OPEN ACCESS

Microbiological & Immunological Communications

DOI: 0.55627/mic.001.01.0181

Research Article

Siderophore Production and its Role as a Therapeutic Agent

Ayaz Ahmed[1*], Shahana Urooj Kazmi[2]

[1]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan.
[2]Women University Swabi, Gulo Dheri, Swabi, Pakistan.
Correspondence: jabees2003@hotmail.com, ayaz.ahmed@iccs.edu

Abstract

Siderophores are iron chelators, which are produced by bacteria under iron-deficient conditions required for their growth. Therefore, siderophores can be used as a carrier to direct drugs into the bacteria and kill them. The present study was designed to screen siderophore production of 17 different gram-positive and negative bacteria by using an iron-deficient medium and chrome azurol S (CAS) assay. The antimicrobial potential (minimum inhibitory concentration) of tetracycline, ceftriaxone, ampicillin, epigallocatechin gallate (EGCG), and acetohydroxamic acid (aHa; synthetic siderophore) were determined against pathogen using micro broth dilution method. In the end, a checkerboard assay was used to determine the synergistic potential of synthetic siderophores with EGCG and antibiotics as a possible therapy. Results demonstrated that all the tested microorganisms produced siderophore under the iron-deprived condition, as evidenced by orange halo zones in CAS agar plates. Gram-negative bacteria produced more siderophores, as evidenced by an intense and large orange color halo zone of 17-22mm as compared to Gram-positive bacteria, which is 13-15mm in size. As compared to antibiotics and EGCG, acetohydroxamic acid (aHa; synthetic siderophore) showed no antibacterial properties (1500 - 6500 µg/ml). The combinatorial approach showed that aHa synergized significantly with tetracycline, ceftriaxone, and EGCG (i.e., FIC index <0.5) against S. typhi, methicillin-resistant or methicillin-resistant or sensitive *Staphylococcus aureus*, and *E. coli*. In conclusion, siderophore may be considered as an approach to deliver drugs within microorganisms as a combinatorial therapy approach against MDR pathogens.

Keywords: Siderophore, checkerboard assay, fraction inhibitory concentration index, synergism, epigallocatechin gallate.

1. Introduction

Iron constitutes about 35% of the earth's mass and is an essential nutrient for humans and microbes. In the host, nutritional immunity restricts iron availability to invading pathogens by binding to various proteins (Arnold 2018, Cassat and Skaar 2013). Due to its oxidation-reduction potential, iron holds a key position in cellular functions such as aerobic respiration, ATP production, heme formation, DNA synthesis, etc. (Abbaspour, Hurrell, and Kelishadi 2014, Raymond, Dertz, and Kim 2003). In normal human serum, the level of free iron is ~10-18 – 10-24 M, which is far below the concentration required by bacterial pathogens to grow and cause infection (Raymond, Dertz, and Kim 2003). A concentration of around 1 mM of iron is needed for optimum growth; however, this concentration varies for different organisms (Cassat and Skaar 2013, Fischbach et al. 2006). In an iron-deprived condition, microorganisms produce low molecular weight siderophores with a strong affinity for iron (Behnsen and Raffatellu 2016, Crosa and Walsh 2002). Siderophore, a Greek word meaning “iron carrier” is produced by

Table-1: Minimum Inhibitory Concentration of Antibiotics, EGCG and Acethydroxamic acid against different Microorganisms

Microorganisms	Minimal Inhibitory Concentration (MIC) (µg/ml)				
	Tetracycline	Amplicillin	Ceftriazone	EGCG	Acetohydoxamic Acid
MRSA	62.5	1000	500	3120.0	1950.0
MSSA	31.25	250	250	190.0	3125.0
Salmonella typhi	31.25	62.5	3.9	1560	3125.0
Staphylococcus epidermitis	62.5	62.5	31.25	6250	1512.0
Escherichia coli	15.62	7.81	1.95	3120	6250.0
Escherichia coli(401)	15.62	62.5	7.81	6250	6250.0
Escherichia coli (UTI)	0.48	31.25	31.25	3120	6250.0
Pseudomonas aeroginosa	15.62	7.81	3.90	1560	1512.0

Methicillin Resistant *Staphylococcus aureus* (MRSA) *and* Methicillin Sensitive *Staphylococcus aureus* (MSSA)

microorganisms having an extremely high affinity for ferric ions (Chu et al. 2010, Schwyn and Neilands 1987a). There are different classes of siderophores like hydroxamates, thiohydroxamates, and catecholates (Ahmed and Holmström 2014, Boukhalfa et al. 2003). Many bacteria can synthesize their own siderophores or utilize other microbial- and plant-siderophores for iron acquisition. The mechanism of iron acquisition is known to be a virulence factor for human and animal pathogenic bacteria (Koh and Henderson 2015).

Siderophores are commonly produced by most aerobic and anaerobic microorganisms(Sana et al., 2021:(Ahmed and Holmström 2014, Koh and Henderson 2015). Different types of siderophores have been identified, such as enterobactin, mycobactin, pyoverdin, and pyochelin (Wilson et al. 2016, Brandel et al. 2012, Raymond, Dertz, and Kim 2003, Rodriguez and Smith 2006b). Depending on the participating chelating group by bacteria and fungi, siderophores have been further classified into Catecholates, Hydroxamates, and Mixed ligands (Sah and Singh 2015).

Siderophores can be used for the selective delivery of antibiotics in antibiotic-resistant bacteria (Negash, Norris, and Hodgkinson 2019, Górska, Sloderbach, and Marszałł 2014, Möllmann et al. 2009). It is a potent antimicrobial approach to utilize bacterial own iron transport system to overcome drug-resistant bacteria. Keeping this in mind present study was designed to investigate the siderophore production ability of multidrug-resistant clinical isolates, especially methicillin-resistant *Staphylococcus aureus* (MRSA) and some enteropathogens. Furthermore, the synergistic capability of synthetic siderophore (Acetohydroxamate) with different drugs or epigallocatechin gallate (EGCG) to treat MDR infections was also determined.

2. Materials & Methods

2.1 Bacterial Strains and Culture Medium Condition

Bacterial cultures such as enteropathogenic and enterotoxigenic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermitis*, *Klebsiella pneumoniae*, *Pseudomonas auroginosa*, *Shigella dysintriae* and different strains of methicillin-resistant *Staphylococcus aureus* were used for siderophore detection. Gram staining and biochemical characterization confirmed the purity of the above-mentioned cultures (data not

Table-2: Siderophore Production and Detection of Different Groups of Microorganisms by Chrome Azurol S Agar Diffusion Assay

Microorganisms	Halo Orange Zone (mm)	
	Iron Deficient Medium	Iron Containing Medium
Escherichia coli	25***	0
Enteropathogenic Escherichia coli	20**	0
Enterotoxigenic Escherichia coli	18*	0
Staphylococcus epidermitis	15	0
Staphylococcus aureus	15	0
Streptococcus pyogens	16	0
Salmonella typhi	20**	0
Salmonella paratyphi A	16	0
Bacillus subtilis	15	0
Klebsiella pneumoniae	14	0
Pseudomonas aeruginosa	21**	0
Corynibacterium diphtheriae	15	0
Enterococcus aerogenes	13	0
Micrococcus luteus	13	0
Shigella dysentriae	18*	0
Proteus Mirabilis	14	0
MRSA strain 1-10	14-19	0

Methicillin-Resistant *Staphylococcus aureus* (MRSA)
Note: * = statistically significant in comparison to gram-positive strains.

shown)(Ahmed et al. 2016, Ahmed A 2019). These cultures were maintained on Tryton Soya agar or broth (TSB; TSA; Oxoid UK) for routine use and incubated overnight at 37°C under aerobic conditions. Iron-deficient medium containing MM9 was prepared as described earlier (Payne 1994). Briefly, solution-1 (KH_2PO_4 (0.3 g), NaCl (0.5 g), NH_4Cl (1 g), NaOH (6 g), and PIPES (30.24 g) in 1 L was mixed and autoclaved, followed by mixing with solution-2 (30 ml of 10% (m/v) iron free casamino acids, (Contaminating iron was removed with 8-hydroxyquinoline (3%) in chloroform), fructose (2.0 g), $MgCl_2$ (1M, 1ml) and of $CaCl_2$ (100mM,1ml) in one liter was prepared, filtered and sterilized.

2.2 Minimum Inhibitory Concentration (MIC) Determination

The minimal inhibitory concentrations (MIC) of ampicillin, ceftriazone, tetracycline, and EGCG were determined by microdilution method according to CLSI standards and, as mentioned earlier (Khan et al. 2017, Wayne 2012). Briefly, Mueller Hinton broth (100µl) was transferred to each well of 96 well plates containing antibiotic (100µl, 1mg/ml) to the first well and two-fold dilutions were prepared to start from well 1 – 10 to achieve 500 – 1.0µg/ml concentration. An

Table-3: Fractional Inhibitory Concentration of Different Combinations of Antibiotics and Acetohydroxamaic Acid

Microorganisms	Combination	FIC Index	Relation
MRSA	Tetracycline + Acetohydroxamic Acid	0.7	Indifferent
MSSA		0.7	Indifferent
Salmonella typhi		0.18	Synergy
Escherichia coli		3	Antagonism
MRSA	Ampicillin + Acetohydroxamic Acid	0.62	Indifferent
MSSA		0.5	Indifferent
Staphylococcus epidermitis		0.76	Indifferent
Escherichia coli		3.0	Antagonism
MRSA	Ceftriazone + Acetohydroxamic Acid	0.37	Synergy
MSSA		0.25	Synergy
Salmonella typhi		0.18	Synergy
MRSA	Green Tea + Acetohydroxamic Acid	0.2	Synergy
MSSA		0.18	Synergy
Salmonella typhi		0.6	Indifferent
Escherichia coli		0.37	Synergy

Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA)

Fractional Inhibitory Concentration (FICI) Index:
Synergism (≤0.5), Additive (>0.5 to ≤1), Indifference (> 1 to ≤4) and Antagonism (>4).

inoculum (5 x 10^5) cells were inoculated in each well except the 12th well, which served as a negative control, whereas the 11th well served as positive control and was incubated at 37°C for 18 to 24 hours under aerobic conditions, and MIC of drugs was recorded as the lowest concentration which inhibited the bacterial growth.

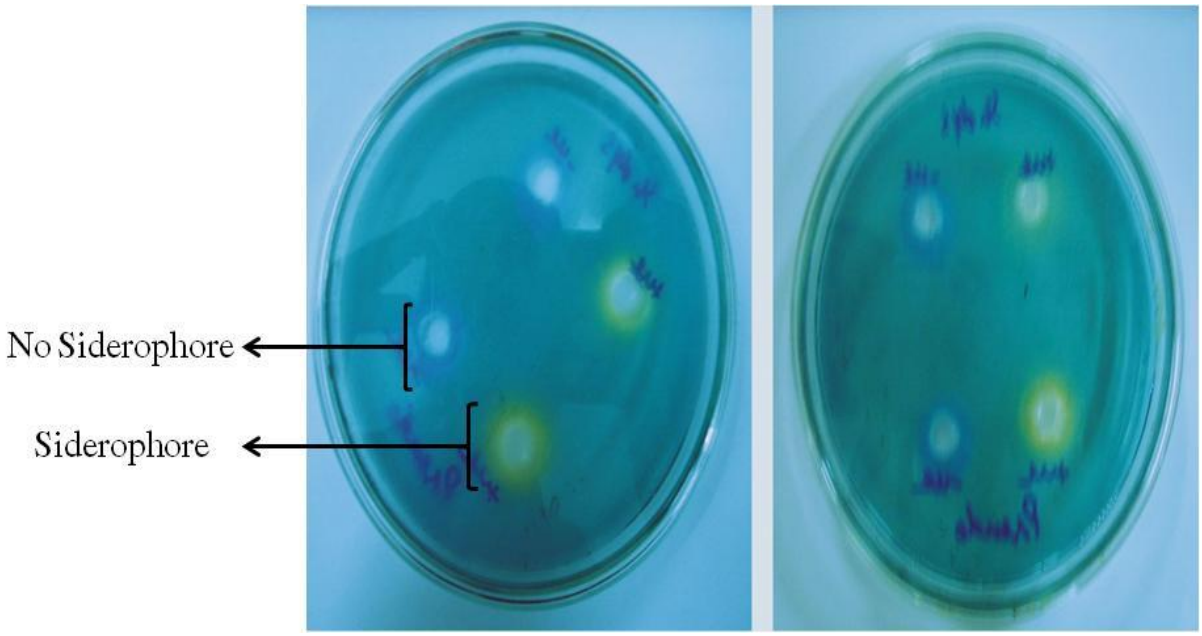
2.3 *Detection of Siderophore Production*

Chrome azurol (CAS) assay was performed to detect siderophore production under the iron-free condition as described before (Payne 1994). The CAS agar plates were prepared by the addition of CAS (60.5mg in 50 ml deionized water) and 10 ml of iron (III) in a mixture ($FeCl_3$-$6H_2O$, 1 mM and HCl, 10 mM). Under stirring, this solution was slowly mixed with HDTMA (72.9mg) dissolved in water (40 ml). The resultant dark blue solution was autoclaved and mixed with an autoclaved mixture (900 ml) of water containing agarose (10g) and Tris (1mM), and the pH was adjusted to 6.8. The plates were poured and sealed with polythene bags and stored in a refrigerator until use. The plates were punched with holes using a borer for the CAS assay. Each hole was properly labeled and filled with cell-free filtrates (50µl) of different bacteria grown in an iron-deficient medium to validate siderophore production and incubated at 37^{o}C for 5 hours. The presence of siderophores was indicated by the appearance of orange halos around the well. Siderophore activity was expressed as the square value of the halo diameter.

2.4 Checkerboard Synergism Assay

The synergistic capability of synthetic siderophore acetohydroxamate (aHa) with tetracycline, ampicillin, ceftriaxone, and EGCG was determined by calculating fractional inhibitory concentration (FIC) index using checkerboard assay (Sopirala et al. 2010). The concentration range of each antimicrobial agent in combination ranged from 1/32 times the MIC (1/32 x MIC). Two-fold dilutions of drugs A and B were made. The initial inoculum was the same as that used for

Figure-1: Siderophore production and detection by Chrome Azurol S Assay.



In Both plates, the clear well represents the absence of siderophore while the yellowish-orange colored halo zone represents siderophore production.

MIC. The FIC index of each antibiotic in combination was calculated by following the formula as described by Holger et al., 2022:

Fractional inhibitory concentration index = FIC of drug A + FIC of drug B

FIC of drug A or B = MIC of the drug in combination / MIC of the Drug alone.

Results were interpreted based on the following scale:

FIC index: ≤0.5 (Synergism), >0.5 to ≤1 (Additive), > 1 to ≤4 (Indifference), and >4 (Antagonism) (Holger et al. 2022).

2.5 Statistical Analysis

The data were represented as mean ± S.D. t-test was used to determine the significance of siderophore production between gram-positive and negative strains.

3. Results & Discussion

The minimal inhibitory concentrations of tetracycline, ampicillin, ceftriaxone, and green tea were recorded between 1-500µg/ml (Table I). Antibiotics were selected based on their susceptibility pattern against the microorganism used (Result not shown). Different microorganisms have different MIC ranges with respect to antibiotics.

Siderophores are commonly produced by most aerobic and anaerobic microorganisms to counter iron-deficient environments. Different strains of microorganisms were grown and tested for siderophore production on the CAS agar well diffusion method. Results showed that all the tested microorganisms were positive for siderophore production (Table 2). Siderophores produced by microorganisms abstracting Fe^{+3} from the blue ternary complex of CAS change color from blue to orange (Guan, Kanoh, and Kamino 2001).

Gram-negative bacteria (*E. coli*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa*, and *S. dysentery*) produced significantly more siderophore (16 – 25

mm) as reflected by orange halo zones around the well (Figure-1) as compared to Gram-positive bacteria (MRSA clinical strain, *S. epidermitis, S. pyogenes* (12-16mm) in the absence of iron as compared to iron sufficient media.

Different studies also verified the production of siderophores by microorganisms in iron-deprived conditions and that pathogen produced more siderophores in iron-limited conditions than Gram-positive bacteria (Faraldo-Gómez and Sansom 2003, Furrer et al. 2002, Lankford and Byers 1973, Palyada, Threadgill, and Stintzi 2004). Among various other methods to detect siderophore, Chrome azurol S agar diffusion assay is one of the universal and cost-effective methods used for detecting siderophore production as described previously (Schwyn and Neilands 1987b). Irrespective of its chemical nature, this assay can detect total siderophores in chemically defined media. The principle involved the extraction of iron from Fe^{+3}-CAS conjugate by siderophore, which has a great affinity for iron and converts blue color to orange(Guan, Kanoh, and Kamino 2001).

Our results demonstrated that resistant and toxin-producing bacteria methicillin-resistant *Staphylococcus aureus*, Escherichia *coli*, enteropathogenic and enterotoxigenic *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella dysentria* produced more siderophores as compared to less virulent strains (*Enterococcus aerogenes, Micrococcus luteus* and *Proteus mirabilis*). A correlation exists that siderophore production is directly related to virulence(Granato et al. 2016). *Pseudomonas* strains positive for siderophore production were more virulent in establishing infection in mice as compared to non-siderophore producers(Little et al. 2018, Takase et al. 2000). Likewise, *Yersinia pestis* siderophore mutant strain was less virulent to cause plague in an animal model as compared to non-mutant strains (Fetherston et al. 2012, Miller et al. 2010), thereby suggesting that siderophore are involved in the virulence of the pathogens.

The checkerboard method was used to evaluate synergistic studies of synthetic siderophore (Acetohyroxamic acid; aHa) with other antibiotics. The combination shows a pattern of synergism, indifference, and antagonism (Table-3). The combination of acetohydroxamic acid and ceftriazone showed synergism (FIC index < 0.5) against methicillin-sensitive or resistant *S. aureus* and *S. typhi* strains. Besides, ceftriazone, and acetohydroxamic acid also showed appreciable synergism with EGCG against methicillin-sensitive or resistant *S. aureus* and *E. coli* (Table-3). This might be important as EGCG itself plays a vital role as an iron chelator and possesses antimicrobial properties (Hatcher et al. 2009, Reznichenko et al. 2006), indicating that siderophores and their analogs have tremendous therapeutic potential (Rodriguez and Smith 2006a, Brandel et al. 2012).). It might be possible that different drugs may be linked with synthetic siderophores, which can be manipulated to enter the cell and exert its function and hence overcome the emerging antimicrobial resistance. These conjugates have selective antimicrobial activity because the microbes recognize specific siderophores (Diarra et al. 1996). Although our study lacks conjugate preparation but provides support that antibiotics can be synergized with siderophore. Other investigators used green tea as an iron chelator with antimicrobial properties, which also showed synergism with antibiotics suggesting its importance to utilize it either as a siderophore or as an antibacterial agent (Hu et al. 2001). The EGCG interacts positively with β-lactam drugs to treat MRSA (Hu et al. 2001). Siderophore drug conjugates such as albomycin inhibited tRNA synthetase and cured bacterial infections in a mouse model(Pramanik et al. 2007). Microcin E422m, an antimicrobial peptide produced by *Klebsiella pneumonia*, contains several catecholate groups that bind Fe3+, internalize through siderophore outer membrane transporters and kill pathogens (Thomas et al. 2004). Such siderophore drugs have been named

sideromycins (Pramanik et al. 2007, Wencewicz et al. 2009).

4. Conclusions

In conclusion, siderophore can be used as a carrier system to deliver drugs and can be used to cure alarming antimicrobial-resistant pathogens. In addition, siderophores can formulate drug conjugates against intracellular or MDR pathogens, which acquire resistance against multiple drugs.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

N/A

Study Approval

Not required

Consent Forms

Not required

Authors Contribution

AA performed the study, wrote the final manuscript, and analyzed the results. SUK conceptualized the study and supervised the whole project.

Acknowledgments

The authors like to acknowledge the Department of Microbiology support staff at the University of Karachi for completing this project.

References

Abbaspour, N., R. Hurrell, and R. Kelishadi. 2014. "Review on iron and its importance for human health." *J Res Med Sci* 19 (2):164-74.

Ahmed A, and Khan A. 2019. "Isolation and characterization of antimicrobial resistant water contaminant and bacteriophage remedy to improve water quality."

Ahmed, Ayaz, Anum Khalid Khan, Ayaz Anwar, Syed Abid Ali, and Muhammad Raza Shah. 2016. "Biofilm inhibitory effect of chlorhexidine conjugated gold nanoparticles against Klebsiella pneumoniae." *Microbial pathogenesis* 98:50-56.

Ahmed, E., and S. J. Holmström. 2014. "Siderophores in environmental research: roles and applications." *Microb Biotechnol* 7 (3):196-208. doi: 10.1111/1751-7915.12117.

Arnold, K. 2018. "What four elements make up almost 90% of the earth? ." 2018.

Behnsen, J., and M. Raffatellu. 2016. "Siderophores: More than Stealing Iron." *mBio* 7 (6). doi: 10.1128/mBio.01906-16.

Boukhalfa, Hakim, Joe Lack, Sean D. Reilly, Larry Hersman, and Mary P. Neu. 2003. "Siderophore Production and Facilitated Uptake of Iron and Plutonium in P. Putida." *AIP Conference Proceedings* 673 (1):343-344. doi: 10.1063/1.1594658.

Brandel, J., N. Humbert, M. Elhabiri, I. J. Schalk, G. L. Mislin, and A. M. Albrecht-Gary. 2012. "Pyochelin, a siderophore of Pseudomonas aeruginosa: physicochemical characterization of the iron(III), copper(II) and zinc(II) complexes." *Dalton Trans* 41 (9):2820-34. doi: 10.1039/c1dt11804h.

Cassat, J. E., and E. P. Skaar. 2013. "Iron in infection and immunity." *Cell Host Microbe* 13 (5):509-519. doi: 10.1016/j.chom.2013.04.010.

Chu, B. C., A. Garcia-Herrero, T. H. Johanson, K. D. Krewulak, C. K. Lau, R. S. Peacock, Z. Slavinskaya, and H. J. Vogel. 2010. "Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view." *Biometals* 23 (4):601-11. doi: 10.1007/s10534-010-9361-x.

Crosa, J. H., and C. T. Walsh. 2002. "Genetics and assembly line enzymology of siderophore biosynthesis in bacteria." *Microbiol Mol Biol*

Rev 66 (2):223-49. doi: 10.1128/mmbr.66.2.223-249.2002.

Diarra, MS, MC Lavoie, M Jacques, I Darwish, EK Dolence, JA Dolence, A Ghosh, M Ghosh, MJ Miller, and F Malouin. 1996. "Species selectivity of new siderophore-drug conjugates that use specific iron uptake for entry into bacteria." *Antimicrobial agents and chemotherapy* 40 (11):2610-2617.

Faraldo-Gómez, José D, and Mark SP Sansom. 2003. "Acquisition of siderophores in gram-negative bacteria." *Nature reviews Molecular cell biology* 4 (2):105-116.

Fetherston, Jacqueline D, Ildefonso Mier Jr, Helena Truszczynska, and Robert D Perry. 2012. "The Yfe and Feo transporters are involved in microaerobic growth and virulence of Yersinia pestis in bubonic plague." *Infection and immunity* 80 (11):3880-3891.

Fischbach, M. A., H. Lin, D. R. Liu, and C. T. Walsh. 2006. "How pathogenic bacteria evade mammalian sabotage in the battle for iron." *Nat Chem Biol* 2 (3):132-8. doi: 10.1038/nchembio771.

Furrer, Jason L, Douglas N Sanders, India G Hook-Barnard, and Mark A McIntosh. 2002. "Export of the siderophore enterobactin in Escherichia coli: involvement of a 43 kDa membrane exporter." *Molecular microbiology* 44 (5):1225-1234.

Górska, Agnieszka, Anna Sloderbach, and Michał Piotr Marszałł. 2014. "Siderophore–drug complexes: potential medicinal applications of the 'Trojan horse'strategy." *Trends in pharmacological sciences* 35 (9):442-449.

Granato, Elisa T, Freya Harrison, Rolf Kümmerli, and Adin Ross-Gillespie. 2016. "Do bacterial "virulence factors" always increase virulence? A meta-analysis of pyoverdine production in Pseudomonas aeruginosa as a test case." *Frontiers in microbiology* 7:1952.

Guan, Le Luo, Kaneo Kanoh, and Kei Kamino. 2001. "Effect of exogenous siderophores on iron uptake activity of marine bacteria under iron-limited conditions." *Applied and Environmental Microbiology* 67 (4):1710-1717.

Hatcher, Heather C, Ravi N Singh, Frank M Torti, and Suzy V Torti. 2009. "Synthetic and natural iron chelators: therapeutic potential and clinical use." *Future medicinal chemistry* 1 (9):1643-1670.

Holger, Dana J, Ashlan J Kunz Coyne, Jing J Zhao, Avnish Sandhu, Hossein Salimnia, and Michael J Rybak. 2022. "Novel Combination Therapy for Extensively Drug-Resistant Acinetobacter baumannii Necrotizing Pneumonia Complicated by Empyema: A Case Report." Open forum infectious diseases.

Hu, Zhi-Qing, Wei-Hua Zhao, Yukihiko Hara, and Tadakatsu Shimamura. 2001. "Epigallocatechin gallate synergy with ampicillin/sulbactam against 28 clinical isolates of methicillin-resistant Staphylococcus aureus." *Journal of Antimicrobial Chemotherapy* 48 (3):361-364.

Khan, A. K., A. Ahmed, M. Hussain, I. A. Khan, S. A. Ali, A. D. Farooq, and S. Faizi. 2017. "Antibiofilm potential of 16-oxo-cleroda-3, 13(14) E-diene-15 oic acid and its five new γ-amino γ-lactone derivatives against methicillin resistant Staphylococcus aureus and Streptococcus mutans." *Eur J Med Chem* 138:480-490. doi: 10.1016/j.ejmech.2017.06.065.

Koh, E. I., and J. P. Henderson. 2015. "Microbial Copper-binding Siderophores at the Host-Pathogen Interface." *J Biol Chem* 290 (31):18967-74. doi: 10.1074/jbc.R115.644328.

Lankford, Charles E, and Benjamin R Byers. 1973. "Bacterial assimilation of iron." *CRC Critical Reviews in Microbiology* 2 (3):273-331.

Little, Alexander S, Yuta Okkotsu, Alexandria A Reinhart, F Heath Damron, Mariette Barbier, Brandon Barrett, Amanda G Oglesby-Sherrouse, Joanna B Goldberg, William L Cody, and Michael J Schurr. 2018. "Pseudomonas aeruginosa AlgR phosphorylation status differentially regulates pyocyanin and pyoverdine production." *MBio* 9 (1):e02318-17.

Miller, M Clarke, Jacqueline D Fetherston, Carol L Pickett, Alexander G Bobrov, Robert H Weaver, Edward DeMoll, and Robert D Perry. 2010. "Reduced synthesis of the Ybt siderophore or production of aberrant Ybt-like molecules activates transcription of yersiniabactin genes in Yersinia pestis." *Microbiology* 156 (Pt 7):2226.

Möllmann, Ute, Lothar Heinisch, Adolf Bauernfeind, Thilo Köhler, and Dorothe Ankel-Fuchs. 2009. "Siderophores as drug delivery agents: application of the "Trojan Horse" strategy." *Biometals* 22 (4):615-624.

Negash, Kokob H, James KS Norris, and James T Hodgkinson. 2019. "Siderophore–antibiotic conjugate design: New drugs for bad bugs?" *Molecules* 24 (18):3314.

Palyada, Kiran, Deborah Threadgill, and Alain Stintzi. 2004. "Iron acquisition and regulation in Campylobacter jejuni." *Journal of bacteriology* 186 (14):4714-4729.

Payne, Shelley M. 1994. "[25] Detection, isolation, and characterization of siderophores." *Methods in enzymology* 235:329-344.

Pramanik, Avijit, Uwe H Stroeher, Juliane Krejci, Alistair J Standish, Erwin Bohn, James C Paton, Ingo B Autenrieth, and Volkmar Braun. 2007. "Albomycin is an effective antibiotic, as exemplified with Yersinia enterocolitica and Streptococcus pneumoniae." *International Journal of Medical Microbiology* 297 (6):459-469.

Raymond, K. N., E. A. Dertz, and S. S. Kim. 2003. "Enterobactin: an archetype for microbial iron transport." *Proc Natl Acad Sci U S A* 100 (7):3584-8. doi: 10.1073/pnas.0630018100.

Reznichenko, L, T Amit, H Zheng, Y Avramovich-Tirosh, MBH Youdim, O Weinreb, and S Mandel. 2006. "Reduction of iron-regulated amyloid precursor protein and β-amyloid peptide by (−)-epigallocatechin-3-gallate in cell cultures: implications for iron chelation in Alzheimer's disease." *Journal of neurochemistry* 97 (2):527-536.

Rodriguez, G Marcela, and Issar Smith. 2006a. "Identification of an ABC transporter required for iron acquisition and virulence in Mycobacterium tuberculosis." *Journal of bacteriology* 188 (2):424-430.

Rodriguez, G. M., and I. Smith. 2006b. "Identification of an ABC transporter required for iron acquisition and virulence in Mycobacterium tuberculosis." *J Bacteriol* 188 (2):424-30. doi: 10.1128/jb.188.2.424-430.2006.

Sah, Stuti, and Rajni Singh. 2015. "Siderophore: Structural and functional characterisation–A comprehensive review." *Agriculture (Pol'nohospodárstvo)* 61 (3):97-114.

Schwyn, B., and J. B. Neilands. 1987a. "Universal chemical assay for the detection and determination of siderophores." *Anal Biochem* 160 (1):47-56. doi: 10.1016/0003-2697(87)90612-9.

Schwyn, Bernhard, and JB Neilands. 1987b. "Universal chemical assay for the detection and determination of siderophores." *Analytical biochemistry* 160 (1):47-56.

Sopirala, Madhuri M, Julie E Mangino, Wondwossen A Gebreyes, Beth Biller, Tammy Bannerman, Joan-Miquel Balada-Llasat, and Preeti Pancholi. 2010. "Synergy testing by Etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant Acinetobacter baumannii." *Antimicrobial agents and chemotherapy* 54 (11):4678-4683.

Takase, Hiroyuki, Hironobu Nitanai, Kazuki Hoshino, and Tsuyoshi Otani. 2000. "Impact of siderophore production on Pseudomonas aeruginosa infections in immunosuppressed mice." *Infection and immunity* 68 (4):1834-1839.

Thomas, Xavier, Delphine Destoumieux-Garzón, Jean Peduzzi, Carlos Afonso, Alain Blond, Nicolas Birlirakis, Christophe Goulard, Lionel Dubost, Robert Thai, and Jean-Claude Tabet. 2004. "Siderophore peptide, a new type of post-translationally modified antibacterial peptide with potent activity." *Journal of Biological Chemistry* 279 (27):28233-28242.

Wayne, PA. 2012. "Clinical and laboratory standards institute (CLSI)." *Performance standards for antimicrobial susceptibility testing*.

Wencewicz, Timothy A, Ute Möllmann, Timothy E Long, and Marvin J Miller. 2009. "Is drug release necessary for antimicrobial activity of siderophore-drug conjugates? Syntheses and biological studies of the naturally occurring salmycin "Trojan Horse" antibiotics and synthetic desferridanoxamine-antibiotic conjugates." *Biometals* 22 (4):633-648.

Wilson, B. R., A. R. Bogdan, M. Miyazawa, K. Hashimoto, and Y. Tsuji. 2016. "Siderophores in Iron Metabolism: From Mechanism to Therapy Potential." *Trends Mol Med* 22 (12):1077-1090. doi: 10.1016/j.molmed.2016.10.005.