecycline, and levofloxacin in *P. mirabilis* isolates. According to the checkerboard synergy results, a synergistic interaction was found between DFO and tigecycline, cefepime and amikacin for *P. mirabilis* isolates.

Conclusions. Among the investigated bacteria, a synergy between antibiotics and DFO was only discovered against *P. mirabilis*. We do not believe that this entirely disproves our hypothesis, though. The production of siderophores triggered by the increased metabolic activity of actively proliferating bacteria at the infection site may provide better results. Therefore, expanding these investigations and developing infection models through animal testing would be advantageous.

Key words: deferoxamine, iron, *Proteus mirabilis*

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Background

Antimicrobial resistance is a major public health concern and has an impact on many facets of medical practice.[1] Resistant bacterial strains pose a considerable obstacle to proper treatment, as few clinically available antibiotics maintain adequate action.[2] Gram-negative bacteria are intrinsically more resistant than Gram-positive bacteria because they have an outer membrane that acts as a permeation barrier.[3] Resistance is very common among Gram-negative organisms such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*, which are hospital-acquired infectious agents.[4]

Iron, a vital element for growth, is necessary for the activity of numerous proteins and enzymes participating in various physiological pathways such as oxygen transportation, gene regulation and nitrogen fixation.[5] In the mammalian host, the majority of intracellular iron is stored in ferritin or bound to heme or heme-containing substances, whereas extracellular iron is bound to transferrin, lactoferrin, hemopexin, and haptoglobin, making it unavailable for bacterial uptake.[6] Bacteria use several strategies to scavenge essential elements such as iron and zinc; therefore, bacteria are in a constant race with the host for micronutrients.[7] Siderophores are low-molecular weight iron binding substances that are secreted and imported by microorganisms for iron acquisition.[8] During infection with bacterial and fungal pathogens, siderophores are thought to be important virulence components.[9] Deferoxamine (DFO) is a semisynthetic drug derived from the bacterial siderophore desferrioxamine B, which has been licensed for medical use for the treatment of iron excess.[10] Iron chelators that have already withstood toxicity and preclinical testing in animals may provide an alternate therapeutic technique in the case of multidrug-resistant bacteria, where entire classes of antibiotics are no longer treatment options.[11] The siderophores may also serve as a facilitator for antibiotics to cross the cell membrane because of the increased permeability induced by iron deprivation.[12]

Objectives

We believe that iron deprivation or interactions with the cell membrane caused by DFO or increased siderophore synthesis may cause inhibition or inactivation of proteins and enzymes necessary for critical processes in bacteria, as well as exhibit synergy with several antibiotics.

Materials and methods

Bacterial isolates

According to the results of the power analysis, a total of 55 isolates were included in the study. Between May 2021 and December 2021, 11 strains of each of the following bacteria: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *Proteus mirabilis* were isolated from clinical samples that were randomly selected. While determining the isolates included in the study, resistance profiles and hospital ward or clinical sample type criteria were not used to ensure randomization. To prevent recurrence, only 1 sample from each patient was included in the study. Of the 55 isolates included in the study, 17 were recovered from the urine, 11 from blood, 10 from sputum, 8 from tracheal aspirates, 7 from wound swabs, and 2 from cerebrospinal fluid. The patients whose samples were included in the study were distributed by departments as follows: 18 from intensive care units, 12 from internal medicine clinics, 6 from pediatrics clinics, 4 from infectious diseases, 4 from urology, 2 from cardiology, 2 from neurosurgery, and 7 from other clinics. Of the isolates included in the study, 73% were susceptible to amikacin, 27% to levofloxacin, 62% to meropenem, 33% to cefepime, 40% to ceftriaxone, and 42% to tigecycline. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains. The Vitek2 (Biomerieux, Marcy-l'Étoile, France) system was used for bacterial identification.

Disc diffusion method

Using the disc diffusion method, we aimed to detect potential synergy between DFO and ceftriaxone, cefepime, meropenem, levofloxacin, amikacin, and tigecycline. Also, we aimed to evaluate changes in the resistance of bacteria to antibacterial drugs at increased iron levels, and investigate the synergistic effects of iron chelator and whether it is reversible with the addition of iron to the environment or not. *Escherichia coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *P. mirabilis* isolates kept in a skim milk storage medium at –20°C were thawed at room temperature and inoculated onto blood agar medium. Bacterial suspensions at a turbidity standard of 0.5 McFarland were prepared with the direct colony suspension method from colonies on agar plates incubated for 24 h. Inoculation was performed by spreading inoculum to the entire surface of the Mueller Hinton–Agar (MHA; Oxoid, Boston, USA) plate with a sterile swab. For each isolate, 6 MHA plates were used for different antibiotics (ceftriaxone, cefepime, meropenem, amikacin, levofloxacin, and tigecycline; Bioanalyse, Ankara, Turkey), and 4 discs were placed on these plates. Control antibiotic discs included a 10 µL antibiotic disc loaded with 10 mg/mL of DFO (Desferal; Novartis, East Hanover, USA), a 10 µL antibiotic disc loaded with ferric iron (Venofer; Vifor, St. Gallen, Switzerland), and a 10 µL antibiotic disc loaded with DFO+ferric iron. The MHA plates were incubated at 35 ±2 °C for 24 h and inhibition zones were measured. The European Antimicrobial Susceptibility Testing Committee (EUCAST) guidelines were followed for the evaluation of zone diameters.[13]

Broth microdilution method

Minimal inhibitory concentration (MIC) values of the commercially available antibacterial drugs ceftriaxone, cefepime, meropenem, levofloxacin, amikacin, and tigecycline and DFO for 55 Gram-negative isolates included in our study were determined with broth microdilution methods according to EUCAST standards (ISO 20776–1:2019). Stock solutions of ceftriaxone, cefepime, meropenem, levofloxacin, amikacin, and tigecycline (Carbosynth, Campton, UK) were prepared in accordance with the manufacturer's instructions. Water was utilized as a solvent for ceftriaxone, meropenem, levofloxacin, amikacin, and tigecycline. The solvent for cefepime was phosphate buffer (pH 6.0, 0.1 mol/L). Twofold concentrations of antibiotics and DFO ranging between 0.06 µg/mL and 64 µg/mL (from 0.06 µg/mL to 512 µg/mL for DFO) were added to microplate wells filled with cation-adjusted Mueller–Hinton broth (CAMHB). Bacterial suspension at the concentration of 5×10^5 CFU/mL was inoculated to the microplate wells. Inoculated microplates were incubated at 35 ±2°C for 24 h. The MIC was defined as the lowest antimicrobial drug concentration that inhibits the visible growth of the microorganism in the microdilution wells.

Checkerboard method

In our study, the checkerboard test, a reference method used for determining the efficacy of combinations of antimicrobial agents, was used to determine the fractional inhibitory concentration index (FIC_i) values of the combinations of DFO with ceftriaxone, cefepime, meropenem, amikacin, levofloxacin, and tigecycline antibiotics, and the results of the combinations for 55 Gram-negative bacterial isolates included in our study. In brief, serial twofold dilutions of the 1st compound (antibiotic) were performed across the columns, and serial twofold dilutions of the 2nd compound (DFO) were performed across the rows of a 96-well plate. Individual wells were inoculated with suspensions of overnight cultures in CAMHB to provide a final inoculum density of 5×10^5 CFU/mL. The plates were incubated for 24 h at 35 ±2°C. The FIC_i value takes into account the combination of antibiotics that produced the largest change from the MIC of each antibiotic. The following equation was used to quantify the interactions between the tested antibiotics (FIC_i):

$$A/MIC_A + B/MIC_B = FIC_A + FIC_B = FIC_i,$$

where A and B are the MIC of each antibiotic in combination (in a single well), and MIC_A and MIC_B are the MIC of each drug individually. If the FIC_i value was ≤0.5, it was considered synergy; values of greater than 0.5 but less than 1 were considered additive, values between 1 and 4 were interpreted as indifferent, and values >4 were considered antagonism.[14] When a MIC for one of the test compounds was off-scale (greater than the highest concentration tested), the MIC was set to the next highest twofold concentration for calculation of the FIC (e.g., if the MIC was 32 µg/mL, the FIC was calculated based on a MIC of 64 µg/mL).[15]

Statistical analyses

Statistical analysis methods were used to evaluate the differences in inhibition zone diameters. The Kolmogorov–Smirnov test and Shapiro–Wilk test were used to test the normality of the subgroups, and the Levene's test was used to evaluate the homogeneity of variance. Normality and variance homogeneity tests are presented in Supplementary Table 1. For statistical analysis, Student's t-test and Wilcoxon rank sum test were used for normal and non-normal distributions, respectively. For descriptive statistics, mean ± standard deviation (M ±SD) were used for normal data, while median, 1st quartile (Q1) and 3rd quartile (Q3) values were used for non-normal data. Statistical analysis was carried out using IBM Statistical Package for Social Sciences (SPSS) for Windows v. 24.0 (IBM Corp., Armonk, USA), and $p < 0.05$ was considered statistically significant. Prior to the study, ethical approval was obtained from Gaziantep University Clinical Research Ethics Committee (approval No. 2021/11 issued on January 27, 2021).

Table 1. Changes in the inhibition zones of antibiotics against *Proteus mirabilis* with and without deferoxamine

Isolate (n = 11)	Antibiotics	Inhibition zone diameters [mm]				Mean increase of inhibition zone diameters (95% CI) control vs. DFO	p-value	t or Z
		control (n = 11)		+DFO (n = 11)				
		mean/median	SD/Q1, Q3	mean/median	SD/Q1, Q3			
Proteus mirabilis	ceftriaxone	28	24, 29	30	28, 33	2.4 (–5.5, 10.3)	0.116[W]	–1.622
	meropenem	25	25, 26	28	28, 29	2.7 (2.0, 3.3)	**<0.001**[W]	–3.914
	amikacin	21	16, 22	29	27, 30	4.8 (2.7, 6.8)	**<0.001**[W]	–3.992
	levofloxacin	23	22, 26	28	27, 29	4.3 (1.3, 7.2)	**0.004**[W]	–2.813
	tigecycline	9.5	±0.8	26.6	±2.1	17.1 (15.6, 18.5)	**<0.001**[t]	24.559
	cefepime	18.7	±3.7	28.5	±1.8	9.8 (7.2, 12.3)	**<0.001**[t]	7.831

DFO – deferoxamine; SD – standard deviation; Q1 – 1st quartile; Q3 – 3rd quartile; 95% CI – 95% confidence interval; [W] – Wilcoxon rank sum test; [t] – t-test. The p-values in bold are statistically significant.

Results

Disc diffusion rest results

No synergistic or antagonistic interactions were observed between DFO and the antibiotics ceftriaxone, cefepime, meropenem, amikacin, tigecycline, and levofloxacin in the *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* isolates. No synergistic or antagonistic interactions were observed between DFO and ceftriaxone in *P. mirabilis* isolates, but the zone diameters of cefepime, amikacin, meropenem, tigecycline, and levofloxacin antibiotics increased statistically significantly with the addition of DFO (Table 1). The changes in the antimicrobial inhibition zone diameters of the isolates included in the study with iron supplementation, DFO supplementation and DFO+iron supplementation are shown in detail in Supplementary Table 2.

In *P. mirabilis* isolates, we observed a significant difference in inhibition zone diameters with the addition of the iron chelator. It was evaluated whether the synergistic effect observed between cefepime, amikacin, meropenem, tigecycline, and levofloxacin antibiotics and DFO was reversible by adding iron to the medium or not. The synergy between DFO and levofloxacin, cefepime, amikacin, meropenem, and tigecycline was reversed with the addition of iron to the medium, while the inhibition zone diameters decreased significantly (Table 2). In addition, significant changes were observed in the inhibition zone diameters of cefepime, meropenem, amikacin, tigecycline, and levofloxacin against *P. mirabilis* isolates in iron-rich and iron-depleted environments (Table 3).

Broth microdilution and checkerboard test results

No bacteriostatic and bactericidal effects were observed in the ranges of human therapeutic doses of DFO (3 mg/kg/day) on the strains tested in our study. Antimicrobial susceptibility results determined using the broth microdilution method and disc diffusion test results were found to be compatible.

According to the checkerboard tests, while no significant synergy was detected in *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* isolates, a synergistic interaction was found between tigecycline and cefepime antibiotics and DFO for all *P. mirabilis* isolates. In addition, a synergy between amikacin and DFO was detected in 72% of the *P. mirabilis* isolates included in the study. The results of the checkerboard synergy tests for *P. mirabilis* isolates are given in Table 4. Also, a comparison of the disc diffusion method and checkerboard synergy tests is presented

Table 2. Reversibility assay to determine whether iron supplementation eliminates the deferoxamine impact on inhibition zones in *Proteus mirabilis* isolates

Isolate (n = 11)	Antibiotic	Inhibition zone diameters [mm]				Mean increase of inhibition zone diameters (95% CI) DFO vs. DFO+iron	p-value	t or Z
		+DFO (n = 11)		DFO+iron (n = 11)				
		mean/median	SD/Q1, Q3	mean/median	SD/Q1, Q3			
Proteus mirabilis	ceftriaxone	30	28, 33	28	24, 29	−2.0 (−8.5, 4.5)	0.243[W]	−1.230
	meropenem	28	28, 29	26	26, 27	−1.7 (−2.2, 1.1)	**<0.001**[W]	−3.493
	amikacin	28.6	±1.8	24.8	±1.6	−3.8 (−5.3, −2.2)	**<0.001**[t]	5.078
	levofloxacin	28	27, 29	26	24, 27	−2.0 (−4.3, 0.3)	**<0.001**[W]	−3.607
	tigecycline	26.6	±2.1	22.8	±2.1	−3.8 (−5.6, −1.9)	**0.001**[t]	4.126
	cefepime	29	27, 30	26	24, 27	−2.7 (−4.2, −1.1)	**0.003**[W]	−2.896

DFO – deferoxamine; SD – standard deviation; Q1 – 1st quartile; Q3 – 3rd quartile; 95% CI – 95% confidence interval; [W] – Wilcoxon rank sum test; [t] – t-test. The p-values in bold are statistically significant.

Table 3. Evaluation of the activity of several different antibiotics against *Proteus mirabilis* isolates in iron-rich and iron-depleted environments

Isolate (n = 11)	Antibiotic	Inhibition zone diameters [mm]				Mean increase of inhibition zone diameters (95% CI) iron vs. DFO	p-value	t or Z
		+iron (n = 11)		+DFO (n = 11)				
		mean/median	SD/Q1, Q3	mean/median	SD/Q1, Q3			
Proteus mirabilis	ceftriaxone	26	22, 29	30	28, 33	−3.4 (−0.6, 7.4)	0.076[W]	−1.782
	meropenem	26	25, 26	28	28, 29	−2.3 (−2.8, −1.7)	**<0.001**[W]	−3.894
	amikacin	20.0	±2.5	28.6	±1.8	−8.6 (−10.4, −6.7)	**<0.001**[t]	8.898
	levofloxacin	23	22, 26	28	27, 29	−4.4 (−7.3, −1.4)	**0.004**[W]	−2.811
	tigecycline	9.5	±0.8	26.6	±2.1	−17.1 (−18.4, −15.7)	**<0.001**[t]	24.559
	cefepime	18.7	±3.3	28.5	±1.8	−9.8 (−12.0, −7.5)	**<0.001**[t]	8.439

DFO – deferoxamine; SD – standard deviation; Q1 – 1st quartile; Q3 – 3rd quartile; 95% CI – 95% confidence interval; [W] – Wilcoxon rank sum test; [t] – t-test. The p-values in bold are statistically significant.

Table 4. Broth microdilution and checkerboard synergy test results for *Proteus mirabilis* isolates

P. mirabilis isolates	DFO MIC [µg/mL]	Ceftriaxone MIC [µg/mL]	Ceftriaxone +DFO FIC_i and Int	Meropenem MIC [µg/mL]	Meropenem+ DFO FIC_i and Int	Amikacin MIC [µg/mL]	Amikacin+ DFO FIC_i and Int	Levofloxacine MIC [µg/mL]	Levofloxacine+ DFO FIC_i and Int	Tigecycline MIC [µg/mL]	Tigecycline+ DFO FIC_i and Int	Cefepime MIC [µg/mL]	Cefepime+ DFO FIC_i and Int
PM1	>512	1	0.53 indifferent	0.125	1.01 indifferent	2	0.26 synergy	1	0.53 indifferent	1	0.06 synergy	0.5	0.13 synergy
PM2	>512	0.25	1.01 indifferent	0.125	1.01 indifferent	1	0.51 indifferent	0.5	0.26 synergy	1	0.06 synergy	2	0.07 synergy
PM3	>512	0.5	0.51 indifferent	0.125	1.01 indifferent	1	0.13 synergy	0.125	1.01 indifferent	1	0.06 synergy	0.25	0.26 synergy
PM4	>512	0.25	1.01 indifferent	0.125	1.01 indifferent	0.5	0.13 synergy	2	1.12 indifferent	1	0.06 synergy	0.5	0.13 synergy
PM5	>512	0.25	1.01 indifferent	0.125	1.01 indifferent	1	0.51 indifferent	0.5	2.06 indifferent	1	0.06 synergy	1	0.13 synergy
PM6	>512	16	0.98 indifferent	0.125	1.01 indifferent	1	0.26 synergy	0.5	1.03 indifferent	1	0.06 synergy	4	0.26 synergy
PM7	>512	0.25	1.01 indifferent	0.125	1.01 indifferent	0.5	0.13 synergy	4	0.62 indifferent	1	0.06 synergy	0.5	0.13 synergy
PM8	>512	0.25	1.01 indifferent	0.125	1.01 indifferent	0.25	0.26 synergy	4	0.62 indifferent	2	0.03 synergy	0.25	0.26 synergy
PM9	>512	0.25	1.01 indifferent	0.125	1.01 indifferent	0.5	0.26 synergy	0.125	1.01 indifferent	1	0.06 synergy	0.5	0.13 synergy
PM10	>512	0.25	1.01 indifferent	0.125	1.01 indifferent	0.5	0.26 synergy	0.125	1.01 indifferent	2	0.03 synergy	16	0.13 synergy
PM11	>512	0.25	1.01 indifferent	0.125	1.01 indifferent	0.5	0.51 indifferent	1	0.53 indifferent	1	0.06 synergy	0.25	0.26 synergy

PM – *Proteus mirabilis*; Int – interpretation; DFO – deferoxamine; FIC_i – fractional inhibitory concentration index.

Table 5. Comparison of synergy testing with disc diffusion and checkerboard method

Antimicrobial agent	Synergy	
	checkerboard method	disc diffusion[a]
Tigecycline	+	+
Cefepime	+	+
Ceftriaxone	–	–
Amikacin	+*	+
Meropenem	–	+
Levofloxacine	–	+

* synergy was detected in 72% of the isolates; [a] synergy was defined as statistically significant increase in inhibition zone diameters.

in Table 5. Broth microdilution and checkerboard assay results for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* were not included in the paper due to negative results, but are available on request.

Discussion

A thorough understanding of host–bacteria relationships during Gram-negative bacterial infections can strengthen our dwindling arsenal of traditional antibiotics with new strategies.[16] Sequestration of iron by chelation may be a beneficial adjunct for the treatment of infections, given the relationship between iron excess or dietary iron supplementation and infection.[17] Excess iron has been shown to aggravate the condition of the patient in various infections, including tuberculosis, malaria, invasive bacterial infections, cystitis, keratitis, and wound infections.[18] We also observed that the in vitro bacterial activity was higher in an iron-rich environment since the inhibition zone diameters were lower when compared with inhibition zone diameters in an iron-depleted environment (Table 3). The overabundance of iron is hazardous to the host not just because of enhanced bacterial growth, but by inducing increased inflammatory activity and epithelial cell stress due to lysosomal damage.[19] Iron is known to catalyze Fenton reactions, which generate highly reactive hydroxyl radicals that can compromise lysosomal membrane integrity, leading to the release of hydrolases and redox-active iron into the cytosol, and subsequent injury or cell death.[20] Similarly, lysosomal dysfunction induced by iron overload causes chronic liver injury through hepatocellular apoptosis, hepatic inflammation and liver fibrosis in mice fed with an iron-rich diet.[21] The DFO, as an iron chelator, functions in aiding the host's intrinsic iron-withholding systems, and appears to be a promising treatment option for local

infections.[12] Iron chelation with DFO improved host cell survival, reduced bacterial proliferation in urothelial cells and reduced autophagy.[19] We have hypothesized that iron limitation conditions may result in increased production of siderophores, specific molecules for transporting iron. Siderophore secretion has the physical outcome of allowing molecules to diffuse away from producers, possibly preventing benefits from being returned to producer cells. Diffusion can still result in significant siderophore loss, putting bacterial fitness at risk.[22] Conformational changes in the outer membrane of the bacteria during both increased secretion and uptake of siderophores may be responsible for vulnerabilities against antimicrobial activity. Moreover, under low iron concentrations, several physiological changes may occur in the bacterial pathogens, including a shift to a planktonic state.[23,24] Bacteria in the planktonic state are known to be more susceptible to certain antimicrobials, suggesting a potential mechanism of iron chelation-induced sensitization to antimicrobials.[1] Because of the increased permeability induced by iron deprivation, siderophores may potentially serve as a facilitator for antibiotics across the cell membrane. Similarly, deprivation of iron reduces the activity of key proteins and enzymes such as cytochromes, which are examples of iron-dependent proteins that are crucial for energy metabolism, and ribonucleotide reductase, which is involved in DNA synthesis. If any of these get disrupted, the multiplication of the microorganism may be halted.[12] Our reversibility assay to determine if iron supplementation decreases the synergistic interaction between DFO and antibiotics revealed that iron supplementation significantly altered DFO's synergistic interaction with all antibiotics tested (Table 2). Therefore, a longer period may be required for permanent changes at a cellular level.

Previous reports revealed that *P. mirabilis* lacks detectable siderophore production.[25,26] The absence of effective siderophores may explain the differences in *P. mirabilis* isolates in our study. Consistent with our study, in the study conducted by Marcelis et al., *Proteus* were the most susceptible bacteria to ethylenediamine-di-ortho-hydroxyphenylacetic acid (EDDA), a synthetic iron chelator, among Enterobacterales.[26] Based on these findings, we could hypothesize that bacteria incapable of producing effective siderophores will be potential targets for iron restriction and iron chelation therapy. Traditionally, siderophore production, or efficient siderophore production, has been considered to be characteristic of aerobic Gram-negative bacteria.[27] Anammox bacteria, which oxidize ammonium with nitrite as the terminal electron acceptor in the absence of oxygen, are anaerobic Gram-negative microorganisms within the phylum of planctomycetes and do not possess genes required for siderophore synthesis.[28,29] Some researchers explain the poor efficacy of siderophore-conjugated antibacterial agents against Gram-positive bacteria with a lack of effective siderophore synthesis.[30] Certain Gram-positive bacteria, such as *Staphylococcus lugdunensis* and *Streptococcus pyogenes*, have been demonstrated to lack endogenous siderophore synthesis.[31,32] However, it is known that some Gram-positive bacteria can produce siderophores, such as staphyloferrins, bacillibactin and corynebactin.[33] Therefore, a species-level examination would be more useful than a general approach in determining iron chelation therapy targets. Furthermore, genetic modifications affect bacteria's ability to produce siderophores as well as their ability to thrive under iron-restricted conditions.[34] Therefore, it may be beneficial to identify genetic modifications at the species level in bacteria for which iron chelation therapy will be preferred in the near future.

Limitations of the study

For the strains included in the study, pulse-field gel electrophoresis may be accompanied by clone analysis, but our resources were limited. The disc diffusion method was unreliable because we lacked the knowledge of whether the disk was saturated with the desired amount of the tested compound. Furthermore, one of the limitations of our study is the inability to compare various bacterial species that lack the ability to produce siderophores.

Conclusions

No synergy was found between antibiotics and DFO against tested microorganisms other than *P. mirabilis*. However, we think that this does not completely rule out our hypothesis. Increased metabolic activities of actively growing bacteria at the site of infection may induce the synthesis of siderophores. We think that it would be beneficial to expand these studies and create infection models with animal experiments.

Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.8132064. The package contains the following files:

Supplementary Table 1. Tests of normality and variance homogeneity test for disc diffusion results.

Supplementary Table 2. Comparison of the changes in the inhibition zones of the antibiotics with iron supplementation and iron restriction.

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