ISSN: 0067-2904

The Correlation Between the Persistence of Methicillin Resistant Staphylococcus Aureus Isolates to Mupirocine and Toxin-Antitoxin Type II Genes

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Received: 4/5/2021 Accepted: 6/7/2021 Published: 30/5/2022

Abstract

Under high concentrations of antibiotics, a fraction of the bacterial population exhibits a phenomenon known as persistence. Toxin- system (TA system) has been reported to be involved in the formation of E. coli, Mycobacterium, and S. aureus persisters. In this study, the ability of thirty Iraqi isolates of MRSA to form in vitro persister cells after exposure to three different antibiotics (Ceftriaxone 30 µg, Mecillinam 10 µg, and Mupirocin 20 µg) was examined by TD test. Additionally, efflux pump inhibitor [Fluphenazine 0.25 mg/ml] was combined with the antibiotic that triggered persister formation. The distribution of mazEF and yefM-yoeB (Type II TA system) in the tested isolates was detected by PCR. 91% of Mupirocin susceptible isolates formed persister cells.42% of the persistent level was reduced when Mupirocin was combined with the Fluphenazine. Genes for homologs of the yefM-yoeB and mazEF TA system were present in 100% of the tested isolates. The prevalence of these genes in the tested isolates suggested a link between persistence and the TA system. Further investigation is required to study the expression of these genes under stress conditions.

Keywords: Persistence, MRSA, Toxin- system, mazEF, yeEF-yoeB

العلاقة بين ثباتية عزلات المكورات العنقودية المقاومة للمثيسيلين الى المضاد الحياتي الميبروسين و جينات النوع الثاتي من النظام السمي و ضد السمي

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الخلاصة

تحت التراكيز العالية من المضادات الحيوية, يظهر جزء من النمو البكتري لظاهرة الاصرار (المثابرة). يعتبر نظام السم و ضد السم احد الانظمة التي تم توثيق دورها في تكوين الخلايا المثابرة في الاشريكية القولونية و المتفطرات و المكورات العنقودية. في هذه الدراسة قابلية ثلاثون عزلة عراقية من المكورات العنقودية المقاومة للمثسلين على تكوين الخلايا المثابرة في المختبر بعد التعرض لثلاث انواع من المضادات الحيوية

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(السفترياكزون 30 مايكروغرام , الميسيلينام 10 مايكروغرام و الميبروسين 20 مايكروغرام) تم التحري عتها باستخدام طريقة .TD test كذلك تم مزج المضاد الحيوي الذي حفز تكوين الخلايا االمثابرة مع مثبط مضخات التدفق(الفلوفينازين 0.25 مليغرام/ مليليتر). تم الكشف عن توزيع جيناتmazEF و yefM– yoeBفي العزلات بواسطة تفاعل البوليميريز المتسلسل.. 91% من العزلات الحساسة لمضاد المبيروسين كونت خلايا مثابرة في منطقة النمو المثبط بعد استبدال قرص المضاد الحيوي بقرص مشبع بالوسط الزرعي. انخفض مستوى بنسبة 42 % عند دمج الميبروسين مع مثبط مضخات التدفق. كانت جينات yefM–yoeBو mazEFموجودة في 100%من عزلات هذه الدراسة. يشيرانتشارهذه الجينات في العزلات إلى وجود صلة بين الثباتية ونظام السم و ضد السم. مزيدا من االدراسات مطلوبة لدراسة التعبير الجيني لجينات نظام السم و ضد السم تحت ظروف الإجهاد.

Introduction

The most common untreatable surgical infections in hospitals are those caused by S. aureus. This bacterium possesses different defense strategies that provide the ability to survive various stress conditions [1]. In a high concentration of antibiotics, some bacterial populations would use one of the resistance mechanisms, such as mutations, and become resistant [2]. While some subpopulation would alter their metabolic process and enter the dormancy state and become persistent [3,4]. After stress factors removal, these persistent cells will resume normal growth and generate viable cells that are just like the original cell´s phenotypes [5,6]. Studies performed on persistent *E. coli* cells revealed an increase in toxin-antitoxin transcripts levels and suggested the implication of the TA system in the formation of persistent cells [7 ,8, 9]. The formation of persistent cells was decreased upon toxin coding genes disruption or deletion, but resulted in more acute pathogenic infections [9,10,11] These observations confirmed the notion that the TA system is implicated in persistent cells formation. Many other studies performed on *E. coli* showed that antibiotics would induce the formation of persistence in a toxin- antitoxin dependent manner [7]. Toxin -antitoxin system is prevalent in various types of bacteria due to their movement among bacterial genome by horizontal gene transfer [12]. This system was first identified in plasmid as an element that enhances plasmid's stability. But then, by homology searches, it was detected in bacterial chromosomes with an ambiguous function [13,14,15]. Later on, many studies have conducted to understand the structure, function, and role of the chromosomal TA system to answer questions regarding the different cellular processes, like stress response, gene regulation, and persistence. At a molecular level, this system consists of two genes: toxin coding gene and antitoxin coding gene. The toxins are stable proteins of various activities (kinase, RNase, DNase, and acetyltransferase), while the antitoxins are unstable and can be either proteins or small RNAs [16,17,18,19]. The reason that the antitoxin proteins are less stable than the toxin proteins is that they are intrinsically disordered and very vulnerable to degradation by ATP-dependent protease or ATP-independent protease [20,21,22,23]. The toxins have certain cellular targets to attack that lead to cell growth inhibition. In normal growth conditions (absence of stress) the neutralize the effect of toxin to make sure that cells are in save of toxins effect [24,25]. Antitoxins neutralize the toxins either by direct interaction or by coupling with other elements of toxins expression regulation [21]. According to how antitoxin neutralizes the activity of toxins, the TA system has been classified into six types (I-VI). Type one and type three TA modules, the antitoxins are small RNA, while the rest of TA Types the antitoxins are proteins (type II, IV, V, and VI) [26]. The TA systems regulation and activation are triggered by stress response. Stress response elements increase the expression of toxin coding genes and increase the activity of toxins by decreasing antitoxin stability [19,20,27,28]. Among the six different types of TA systems, mazEF and yefM-yoeB type II TA systems are the well-studied modules, especially in E. coli and Staphylococcus aureus [29, 30]. The mazEF operon

consists of mazF gene locates downstream of mazE gene [31]. mazF is a stable ribosome-independent mRNA cleavage (activity is independent on coupling with ribosome) [32]. mazE is an unstable protein that is vulnerable to proteases. YoeB toxin is a ribosome-dependent mRNA sequence specific interferes that inhibits the initiation of the translation process. The action of yoeB toxin is counteracted by yefM anti-toxin which binds to the yefM-yoeB operon´s promoter and represses the transcription process [33]. The TA system, as mentioned above, has been associated with the formation of persister cells in gram-negative bacteria, but its role in Staphylococcus is controversial. So, this study aimed to investigate the ability of methicillin resistance S. aureus isolated from Iraqi patients to form persister cells using TD test (Tolerance and persistence Disk Test) which promotes the growth of persistent cells after removing the inducer agent (antibiotic disks), in addition, the association with the presence mazEF and yefM-yoeB chromosomal loci genes.

2. Materials and Methods

2.1. Bacterial isolates

Thirty isolates of *Staphylococcus aureus* were isolated from the noise, blood, and wounds of Iraqi patients. *Staphylococcus aureus* isolates were identified by biochemical tests oxidase, coagulase, and catalase [34]. The isolates were confirmed by PCR for the presence of *nuc* and *mec*A genes.

2.2. Persister cells detection

2.2.1. Antibiotic sensitivity test

With a sterile loop, four morphologically similar colonies were transferred to Mueller Hinton broth. Inoculated media was incubated at (35-37 °C) until the visible turbidity is equal to 0.5 McFarland. The inoculum was spread evenly over the entire surface of the Muller Hinton agar. Discs with defined concentrations CRO, Ceftriaxone (30 µg), Mupirocin, MUP (20 µg), and Mecillinam, MEC (10 µg) were applied to the surface of an agar plate. Plates were incubated at 37 ˚C for 18hr.

2.2.2. Persistence disk test

After incubation at 37 °C for 18hr, the inhibition zones were measured and determined for each isolate and antibiotic, the antibiotic disks were carefully removed with sterile forceps, and disks containing a sufficient amount of nutrient broth were substituted. The substituted disks were prepared by pipetting 100 µl of nutrient broth onto the blank disks. These disks were used immediately after their preparation. Then the plates were incubated at 37 °C for 18hr.

2.2.3. Spot plating Assay

Exponential growing cells were exposed to Mupirocin (20 µg/ml) and Ceftriaxone(30µg/ml) for four hours at 37 ºC. 2 µl of each isolate was plated to determine the number of survived cells (level of persistence).

2.2.4 Efflux pumps inhibitor

Exponential growing cells were grown in nutrient broth containing Mupirocin (20 µg/ml) only and in nutrient broth containing Mupirocin (20 µg/ml) and Fluphenazine with final concentration (0.25 mg/ml). the cultures were incubated for 4hr at 37Cº. Cultures were centrifuged and re-suspended in nutrient broth and 2µl of suspended cells was spotted in nutrient agar media and incubated at 37 Cº for 18hr.

2.3. DNA Isolation.

Extraction of DNA from 1 ml of overnight culture using Promega DNA extraction kit supplemented with (30 µg/ml) lysozyme enzyme.

2.4. PCR Detection of mazEF and yefM-yoeb loci.

1µl of 100ng DNA was used as a template for PCR. mazEF loci presence among the extracted DNA of isolates was determined by polymerase chain reaction by using forward primer (5'-AGCTACTGCATTCAGCCCTA-3') and reverse primer (5'-

AGGGATCTGAACAAGGGGGA-3'), which will yield 300 bp fragment. yefM-yoeB loci were detected by using forward primer (5'-CGACCCTGTGCTGATGGTTA-3') and reverse primer (5'-CACTAGCCCTACAGAAGCGAG-3'), which will yield 451 bp fragments. 25 µl of PCR reaction contains 1x master mix buffer, 10 pmol/µl forward and reverse primers, 200 ng/µl DNA, and water. The PCR reaction was carried out with the following parameters: initial denaturation at 94 °C for 5min, second denaturation at 94 °C for 1 min, 1 min of annealing at 56 °C and 55 °C for mazEF and yefM-yoeB, respectively, extension at 72 °C for 50 sec. 30 cycles of amplification were applied. To analyze the PCR products, 10 µl of PCR mixture was loaded to 2% agarose in the presence of 100 bp DNA ladder. After performing gel electrophoresis, the gel was exposed to UV by using UV Transilluminator.

3. Results and Discussion

3.1. Cells with distinct phenotypes were detected in the inhibition zone of Mupirocin-susceptible Isolates after the Substitution of the antibiotic disk with nutrient broth disk.

Bacterial populations are genetically and phenotypically heterogeneous. The phenotypic heterogeneity is due to the presence of normal growing cells and slow growing cells in a dormant state (persister cells). Persistent cells can survive stress conditions like antibiotics due to their low metabolic activity [35, 36]. To analyze the failure of antibiotic treatments, susceptibility testing usually focuses only on identifying resistance levels neglecting the possibility of persister cells presence. Studying the persistence in *Pseudomonas* and *E. coli* concluded that there is a positive correlation between persistence and resistance. Once the bacteria have the ability to evolve persister cells, it will increase the chance to evolve resistant either by mutation or horizontal gene transfer [37]. The minimum inhibitory concentration (MIC) of persister cells is unchangeable, therefore, they will be neglected if the MIC assay is used only to detect isolates with higher values. Standard Disk diffusion assay is not efficient in distinguishing persistent from susceptible. Therefore, Persistence in isolates of methicillin resistant *S. aureus* was detected by TD test. In this study, to detect the presence of cells with distinct phenotype among the population of tested Iraqi isolates of MRSA (Methicillin Resistant *S. aureus*), the isolates were exposed to three different antibiotics. Among the three tested antibiotics, Mupirocin was more effective in inhibiting the growth of isolates.73% of isolates were susceptible to Mupirocin Whereas, 60% and 100% of isolates were resistant to Ceftriaxone and Mecillinam, respectively [Figure 1] and [Figure 2]. To detect the presence of survival persisters inside the inhibition zone of Mupirocin and Ceftriaxone susceptible isolates, the antibiotic disk was substituted with nutrient broth saturated disk. After 18hr of incubation at 37C, detectible colonies were obtained [Figure 4]. Of the 22 Mupirocin-susceptible isolates, 91% (20 isolates) were triggered to form persisters, while no persistence was observed in the inhibition zone of 9% (2 isolates) (non-persistent susceptible isolate) [Figure 3]. All Ceftriaxone susceptible isolates formed persister cells [Figure 3]. The number of persister cells generated in the inhibition zones after disk substitution (persistence level) was ranged from medium (tens to hundreds of colonies in the inhibition zone) to high (Bacterial lawn in the inhibition zone) [Figure 4]. 70% (14 isolates) of the Mupirocin susceptible isolates formed lawn in the inhibition zone, while 30% (6 isolates) formed hundreds of colonies. Exponential growing cells exposed to Ceftriaxone had lesser persister frequency compared to when exposed to Mupirocin 20 µl [Figure 5].

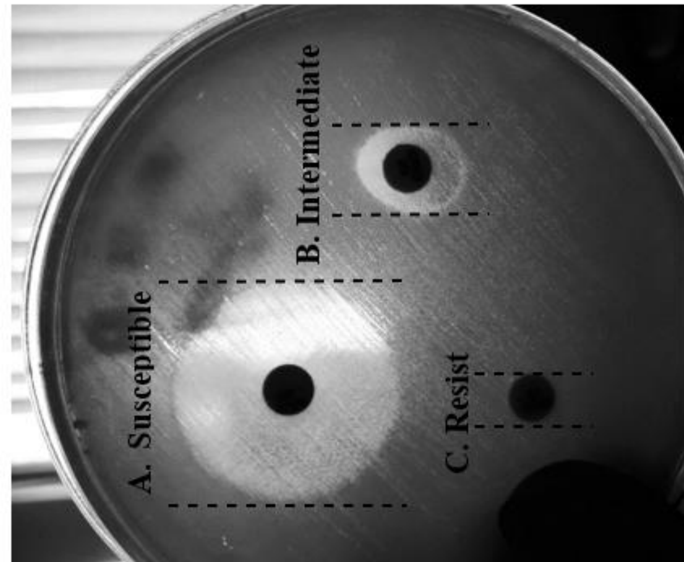

Figure - 1 Standard Disk diffusion assay of three different antibiotics Ceftriaxone (CRO) 30 mcg, Mecillinam (MEC) 10 mcg, and Mupirocin (MUP) 20 mcg. A. The isolate was susceptible to MUP, B. Intermediate to CRO, and C. Resistant to MEC.

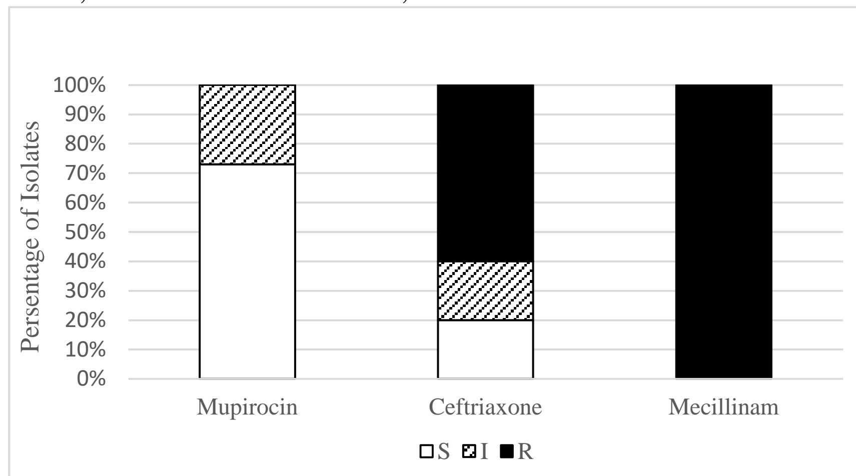

Figure -2 Antibiotic susceptibility disk diffusion assay of methicillin resistant *Staphylococcus aureus*.S: Susceptible, I: Intermediate, R: Resistant.

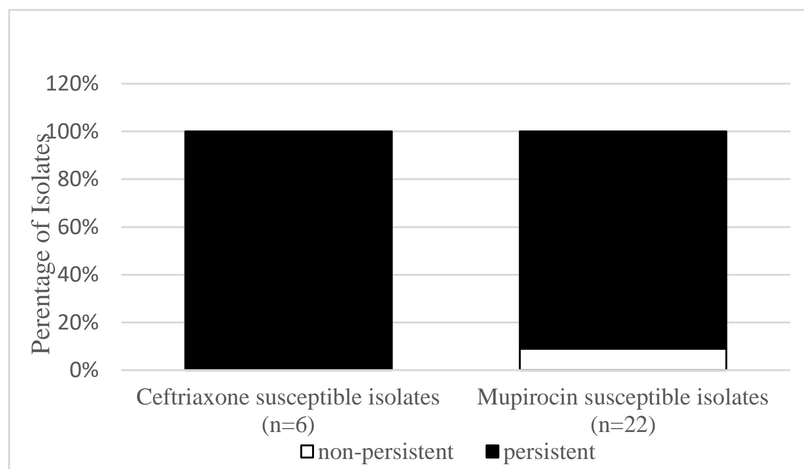

Figure – 3 TD test for persistence detection in Mupirocin and Ceftriaxone susceptible isolates. The number of isolates detected in this test is indicated

Mupirocin has been used as an excellent agent to treat *Staphylococcus* infections [38]. Based on the outcome of this study´s TD test, Mupirocin is not free of persistent cells. The substitution of the antibiotic disk with nutrient broth saturated disks allowed the detection of late growing cells that managed to survive the effect of antibiotic in the inhibition zone of Mupirocin susceptible isolates and Mupirocin and Ceftriaxone intermediate isolates. To make sure that these cells are persisters, colonies inside the inhibition zones were picked and the TD test was repeated, same susceptibility results were obtained. The level of persistence, represented by the number of colonies inside the inhibition zones and the number of colonies obtained after exposing exponential growing cells to antibiotics, was varying between isolates and antibiotics from medium to high. Isolates treated with the same antibiotic showed different persistence levels which can be attributed to cells growth rates. Since antibiotics target active mechanisms within bacterial cells, fast growing cells would be attacked by antibiotics faster than slow growing cells.

Therefore, the low growing isolates will form a higher level of persister cells. The persistence level is affected by the type of antibiotics. Exposing isolates to Mupirocin resulted in a higher level of persistence compared to Ceftriaxone. Mupirocin triggers the formation of persisters through inhibiting the translation process. Mupirocin mimics Isoleucine and binds to the active sites of Isoleucyl –tRNA synthetase resulting in the latter inactivation. The accumulation of Isoleucyl-tRNA synthetase molecules within cells activates the ppGpp alarm one synthesis which in turn activates regulatory pathways and mechanisms implicated in stress-related phenomena like persistence [39].

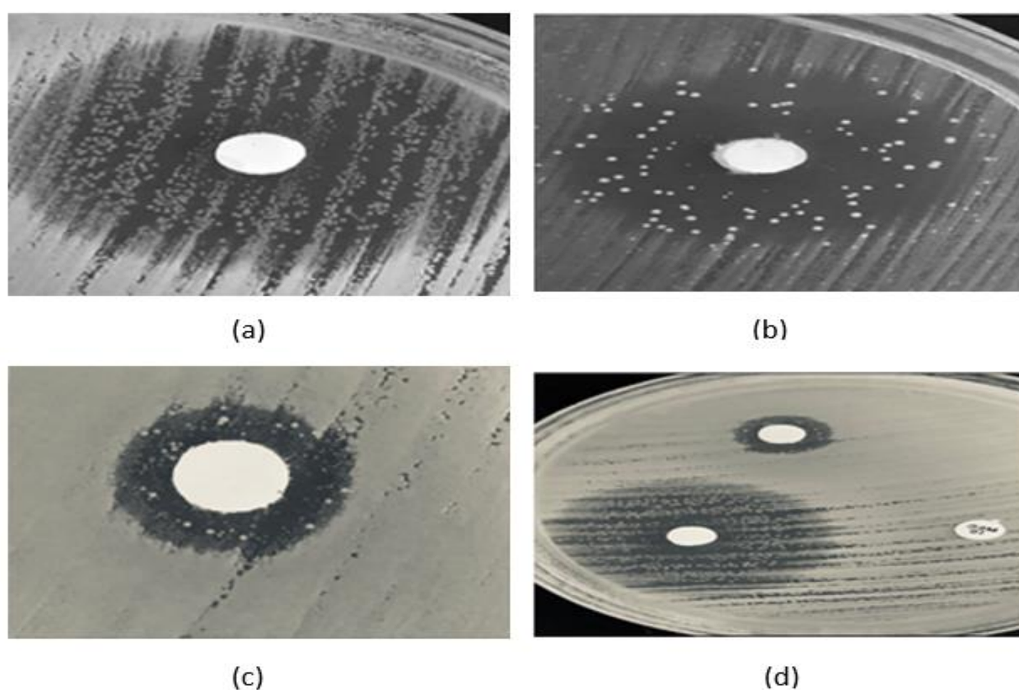

Figure 4-Different levels of persistence detected by TD test. Regrowth of colonies of Mupirocin and Ceftriaxone Persistent Methicillin resistant staphylococcus aureus. Colonies in the inhibition zone after the substitution of Mupirocin and Ceftriaxone disks with nutrient broth disk. The result was obtained after the 18hr of incubation at 37 ºC. (a, c, and d) High-persistence. (b) Medium persistence

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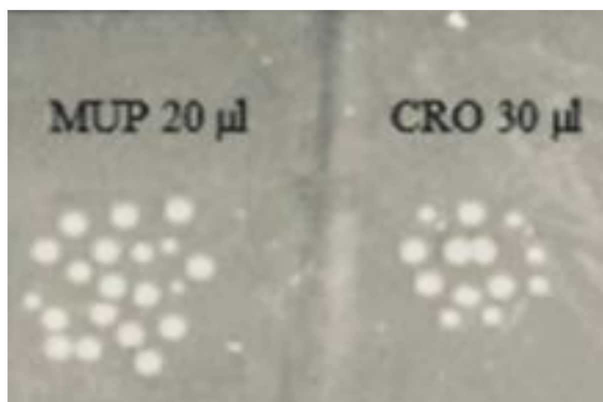
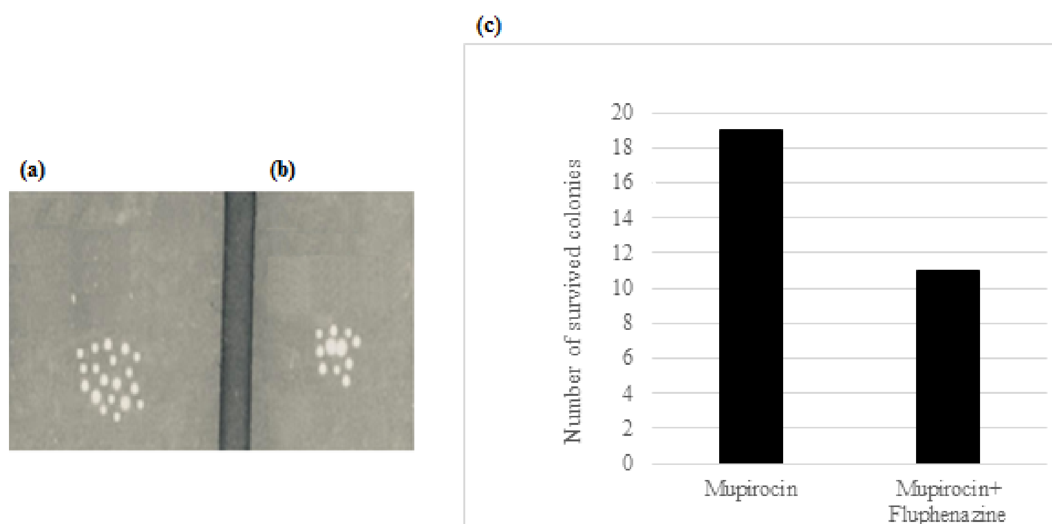


Figure- 5 Exponential growing cells were exposed to two different antibiotics (Mupirocin and Ceftriaxone) for4hr at 37 ºC. 2 µl of treated bacteria was plated on antibiotic free nutrient agar for 12 hr at 37 Cº. next day, the number of viable persister cells was counted.

3.2. The effect of Fluphenazine [Efflux Pumps Inhibitor] on the persistence of bacteria to Mupirocin

Toxin - antitoxin system is not the only strategy for persistence, efflux pumps are another strategy. Studying persistence in E. coli and Mycobacterium tuberculosis concluded that toxin - antitoxins system and efflux pumps are implicated in persister cells formation [40]. Relying on our previous work about the impact of efflux pumps inhibitor Fluphenazine on the ability of MRSA isolates to form a biofilm, Fluphenazine (0.25 mg/ml) was used to investigate whether the ability of the tested isolates to form persister cells is conferred by TA system or efflux pumps. The persistence of isolates to Mupirocin in the presence of efflux pumps inhibitor was tested. Combining Mupirocin with efflux pumps inhibitor resulted in a 42% reduction in the number of persister colonies compared to number of survived persister colonies when Mupirocin was applied alone.



F igure - 6 Efflux pumps inhibitor and persistence. Exponential growing cells were exposed to Mupirocin (a) and Mupirocin + Fluphenazine (b) for 4hr at 37 ºC. 2 µl of treated cells was plated in nutrient agar and incubated for 18hr at 37 ºC. The number of viable persister colonies of the two treatments was counted and compared (c).

3.3. The loci of mazEF and yoeB/yefM in the genome of tested isolates.

Although the efflux pump inhibitor enhanced the effect of Mupirocin, the isolate formed persister cells, confirming that tested isolates depend on different mechanisms to promote dormancy state to survive the effect of antibiotics. Additionally, many reliable studies attribute the formation of persister cells to TA system that usually is triggered by stress responses. PCR was used to detect the prevalence of Two type II Toxin - antitoxin systems *(mazEF and yefm-yeob*) among the tested isolates. Results consider positive if a distinct band at 300 bp and 451 bp for mazEF and yoeB/ yefM chromosomal loci, respectively, is observed on 1.5 % agarose gel. Results showed the presence of these two loci in 100% of the investigated isolates [Figure 7]. The prevalence of these genes among the Iraqi MRSA isolates and the ability of these isolates to evolve persister cells urged the need for new antibiotics that target the TA system

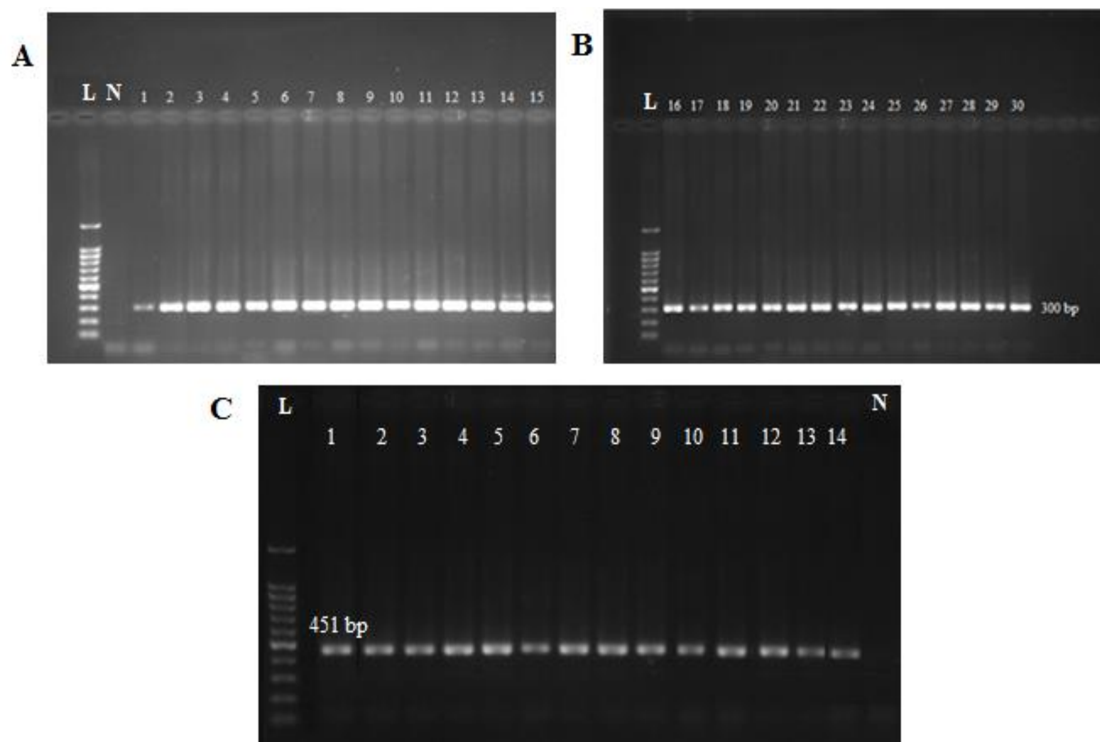


Figure -7 Polymerase chain reaction screen for *mazEF* (A and B) and *yeEF-yoeB* loci (C). lane L: 100 bp DNA ladder. Lane N negative control. The number above the lanes represented the screened isolates. *mazEF* loci and *yeEF-yoeB* loci are present in all the screened isolates.

4. Conclusion

The incorrect use of antibiotics resulted in the development of bacterial resistance that led to an increase in the number of cases of failed antibiotic treatments. Methicillin resistant Staphylococcus aureus emerged in society as a result of the unnecessary or an inappropriate use of antibiotics. Persistence is another cause of treatment failure. Detecting the ability of bacteria to evolve persister cells is very important to choose the right antibiotic or anti-persistent therapies to treat infectious diseases. Different strategies have been made to prevent the formation of persister cells and to eradicate their presence. Exposing tested isolates to Mupirocin resulted in a high level of persistence. The lethal effect of Mupirocin was enhanced by combining efflux pumps inhibitor. This urged the need to discover agents targeting genes code for efflux pumps and suitable inhibitors to be combined with an

antibiotic. Due to its Prevalence distribution among pathogenic bacteria, the TA system has been considered as a promising therapeutic target.

5. Ethical statement

All experiments followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000.

References

[1] S. S. Magill et al., "Changes in Prevalence of Health Care–Associated Infections in U.S. Hospitals,"*N Engl J Med*, vol. 379, no. 18, pp. 1732–1744, Nov. 2018, doi: 10.1056/NEJMoa1801550.

[2] Gary P. Wormser, Henry F. Chambers, *The Antimicrobial Drugs*, Second Edition by Eric Scholar and William Pratt New York: Oxford University Press, 2000. 607 pp., illustrated. Clinical Infectious Diseases, Volume 32, Issue 3, 1 February 2001, Page 521, https://doi.org/10.1086/318515.

[3] JosephW. Bigger, "TREATMENT OF STAPHYLOCOCCAL INFECTIONS WITH PENICILLIN BY INTERMITTENT STERILISATION," *The Lancet*, vol. 244, no. 6320, pp. 497–500, Oct. 1944, doi: 10.1016/S0140-6736(00)74210-3.

[4] O. Gefen and N. Q. Balaban, "The importance of being persistent: heterogeneity of bacterial populations under antibiotic stress," *FEMS Microbiol Rev*, vol. 33, no. 4, pp. 704–717, Jul. 2009, doi: 10.1111/j.1574-6976.2008.00156.x.

[5] S. W. Newsom, "Staphylococcal persisters grown from empyema fluid on L-form medium," *J Med Microbiol*, vol. 3, no. 4, pp. 669–673, Nov. 1970, doi: 10.1099/00222615-3-4-669.

[6] R. A. Fisher, B. Gollan, and S. Helaine, "Persistent bacterial infections and persister cells," *464*, May 2017, doi: 10.1038/nrmicro.2017.42.(2017).

[7] T. Dörr, M. Vulić, and K. Lewis, "Ciprofloxacin Causes Persister Formation by Inducing the TisB toxin in Escherichia coli," *PLOS Biology*, vol. 8, no. 2, p. e1000317, Feb. 2010, 10.1371/journal.pbio.1000317.

[8] S. B. Korch, T. A. Henderson, and T. M. Hill, "Characterization of the hipA7 allele of Escherichia coli and evidence that high persistence is governed by (p)ppGpp synthesis," *Molecular Microbiology*, vol. 50, no. 4, pp. 1199–1213, 2003, doi: https://doi.org/10.1046/j.1365-2958.2003.03779.x.

[9] E. Maisonneuve, L. J. Shakespeare, M. G. Jørgensen, and K. Gerdes, "Bacterial persistence by RNA endonucleases," *Proc Natl AcadSci USA*, vol. 108, no. 32, pp. 13206–13211, Aug. 2011, doi: 10.1073/pnas.1100186108.

[10] D. Ma *et al.*, "The Toxin-Antitoxin MazEF Drives Staphylococcus aureus Biofilm Formation, Antibiotic Tolerance, and Chronic Infection," *mBio*, vol. 10, no. 6, Nov. 2019, doi: 10.1128/mBio.01658-19.

[11] N. Wu *et al.*, "Ranking of persister genes in the same Escherichia coli genetic background demonstrates varying importance of individual persister genes in tolerance to different antibiotics," *Front Microbiol*, vol. 6, Sep. 2015, doi: 10.3389/fmicb.2015.01003.

[12] B. C. M. Ramisetty and R. S. Santhosh, "Horizontal gene transfer of chromosomal Type II toxin–antitoxin systems of *Escherichia coli*," *FEMS Microbiology Letters*, vol. 363, no. 3, p. fnv238, Feb. 2016, doi: 10.1093/femsle/fnv238.

[13] T. Ogura and S. Hiraga, "Mini-F plasmid genes that couple host cell division to plasmid proliferation.," *Proc Natl AcadSci U S A*, vol. 80, no. 15, pp. 4784–4788, Aug. 1983, Accessed:Apr.03,2021

[14] A. Gupta, "Killing activity and rescue function of genome-wide toxin–antitoxin loci of *Mycobacterium tuberculosis*," *FEMS Microbiology Letters*, vol. 290, no. 1, pp. 45–53, Jan. 2009, doi: 10.1111/j.1574-6968.2008.01400.x.

[15] H. Sberro*et al.*, "Discovery of functional toxin/antitoxin systems in bacteria by shotgun cloning," *Mol Cell*, vol. 50, no. 1, pp. 136–148, Apr. 2013, doi:10.1016/j.molcel.2013.02.002.

[16] O. Vesper *et al.*, "Selective Translation of Leaderless mRNAs by Specialized Ribosomes Generated by MazF in Escherichia coli," *Cell*, vol. 147, no. 1, pp. 147–157, Sep. 2011, doi: 10.1016/j.cell.2011.07.047.

[17] M. G. Jørgensen, D. P. Pandey, M. Jaskolska, and K. Gerdes, "HicA of Escherichia coli Defines a Novel Family of Translation-Independent mRNA Interferases in Bacteria and Archaea," *J Bacteriol*, vol. 191, no. 4, pp. 1191–1199, Feb. 2009, doi: 10.1128/JB.01013-08.
[18] D. Castro-Roa, A. Garcia-Pino, S. De Gieter, N. A. J. van Nuland, R. Loris, and N. Zenkin, "The Fic protein Doc uses an inverted substrate to phosphorylate and inactivate EF-Tu," *Nat ChemBiol*, vol. 9, no. 12, Dec. 2013, doi: 10.1038/nchembio.1364.
[19] I. Cherny, L. Rockah, and E. Gazit, "The YoeB toxin is a folded protein that forms a physical complex with the unfolded YefM antitoxin. Implications for a structural-based differential stability of toxin-antitoxin systems," *J BiolChem*, vol. 280, no. 34, pp. 30063–30072, Aug. 2005, doi: 10.1074/jbc.M506220200.
[20] M. Muthuramalingam, J. C. White, and C. R. Bourne, "Toxin-Antitoxin Modules Are Pliable Switches Activated by Multiple Protease Pathways," *Toxins (Basel)*, vol. 8, no. 7, Jul. 2016, doi: 10.3390/toxins8070214.
[21] E. Maisonneuve, L. J. Shakespeare, M. G. Jørgensen, and K. Gerdes, "Bacterial persistence by RNA endonucleases," *Proc Natl AcadSci USA*, vol. 108, no. 32, pp. 13206–13211, Aug. 2011, doi: 10.1073/pnas.1100186108.
[22] A. Harms, D. E. Brodersen, N. Mitarai, and K. Gerdes, "Toxins, Targets, and Triggers: An Overview of Toxin-Antitoxin Biology," *Molecular Cell*, vol. 70, no. 5, pp. 768–784, Jun. 2018, doi: 10.1016/j.molcel.2018.01.003.
[23] Y. Shan, A. Brown Gandt, S. E. Rowe, J. P. Deisinger, B. P. Conlon, and K. Lewis, "ATP-Dependent Persister Formation in *Escherichia coli*," *mBio*, vol. 8, no. 1, pp. e02267-16, /mbio/8/1/e02267-16.atom, Mar. 2017, doi: 10.1128/mBio.02267-16.
[24] L. Van Melderen and M. Saavedra De Bast, "Bacterial Toxin–Antitoxin Systems: More Than Selfish Entities?," *PLoS Genet*, vol. 5, no. 3, p. e1000437, Mar. 2009, doi: 10.1371/journal.pgen.1000437.
[25] K. Gerdes, "Toxin-Antitoxin Modules May Regulate Synthesis of Macromolecules during Nutritional Stress," *J Bacteriol*, vol. 182, no. 3, pp. 561–572, Feb. 2000, Accessed: Apr. 03, 2021.
[26] A. M. Hall, B. Gollan, and S. Helaine, "Toxin–antitoxin systems: reversible toxicity," *Current Opinion in Microbiology*, vol. 36, pp. 102–110, Apr. 2017, doi: 10.1016/j.mib.2017.02.003.
[27] M. F. Traxler, S. M. Summers, H.-T. Nguyen, V. M. Zacharia, J. T. Smith, and T. Conway, "The global, ppGpp-mediated stringent response to amino acid starvation in Escherichia coli," *MolMicrobiol*,vol.68,no.5,pp.1128–1148,Jun.2008,doi:10.1111/j.1365-2958.2008.06229.x.
[28] E. Germain, D. Castro-Roa, N. Zenkin, and K. Gerdes, "Molecular mechanism of bacterial persistence by HipA," *Mol Cell*, vol. 52, no. 2, pp. 248–254, Oct. 2013, doi:10.1016/j.molcel.2013.08.045.
[29] K. Gerdes, S. K. Christensen, and A. Løbner-Olesen, "Prokaryotic toxin-antitoxin stress response loci," *Nat Rev Microbiol*, vol. 3, no. 5, pp. 371–382, May 2005, doi: 10.1038/nrmicro1147.
[30] S. Yoshizumi, Y. Zhang, Y. Yamaguchi, L. Chen, B. N. Kreiswirth, and M. Inouye,"Staphylococcus aureus YoeB Homologues Inhibit Translation Initiation," *Journal of Bacteriology*, vol. 191, no. 18, pp. 5868–5872, Sep. 2009, doi: 10.1128/JB.00623-09.
[31] I. Marianovsky, E. Aizenman, H. Engelberg-Kulka, and G. Glaser, "The regulation of the Escherichia coli mazEF promoter involves an unusual alternating palindrome," *J Biol Chem*, vol. 276, no. 8, pp. 5975–5984, Feb. 2001, doi: 10.1074/jbc.M008832200.
[32] W. T. Chan, M. Espinosa, and C. C. Yeo, "Keeping the Wolves at Bay: Antitoxins of Prokaryotic Type II Toxin-Antitoxin Systems," *Front MolBiosci*, vol. 3, Mar. 2016, doi: 10.3389/fmolb.2016.00009.
[33] Y. Zhang and M. Inouye, "The Inhibitory Mechanism of Protein Synthesis by YoeB, an Escherichia coli Toxin," *J BiolChem*, vol. 284, no. 11, pp. 6627–6638, Mar. 2009, doi: 10.1074/jbc.M808779200.
[34] American Society for Microbiology., A. S. for Microbiology, D. H. Bergey, and R. S.

Breed, *Bergey's manual of determinative bacteriology*, 7th ed. Baltimore: Williams & Wilkins Co., 1957, pp. 1–1130.

[35] B. Claudi*et al.*, "Phenotypic Variation of Salmonella in Host Tissues Delays Eradication by Antimicrobial Chemotherapy," *Cell*, vol. 158, no. 4, pp. 722–733, Aug. 2014, doi: 10.1016/j.cell.2014.06.045.

[36] K. R. Allison, M. P. Brynildsen, and J. J. Collins, "Heterogeneous Bacterial Persisters and Engineering Approaches to Eliminate them," *CurrOpinMicrobiol*, vol. 14, no. 5, pp. 593–598, Oct. 2011, doi: 10.1016/j.mib.2011.09.002.

[37] T. Vogwill, A. C. Comfort, V. Furió, and R. C. MacLean, "Persistence and resistance as complementary bacterial adaptations to antibiotics," *J EvolBiol*, vol. 29, no. 6, pp. 1223–1233, Jun. 2016, doi: 10.1111/jeb.12864.

[38] J. E. Coia*et al.*, "Guidelines for the control and prevention of meticillin-resistant Staphylococcus aureus (MRSA) in healthcare facilities," *J Hosp Infect*, vol. 63 Suppl 1, pp S1-44, May 2006, doi: 10.1016/j.jhin.2006.01.001.

[39] T. Nakama, O. Nureki, and S. Yokoyama, "Structural Basis for the Recognition of Isoleucyl-Adenylate and an Antibiotic, Mupirocin, by Isoleucyl-tRNASynthetase," Journal of Biological Chemistry, vol. 276, no. 50, pp. 47387–47393, Dec. 2001, doi: 10.1074/jbc.M109089200.

[40] Y. Pu *et al.*, "Enhanced Efflux Activity Facilitates Drug Tolerance in Dormant Bacterial Cells," *Mol Cell*, vol. 62, no. 2, pp. 284–294, Apr. 2016, doi: 10.1016/j.molcel.2016.03.035.