International Journal of Research in Medical Sciences
Abhirami et al. Int J Res Med Sci. 2025 Dec;13(12):5353-5360
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20253963

Original Research Article

Exploring Deferoxamine-B for resensitization and antimicrobial resistance mitigation in drug resistant *Klebsiella pneumoniae* and *Staphylococcus aureus*: an *in vitro* study

Abhirami[1], Arundhathi Satheesh[1], Adheena Joemon[1], Sajmi Sathar[1], Silpa Kannanpallil Narayanan[2], Harish Kumar Kaleeckal Sadanandan[1]*

[1]Department of Medical Microbiology, School of Medical Education, Centre for Professional and Advanced Studies, Kottayam, Kerala, India
[2]Department of Microbiology, St. Mary's Hospital, Thodupuzha, Kerala, India

Received: 10 October 2025
Revised: 13 November 2025
Accepted: 19 November 2025

***Correspondence:**
Dr. Harish Kumar Kaleeckal Sadanandan,
E-mail: drharishkumarks@gmail.com

ABSTRACT

Background: The rising incidence of antimicrobial resistance (AMR) in pathogens like *Klebsiella pneumoniae* and *Staphylococcus aureus* is a major global concern. Resistance mechanisms such as extended spectrum β-lactamases (ESBLs), metallo β-lactamases (MBLs), and methicillin resistance limit treatment options. Iron-chelating siderophores like DFO-B may disrupt bacterial iron metabolism and potentially resensitize resistant strains to antibiotics.
Methods: This *in vitro* cross-sectional study assessed the adjunctive effect of DFO-B with selected antibiotics against clinical isolates of MDR *K. pneumoniae* and *S. aureus*. Isolates were identified, and antibiotic susceptibility was determined using disc diffusion and micro broth dilution. Synergy between DFO-B and antibiotics was evaluated by disc diffusion enhancement and MIC reduction assays.
Results: Among 100 *K. pneumoniae* isolates, 54% were multidrug-resistant and 46% extensively drug-resistant; 83% produced ESBLs and 70% showed MBL activity, with high resistance to third-generation cephalosporins. DFO-B alone showed no antibacterial effect and did not enhance antibiotic activity, displaying indifferent interactions and mild antagonism with imipenem in ESBL producers. Among 128 *S. aureus* isolates, tigecycline showed 100% susceptibility in both MSSA and MRSA, with no additional effect from DFO-B. Cefdinir and ampicillin showed higher resistance in MRSA, but their activity improved in the presence of DFO-B, with cefdinir showing a marked increase in MRSA susceptibility and ampicillin showing modest improvement.
Conclusion: DFO-B did not enhance antibiotic activity against MDR *K. pneumoniae*, but it showed some potential to increase susceptibility in selected *S. aureus* strains. These results highlight the complex role of siderophore-mediated iron chelation in AMR and the need for further research to optimize such combination therapies.

Keywords: *Klebsiella pneumoniae*, *Staphylococcus aureus*, AMR, MDR, XDR, ESBL, MBL, Siderophores, Deferoxamine-B

INTRODUCTION

The world is steadily approaching a post-antibiotic era, where once-treatable infections by pathogens such as *Klebsiella pneumoniae* and *Staphylococcus aureus* could again become life-threatening. The alarming rise in multi-drug resistant (MDR) strains driven by mechanisms such as β-lactamase production, altered drug targets, and plasmid-mediated resistance has rendered many conventional antibiotics ineffective.[1] Without effective therapeutic options, common surgical procedures, routine medical care, and treatment of minor infections could carry

substantial risk, echoing the pre-antibiotic mortality rates of the early 20th century. This growing crisis threatens not only individual patient outcomes but global health security, underscoring the urgent need for innovative antimicrobial strategies before the medical world loses one of its most fundamental pillars of treatment.[2] The increasing prevalence of antimicrobial resistance among major bacterial pathogens such as *K. pneumoniae* and *S. aureus* poses a significant threat to global public health.[3] *K. pneumoniae* has emerged as a major MDR organism owing to the production of extended-spectrum β-lactamases (ESBLs), carbapenemase resistant *K. pneumoniae* (CRKP), and the presence of plasmid-mediated resistance determinants.[4] Similarly, *S. aureus* has developed MDR through mechanisms including β-lactamase production, alteration of penicillin-binding proteins, and acquisition of the mecA gene leading to methicillin resistance.[5] The adaptability of these organisms not only limits therapeutic options but also contributes to increased morbidity, mortality, and treatment costs, emphasizing the urgent need for novel antimicrobial strategies.

As iron is a vital element for the growth of bacteria, iron-chelating agents (siderophores) can be used to arrest their multiplication.[6] Deferoxamine-B (DFO-B), a naturally occurring tri-hydroxamate siderophore produced by *Streptomyces pilosus*, is clinically used as an iron-chelating agent for the treatment of iron overload conditions like hemochromatosis and thalassemia.[7] While DFO-B primary clinical application is not as an antimicrobial, its ability to interfere with bacterial iron metabolism offers a unique avenue for repurposing or investigating its anti-virulence properties.[8] This study evaluated siderophore DFO-B as adjunct with antibiotics against common clinically encountered drug resistant pathogens- *K. pneumoniae* (ESBL and metallo-β-lactamase (MBL)- positive) and *S. aureus* (methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin – susceptible *Staphylococcus aureus* (MSSA)) using micro broth dilution method.

METHODS

The present cross-sectional study was conducted at the School of Medical Education (SME), Kottayam, Kerala, India from June 2024 to June 2025. During this period, study isolates of *K. pneumoniae* and *S. aureus* were collected from St. Mary`s Hospital, Thodupuzha, Kerala, India. All *K. pneumoniae* and *S. aureus* isolates which were deemed clinically significant were included in the study. Isolates that did not show clinically significance were excluded from the study.

Identification of isolates and antimicrobial susceptibility testing

All the isolates identified by routine biochemical tests and antimicrobial susceptibility testing was done as prescribed by Clinical Laboratory Standards Institute (CLSI) guidelines M02-A13. Antibiotics tested for both *K. pneumoniae* and *S. aureus* included Ciprofloxacin (5 µg), Gentamicin (10 µg), Tetracycline (30 µg), Cefoxitin (30 µg) and Cotrimoxazole (25 µg). Amoxicillin/clavulanate (20/10 µg), Cefuroxime (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Cefepime (30 µg), Amikacin (30 µg), Imipenem (10 µg), Aztreonam (30 µg), Cefoperazone/ sulbactam (75/30 µg), Meropenem (10 µg), Piperacillin-tazobactam (100/10 µg), Ceftazidime-avibactam (30/20 µg), Tigecycline (30 µg) was only for *K. pneumoniae*, while Vancomycin (10 µg) erythromycin (15 µg) clindamycin (2 µg) penicillin (10 µg) linezolid (30 µg) and nitrofurantoin (300 µg) was tested for *S. aureus*. Based on the recommendations of the Centre for Disease Control and Prevention (CDC) and European Centre for Disease Prevention and Control (ECDC), isolates were termed as MDR which are non-susceptible to at least one agent in three or more antimicrobial categories. XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (bacterial isolates remain susceptible to only one or two categories). Non-MDR (non-multidrug-resistant) is defined as susceptibility to all the agents in all antimicrobial categories. Methicillin resistance in *S. aureus*, ESBL and carbapenemase producing *K. pneumoniae* was detected by the guidelines of CLSI M100^{TM} 34th edition.[9] All culture media, reagents and antibiotic disc was purchased from Hi-Media laboratories Pvt. Ltd India and Deferoxamine-B from Novartis, India.

Antibiotic/DFO-B disc diffusion synergy test for CRKP, ESBL-KP, MRSA and MSSA

The antibiotic siderophore disc diffusion test was done as per Gokarna and Pal with modification.[10] Briefly for ESBL-KP and CRKP, ampicillin (10 µg), imipenem (10 µg) and meropenem (10 µg) discs were used. For MRSA, ampicillin (10 µg), tigecycline (30 µg) and cefdinir (30 µg) discs were used. On each antibiotic disc, 10 µl of 10 mg/ml DFO-B solution was loaded. As a negative control, 10 µl of sterile distilled water was loaded on the antibiotic discs. Culture suspensions of CRKP, ESBL-KP, MRSA and MSSA isolates adjusted to 0.5 McFarland standard were lawn cultured on to MHA plate. The sterile antibiotic discs were placed on the surface of the seeded agar plate. After incubation at 37°C for 18 hours, the zone of inhibition around each disc was measured.

Antibiotic/DFO-B micro broth dilution synergy test for CRKP, ESBL-KP, MRSA and MSSA

For each isolate, minimum inhibitory concentration (MIC) was determined by micro-broth dilution method in triplicates in 96-well microtiter plates using cation adjusted Mueller–Hinton broth (MHB). The total volume in each well was 200 µl and the plates were incubated at 37ºC for 24 hours. To determine MIC of siderophores, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096 and 10 mg/ml of DFO-B was used individually and in combination with amoxicillin for

CRKP, ESBL-KP, MRSA and MSSA. The concentration of amoxicillin was kept constant at 0.01 mg/ml as recommended by CLSI M100^{TM} 34th edition. Medium control contained 200 µl MHB without any isolate, growth control contained 200 µl MHB with each isolate, and amoxicillin control contained 200 µl MHB+ amoxicillin with each isolate. Ferric ammonium citrate at a final concentration of 0.5 mg/ml was used for each isolate as "Fe" control. Each experiment mentioned above was carried out three times. The turbidity of each well at the end of 24 hours at 37°C was visually read. The lowest concentration of the DFO-B that inhibit the growth of the isolates was the MIC for the siderophores. To determine whether the siderophore concentration had a bacteriostatic or a bactericidal effect, a loopful of broth from each well showing no growth after 24 hours was streaked onto MHA plates and incubated at 37°C for 24 hours.

Statistical analysis

All data and graphs were processed using Microsoft Excel and appropriate statistical analysis were performed. The study was approved by the Institutional Ethical Committee (IEC) at the School of Medical Education, Kerala, India.

RESULTS

In the present study, a total of 228 isolates were used including 100 isolates of *K. pneumoniae* and 128 isolates of *S. aureus*, sourced from various clinical samples. Based on demographic distribution, *K. pneumoniae* was isolated from 64 females and 36 males, while *S. aureus* was isolated from 58 females and 70 males, indicating a slightly higher prevalence of *S. aureus* among male patients (Table 1). *K. pneumoniae* was most frequently isolated from urine samples (74), followed by blood (14), sputum (1), pus (6), and endotracheal aspirate (2) bronchial wash (4) nasal swab (0). In contrast, *S. aureus* was predominantly isolated from pus (82), bronchial wash (14), nasal swabs (12), sputum (4), urine (6), blood (10) and endotracheal aspirates (0) (Figure 1). This data indicates that urinary tract specimens were the main source for *K. pneumoniae*, while pus and swab samples were most common for *S. aureus* isolates.

Table 1: Gender-wise distribution of *Klebsiella pneumoniae* and *Staphylococcus aureus* clinical isolates.

Gender	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Female	64	58
Male	36	70
Total	100	128

Antimicrobial susceptibility pattern of K. pneumoniae and S. aureus

In the current study of *K. pneumoniae*, amoxicillin/clavulanic acid showed poor efficacy, with only 7% (n=7) of isolates sensitive and 93% (n=93) resistant, with no intermediate susceptibility. Cefuroxime exhibited even lower sensitivity at 3% (n=3), and a high resistance rate of 97% (n=97), with no intermediate responses. Ciprofloxacin showed 20% (n=20) sensitivity and 80% (n=80) resistance. Cotrimoxazole demonstrated a sensitivity of 26% (n=26), with resistance in 74% (n=74) of isolates. Tetracycline showed 27% (n=27) sensitivity, while the remaining 73% (n=73) were resistant. Ceftazidime and cefotaxime were completely ineffective, both showing 0% (n=0) sensitivity and 100% (n=100) resistance. Cefepime showed 3% (n=3) sensitivity and 97% (n=97) resistance, with no intermediate cases. Among aminoglycosides, amikacin exhibited 39% (n=39) sensitivity and 61% (n=61) resistance, while gentamicin demonstrated slightly higher sensitivity at 46% (n=46), with 54% (n=54) resistance. Piperacillin/Tazobactam showed 48% (n=48) sensitivity and 52% (n=52) resistance. Cefoperazone-sulbactam combination also exhibited equal sensitivity and resistance, both at 50% (n=50), without any intermediate susceptibility. Carbapenems such as imipenem and meropenem showed moderate activity, with both drugs having 50% (n=50) sensitivity and 50% (n=50) resistance. Aztreonam displayed slightly higher sensitivity at 51% (n=51), with 49% (n=49) resistance. Cefoxitin showed a sensitivity of 46% (n=46) and resistance of 54% (n=54). Ceftazidime-avibactam combination showed 54% (n=54) sensitivity and 46% (n=46) resistance, indicating slightly improved efficacy compared to ceftazidime alone. Tigecycline exhibited the highest sensitivity among all agents tested, with 59% (n=59) of isolates being sensitive and 41% (n=41) resistant. No intermediate susceptibility was observed for any of the tested antibiotics. These findings highlight significant resistance patterns among the isolates, with tigecycline, ceftazidime-avibactam, aztreonam, and carbapenems demonstrating relatively better activity.

In the study of *S. aureus*, Vancomycin and cotrimoxazole demonstrated 100% sensitivity (n=128), with no intermediate or resistant isolates, indicating their continued efficacy. Tetracycline showed high sensitivity at 93.7% (n=120), with intermediate and resistant responses observed in 3.1% (n=4) each. Linezolid and nitrofurantoin also exhibited high sensitivity rates of 92.1% (n=118) and 89% (n=114) respectively, with no intermediate susceptibility, and resistant isolates accounting for 7.8% (n=10) and 10.9% (n=14). Clindamycin showed 84.3% sensitivity (n=108), with 15.6% (n=20) intermediate susceptibility and no resistant cases. Gentamicin demonstrated 87.5% sensitivity (n=112), with 1.5% (n=2) intermediate and 10.9% (n=14) resistant isolates. Ciprofloxacin showed moderate sensitivity at 67.1% (n=86), with no intermediate responses and 32.8% (n=42) resistance. Erythromycin revealed a sensitivity rate of 65.6% (n=84), with 34.3% (n=44) resistant isolates and no intermediates. Cefoxitin displayed lower sensitivity at 57.8% (n=74), with a significant resistance rate of 42.1% (n=54). Penicillin exhibited the lowest sensitivity at 45.3%

(n=58), with no intermediate responses and the highest resistance rate of 54.6% (n=70). These findings highlight vancomycin and cotrimoxazole as the most effective antibiotics among the tested agents, whereas penicillin and cefoxitin showed notably higher resistance rates. Based on the antimicrobial susceptibility profiles of 228 isolates, among the 100 isolates of *K. pneumoniae* showed 54% (n=54) as MDR and 46% (n=46) as XDR. In 128 isolates of *S. aureus* exhibited 38% (n=48) as MDR and 62% (n=80) as non-MDR. Prevalence of ESBL production in *K. pneumoniae*, 60 isolates were tested out of which 83% (n=50) were identified as ESBL producers, while the remaining 17% (n=10) were non- ESBL producers and the prevalence of carbapenemase production total of 71 isolates tested the majority constituted 70% (n=50) of the isolates were MBL producers and in contrast, serine carbapenemase producers make up the remaining 30% (n=21). In this study, 42% of the isolates were identified as MRSA, indicating resistance to cefoxitin, while 58% were classified as MSSA, showing sensitivity to cefoxitin.

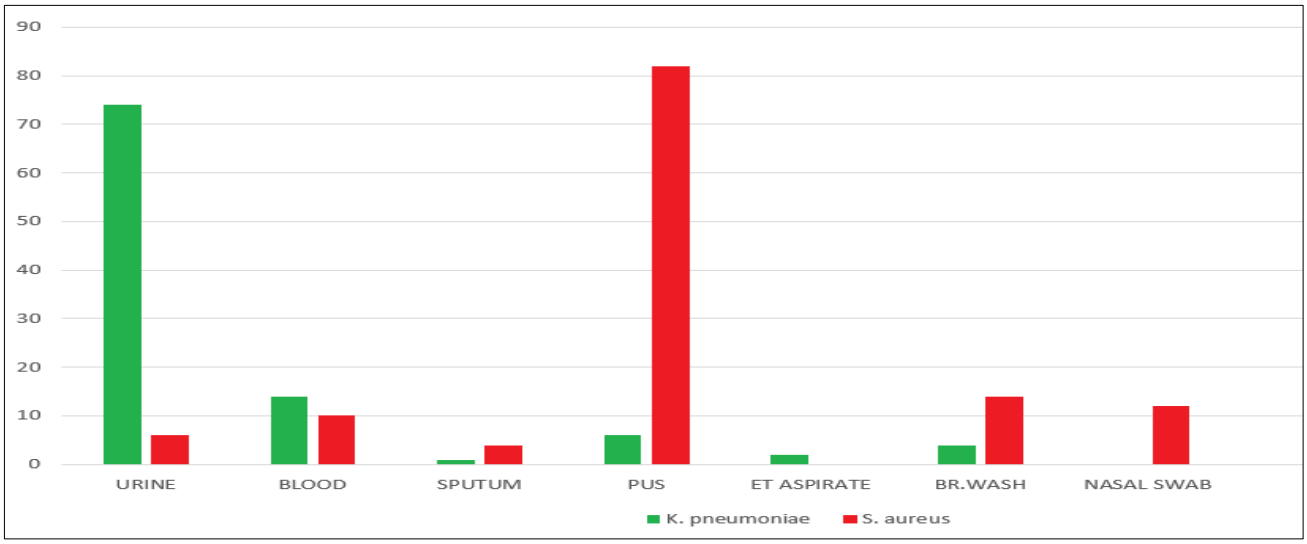

Figure 1: Distribution of clinical isolates by sample type.

Antibiotic/DFO-B disc diffusion synergy test for CRKP, ESBL-KP, MRSA and MSSA

The present study of *K. pneumoniae*, exhibited no synergistic effect between meropenem and DFO-B in test and control groups against ESBL-KP were both groups show 100% (n=50) sensitivity, indicating that meropenem remains highly effect with or without DFO-B i.e., indifference (Figure 2). Similarly, ampicillin showed complete resistance in both test and control group 100% (n=50) resistant, which demonstrates indifference, as DFO-B had no effect on its performance. In contrast, Imipenem showed a slight reduction in activity when combined with DF0-B, in the control group 9 isolates were sensitive, 27 were resistant and 14 were intermediate whereas in the test group sensitivity decreased to 8 isolates, resistance increased to 30 and intermediate decreased to 12. This shift in susceptibility patterns indicates a mild antagonistic effect, suggesting that DFO-B slightly impairs the activity of imipenem against ESBL-KP. In contrast, the synergistic activity of DFO-B in combination with Meropenem, Imipenem, Ampicillin in MBL producing *K. pneumoniae* and all the strains were indifference i.e., there is no change in resistance that as to either intermediate or sensitive.

The current study of *S. aureus*, evaluated the synergistic activity of DFO-B in combination with tigecycline, cefdinir, ampicillin in combination with DFO-B against MRSA all the strains were indifference i.e., there is no change in resistance that as to either intermediate or sensitive. In contrast to MSSA, exhibited synergistic activity with DFO-B in association with Ampicillin for 10 strains, the remaining 64 strains exhibited resistance. In case of cefdinir indifference was observed as no synergistic activity between cefdinir and DFO-B was detected. In case of Tigecycline, 12 strains converted to sensitive i.e., a synergistic effect between DFO-B and tigecycline were observed.

Antibiotic/DFO-B micro broth dilution synergy test for CRKP, ESBL-KP, MRSA and MSSA

In this study, the comparative MIC reduction pattern of different bacterial groups when treated with DFO-B alone and in combination with Amoxicillin (Figure 3). For *K. pneumoniae*, DFO-B+MBL, DFO-B+ MBL+ Amoxicillin, DFO-B+ESBL-KP, and DFO-B+ ESBL-KP+ Amoxicillin groups, all 50 isolates showed no MIC reduction. For in case of *S. aureus*, DFO-B+MRSA, 46 isolates showed no MIC, and 8 showed MIC 1/2 reduction, while DFO-B+ MRSA+ Amoxicillin exhibited 46 with no MIC and 4 isolates each with MIC 1/4 and MIC 1/8 reductions. In DFO-B+MSSA, 62 isolates showed no MIC and 12 showed MIC 1/2 reduction, whereas DFO-B+ MSSA+ Amoxicillin showed 58 with no MIC and 16 with MIC 1/4 reduction. This indicates that the combination of

DFO-B with Amoxicillin showed enhanced activity particularly against MRSA and MSSA strains, while no synergistic effect was observed in MBL and ESBL-KP groups.

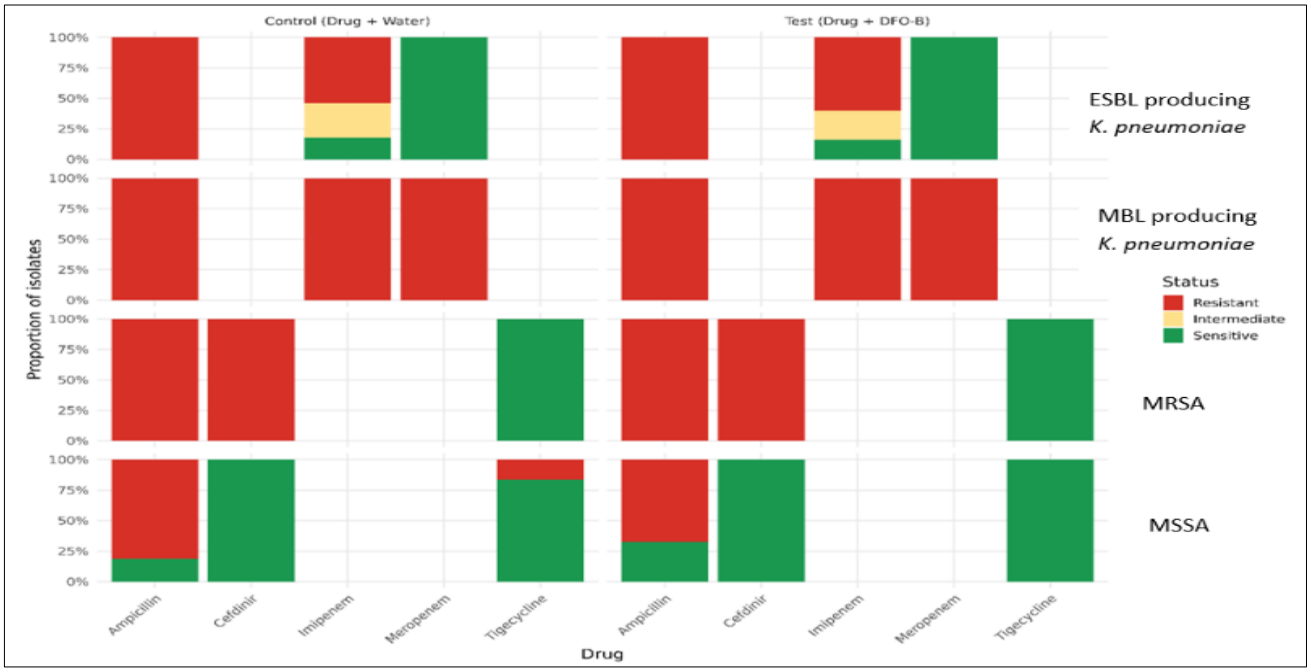

Figure 2: Antibiotic/DFO-B disc diffusion synergy test for CRKP, ESBL-KP, MRSA and MSSA.

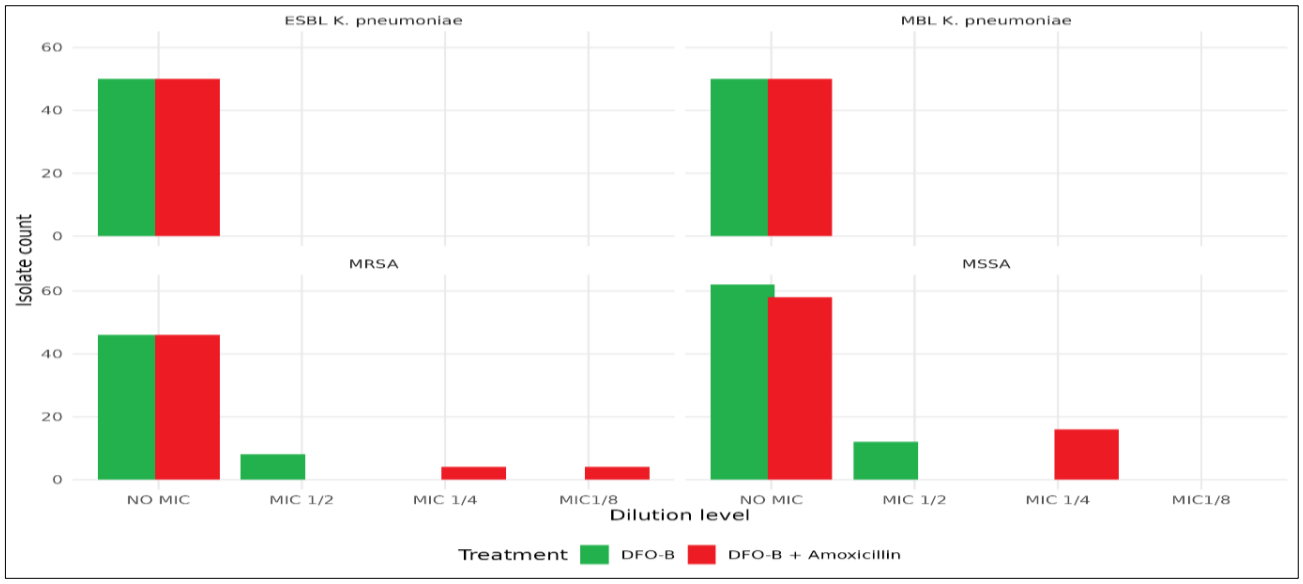

Figure 3: Antibiotic/DFO-B micro broth dilution synergy test for CRKP, ESBL-KP, MRSA and MSSA.

DISCUSSION

Antimicrobial resistance driven by ESBL-KP and CRKP, alongside MRSA, presents a grave global health challenge, particularly in India where resistance rates are notably high in both community and hospital settings.[11] Overuse of antibiotics, inadequate infection control, and plasmid-mediated gene transfer accelerate this resistance, emphasizing the urgent need for alternative therapeutic strategies.[12] DFO-B, an iron-chelating siderophore, has been studied for its potential to disrupt bacterial iron metabolism and restore antibiotic susceptibility in both *K. pneumoniae* and *S. aureus*.[13] While in vitro studies have shown DFO-B's limited resensitizing effects against ESBL and MBL-producing *K. pneumoniae*, its interaction with *S. aureus* is complex; although DFO-B can inhibit growth and disrupt biofilms, *S. aureus* may paradoxically exploit DFO-B to acquire iron, possibly enhancing infection persistence in iron-rich environments.[14] These findings highlight the promise and challenges of

repurposing DFO-B as an adjunctive approach to mitigate antimicrobial resistance, underlining the necessity for innovative strategies targeting bacterial iron acquisition and biofilm formation.

A study conducted by Gokarna and Pal investigated the synergistic activity of DFO-B in combination with selected antibiotics against MBL producing *P. aeruginosa* and *A. baumannii* isolates.[10] A total of 15 MBL-producing *P. aeruginosa* and *A. baumannii* strains each were tested using the disc diffusion method against ampicillin, meropenem, and imipenem. In *P. aeruginosa*, synergy between DFO-B and ampicillin was observed in 33.3%, DFO-B with meropenem was 40%, and DFO-B with imipenem was 53.3%. In *A. baumannii*, synergy between DFO-B and ampicillin was noted in 40%, DFO-B with meropenem was 67%, and also DFO-B with imipenem was 53.3%. In the present study, 100 XDR *K. pneumoniae* isolates including 50 MBL producing strains were tested using the same antibiotic discs ampicillin, meropenem, and imipenem against *K. pneumoniae*, and all the strains exhibited indifference when DFO-B was tested in combination with these antibiotics. To best of our knowledge, this is a novel study in which ESBL (n=50) was tested against same antibiotics, ampicillin and meropenem when combined with DFO-B, also exhibited an indifferent effect. However, the combination of imipenem with DFO-B against ESBL producers demonstrated a minimal antagonistic effect, as evidenced by a reduction in the zone of inhibition compared to imipenem alone. In their study 20% of *P. aeruginosa* isolates showed a detectable MIC DFO-B alone, but in case of DFO-B in combination with ampicillin showed 33% of synergistic effect on the isolates. While in 27% of *A. baumannii* isolates exhibited a detectable MIC but in case of DFO-B combination with ampicillin showed 40% synergistic effect on isolates. In the present study of *K. pneumoniae*, for MIC determination with amoxicillin in combination with DFO-B and DFO- B alone no measurable MIC values were obtained for any of the isolates. This contrasts with the synergistic effects reported by Gokarna and Pal for *P. aeruginosa* and *A. baumannii*. Gokarna and Pal focused on MBL-producing *A. baumannii* and *P. aeruginosa* both belonging to the non-fermenter group and reported notable synergistic effects between DFO-B and certain antibiotics in both disc diffusion and MIC testing. The present study also targeted MBL-producing *K. pneumoniae*, a highly resistant member of the Enterobacteriaceae family and distinct from the non-fermenter group in terms of metabolic properties, resistance mechanisms, and antibiotic permeability. This fundamental difference in bacterial physiology, coupled with the multiple resistance mechanisms like efflux pump, porin mutation profile of *K. pneumoniae*, likely explains why no synergistic effect was observed in either disc diffusion or MIC testing in the current study, even when using similar antibiotic combinations. In the present study of *S. aureus*, disc diffusion assays were performed using cefdinir, tigecycline, and ampicillin discs on 64 MRSA isolates, revealing indifference across all tested strains; no synergistic interactions were observed with any of these antibiotic combinations. In their study, 10% of isolates exhibited a detectable MIC for DFO- B alone, but in case of DFO-B combination with ampicillin showed 50% synergistic effect in isolates. In the present study in which MRSA (n=64) was tested against DFO-B alone and found 12.5% of strains showed antibacterial effect and in contrast, antibiotic-DFO-B combination found 6.25 % of strains exhibited synergy. To best of our knowledge, this is a novel study in which MSSA (n=74) was tested against DFO-B alone showed 16.21% antibacterial effect, but in case of DFO-B combination with amoxicillin found 21.62% of strains exhibiting synergy.

In the study of Temel and Aksoyalp all three carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates showed no measurable zone of inhibition to DFO-B alone, indicating complete resistance or lack of activity.[15] When treated with imipenem alone, the zones of inhibition measured 12 mm, 11 mm, and 11 mm for the three isolates respectively. However, the combination of imipenem with DFO-B did not enhance activity; in fact, inhibition zones were reduced to 9 mm, 8 mm, and 10 mm respectively, suggesting a lack of synergy or even an antagonistic effect. Colistin alone produced consistent inhibition zones of 13 mm for all three isolates. The addition of DFO-B to colistin resulted in slightly increased zones of 15 mm, 15 mm, and 14 mm respectively, indicating a modest enhancement of colistin's activity when combined with DFO-B. These findings highlight that, while DFO-B alone was ineffective, its combination with colistin provided minor improvements against CRAB, whereas combining DFO-B with imipenem appeared to decrease antibacterial efficacy. In the present study of MBL and ESBL-producing *K. pneumoniae*, isolates were evaluated for their susceptibility to ampicillin, imipenem, and meropenem using the disc diffusion method. For MBL-producing strains, the addition of DFO-B to any of the antibiotics resulted in an indifference response across all isolates, indicating no significant change in antibacterial activity. Among ESBL-producing isolates, both ampicillin and meropenem, when combined with DFO-B, also exhibited an indifference effect. However, the combination of imipenem with DFO-B against ESBL producers demonstrated a minimal antagonistic effect, as evidenced by a reduction in the zone of inhibition compared to imipenem alone. These findings suggest that while DFO-B does not enhance the activity of these antibiotics against MBL and ESBL strains, its combination with imipenem may slightly reduce efficacy in ESBL-producing *K. pneumoniae*. In contrast, *S. aureus* studying the synergistic effect of DFO-B with vancomycin and amoxicillin exhibited a modest synergistic effect; an increase of 2 mm each in 3 MRSA isolates. They also found no individual antibacterial activity of DFO-B which is similar to the present study of *S. aureus* and the percentage of difference between the studies can be attributed to the number of isolates used.

In the study of Hartzen et al using DFO-B as a potential siderophore inhibitor in combination with ascorbic acid on *S. aureus* found no activity for DFO-B and ascorbic acid individually but found a synergistic effect in 50% of isolates by time kill curve at 6 hours, but the organism overcame the inhibitory effect at 24 hours.[16] In the present study of both *K. pneumoniae* and *S. aureus*, growth inhibition was noted only after overnight incubation, which could be a reason for lesser synergistic activity, and also an addition of an agent like ascorbic acid to DFO-B antibiotic combination could yield a higher synergistic effect.

Our study has some limitations, the study is limited by its focus on a single *Klebsiella* species (*K. pneumoniae*) and *Staphylococcal* species (*S. aureus*) and by the single-centre design, which may reduce generalizability. Additionally, experiments need to be conducted *in vitro*, and the genotypic basis of carbapenem resistance in *K. pneumoniae* was not evaluated. MIC-based synergy assays like, checkerboard assay were not performed for all antibiotic combinations, and clinical efficacy was not assessed. Future multicentric studies incorporating genotypic analysis, animal infection models, and clinical outcome data are warranted to better define the therapeutic potential of DFO-B as a repurposed adjunct against drug-resistant *K. pneumoniae* and *S. aureus*.

CONCLUSION

This study investigated the adjunctive potential of DFO-B, a natural iron-chelating siderophore, to restore antimicrobial efficacy against clinical isolates of both MRSA, MSSA, MDR ESBL and MBL-producing *K. pneumoniae*. In *K. pneumoniae*, DFO-B failed to produce meaningful improvement in antibiotic susceptibility under tested *in vitro* conditions, underscoring the pathogen-specific challenges of siderophore-based approaches. In contrast, against *S. aureus*, DFO-B demonstrated a complex dual role in iron metabolism, showing biofilm inhibition and increased susceptibility of certain MSSA strains to antibiotics such as ampicillin and tigecycline, though synergistic effects in many MRSA isolates were limited. These findings highlight both the promise and limitations of repurposing DFO-B as an adjunctive strategy and emphasize the need for future research into optimized combination therapies integrating siderophore analogues with agents that disrupt efflux, alter membrane permeability, or enhance antibiotic uptake to more effectively combat resistant Gram-positive and gram-negative pathogens.

ACKNOWLEDGEMENTS

Authors would like to thank Mrs. Rajumol B. Zacharia for her technical assistance.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Eftekhar F, Naseh Z. Extended-spectrum β-lactamase and carbapenemase production among burn and non-burn clinical isolates of Klebsiella pneumoniae. Iran J Microbiol. 2015;7(3):144-9.
2. Zhang Y, Wang M, Li Z, Peng Y, Yang Y, Liu X, et al. Characteristics of ESBL-positive Klebsiella pneumoniae isolated from paired children with and without diarrhea. Gut Pathog. 2025;17(1):36.
3. Li J, Shi Y, Song X, Yin X, Liu H. Mechanisms of Antimicrobial Resistance in Klebsiella: Advances in Detection Methods and Clinical Implications. Infect Drug Resist. 2025;18:1339-54.
4. Martin RM, Bachman MA. Colonization, Infection, and the Accessory Genome of Klebsiella pneumoniae. Front Cell Infect Microbiol. 2018;8:4.
5. Chambers HF, DeLeo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol. 2025;18(12):711-25.
6. Bellotti D, Remelli M. Deferoxamine B: A Natural, Excellent and Versatile Metal Chelator. Molecules. 2021;26(11):3255.
7. Van Asbeck BS, Marcelis JH, Marx JJ, Struyvenberg A, van Kats JH, Verhoef J. Inhibition of bacterial multiplication by the iron chelator deferoxamine: potentiating effect of ascorbic acid. Eur J Clin Microbiol. 1983;2(5):426-31.
8. Erinmez M, Zer Y. In vitro effects of deferoxamine on antibiotic susceptibility in Gram-negative bacteria. Adv Clin Exp Med. 2024;33(5):491-7.
9. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 35th Edition. 2025.
10. Gokarn K, Pal RB. Activity of siderophores against drug-resistant Gram-positive and Gram-negative bacteria. Infect Drug Resist. 2018;11:61-75.
11. Yan W, Liang J, Liu M, Hu X, Zhang H, Guo J, et al. Clinical and Antibiotic Resistance Features for Extended-Spectrum Beta-lactamase-Producing Escherichia coli and Klebsiella pneumoniae Bloodstream Infections and Predictors of Poor Prognosis in Neonatal Patients. Infect Drug Resist. 2025;18:3907-18.
12. Ali Abdel Rahim KA, Ali Mohamed AM. Prevalence of Extended Spectrum β-lactamase-Producing Klebsiella pneumoniae in Clinical Isolates. Jundishapur J Microbiol. 2014;7(11):e17114.
13. Kim CM, Shin SH. Effect of iron-chelator deferiprone on the in vitro growth of staphylococci. J Korean Med Sci. 2009;24(2):289-95.
14. Haider MH, McHugh TD, Roulston K, Arruda LB, Sadouki Z, Riaz S. Detection of carbapenemases blaOXA48-blaKPC-blaNDM-blaVIM and extended-spectrum-β-lactamase blaOXA1-blaSHV-blaTEM

genes in Gram-negative bacterial isolates from ICU burns patients. Ann Clin Microbiol Antimicrob. 2022;21(1):18.
15. Temel A, Aksoyalp ZŞ. A Preliminary Study on the Effect of Deferoxamine on the Disruption of Bacterial Biofilms and Antimicrobial Resistance. Turk J Pharm Sci. 2024;21(4):267-73.
16. Hartzen SH, Frimodt-Møller N, Frølund Thomsen V. The antibacterial activity of a siderophore. 1. In vitro activity of deferoxamine alone and in combination with ascorbic acid on Staphylococcus aureus. APMIS. 1989;97(5):419-24.

Cite this article as: Abhirami, Satheesh A, Joemon A, Sathar S, Narayanan SK, Sadanandan HKK. Exploring Deferoxamine-B for resensitization and antimicrobial resistance mitigation in drug resistant *Klebsiella pneumoniae* and *Staphylococcus aureus*: an *in vitro* study. Int J Res Med Sci 2025;13:5353-60.