Nat. Volatiles & Essent. Oils, 2021; 8(6): 4500-4514

NVEO
Natural Volatiles &
Essential Oils

The Influence Of Iron In The Growth And Bio Film Formation Of Klebsiella Pneumoniae And ESBL Producing Klebsiella Pneumoniae

Lyndia Effendy[1] , Eddy Bagus Wasito[1] , Agung Dwi Wahyu Widodo[1]

[1]Department of Medical Microbiology Faculty of Medicine Universitas Airlangga

Abstract

Background: The aim of this study was to investigate the impact of different iron concentrations in the growth and biofilm formation betweenKlebsiella pneumoniae and ESBL producingKlebsiella pneumoniae

Methods: An experimental study used a sample of 12 clinical isolates of ETT aspirates from the Unit of Clinical Microbiology,Dr. Soetomo hospital, starting from October 2020 to March 2021. A growth measurement and a biofilm formation test were performed at different concentration of iron sulphate.Statistical analysis was performed using SPSS, one-way ANOVA test and post hoc LSD, with significancy level $p<0.05$.

Results: In the absence of iron, 0 µMol, classicK. pneumoniae showed a slower peak attainment of growth (5 McFarland), which occurred at 5 hours incubation, one hour later than the ESBL producing K. pneumoniae and ATCC 700603. With the addition of low iron concentration (1 µMol), it increased the time of peak achievement of growth rate for all strains, classic K. pneumoniae, ESBL producing K. pneumoniae and ATCC 700603. An intermediate iron sulphate concentration, 10 µMol, classic Klebsiella pneumoniaeshowed a slower rate of two peaks attainment for its log growth fase, the first peak (4 McFarland) was after five hours incubation and then the second (4.5 McFarland)occurred after 12 hours incubation, whencompare toATCC 700603. At high concentration of iron sulphate (100µMol), all strains formed gas, therefore the normal peak of log growth phase was not achieved instead an irregular fluctuating growth density curve was observed.After 24 hours incubation (day one), in the absence of iron, classic K. pneumoniae formed a moderate density (1 unit) of biofilm compare to slightly more moderate (1.3 unit) biofilm formation by ESBL producing K. pneumoniae ($p=0.024$) and a stronger (1.6 unit) biofilm density of ATCC 700603 ($p=0.014$). These biofilm densities showed the same pattern of density escalation in day two. Low (1µMol) and intermediate (10 µMol) induced all three strains into forming stronger density biofilm, whereas, high iron concentration, 100 µMol, did limit their biofilm formation.

Conclusion: ESBL producing Klebsiella pneumoniae and ATCC 700603 showed a faster growth rate and significantly stronger biofilm formation compare to classic K. pneumoniae in the depletion of iron concentration (0 µMol) ($p < 0.05$).Low (1 µMol) iron concentrations had proven their effect as inducer of growth and significantly forming stronger and more stable biofilm in all three strains ($p> 0.05$). Whereas an intermediate (10 µMol)iron concentration showed similar inducing effect in classic Klebsiella pneumoniae and ESBL producing Klebsiella pneumoniae, except ATCC 700603 tends to show more increased planktonic growth and decreasing biofilm stability in day 2 incubation ($p < 0.05$). A 100 µMol concentration of iron sulphate is considered high and showed an increased rate of gas formation effect which resulted in disrupted pattern of

growth and a significantly slower and weaker biofilm formation in all three strains (p> 0.05, there was no significant different among three groups).

Keywords: Iron, Cellular Growth, Biofilm, Klebsiella pneumoniae

INTRODUCTION

Klebsiella pneumoniaeisrecently identified as aleading bacterial cause of ventilated associated pneumoniaoccurs in ICU patients requiring mechanical ventilation in hospitals after surgery or various critical illness (Zheng et al, 2018, Nirwatiet al, 2019, Buffet et al, 2021, Schulze et al, 2021). Surveillance studies from Dr Soetomo Hospital, between 2019-2020, showed Klebsiella pneumoniae as the main Gram-negative bacteria causing ventilator-related nosocomial infection (VAP) in ICU patients. The long-term use of a medical device, such as an endotracheal tube, is known to provide a non-shedding surface in non-sterile liquid or wet environment, impaired mucociliary clearance, and other impaired host defenses, promote accelerated growth of this normal flora colonization into outgrow dysbiosis and persistence biofilm infection as well as its high antibiotics resistance (Summaiyaet al, 2012, Jefferson, 2004, Pope et al, 2019).

Apart from poor antibiotic perfusion through biofilm matrix, Klebsiella pneumoniaeisalso recognized for carrying plasmids that encode resistance mechanism to counter aminoglycoside, fluoroquinolone, and ESBL classes(Tilleet al, 2017). This mode of resistance occurred due previous exposure to this broad-spectrum antibiotics and selective pressure phenomenon, therefore, Klebsiella pneumoniae attempts to increase it biological fitness cost by increasing its virulence, its transmission and its growth rate(Tilleet al, 2017, Johnson, 2018). Therefore, this study further investigated the effect of iron on the growth and biofilm formation of ESBL producing Klebsiella pneumoniae and its ATCC 700603.

Iron is highly abundant minerals found in environment and accumulativelly available in human gut due to lack of excretory pathway (Oliveira et al, 2021). This remaining not absorbed iron by the human enterocytes, enters the colon where potentially pathogenic bacteria, such as natural inhabitant Enterobacteriacea family, Klebsiella pneumoniae, can utilise it for growth and biofilm formation as their natural lifecycle(Burretet al, 2019). The iron availability in respiratory mucosal epithelial cell is from the release of iron from the host transferrin and ferritin from lung tissue to the respiratory lining fluid for clearance by mucociliary pathway or to the reticuloendo the lial system for long term storage (Ghioet al, 2006). A study from Gallo, 2012 showed that Klebsiella speciesis able to grow on Fe(III) citrate as energy source, yielding acetic acid and CO2 coupled with Fe(III) reduction to Fe(II) and produces an exopolysaccharide (EPS) having a high rhamnose content and abiotic surface-binding properties, whose enhances its ability of biofilm formation. The ability to obtain iron is therefore an important virulence factor and in response to iron deprivation, Klebsiella pneumoniae is known to produce small iron-chelating compounds called siderophores, up to four siderophores: enterobactin, salmochelin, aerobactin and yersiniabactin, and membrane receptor protein(Lodge et al, 1986, Bengoecheaet al, 2019, Parmanadaet al, 2019, Effahet al, 2020). This high affinity iron uptake system is the key virulence factor of Klebsiella pneumoniaein surviving during nutrient and iron limiting condition of infection (Lodge et al, 1986, Parmanadaet al, 2019, Effahet al, 2020). Once succeed in mucosal surface colonization, along with Klebsiella pneumoniae natural life cycle, form biofilm to survive and persist on site (Summaiyahet al, 2012, Jefferson, 2004, Tilleetal, 2017, Johnson, 2018, Pope et al, 2019). Most importantly, Klebsiella pneumoniae biofilm may disperse

their cells by quorum sensing signaling thus may efficiently achieve translocated colonization, combine with subverting from the host immune response and may cause further bloodstream infection (Buffet et al, 2021, Schulze et al, 2021).

Thereis still limited study on the role of iron inKlebsiella pneumoniae biofilm formation(Hu et al, 2020). Therefore, this present study aimed to evaluate in vitro the comparative effect of iron for cellular growth and biofilm formation of classic Klebsiella pneumoniae and ESBL producing Klebsiella pneumoniae. The results were intended to provide a proper infection management advice.

METHODS

Bacteria, media and culture conditions

Sample used in this study were collected from six clinical isolates of classicKlebsiella pneumoniae, six clinical isolates of ESBL producingKlebsiella pneumoniae of ETT aspirates specimen and a control ofESBL producingKlebsiella pneumoniae ATCC 700603. All isolates were then stored in cryotubes containing Trypticase Soy Broth. Several experimental growth media were then made by providing some TSB experimental bottles containing each an additional 2ml of zero µMol iron sulphate (absence of iron), 1µMol iron sulphate considered as a low iron concentration, 10µMol iron sulphate as an intermediate iron concentration, and 100µMol iron sulphate as a high iron concentration. Klebsiella pneumoniae suspension were prepared by diluting 0.5 McFarland Klebsiella pneumoniae colonies into 2ml sterile NaCl. Each of these diluted bacterial suspensions were then added to TSB media broth containing different concentrations of iron sulphate.

Growth measurement

The growth measurement was performed by measuring and recording the densities at 0 hour after adding bacterial suspension to each culture TSB media (plus iron sulphate) to ensure an initial homogenized measurement data. Then every hour of incubation at 37^0C incubator, the bottle containing mixture of TSB plus iron sulphate concentration and bacterial suspension were observed and measured the densities using BD neflometer. This observation was completed with an every hour measurement and recordup to 24hours length study. A measured density from BD Neflometer of 1 McFarland is approximately equal to cell density 3 x 10^8CFU/ml (Rumbaugh et al, 2020).

Biofilm detection

Biofilm formation in fluid phase was detected by microtiter plate methods and spectrophotometry counting. Briefly a total of freshly made 0.5 McFarland of classic Klebsiella pneumoniae, ESBL producing Klebsiella pneumoniae and ATCC 700603 were prepared. Afterwards, each microwell of the microtiter plate were filled with 180µl TSB with added several different concentrations of iron sulphate (0,1,10,100µMol) and 20µl of bacterial suspension were further added. The microwell plates were covered to avoid evaporation bias and incubated at 37^0C incubator for 24-hour and made an extra similar plate for 48hours incubation. After incubation, the microwell plate were washed three times in phosphate buffer saline (pH 7.2) and air-dried in inverted position. When fully dried, 150µl methanol were added into each well to ensure the biofilm were formed and fixated at the bottom of microwell. After 20 minutes of methanol fixation, each microwell would be emptied by inverting the microwell plate and tapping the fluid out. Then 0,1% crystal violet were added and leaved for five minutes then washed gently the excess crystal violet. The biofilm was quantified by solubilizing crystal violet with a mixture of ethanol and acetone (80: 20 v/v) and by determining the

absorbance of the sample at 595nm using BioRad spectrophotometer. Biofilm producer Klebsiella pneumoniae was determined by its optical density cut off more than 0.4. A weak biofilm producer when 0.4 ≤ OD 595 < 0.8, moderate biofilm producer when 0.8≤ OD 595 < 1.6. A high biofilm producer when OD≥ 1.6

Data analysis

Statistical analysis was performed using SPSS, the normality test with Kolmogorov Smirnov, one-way ANOVA test and post hoc LSD, $p<0.05$ were considered significant. The graphs were set up using SPSS general lineal model.

RESULTS

Growth ofclassic Klebsiela pneumoniae, ESBL producing Klebsiella pneumoniae and ATCC 700603 under different iron concentrations

The mean difference of the growth rate between classic K. pneumoniae-ATCC 700603 and classic K. pneumoniae – ESBL producing K. pneumoniae were not significantly different statistically, with p value > 0.05. All strains showed similar growth patterns. However, when examined into detail with the use of graphics, there were slight differences among three strains in the speed of bacterial growth time. For instance, in the absence of iron, 0 µMol, classic K. pneumoniae showed a slower peak attainment of growth (5 McFarland), which occurred at 5 hours incubation, one hour later than the ESBL producing K. pneumoniae and ATCC 700603. With the addition of low iron concentration (1 µMol), it fasten the time of peak achievement of growth ratefor all strains, classic K. pneumoniae, ESBL producing K. pneumoniae and ATCC 700603. The peak level of growth for both classic K. pneumoniae and ATCC 700603 were 4 McFarland occurred after 4 hours incubation, whilst, for ESBL producing K. pneumoniae was 4.5 McFarland after 4 hours incubation. Apart from bringing all three strains to a common speed of growth rate, this low iron concentration seems to induce the ATCC 700603 to generate a second peak of growth after 14 hours incubation. An intermediate iron sulphate concentration, 10 µMol, classic Klebsiella pneumoniaeshowed a slower rate of two peaks attainment for its log growth fase, the first peak (4McFarland) was after five hours incubation and then the second (4.5 McFarland)occurred after 12 hours incubation, when compare toATCC 700603, the first peak 4 McFarland at four hours incubation and the second 5 MacFarland after 13 hours incubation. Whilst, ESBL producing Klebsiella pneumoniaeseem to have just one peak of log phase (4 McFarland) after 4 hours incubation with a declining stationary phase. The most visible different was observed at high concentration of iron sulphate (100µMol), all strains formed gas, obstructing the BD nephlometer optical density reading, therefore the normal peak of log growth phase was not achieved instead an irregular fluctuating growth density curve was observed.

Biofilm formation ofclassic Klebsiela pneumoniae, ESBL producing Klebsiella pneumoniae and ATCC 700603 under different iron concentrations

After 24 hours incubation (day one), in the absence of iron, classic K. pneumoniaeformed a moderate density (1 unit) of biofilm compare to slightly more moderate (1.3 unit) biofilm formation by ESBL producing K. pneumoniae ($p=0.024$) and a stronger (1.6 unit) biofilm density of ATCC 700603 ($p=0.014$). These biofilm densities showed the same pattern of density escalation in day two. Biofilm formed by classic K. pneumoniae and ESBL producing K. pneumoniae, both showed enhancement to strong biofilm (1.6 unit) ($p=0.718$), compare to ATCC 700603 even to a stronger biofilm (2.5 unit)

(p=0.004). Low iron concentration (1 µMol) induced all three strains ability to form a strong biofilm (> 1,6 unit) (p=0.371 and p=0.816) in day one and their biofilm enhancement densities (> 1.7 unit) in day two (p=0.793 and p=0.304), as shown in the figures. At intermediate iron level, 10 µMol, induced both classic K. pneumoniae and ESBL producing K. pneumonias trains into forming strong biofilm (> 1,6 unit), and getting slightly strong in day 2 (p=0.146), except ATCC700603 showed a decreasing stability into a moderate only biofilm density (p=0.023). At high iron concentration, 100 µMol, day one, the biofilm density of both, ATCC 700603 with < 0.4 unit, ESBL producing K. pneumoniae (< 0.4 unit), were weak, whilst classic K. pneumoniae succeed in forming a moderate density biofilm (0.7 unit) (p=0.014, p=0.24). In day two, the biofilm density increased for all three strains,classic K. pneumoniae turning into a strong biofilm (> 1.6 unit), ESBL producing K. pneumoniae also turning into a strong biofilm (> 1.6 unit) (p=0.718), ATCC700603 turning into a moderate biofilm (0.6 unit) (p=0.004).

DISCUSSION

This present study showed that nutrient broth in the absence of iron is able to support to growth and biofilm formation of all three strains of classic Klebsiella pneumoniae, ESBL producing K. pneumoniae and ATCC 700603eventhough, the growth rate and biofilm formation rate of classic K. pneumoniae is slightly slower. Low (1 µMol)and intermediate (10 µMol) concentrations of iron sulphate have proven their inducing effect of growth for all three strainclassic Klebsiella pneumoniae, ESBL producing Klebsiella pneumoniae, ATCC 700603.There is assumption that iron is critical and required in micro-concentration for the growth and synthesize of these bacterial DNA. In this low concentration, it was also observed that ATCC 700603 has presumably the capability of survive longer in stationary phase, due to activation of HIF (hypoxia inducible factor)-1α protein, in order to persist in nutrient and oxygen scarce environment(Holden et al, 2016). The same survival phenomenon was also seen in intermediate iron concentration for classic Klebsiella pneumoniae and ATCC 700603.Whereas, a high concentration iron sulphate,100µMol does not support the planktonic growth, due to theirbubble gases metabolism, resulting in weaker and slower speed biofilm formation, similar to what happened with other Gram-negative bacteria, Pseudomonas aeruginosa (Berluttiet al, 2005, Rumbaugh et al, 2020). It can be sum up that, both in the absence and the presence of low and moderate iron concentration, Klebsiella pneumoniae pose a huge danger in undernourished and immune compromised patient who losses their epithelial barrier immunity, losses cellular immune competent function to clear the pathogen combined with antibiotic usagewhich then leads to dysbiosis loss of beneficial microbial organism, expansion of potentially harmful Klebsiella, losses of overall microbial diversity and progression to develop opportunistic nosocomial biofilm related infection, whereas at high iron concentration support these bacteria planktonic growth with production of bubble gases and cause planktonic dispersal infection.

CONCLUSIONS

ESBL producing Klebsiella pneumoniae and ATCC 700603 showed a faster growth rate and significantly stronger biofilm formation compare to classic K. pneumoniae in the depletion of iron concentration (0 µMol) ($p < 0.05$). Low (1 µMol) iron concentrations had proven their effect as inducer of growth and significantly forming stronger and more stable biofilm in all three strains ($p > 0.05$). Whereas an intermediate (10 µMol)iron concentration showed similar inducing effect in classic Klebsiella pneumoniae and ESBL producing Klebsiella pneumoniae, except ATCC 700603 tends to show more increased planktonic growth and decreasing biofilm stability in day 2 incubation ($p <$

0.05). A 100 µMol concentration of iron sulphate is considered high and showed an increased rate of gas formation effect which resulted in disrupted pattern of growth and a significantly slower and weaker biofilm formation in all three strains (p> 0.05, there was no significant different among three groups).

ACKNOWLEDGMENTS

The authors would like to thank numerous people who are in the Clinical Microbiology Unit of Dr. Soetomo hospital, all technicians and staffs in microbiology laboratory in Dr Soetomo's hospital, for theirs valuable technical support on this project. Special gratitude should be given to Dr. dr. Eko Budi Koendhori, MKes, SpMK(K), the head of clinical microbiology department, and to Prof. Dr. dr. Ni Made Mertaniasih, MS, SpMK(K), the coordinator of clinical microbiology department, for the permission to begin this study. An ethical clearance for this project was approved from Dr Soetomo's hospital ethical committee, Ref no:0368/LOE/301.4.2/II/2021.

AUTHORS CONTRIBUTION

All authors contributes equally in this manuscript.

APPENDICES

Figure 1. The effect of 0µMol iron concentration on the growth of the three strains of Klebsiella pneumoniae

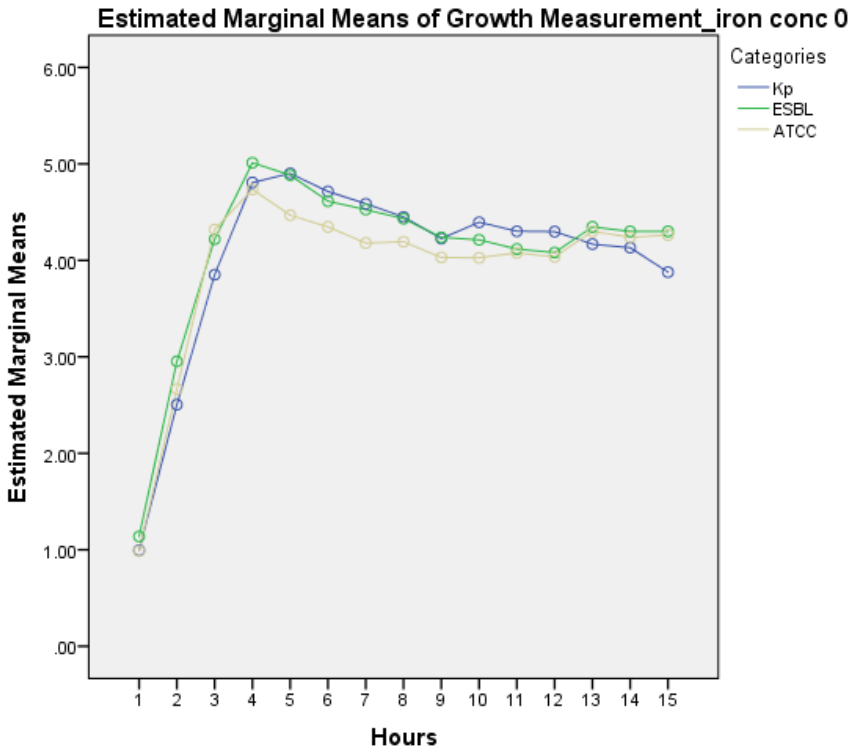

Figure 2. The effect of 1µMol iron concentration on the growth of the three strains of Klebsiella pneumoniae

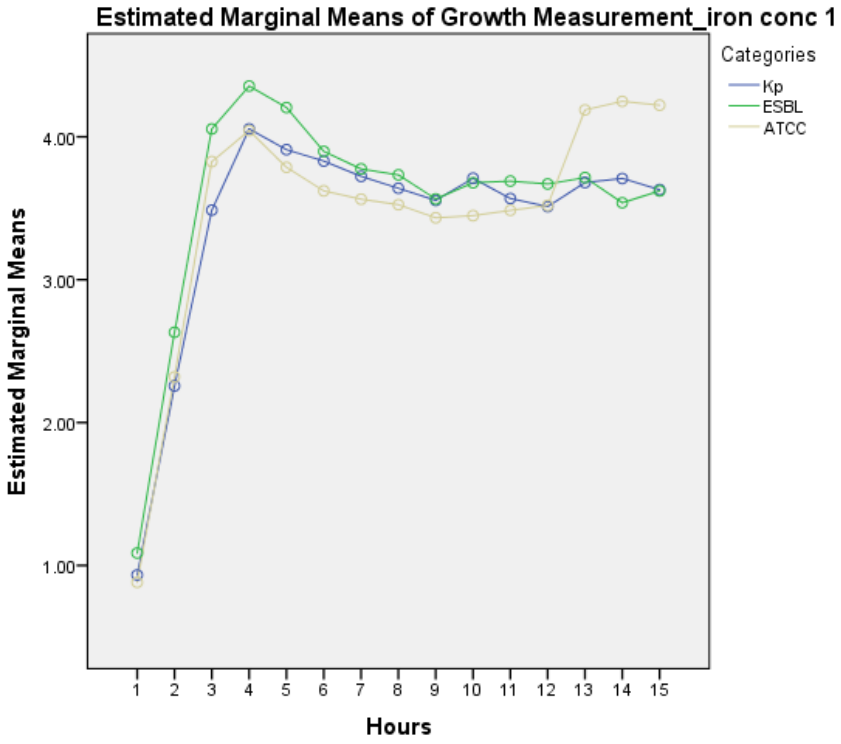

Figure 3. The effect of 10µMol iron concentration on the growth of the three strains of Klebsiella pneumoniae

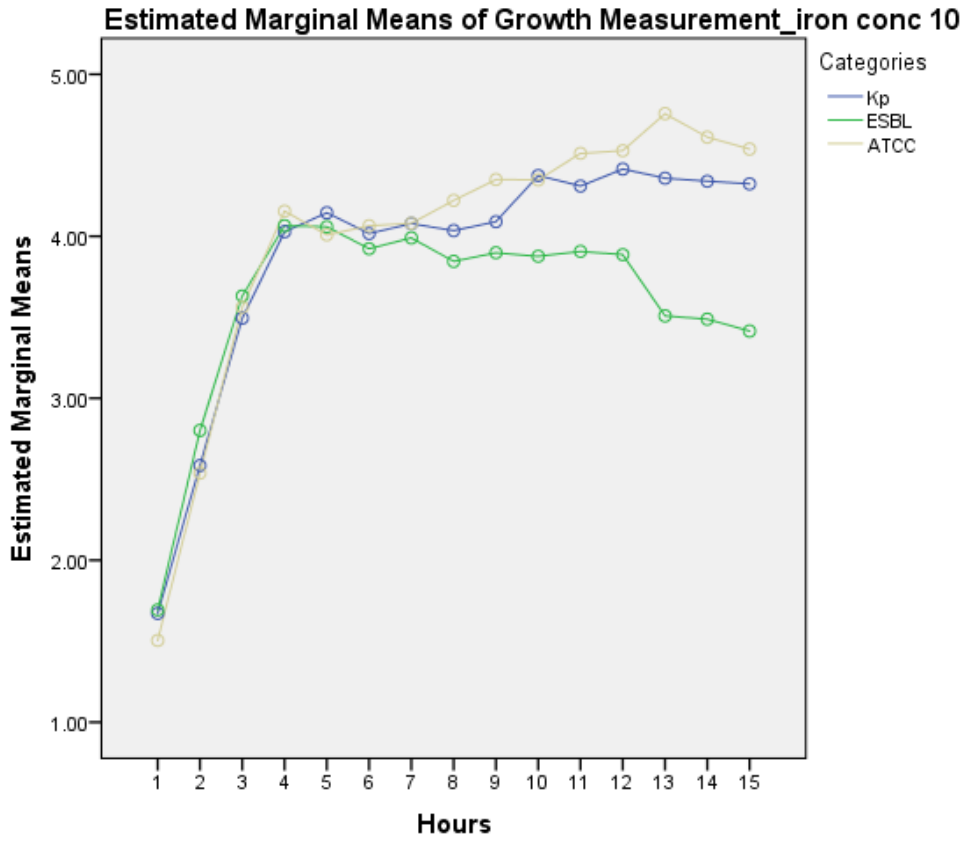

Figure 4. The effect of 100µMol iron concentration on the growth of the three strains of Klebsiella pneumoniae

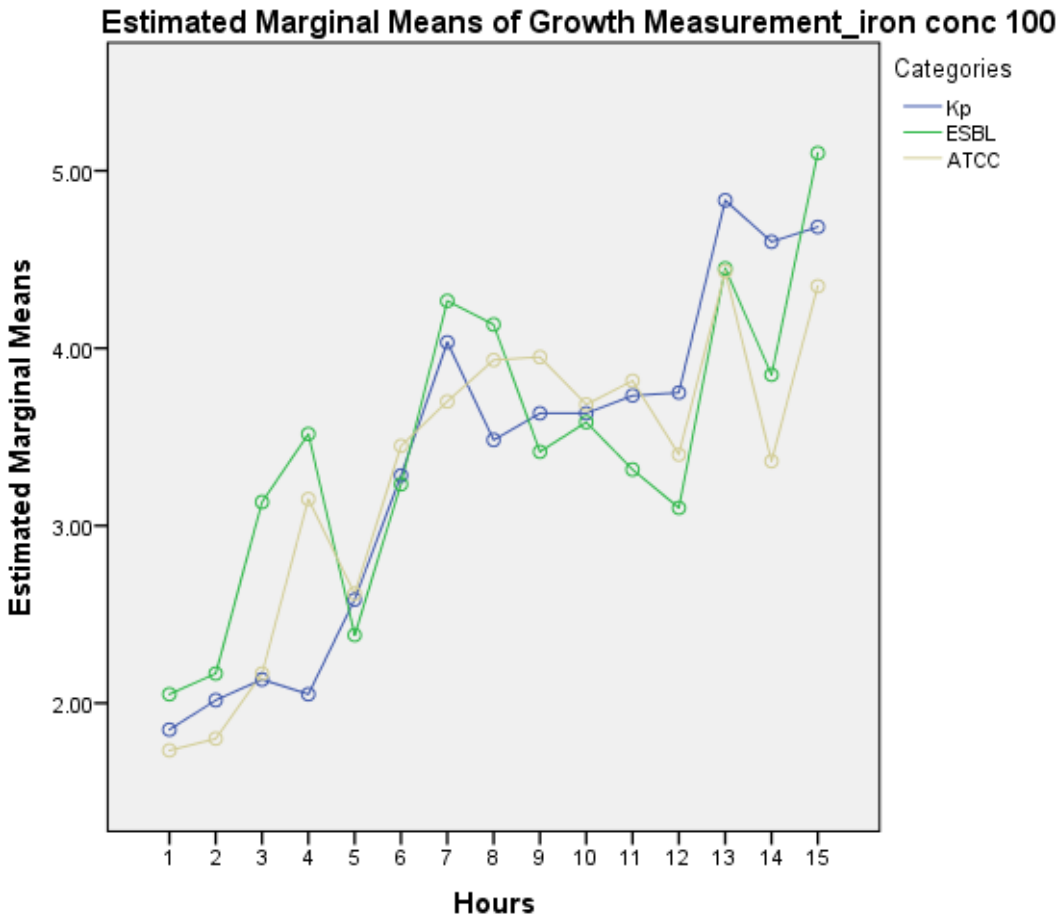

Figure 5. The effect of 0µMol iron concentration on the biofilm formation of the three strains of Klebsiella pneumoniae at 24-hour (day 1) and 48-hour (day 2) observation

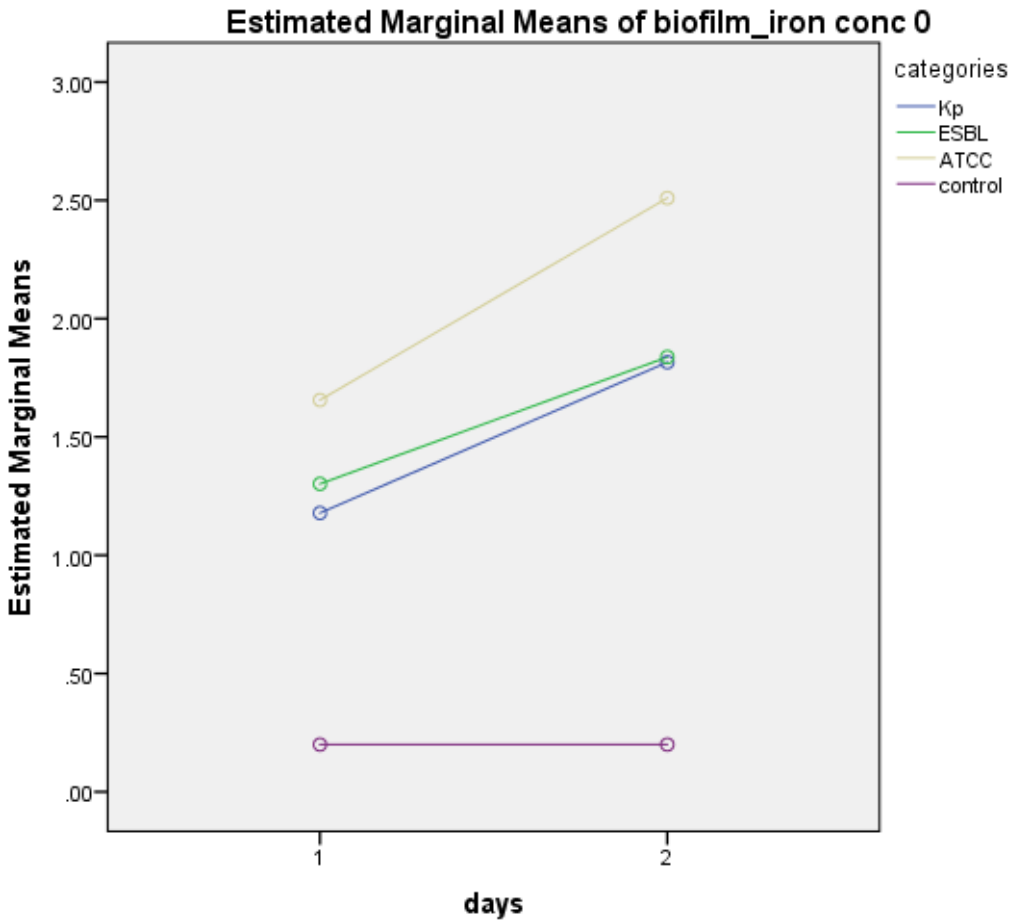

Figure 6. The effect of 1µMol iron concentration on the biofilm formation of the three strains of Klebsiella pneumoniae at 24-hour (day 1) and 48-hour (day 2) observation

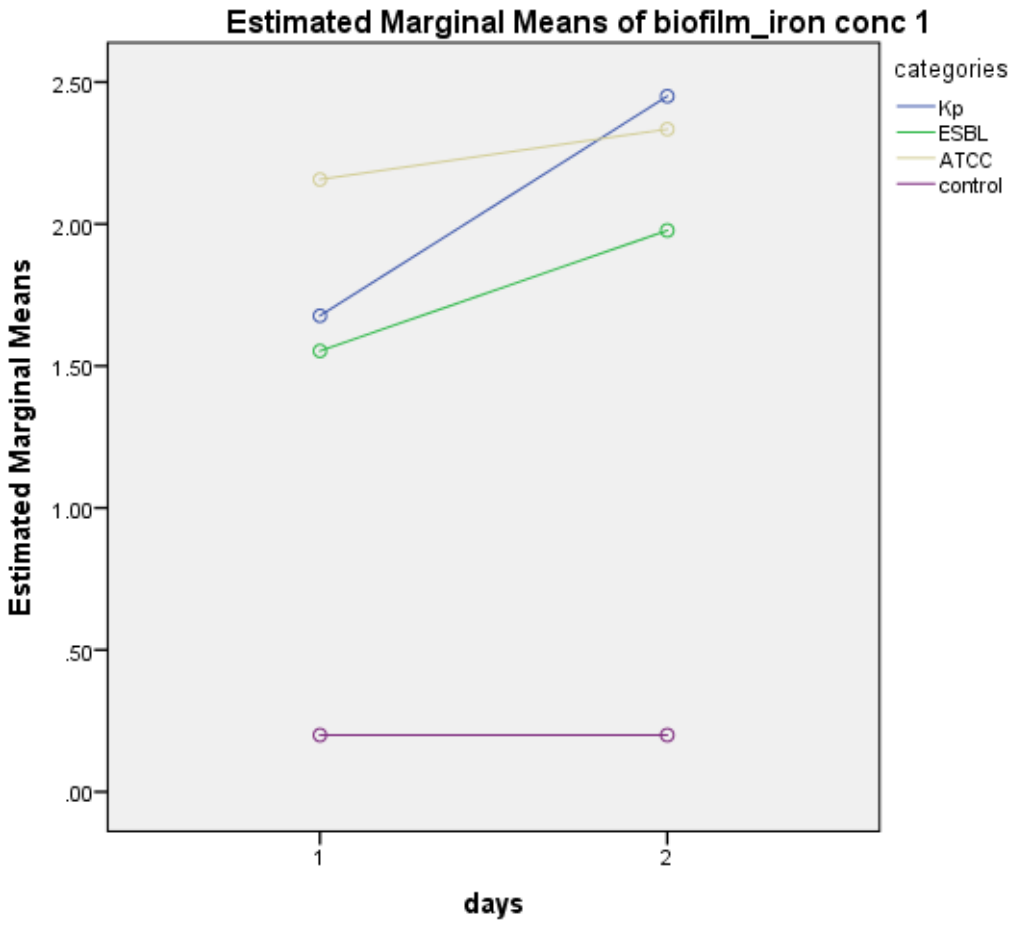

Figure 7. The effect of 10µMol iron concentration on the biofilm formation of the three strains of Klebsiella pneumoniae at 24-hour (day 1) and 48-hour (day 2) observation

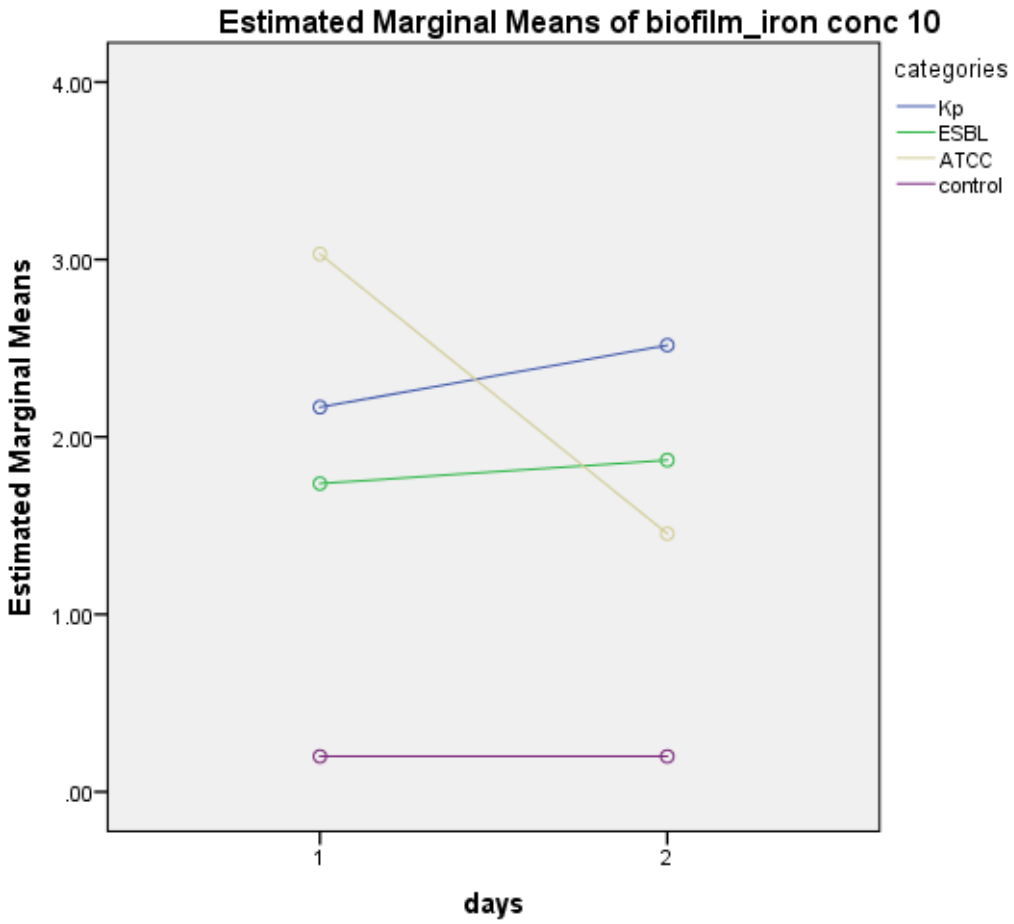

Figure 8. The effect of 100µMol iron concentration on the biofilm formation of the three strains of Klebsiella pneumoniae at 24-hour (day 1) and 48-hour (day 2) observation

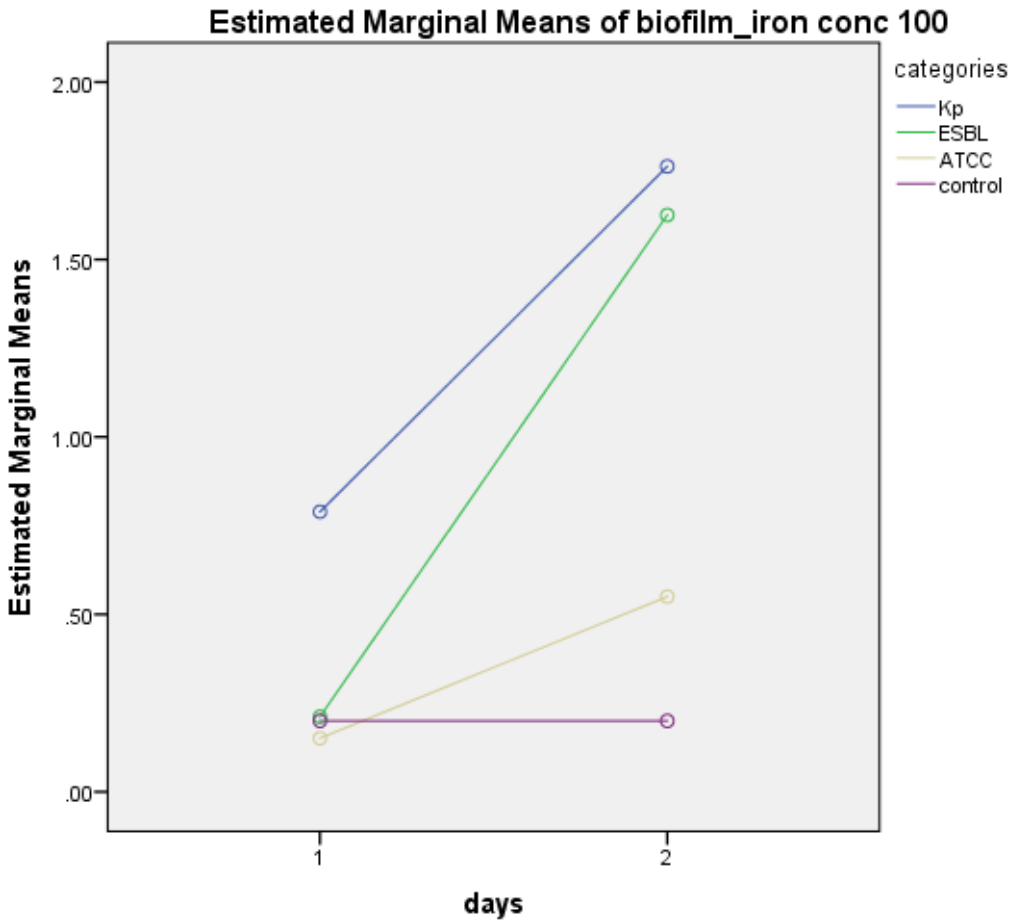

Table 1. One way Annova Post Hoc LSD test – Bacterial Growth Rate

	Control Positive K. pneumoniae – ATCC 700603		Test K. pneumoniae – ESBL producing K.pneumoniae	
Iron Concs. (µMol)	Mean Difference ± SD	Significance 95% CI	Mean Difference ± SD	Significance 95% CI
0	0.089 ± 0.208	0.675	0.077 ± 0.208	0.715
1	0.610 ± 0.191	0.754	0.135 ± 0.191	0.492
10	0.101 ± 0.236	0.675	0.285 ± 0.236	0.245
100	0.502 ± 0.189	0.795	0.933 ± 0.189	0.630

Table 2. One way Annova Post Hoc LSD test – Bacterial Biofilm Formation

	Control Negative K. pneumoniae-control negative	Control Positive K. pneumoniae-ATCC 700603	Test K. pneumoniae-ESBL producing K. pneumoniae

Iron Concs. (µMol)	Day 1		Day 2		Day 1		Day 2		Day 1		Day 2	
	Mean Diff ± SD	Sig. 95% CI	Mean Diff ± SD	Sig. 95% CI	Mean Diff ± SD	Sig. 95% CI	Mean Diff ± SD	Sig. 95% CI	Mean Diff ± SD	Sig. 95% CI	Mean Diff ± SD	Sig. 95% CI
0	0.589 ± 0.237	0.022	1.562 ± 0.373	0	0,638 ± 0.237	0.014	1.212 ± 0.373	0.004	0.578 ± 0.237	0.024	0.137 ± 0.373	0.718
1	1.497 ± 0.524	0.010	2.27 ± 0.448	0	0.480 ± 0.524	0.371	0.119 ± 0.448	0.793	0.124 ± 0.524	0.816	0.473 ± 0.448	0.304
10	1.988 ± 0.513	0.001	2.337 ± 0.428	0	0.863 ± 0.513	0.108	1.058 ± 0.428	0.023	0.431 ± 0.513	0.411	0.648 ± 0.428	0.146
100	0.609 ± 0.237	0.018	1.582 ± 0.373	0	0.639 ± 0.237	0.014	1.212 ± 0.373	0.004	0.578 ± 0.237	0.24	0.137 ± 0.373	0.718

REFERENCES

1. Zheng, J. X., Lin, Z. W., Chen, C., Chen, Z., Lin, F. J., Wu, Y., Yang, S. Y., Sun, X., Yao, W. M., Li, D. Y., Yu, Z. J., Jin, J. L., Qu, D., & Deng, Q. wen. 2018. Biofilm formation in Klebsiella pneumoniae bacteremia strains was found to be associated with CC23 and the presence of wcaG. Frontiers in Cellular and Infection Microbiology, 8(FEB). https://doi.org/10.3389/fcimb.2018.00021
2. Buffet A, Rocha EPC, Rendueles O. Nutrient conditions are primary drivers of bacterial capsule maintenance in Klebsiella. Proc Biol Sci. 2021 Mar 10;288(1946):20202876. doi: 10.1098/rspb.2020.2876. Epub 2021 Mar 3. PMID: 33653142; PMCID: PMC7935059.
3. Schulze, A. Mitterer, F. Pompo, JP. Schild, S. 2021. Biofilms by bacterial human pathogens: Clinical Relevance-development, composition and regulation-therapeutical strategies. Microbial Cell 8:2. http://microbialcell.com/researcharticles/2021a-schulze-microbial-cell/
4. Nirwati, H., Sinanjung, K., Fahrunissa, F. et al. 2019. Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC Proc **13,** 20. https://doi.org/10.1186/s12919-019-0176-7
5. BMJ. 2021. Overview of pneumonia. BMJ Open Respiratory Research
6. Summaiya, AM. Urmi, NJ. 2012. Assessment of biofilm formation by the causative organisms of ventilator associated pneumoniae at intensive care unit of a tertiary care hospital. National Journal of Medical Research, 2 (1 Jan-March)
7. Jefferson, KK. 2004. Mini Review: What drives bacteria to produce biofilm. FEMS Microbiology Letters 236 (2004) 163–173
8. Tille, P.M. 2017. Enterobacteriaceae: Klebsiella pneumoniae. Bailey & Scott's Diagnostic Microbiology 14th edition : pp.329-354. St Louis, Missouri: Elsevier
9. Johnson, D.I., 2018. Klebsiella spp. In Bacterial Pathogens and Their Virulence Factors (pp. 269-277). Springer, Cham.

10. Pope, J,L, Yang, Y. Newsome, R.C. Sun, W. Sun, X. Ukhanova, M. Neu, J. Issa, J.P. Maiz, V. Jobin, C. 2019. Microbial colonization coordinates the pathogenesis of a Klebsiella pneumoniae infant isolates. Nature Scientific Reports 9:3380. https://doi.org/10.1038/s41598-019-39887-8
11. Ghio, AJ.Turi, JL. Yang , F. Garrick, LM. Garrick, MD. 2006. Iron homeostasis in the lung. Biol. Res 39 (1). http://dx.doi.org/10.4067/S0716-97602006000100008.
12. Gallo, G.Baldi, F. Renzone, G. Gallo, M. Cordaro, A. Scaloni, A. Puglia, A.M. 2012. Adaptive biochemical pathways and regulating networks in Klebsiella oxytoca BAS-10 producing a biotechnologically relevant exopolysaccharide during Fe(III)-citrate fermentation. Microbial Cell Factories,11: 152. http://www.microbialcellfactories.com/content/11/1/152
13. Lodge, J.M.T. Williams, P. Brown,M.R.W.1986.Journal of Bacteriology 165;2:353-356.
14. Bengoechea, J.A. Sa Pessoa, J. 2019. Klebsiella pneumoniae infection biology: living to counteract host defenses. FEMS Microbiology Reviews. 43: 123-144. doi: 10.1093/femsre/fuy0437
15. Effah, C.Y. Sun, T. Liu, S. Wu, Y. 2020. Klebsiella pneumoniae: an increasing threat to public health. Ann Clin Microbiol Antimicrob, 19:1. https://doi.org/10.1186/s12941-019-0343-8
16. Parmanada, BA. Kellingray, L. Gall, GL. Basit, AW. Fairweather-Tait, S. Narbad, A. 2019. A decrease in iron availability to human gut microbiome reduces the growth of potentially pathogenic gut bacteria; an in vitro colonic fermentation study. Journal of Nutriotional Biochemistry 67: 20-27. https://doi.org/10.1016/j.jnutbio.2019.01.010
17. Oliveira, F. Rohde, H.Vilanova, M. Cerca, N. 2021. The emerging role of iron acquisition in biofilm-associated infections. Trends in Microbiology, 29: 9. https://doi.org/10.1016/j.tim.2021.02.009
18. Buret AG, Motta JP, Allain T, Ferraz J, Wallace JL. Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: a role for iron?. Journal of biomedical science. 2019 Dec;26(1):1-4.
19. Hussain, A. Ansari, A. Ahmad, R. 2020. Microbial biofilms: Human mucosa and intestinal microbiota. New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Biofilms, 47–60. doi:10.1016/B978-0-444-64279-0.00004-9
20. Hu, Y. Anes, J. Devineau, S. Fanning, S. 2020. Klebsiella pneumoniae: prevalence, reservoirs, antimicrobial resistance, pathogenicity, and infection: a hitherto unrecognized zoonotic bacterium. Foodborne pathogens and disease, 17(12). Doi: 10.1089/fpd.2020.2847
21. Liu, Y. Zhang, B. Cao, Q. Huang, W. Shen, L. Qin, X. 2009. Two clinical strains of Klebsiella pneumoniae carrying plasmid-borne blaIMP-4, bla SHV-12 and armA isolated at a pediatric center in shanghai, china. Antimicrobial Agents Chemother, 53: 1642-1644
22. Curiao, T. Morosini, M.I. Ruiz-Garbajosa, P. Robustillo, A. Baquero, F. Coque, T.M. et al.2010. Emergence of bla KPC-3-Tn4401a associated with a pKPN3/4-like plasmid within ST384 and ST388 Klebsiella pneumoniae clones in Spain. J. Antimicrob Chemother 65: 1608-1614
23. Cuzon, G. Naas, T. Lesenne, A. Benhamou, M. Nordman, P. Queenan, A.M. et al. 2010. Plasmid-mediated carbapenem hydrolising OXA-48 beta-lactamase in Klebsiella pneumoniae from Tunisia. Int J Antimicrob Agents; 36:91-93
24. Chen, L. Matherna, B., Chavda, K.D. DeLeo, F.R. Bonomo, R.A. Kreiswirth, B.N. 2014b. Carbapenemase-producing Klebsiella pneumoniae:Molecular and genetic decoding. Trends in Microbiology; 22: 686-696
25. Hudson, C.M. Bent, Z.W. Meagher, R.J. Williams, K.P. 2014. Resistance determinants and mobile genetic elements of an NDM-1-encoding Klebsiella pneumoniae strain. PLos One; 9:e99209

26. Jin, Y. Shao, C. Li, J. Fan, H. Wang, Y. 2015. Outbreak of multidrug resistant ndm-1-producing Klebsiella pneumoniae from a neonatal unit in Shandong province, china, PLos One; 10:e0119571
27. Lee, G.C., Burgess, D.S. 2012. Treatment of Klebsiella pneumoniae carbapenemase (KPC) infections: a review of published case series and case reports. Ann Clin Microbiol Antimicrob; 11:32
28. Mathers, A.J. Peirano, G. Pitout, J.D.D. 2015. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev; 28: 565-591
29. Pournaras, S. Poulou, A. Voulgari, E. Vrioni, G. Kristo, I. Tsakris, A. 2010. Detection of the new metallo-B-lactamase VIM-19 along with KPC-2, CMY-2 and CTX-M-15 in Klebsiella pneumoniae. J Antimicrob Chemoter; 65: 1604-1607
30. Prior, A.R., Roche, C. Lynch, M. Kelly, S. O'Rourke, K. Crowley, B. 2010. First identified case of VIM-producing carbapenem resistant Klebsiella pneumoniae in the Republic of Ireland associated with fatal outcome. Eurosurveillance; 15: 19752
31. Tzouveleki, L.S. Tzelepi, E. Tassios, P.T. Legakis, N.J. 2000. CTX-M type-B-lactamase: an emerging group of extended-spectrum enzymes Int J Antimicrob Agents; 14: 137-142
32. Tzouveleki, L.S. Markogiannakis, A. Psichougious, M. Tassios, P.T. Daikos, G.L. 2012. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: An evolving crisis of global dimensions. Clin Microbiol Rev; 25: 682-707
33. Zhang, X. Y. Li, X.P. Wang, M. Yue, H.J. Li, P.L. et al. 2015. Outbreak of ndm-1-producing Klebsiella pneumoniae causing neonatal infection in a teaching hospital in mainland China. Antimicrob Agents Chemother; 59: 4349-4351
34. Zheng, R. Zhang, Q. Guo, Y. Feng, Y. Liu, L. Zhang, A, et al. 2016. Outbreak of plasmid-mediated NDM-1-producing Klebsiella pneumoniae ST105 among neonatal patients in Yunnan, China. Ann Clin Microbiol Antimicrob; 15:10
35. Huang, H. Weaver, A. Wu, E. Li, Y. Gao, H. Fan, W. et al. 2013. Lipid based signaling modulates DNA repair response and survival against Klebsiella pneumoniae infection in host cells and in mice. Am J Respir Cell Mol Biol; 49: 798-807
36. Hennequin, C. Robin, F. 2016. Correlation between antimicrobial resistance and virulence in Klebsiella pneumoniae. Eur J Clin Microbiol Infect Dis: 35: 1-9
37. Liu, Y.Y. Wang, T.R. Yi, L.X. Zhang, R. Spencer, J. et al. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study". Lancet Infect Dis 16: 161-168
38. Choi, M-J. Kim, S. Ko, K.S. 2016. Pathways regulating the pbgP operon and colistin resistance in Klebsiella pneumoniae strains. J Microbiol Biotechnol 26: 1620-1628
39. Holden, VI. Breen, P. Houle, S. Dozois, CM. Bachman, MA. 2016. Klebsiella pneumoniae siderophores induce inflammation, bacterial dissemination, and HIF-1α stabilization during pneumonia. mBio 7 (5): e01397-16.doi:10.1128/mBio.01397-16
40. Berlutti, F. Morea, C. Battistonp, S. Sarli, P. Cipriani, F. Superti, MG. Ammendolia. Valenti, P. 2005. Iron availability influences aggregation, biofilm, adhesion and invasion of Pseudomonas aeruginosa and Burkholderia Cenocepacia. International Journal of Immunopathology and pharmacology vol 18 no 4: 661-670
41. Rumbaugh, KP. Sauer, K. 2020. Review: Biofilm dispersion. Nature Review Microbiology. https://doi.org/10.1038/s41579-020-0385-0