مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الخامس والعشرون - المجلد الثاني عشر - علوم الحياة - آب 2025 م
doi.org/10.52866/esj.2023.12.25.23

The Evaluation of Efflux Pump Genes *norA*, *norB*, *norC*, *lmrS* and *mepA* Related to Antibiotic Resistance in *Staphylococcus aureus*

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Abstract :

Staphylococcus aureus (*S. aureus*) is a common bacterium found on the skin and in the upper respiratory tract. Although it is part of the normal flora, it can become an opportunistic pathogen responsible for a wide range of infections, which are often difficult to treat due to the presence of virulence and resistance genes. In recent years, resistance to ciprofloxacin in S. aureus has emerged as a significant clinical challenge, largely attributed to the activity of efflux pumps. This study aimed to evaluate the prevalence of efflux pump genes (norA*, norB, norC, LmrS,* and *mepA*) and their association with ciprofloxacin resistance in clinical isolates of *S. aureus*. A total of 150 clinical samples were collected from patients attending Al-Zahraa and Al-Karamah Teaching Hospitals. *S. aureus* isolates were identified using standard microbiological methods and confirmed by molecular analysis targeting the 16S rRNA and mecA genes. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method in accordance with CLSI guidelines, involving sixteen antibiotics. The antibiotic susceptibility profiles revealed high resistance rates, with 100% resistance to both cefoxitin and ciprofloxacin, followed by oxacillin (88.88%), meropenem (74%), amoxicillin (70.37%), piperacillin (55.55%), tetracycline (51%), and other varying levels of resistance. In contrast, all isolates showed 100% sensitivity to levofloxacin, vancomycin, ceftriaxone, and amikacin. PCR analysis showed that norA, norB, and norC genes were detected in 100% of resistant isolates, while LmrS and mepA were present in 95% and 90% of isolates, respectively. These findings suggest that norA, norB, and norC efflux pump genes play a critical role in the development of ciprofloxacin resistance among *S. aureus* clinical isolates. Detection of these genes could provide valuable insights for guiding effective therapeutic strategies against drug-resistant *S. aureus* infections.

Keywords: *Staphylococcus aureus*, Antibiotic resistance, Efflux pumps, Resistance genes.

تقييم جينات مضخه التدفق *mepA* و *norA , norB , norC , lmrS* المرتبطه بمقاومه المضادات الحيويه في المكورات العنقودية الذهبيه

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مستخلص:

المكورات العنقودية الذهبيه هي بكتيريا شاعه موجودة في الجلد والجهاز التنفسي العلوي .بالرغم من أن البكتيريا جزء من بكتريا طبيعية، إلا أنها تُصبح مُمرضة انتهازية مسؤاله عن كثيرمن العدوىالتي غالبا تُصعّب علاجها بسبب وجود جين الضراوة و المقاومه. في الآونة الأخيرة، أصبحت مقاومة سلالات المكورات العنقودية الذهبية للسيبروفلوكساسين، بسبب مضخات التدفق. هذه الدراسة هدف لتقييم تواتر جينات مضخة التدفق norA وnorB وnorC وLmrS وMepA) ومايرافقها من مقاومة السيبروفلوكساسين في العزلات السريرية من المكورات العنقودية الذهبية. جُمعت مائة وخمسون عينة سريرية من المرضى المراجعين في مستشفى الزهراء التعليمي ومستشفى الكرامة التعليمي، وتم تحديد عزلات المكورات العنقودية الذهبية من خلال الاختبارات الميكروبيولوجية القياسية و تاكيدها من خلال تحليل الجزئي (16S rRNA و mecA).اختبار أأنماط حساسية مضادات الميكروبات بطريقة (Kirby-Bauer) باستخدام أقراص انتشار في إرشادات CLSI ، يتضمن سته عشره مضادا حيويا. أظهرت نتائج اختبار حساسية المضادات الحيوية مستوى مقاومة مرتفع بنسبة (100٪) تجاه سيفوكسيتين (FOX) وسيبروفلوكساسين (CIP) يليه أوكساسيلين (88.88٪) (OX)، ميروبينيم (74٪) (MEP)، أموكسيسيلين (70.37٪) (AMC)، بايبراسيلين (55.55٪) (PRL)، تيتراسايكلين (51٪) (TET)، أزيثروميسين (25٪ (AZM، 37٪ AMS)، إريثروميسين (29.62٪) (E)، سيفترياكسون ((CRO 25.92٪)) على التوالي، بينما كانت الحساسية عالية تجاه ليفوفلوكساسين (LE)، فانكومايسين (V)، سيفترياكسون (C) وأميكاسين (AK) بنسبة مسجلة (100٪). ظهرات باستخدام طريقة تفاعل البوليميراز المتسلسل لكشف وُجدت جينات norA وnorB وnorC في 100٪ بينما كانت جين lmrS و mepA بنسبة 95٪ و بنسبة 90٪ على التوالي.تشير هذه الدراسة تلعب مضخات التدفق norA وnorB وnorC دورًا هامًا في تطور مقاومة المضادات الحيوية في العزلات السريرية لبكتيريا المكورات العنقودية الذهبية. وقد يُفيد اكتشاف هذه الجينات في اقتراح نموذج علاج فعال للعدوى التي تُسببها المكورات العنقودية الذهبية.

الكلمات المفتاحية: المكورات العنقودية الذهبية، مقاومة المضادات الحيوية، مضخة التدفق.

Introduction

Staphylococcus aureus (S.aureus) is one of the most important and prevalent pathogens found in human and animal. It is associated with a wide range of diseases, ranging from minor infections of the skin and soft tissue to life-threatening injuries. Its potential virulence is markedly associated with a wide diversity of its resistance mechanisms to various antimicrobial compounds[1] The most serious and challenging type is the methicillin-resistant Staphylococcus aureus (MRSA). MRSA causes major outbreaks in nosocomial environments with a significant therapy challenge particularly in the intensive care units (ICUs) that urgently necessitates finding effective therapeutic methods to lessen its threat to the health care systems [2].MRSA strains generate a substitute penicillin-binding protein (PBP), PBP2a, which is encoded by the mecA gene. The PBP2a protein has reduced affinity to multiple beta-lactam antibiotics making MRSA strains extensively resistant to almost all these antibiotics and warrants searching for other effective antibiotics [3].

Bacteria can use different mechanisms of resistance to antibiotics, which include degradation or modification qof the antibiotic; alteration of the bacterial target of the antibiotic; target protection and reduction of the intracellular concentration of the antibiotic, either by a decreased permeability of the cell wall or by the efflux of the antibiotic from the cell. Efflux-mediated resistance has been overshadowed in contrast with the other mechanisms known. However, it has been gathering more interest, as we recognize that many bacterial efflux pumps are able to extrude several, unrelated classes of antimicrobial compounds from the cell, promoting the appearance of multidrug resistance phenotypes [4].

Presently, lower than 90% of S.aureus strains resist most penicillin derivatives and ordinary antibiotics like's tetracycline, fluoroquinolones, macrolides, aminoglycosides and chloramphenicol [5]. Efflux pumps have been identified for S.aureus encoded by chromosome or plasmids. The efflux pump gene lmrS, norA,norB,norC and mepA encoded by chromosome [6][7].

[8]reported that over expression of mepA convery a pattern of resistance to fluoroquinolones, tetracyclines, disinfectant, and dyes. The physiological role of efflux pumps in bacteria has been related to the elimination of endogenous metabolites that are noxious to the cell, the secretion of virulence determinants, and in cell stress responses, suggesting that the drugs are "accidental substrates" of these transporters [9].

Materials and methods

Sample Collection : From June to September 2024 a total of one hundred and fifty specimen obtained from different clinical sources (wound, burns, and blood) were collected from patients attending Al Zahraa Teaching Hospital and Al Kramah Teaching Hospital. The samples were collected placed into a transport medium and then were cultured immediately on blood Agar and mannitol salt agar, after incubation at 37co for 18-24 hour, cultural and morphological features of the colonies were evaluated. All clinical samples were primarily examined by gram stain and by biochemical catalase test, coagulase test, and urease test, according to Bergey's manual[10].

Antibiotics susceptibility test : The antimicrobial susceptibility profiles to the main classes of antibiotics were determined by using (Kirby–Bauer method) as described by[11].All S.aureus isolates were transferred with sterile swabs to tubes of sterile saline to achieve turbidity equal to that of a 0.5 McFarland standard. Cell suspensions and sterile swabs were used to inoculate the surface of Mueller-Hinton agar plates tested against several types of antibiotic including: Cefoxitin and Ciprofloxacin followed by Oxacillin Meropenem Amoxicillin, Piperacillin, Tetracyclin,

Azithromycin ,Erythromycin ,ceftriaxone respectively, while high sensitivity toward Levofloxacin(LE), Vancomycin(V) ,Ceftriaxone(C) and Amikacin(AK).Antibiotic resistance was determined after Inoculated plates were incubated for 24 h at 37°C, after which the diameters of inhibition zones were measured in millimeters following the manufacturer's instructions to assess resistance, intermediate, or susceptibility. The results of susceptibility were interpreted according to

the guidelines of the Clinical and Laboratory Standards Institute[11]

Molecular identification of *S.aureus* : In this study, DNA sample of S.aureus isolates has been selected to detect the 16S rRNA and mecA diagnostic gene .The genomic DNA of S.aureus isolates was extracted by using a commercial genomic DNA purification kit (Promega, USA).Then, the concentration and purity of extracted it of S.aureus was determined by using the Nanodrop. It of S.aureus isolates was extracted according to manufacturer (Promega/USA)

Primer selection : The specific primer was used for detection 16srRNA,mecA genes and efflux pump genes(LmrS, mepA, norA, norB and norC) as Table(1).A primer stock solution was prepared From the lyophilized primers according to manufacturing of (Promega/USA). The set of 7 primers With the Sequences and shown in Table(1).

These primers were utilized in a 25 µl reaction containing 12.5 µl of Go Taq®Green Master Mix (Promega, USA), 1 µl of each primer (10 pmol), 7.5 µl of DNase RNase free water and 3 µl of the purified DNA template. The PCR cycles conditions for each genes were included: Initial denaturation at 95°C for 5 min, thirty cycles of (denaturation at 94°C for 30 sec, annealing 53°C for 30sec, extension at 72°C for 30 sec) and final extension 72°C for 5 min. The PCR products were separated by gel electrophoresis using 1% agarose gel (Promega/ USA) and visualized by gel documentation system using ethidium bromide. A 1000 bp DNA Ladder (Promega/USA) was used to detect the correct amplicon size.

Table (1):The primers, sequencings for detection of genes in *S. aureus*

Primer name		Sequencings	Molecular weight	Reference
16S RNA	F	ATGGTTCAAAAGTGAAAGACG	646pb	(12)
	R	ACACTTAGCACTCATCGTTTA		
MecA	F	TCTCTGGAAAATAACGTCGAA	323pb	(12)
	R	TAAACGTCAAAAGTCTTCAGC		
NorA	F	AGATTTGGGGTTAACTGGTAG	378pb	Newly designe
	R	TACGATGTGAAACTTCTGACA		
NorB	F	TTCTCCGACAATAAGAACTCC	511pb	Newly designe
	R	AATTTCACTAATTGCGCTGTT		
NorC	F	TAAGAAGTTCGAAATCGTTGC	235pb	Newly designe
	R	ACACATAAATTTGACGTTGCA		
LmrS	F	ACACATAAATTTGACGTTGCA	204pb	Newly designe
	R	TGTTGTGTTTCTTGCATACAG		
MepA	F	ATGACCAAATGACACCTGTTA	305pb	Newly designe
	R	AGCAGTTATCATGTCTATCGG		

Statistical analysis : The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study [12].

Result and discussion

Bacterial isolation and identification : The Specimens were collected were inoculated on blood agar plates (Hi media /India) and incubated aerobically at 37 °C for 24 hours. The standard conventional methods for diagnosis including Gram-positive cocci on Gram staining, golden yellow colonies on mannitol salt agar (Hi media/ India) due to mannitol fermentation, positive catalase and coagulase tests, and complete hemolysis on blood agar were utilized to identify and recognize S. aureus in the isolates. Also, the other biochemical diagnosis of S.aureus isolates of the present study Positive for (oxidase test, catalase test, Indole test,

Methyl red test(MR-T) Vogas– proskuar test (VP- T), Simmon Citrate test, Motility test and Urease test(13,14).All isolates were kept in tryptic soy broth (TSB) supplemented with 15% glycerol and stored at -20 °C until further processing.

Antibiotic susceptibility testing : In total,54 isolates of S. aureus bacteria showed highly sensitive toward Cefoxitin(FOX)and Ciprofloxacin(CIP) followed by Oxacillin (OX) (88.88%), Meropenem (MEP) (74%), Amoxicillin (AMC)(70.37%), Piperacillin(PRL)(55.55%), Tetracyclin(TET)(51%),Azithromycin(AZM)25%,AMS(37%),Erythromycin(E)(29.62%),ceftriaxone (CRO) (25.92%) respectively, while highly sensitivity toward Levofloxacin(LE), Vancomycin(V), Ceftriaxone(C) and Amikacin(AK) with percentage recorded (100%) as table (2).

Table (2): Antibiotics suitability pattern of S.aureus

antibiotic														
VAN	ME P	CR O	PR L	A K	TE	OX	C	LE	AM	E	FO X	AM S	CI P	AZ M
100	18,5	62.0	40.7	100	37.0	0	100	100	29.6	51.8	0	40.7	0	66.6
0	74.0	25.9	55.5	0	51.0	88.8	0	0	70	29.6	100	37.0	100	25.9
0	7.47	18.5	55.5	0	11.1	11.1	0	0	0	29.6	0	37.0	0	25.9

S=Senstive ,R=resist,I=intermediate

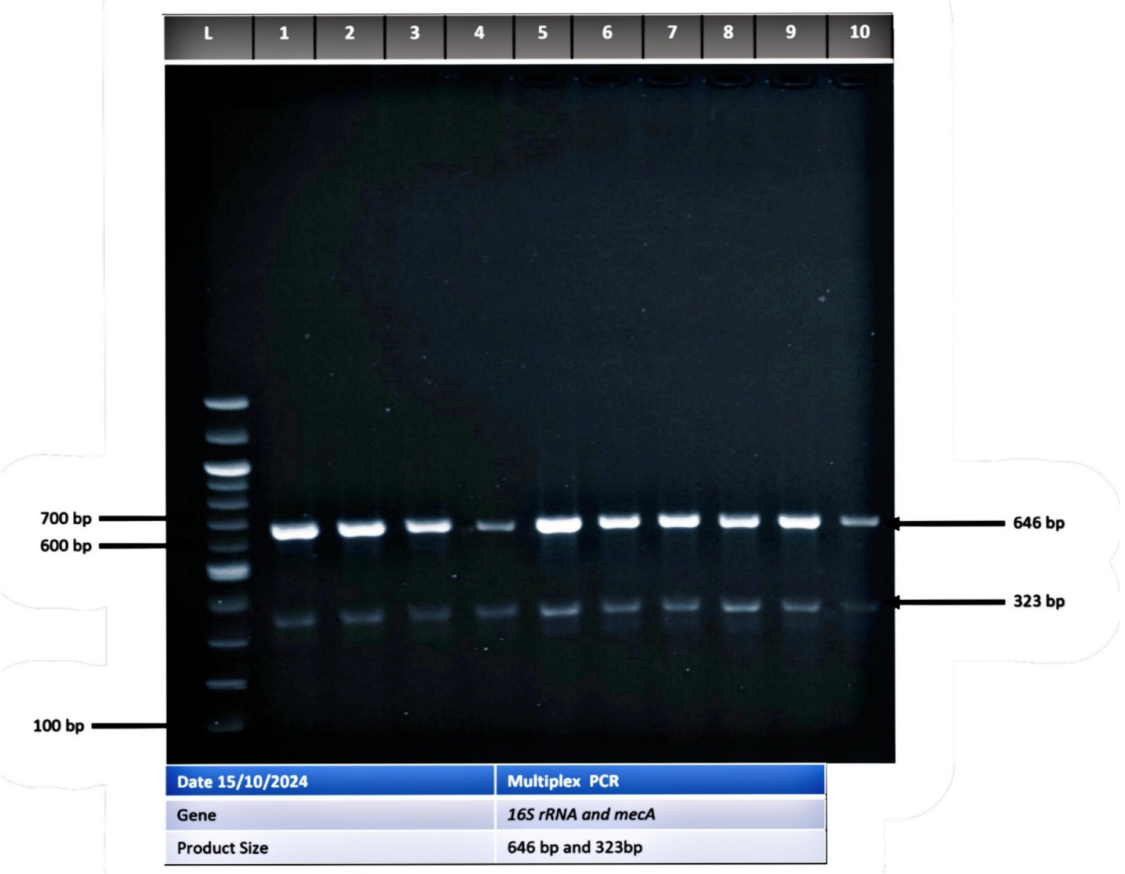
In the present study all of S.aureus isolates have shown highest resist to Cefoxitin and Ciprofloxacin recorded 100% while highly sensitive to Vancomycin,Amikacin and Ceftriaxone were recorded (100%).

The resistance rate was similar with other study reported by [15] who revealed that (100%) of the of S.aureus isolates resistant to this Cefoxitin. However, a study done by [17] in Iran that has found S.aureus isolates have low resistance to Erythromycin (52.8%). while [16,17]reported the highest resistance rate was reported for erythromycin (324, 81.82%) while the highest sensitivity rate was found for levofloxacin (69.4%). Additionally, gender-based differences in antimicrobial susceptibility were negligible ($p > 0.05$), but generally, males had a slightly higher resistance rate compared to females.

Our results were similar to the report by [19] who found that MRSA isolates showed high resistance to ciprofloxacin (70%), clindamycin (63.3%), erythromycin (58.3%) and moderate resistance to doxycycline (28.3%). Similarly, [20] in Brazil reported that MRSA isolates were highly resistant to erythromycin (74.2%), ciprofloxacin (64.5%), clindamycin (46.1%) and tetracycline (14.3%); and highly susceptible to linezolid (100 %). Moreover, [21] found that the isolated MRSA showed high resistance to erythromycin (61.5%), ciprofloxacin (61.5%) and gentamicin (53.8%).[22] found high resistance for ciprofloxacin with a ratio of 75%, followed by levofloxacin with a ratio of 72%, and high sensitivity was shown for norfloxacin with an 85% and chloramphenicol with an 80%. These variations in the antimicrobial drug resistance rates among different countries might be attributed to the availability and indiscriminate use of these antibiotics in certain areas which indicates that the antibiotic resistance patterns vary according to regional and geographical factors. In a study conducted by[23]found resistance to ciprofloxacin and ofloxacin in S. aureus isolated from blood infection was reported as 92.5% and 80.4%, respectively . [24]found among one hundred clinical samples, thirty six S. aureus isolates were recovered and the results of antibiotic susceptibility tests

showed that twenty of them were resistant to ciprofloxacin. 15 isolates were resistant to norfloxacin and one isolate was resistant to ofloxacin. Moreover, the norA, norB, and norC genes were found in 58%, 30%, and 41% of ciprofloxacin-resistant isolates, respectivey

Molecular Detection of *S.aureus* by PCR : In this study, DNA sample of S. aureus isolates has been selected to detect the mecA a gene. The PCR products have been confirmed by the analysis of the bands on gel electrophoresis and by comparing their molecular weight with 1000bp DNA ladder. The results of PCR reaction showed that mecA gene (605 and 323 bp) exists in all S. aureus isolates as Figure (1).In the present study found 16SrRNA and mecA gene in all S. aureus isolates. These findings agreed with those of studies by [24] which illustrated that the 16SrRNA and mecA was detected in (100%) of S. aureus isolates.



Figure(1): Gel electrophoresis of 16sRNA gene and MecA gene of *S. aureus* isolates using 1.5% agarose gel electrophoresis at 90 volts for 60 mincts.

Molecular Detection of efflux pump genes by PCR : The molecular detection efflux pump genes (norA,n-prB,norC ,lmrS and mepA) was done by using specific primers set from a total of 54 extracted DNA samples of *S.aureus*. Our study demonstrated that norA, norB, norC, LmrS and mepA genes were present in 100%,100% ,100%, 90% and 90% of the *S.aureus* isolates, respectively as Figure (2,3).

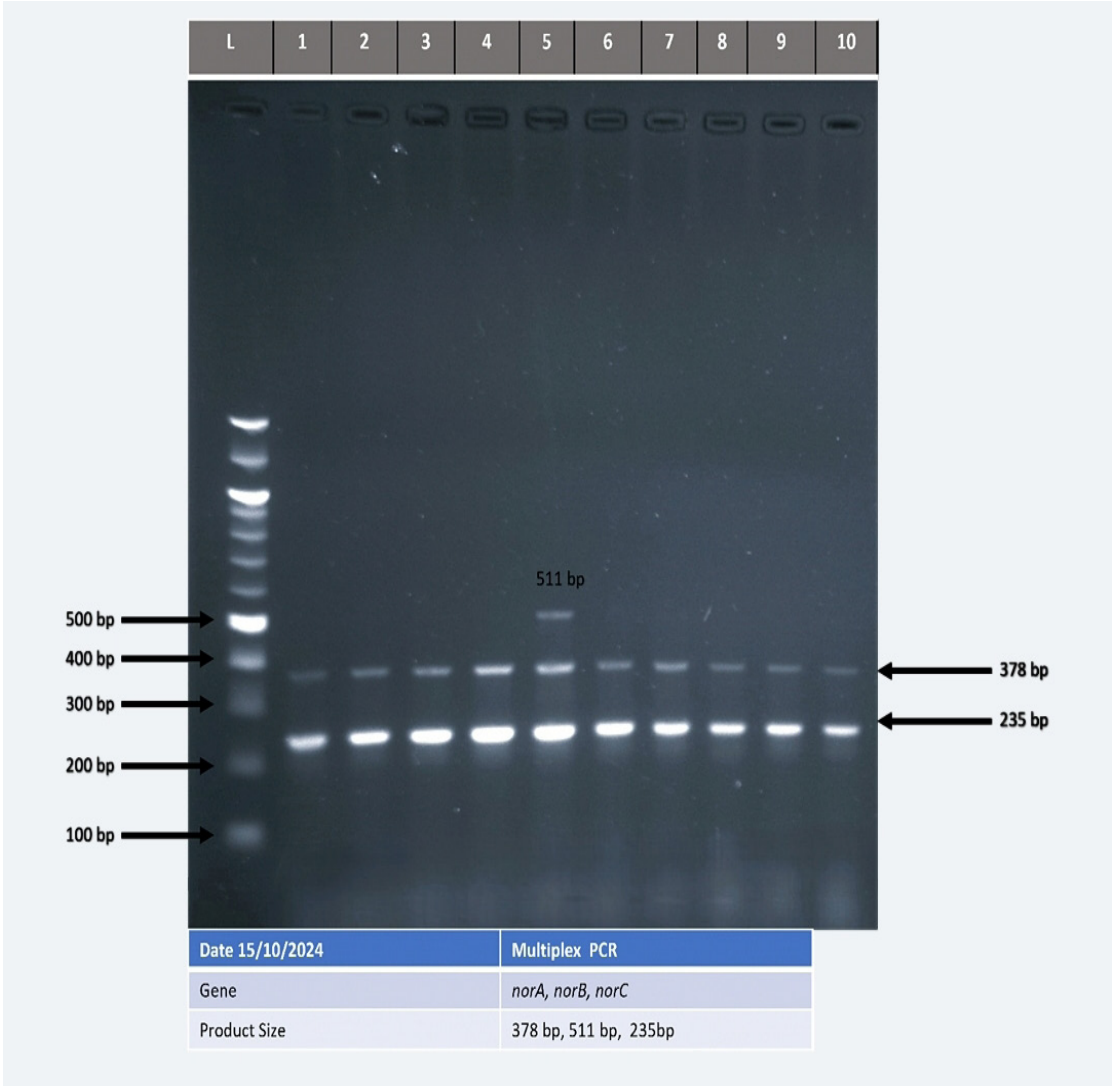


Figure (2): Gel electrophoresis of PCR products of norA, norB, norC gene at 1%Agarose, 90 V and for 1 hr.

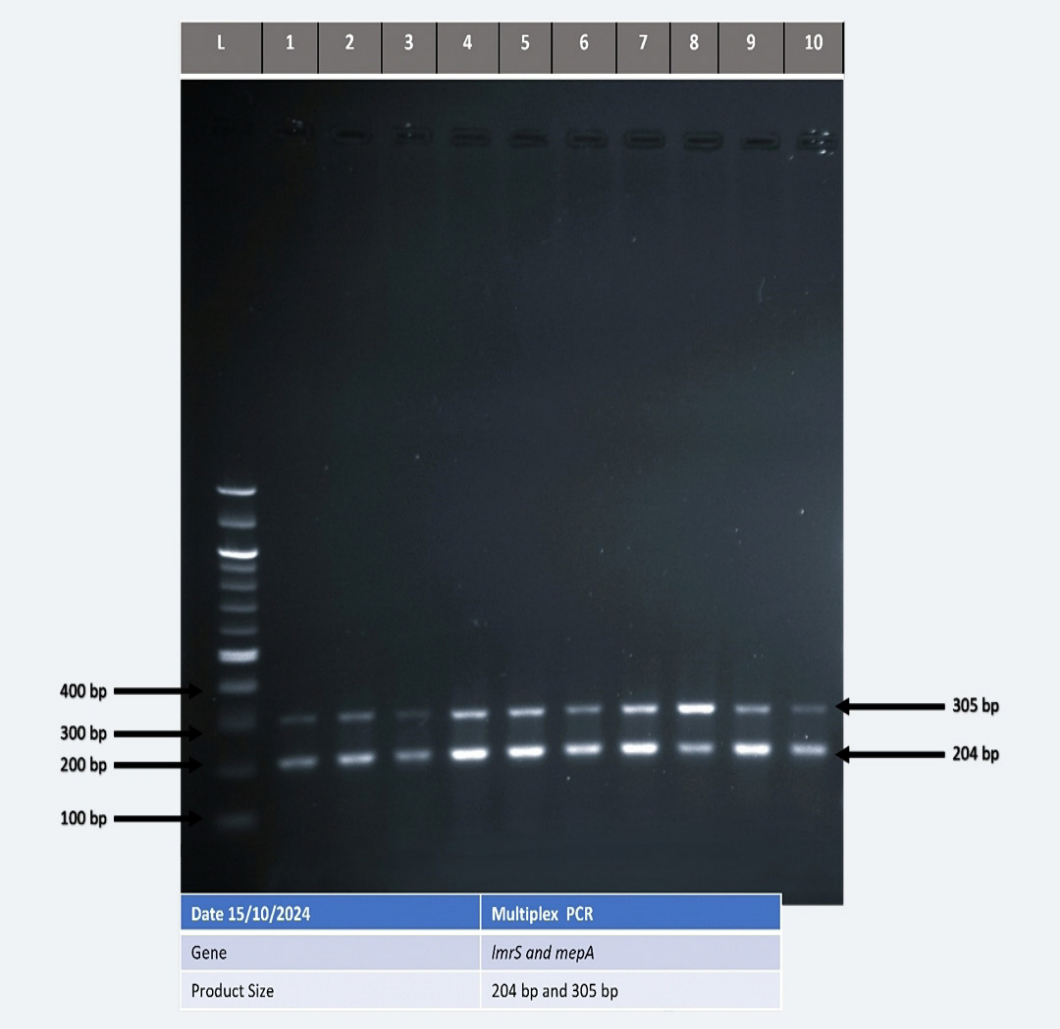


Figure (3): Gel electrophoresis of PCR products of LmrS and mepA gene at 1%Agarose, 90 V and for 1 hr.

The rate of norA, norB and norC gene in this study was 100% while in another study by [20] found the norA and norB genes were present in 77 (80.21%) and 54 (56.25%) respectively of isolates, while the gene norC was present in 17 (17.81%) of the total isolates.

While, in the present research the prevalence of lmrS gene in this study 95% while in other study recorded 68.42% [24]. 90% mepA gene were gave a specific band with a molecular size of 255bp after electrophoresed on 1% agarose gel In a study conducted by [21] indicated that 60% of S.aureus

isolates have this gene.

Another study by [19] showed 56% of S. aureus isolated from eye infection were phenotypically resistant to ciprofloxacin. Also, found 21.42% of the resistant isolates have high experssion in norB gene .

Study by[20] showed that among the strains with activated pumps, almost half of them (48%) had increased expression. 57% showed increased expression in the gene of a single pump, usually norA, while the remaining 43% isolates showed increased expression in 2 or more efflux pumps, most of which were norB

/C. A study by [21]of 52 ciprofloxacin-resistant S. aureus bacterial isolates showed that the activity of efflux pumps increased in 23% of cases, which is associated with increased bacterial resistance to fluoroquinolones and concluded norA, norB, and norC were detected in 75%, 35% and 55% of the MRSA isolates respectively.

The efflux pump MepA was identified in studies with S. aureus norA disrupted mutants . MepA is encoded by the chromosomal gene mepA and it was the first multidrug transporter from the MATE family to be described in S. aureus. The mepA gene is integrated in the mepRAB operon. Se-quence analysis revealed that the encoded protein MepR is similar to regulatory proteins from the MarR family. No significant similarity was found between MepA and any other protein with known function and no association was found between MepA and MDR phenotypes [22]

Conclusions

In conclusion, our findings indicate that S.aureus isolates showed a high resistance to antibiotics. pump efflux is one of the important mechanisms of resistance to fluoroquinolone antibiotics such as ciprofloxacin.Finally, it is suggested that further studies be performed to produce and extend the molecules of efflux pump inhibitors, and the optimization of antimicrobial use and control of infection is recommended to prevent the increase in the population of drug resistant organisms in the hospitals of Baghdad city.

References

Costa SS, Viveiros M, Amaral L, Couto I [2013]. Multidrug efflux pumps in

Staphylococcus aureus: an update. Open Microbiol J 7: 59-71.

2. Dulon M, Haamann F, Peters C, Schablon A, Nienhaus A [2011] MRSA prevalence in European healthcare settings: a review. BMC Infect Dis 11: 138.

3. Deurenberg RH, Vink C, Kalenic S, Friedrich A, Bruggeman CA, Stobberingh EE [2007] The molecular evolution of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect 13: 222-235.

4. Piddock LJV. Clinically relevant chromosomally encoded mul-tidrug resistance efflux pumps in bacteria. Clin Microbiol Rev 2006; 19: 382-402.

5. Kosmidis C, Schindler BD, Jacinto PL, Patel D, Bains K, Seo SM and Kaatz GW. Expression of multidrug resistance efflux pump genes in clinical and environmental isolates of Staphylococcus aureus. International journal of antimicrobial agents, 2012; 40(3): 204-209.

6. Floyd JL, Smith KP, Kumar SH, Floyd JT and Varela MF. LmrS is a multidrug efflux pump of the major facilitator superfamily from Staphylococcus aureus. Antimicrobial agents and chemotherapy, 2010; 54(12): 5406-5412.

7. Poole K. Efflux pumps as antimicrobial resistance mechanisms. Annals of medicine, 2007; 39(3): 162-176.

8. Vali L, Davies SE, Lai LL, Dave J and Amyes SG. Frequency of biocide resistance genes, antibiotic resistance and the effect of chlorhexidine exposure on clinical methicillin-resistant Staphylococcus aureus isolates. Journal of antimicrobial chemotherapy, 2008; 61[3]: 524-532.

9. Poole K. Bacterial multidrug efflux pumps serve other functions. Microbe 2008; 3: 179-85.

10. Bergey, D. H., & Holt, J. G. [2000]. Bergey's manual of determinative bacteriology. 9th ed. Lippincott Williams & Wilkins.

11. CLSI[2018]. Performance Standards for Antimicrobial Disk Susceptibility Testing.28th ed. CLSI standard M100. Clinical and Laboratory Standards Institute, 2018 .

12. Rocchetti,T.T.; Martins,K.B.;

Martin.P.Y.[2018]. Detection of the mecA gene and identification of Staphylococcus directly from blood culture bottles by multiplex polymerase chain reaction. Braz.j. infect dis. 2 0 1 8;2 2[2]:99–105 .

13. SAS. [2018]. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

14. Brooks, G.; Carroll, K.; Butel, J. and Morse, S. [2007]. Melnick and Adelbergs Medical Microbiology (24th ed.). The McGraw-Hill Companies, Inc.; New York. P. 224-232..

15. Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. [2007]. Bailey and Scott's diagnostic microbiology (12th ed.). Mosby Elsevier. (pp. 334-339)..

16. Marangon, F.B.; Miller,D.; Muallem,M.S.; Romano,A.C. and Alfonso,E.C.[2004]. Ciprofloxacin and levofloxacin resistance among methicillin-sensitive staphylococcus aureus isolates from keratitis and conjunctivitis.American Journal of Ophthalmology, Volume 137, Issue 3, 453 – 458.

17. Zheng X-Y, Choy BNK, Zhou M-M and Zhao Z-Y [2021] Antibiotic Resistance Pattern of Staphylococcus Aureus Isolated From Pediatrics With Ocular Infections: A 6-Year Hospital-Based Study in China. Front. Pediatr. 9:728634

18.ALMarjani,M.F.;Kadhim,K.A.; Kadhim,A.A.;Kinani,A.Y.[2015].Ciprofloxacin Resistance in Staphylococcus aureus and Pseudomonas aeruginosa Isolated from Patients in Baghdad.

19.Hussein,N.R.;Naqid,I.A.; Ibrahim,N.; Ahmad,S.I.;Daniel,S.S.; Ali H. Yahya,A.H. and Abdulwahab S. Muhammed,A.S.[2023]. Trends in Antimicrobial Resistance of Staphylococcus aureus Uropathogens: A Retrospective Study From Duhok Province, Kurdistan Region, Iraq.

20.Hassanzadeh S, Mashhadi R, Yousefi M, Askari E, Saniei M, Pourmand MR [2017] Frequency of efflux pump genes mediating ciprofloxacin and antiseptic resistance in methicillin-resistant Staphylococcus aureus isolates. Microb Pathog 111: 71-74.

21.Rossato AM, Primon-Barros M, Rocha L da L, Reiter KC, Dias CAG, d'Azevedo PA [2020] Resistance profile to antimicrobials agents in methicillin-resistant Staphylococcus aureus isolated from hospitals in South Bra-

zil between 2014-2019. Rev Soc Bras Med Trop 53: e20200431.

22.Tsige Y, Tadesse S, G/Eyesus T, Tefera MM, Amsalu A, Menberu MA, Gelaw B [2020] Prevalence of methicillin-resistant Staphylococcus aureus and associated risk factors among patients with wound infection at referral hospital, northeast Ethiopia. Journal of Pathogens 2020: 1-7.

23. Hamza ENH, Fazaa SA. Molecular investigation of quinolone-resistant genes among clinical Staphylococcus aureus isolates in Babylon hospitals. Med J Babylon 2023;20:553-7.

24. Dalir F, Morovvati A, Javadi A, Afkhami H, Khaledi M, Fathi J, Mohsenipour Z, Dirbaziyan A, Zargar M.[2023].The Evaluation of Efflux Pump Genes norA, norB and norC Related to Fluoroquinolones Resistance in Staphylococcus aureus Strains Isolated from Blood Infection. JABS. 2023;13(1): 81-87.

25. Gade ND, Qazi MS.[2013]. Fluoroquinolone Therapy in Staphylococcus aureus Infections: Where Do We Stand? J Lab Physicians. 2013;5(2):109–12.

26. Neamah, A. J.; Ayyez, H. N.; Klaif, S. F.; Khudhair, Y. I. and Hussain, M. H. [2019]. Molecular and phylogenetic study of Staphylococcus aureus isolated from human and cattle of Al-Qadisiyah Governorate, Iraq. Veterinary World, 12(9): 1378- 1382.

27. Shamkhi,GF.;Saadedin,S.M,K, and Jassim,K.A.[2019].Detection the Prevalence of Some Chromosomal Efflux Pump Genes in Methicillin Resistant Staphylococcus aureus Isolated from Iraqi Patients .Iraqi Journal of Biotechnology, December 2019, Vol. 18, No. 3, 33-42

28. Kumar LR, Kasim AK, Lekshmi M, Nayak BB and Kumar S. Incidence of methicillin-resistant staphylococci in fresh seafood. Advances in Microbiology, 2016; 6(06): 399-406.

29. Hassanzadeh S, Mashhadi R, Yousefi M, Askari E, Saniei M and Pourmand MR. Frequency of efflux pump genes mediating ciprofloxacin and antiseptic resistance in methicillin-resistant Staphylococcus aureus isolates. Microbial pathogenesis, 2017; 111: 71-74.

30. Afzal M, Vijay AK, Stapleton F, Willcox M.[2022]. The Relationship between Ciprofloxacin Resistance and

Genotypic Changes in S. aureus Ocular Isolates. Pathog (Basel, Switzerland). 2022;11(11):11.

31. Haddadi Zahmatkesh MA, Laripoor M, Mirzaie A, Ashrafi F.[2016]. Prevalence of norA and norB efflux pump genes in clinical isolates of Staphylococcus aureus and their contribution in ciprofloxacin resistance TT. Iran-J-Med-Microbiol. 2016 1;10(5):20–30.

32. Hamady,A.B.; Abd El-Fadeal,N.M.; Imbaby, S.;Nassar,H.M.; Sakr,M.G. and Marei1,Y.E.(2024).Expression of norA, norB and norC efflux pump genes mediating fluoroquinolones resistance in MRSA isolates. J Infect Dev Ctries 2024; 18(3):399-406.

33.Kaatz GW, McAleese F, Seo SM. Multidrug resistance in Staphy-lococcus aureus due to overexpression of a novel multidrug and toxin extrusion (MATE) transport protein. Antimicrob Agents Chemother 2005; 49: 1857-64.