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3 Molecular Detection of *SpnI/SpnR* and AHL-Mediated Quorum Sensing Signals Genes in *Serratia Marcescens*

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ABSTRACT

Quorum sensing process, bacteria connect with each other using secreted chemical signaling molecules called autoinducers. a quorum-sensing signaling molecule suggested to be involved in related species communication. Twelve isolates *Serratia marcescens* were collected from Baghdad hospitals. Of the 12 *S. marcescens* isolates examined, only 8 (66.66%) produced prodigiosin pigment. This work describes a high frequency of isolation of pigmented *S. marcescens* from clinical specimens, indicating that pigmented strains become clinically significant. The antibiotic sensitivity test showed multi drug resistant against (8- 18) antibiotics, all isolates were resistant (%100) against Beta lactam group, an indication of the comprehensive resistance to beta-lactam antibiotics, as a result of the irresponsible use of these antibiotics, which made them lose the effective therapeutic role that they previously required. In another hand *S. marcescens* 100% sensitive for to Meropenem and Imipenem and varied in their response to other antibiotics used in this study. The results showed a different distribution in *S. marcescens (AI-2 kinase*, *Tsq*, *rpoS* and *SpnI/SpnR*) genes. Eleven out of (92%) isolates namely were negative for *AI-2 kinase* gene, while (100%) 12/12 isolates observed positive for *Tsq* gene. 1\12 (8%) were positive for the presence of *rpoS* gene, whereas 12/12 isolates were positive, *spnI/spnR* gene was found with the highest percentage ratio (100%) when only one isolate were harboring all sets of genes. Our study suggested that expression of *spnI/spnR* by S. *marcescens* is independent of AHL signals synthesis.

Key words: Serratia marcescens, Quorum sensing, AI-2 kinase, Tsq, rpoS and SpnI/SpnR

INTRODUCTION

Serratia marcescens is a Gram-negative bacterium that causes infections in many organisms including humans [1]. There are two types of *Serratia marcescens*; pigmented (red) and non pigmented (white) strains, Pigmented *Serratia marcescens* strains have been shown to cause infections in much less frequency than non-pigmented strains, thus reducing the risk of infection during mass production of pigment [2].The ability of *S. marcescens* to adapt to and survive in both hostile and changing environments also relates to the bacterial capacity to express a wide range of secreted enzymes, including lipases, phospholipases, chitinases, proteases, and nucleases [3].*S. marcescens* is able to develop biofilm associated with either

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biotic or abiotic surfaces [4]. This ability is associated with the capacity of Serratia to colonize and persist in medical devices, such as catheters or prostheses [5], and to enhance bacterial resistance to antibiotics[6]. The treatment of infections caused by some strains of *Serratia marcescens* infections is problematic due to its high resistance to many antibiotics including β-lactam, aminoglycosides in addition to fluoroquinolone antibiotics [7].

S. marcescens utilizes the quorum sensing (QS) system to regulate the production of prodigiosin pigment, virulence factors such as proteases, lipase, nuclease and hemolysin in addition to swimming and swarming motilities and biofilm formation [3]. In *Serratia marcescsens*, SmaI/SmaR is the main type of Q.S signals in [8]. *Serratia* strains utilize AHLs like C4-homoserine lactone (C4-HSL), and C6-HSL to manage the expression of genes encoding virulence and biofilm formation and play important role in the increasing of antibiotic resistance of the bacteria [9]. S*erratia marcescens* may also use spnI/spnR as alternative system for Q.S instead of smaI/smaR system, The SpnI/R system regulates flagellum-independent population surface migration (sliding) and synthesis of biosurfactant, prodigiosin, and nuclease in *S. marcescens* [10]. Many genes also play vital role in the process of signal transduction in *S.marcescens* including Lsrk, TsqA and rpoS. Therefore, this study aims to investigation of Quorum sensing genes and AHL-mediated quorum sensing signals genes, in the other hand study their correlation with *S. marcescens* pathogenesis.

Material and Methods

Bacterial isolates. The study was performed with *S. marcescens* isolates that had been recovered from the four hospitals in Baghdad. Of the 12 isolates of the organism identified over the whole period, from 1st October 2019 – 1 st March 2020 were maintained and included in the present analysis. Presumptive Identification was made by determination of DNase on DNase-test medium , Nutrient agar, and MacConkey agar (Himedia, India), microscopic examination after Gram staining, and biochemical tests according to the manufacturer's recommendations. Bacteria were stored at –4°C in aliquots of 1.5 ml brain heart infusion broth (Biolife, Italy) containing 15% glycerol (as stock culture).

Biofilms assay. The test was done by using congo red agar test, it was prepared by Suspending 47 g of BHIA in 1000 ml of distilled water with 0.08% (w/v) Congo red supplemented with 5% (w/v) sucrose. It is Recommended for detection of biofilm formation [11]

Antimicrobial agents. Antibacterial susceptibility of *S. marcescens* was tested on Mueller-Hinton agar by the disk diffusion method according Patel *et al* (2017)[12]. The following antibiotic used are obtained from Mast disc Company, UK: Meropenem, Imipenem, Ceftazidime, Gentamicin, Amikacin, Netlmicin, Tetracycline, Doxycyclin, Ciprofloxacin, Levofloxacin, Ampicillin, Amoxicillin, Amoxicillin/clavulenic acid, Ampicillin/Salbctum, Cefixime,

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Ceftriaxone, Cefotaxime, Cefepime, Sulfamethoxazol\Trimethoprim, Colistin and compared the result with standard according to CLSI (2020). Each isolate was tested on three separate occasions.

DNA extraction of *S.marcescens* isolates. The DNA *S. marcescens* was extracted by using boiling method, in this method two colonies of overnight growth bacteria were used. The colonies were put in a test tube containing one ml of distilled water and boiled for 10 minutes in a water bath, and then were centrifuged for five minutes at 1000 rpm. Five microliters of the supernatant were used for the PCR [13].

Protocol of gene amplification. Conventional PCR technique was carried out to amplify of *spnI/ spnR*,AI-2 kinase, *TsqA* and *rpoS* genes ,(Table; 1) . Each PCR mixture consisted of 12.5 µl of master mix, 1µl of each forward and reverse primer, 5.5 µl of extracted DNA and 5 µl of deionized water to reach 25 µl as a total volume, and then the PCR mixture was transported to a thermal cycler. We used gradient PCR to all genes to reach the optimum temperature for annealing at temperature degree ranging from 51 C to 63 C.

Table (1) primer sequences and the product size for each one.

Gene ID	Reference	Product Size	Primers sequence (5`→3`)	Primers	
NZ_HG3 262231	This study	**472 bp**	GATTTGCTGCCAGAAGGTGC GAAAGAGCTGCACAACCACG	***Primer-1 f*** ***Primer-1 r***	***AI-2 Kinase***
NZ_HG3 262231	This study	**520 bp**	TGGGGTGATCGTCTGGATCT CCGCCGCGATTTATCCTTTG	***Primer-1 f*** ***Primer-1 r***	***TsqA***
[14]	This study	**354 bp**	AACGGTATTGGCCTTGCCTT TGCCCCTGATGGCGATAACTG	***Primer-1 f*** ***Primer-1 r***	***rpoS***
AB23486 91	This study	**268 bp**	CGTCCCATGCAAAGGGAGAT GACTGCAACGGAGCCTGTAT	***Primer-9 f*** ***Primer-9 r***	***spnI/spnR***

Agarose Gel Electrophoresis. Bacterial DNA electrophoresis was accomplished as it is described by Ream *et al.* (2013)[15]

RESULTES:

1.Identification of S. marcescens isolates

A total of 12 different clinical sources isolates were collected from patients whom visited as “out-patients” at Baghdad city which were cultured on Nutrient agar,

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MacConkey agar and DNase agar. Twelve isolates identified as *S.marcescens*, The identification and characterization of the isolates were carried out according to certain morphological, cultural and biochemical tests as described by Brooks *et al*)2001(.

2.Antimicrobial susceptibility testing.

The antibiotic sensitivity test was done by using (20) antibiotics, Bacterial isolates showed that *S.maacescens* 100% sensitive for to Meropenem and Imipenem, and showed high levels of resistaence by percentage of 100% to ampicillin ,amoxicillin, amoxicillin\clavulenic acid, cefipim, ceftazidime, cefotaxime and doxycycline, it was also have resistence to ampcillin\salbactum, , cefixim, ceftriaxone, ciprofloxacine, levofoxacine ,amikacin, gentamicin netlmicin,tetracycline, and co-trimaxazol by percentage 33%, 66%, 58%, 8%, 8%, 8%, 33%, 25%, 41%, 41% respectively. Urin isolates showed highest antibiogram resistance than other isolating sources. results revealed that 33.3% and 66.6% were MDR and non-MDR of total isolates respectively.Figure 1.

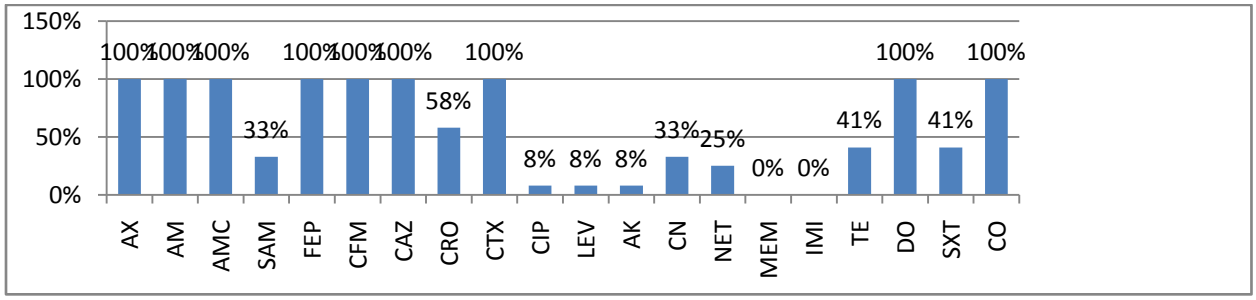

Figure (1) Antimicrobial resistance of S. marcescens isolates.

3.Biofilm assay. Results obtained by Congo red agar method in this study showed that 59% of *S.marcescens* isolates had the ability to produce biofilms.

4.Antibiogram typing. According to dendrogram in the figure (2), we classifed 12 isolates of *S.marcescens* into two cluster I and F , only one isolate S7 was consider out of the group. The cluster I classifed into three group A, B and cluster F contain only one group C according data of antimicrobial sensitivity test. The group A isolates were charachterized by high resistance to Gentamicin, ,Tetracycline, Sulfamethoxazol\Trimethoprim and Colistin. group B isolates charachterized by highly resistance to Ampicillin, Amoxicillin, Amoxicillin/clavulenic acid and Cefixime.. The group C were charachterized by resistance to Ceftazidime, Cefotaxime, Cefepime and Amoxicillin. The out group isolate name S7 was extremely drug resistant (XDR) highly resistance to all types of antibiotics except meropeneme and imipeneme.

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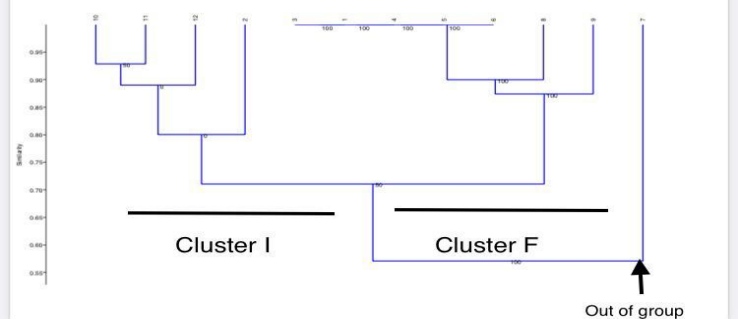


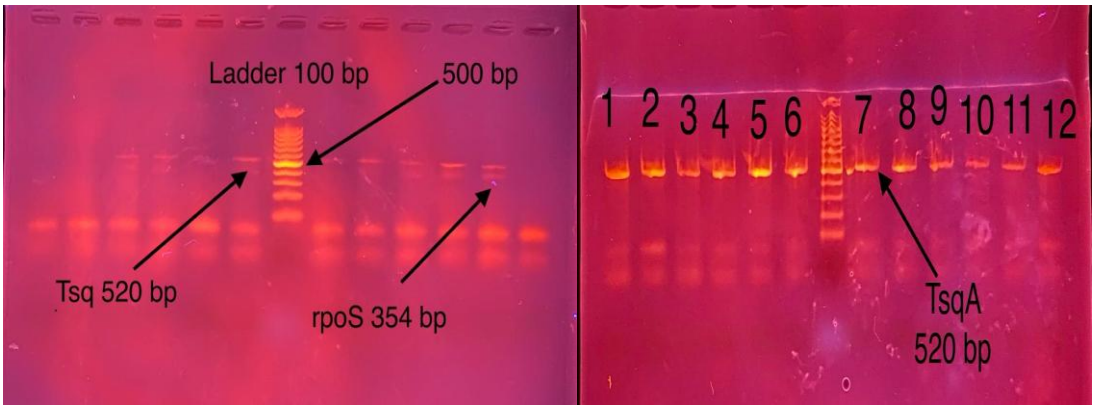
Figure (2) Dendrogram analysis according to antibiogram data

5.Detection of some quorum sensing genes in *S.marcescens* by PCR. The results showed a different distribution in *S. marcescens (AI-2 kinase, Tsq, rpoS* and *SpnI/SpnR* genes) (Table; 2). Eleven out of (92%) isolates namely were negative for *AI-2 kinase* gene, while (100%) 12/12 isolates observed positive for *Tsq* gene. 1\12 (8%) were positive for the presence of *rpoS* gene, whereas 12/12 isolates were positive, *spnI/spnR* gene was found with the highest percentage ratio (100%) when only one isolate were harboring all sets of genes.

Table (2): The percentage and number of *S. marcescens* isolates positive for the presence of screened AHL- mediated quorum sensing and QS genes.

QS genes	NO. of isolates	precentage
Autoinducer- kinase	1	(8%)
TsqA	12	(100%)
rpoS	1	(8%)
spnI/spnR	12	(100%)
spnI/spnR, AI-2 kinase , rpoS and TsqA	1	8(%)

(A) (B)



Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 5274 - 5285
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(C) (D)

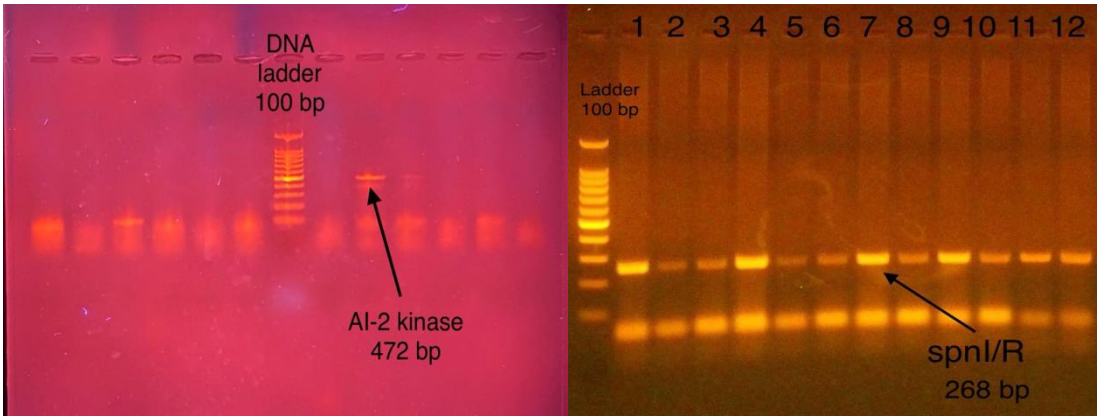

Figure (3)Agaros gel electrophoresis 1% for for (A)TsqA 520 bp and rpoS 354 bp, (B) TsqA 520 bp (C), AI-2 kinase 472 bp, (D) spnI/spnR 268 bp of *Serratia marcescens* compared with (100 bp) DNA ladder lane.

DISCUSSION :

S. marcescens isolates grown on MacConkey agar appeared pale color result from non-lactose fermentation in the medium [16]. *Serratia marcescens* have ability to produce red non-soluble pigment which is called Prodigiosin which differ from lactose fermentation colonies on MacConkey agar. , in the same manner Srimathi *et al* (2017) [17] mentioned that non- lactose fermenting is the reason where *S . marcescens* appeared in yellow color on the MacConkey agar. *S. marcescens* isolates differ in their ability to produce Prodigiosin pigment on nutrient agar medium at different temperatures, 6(50%) of the isolates were pigment producers at both of 28 and 37 °C, while 6(50%) of *S. marcescens* isolates were non pigment producers at same temperatures. Similar results were obtained by other researchers, a study by Ali *et al* (2008) [18]noticed that their *S. marcescens* isolates had the ability to produce prodigiosin at 30°C and the rate was reduced as the temperature increases, Arivizhivendhan *et al* (2015) [19] Noticed most *S. marcescens* demonstrated the ability to prodigiosin production at 29 C. *S.marcescens* was positive for DNAase activity , and have been changed the colour from blue to pink purple around the growth , in the same line Ryan and Ray (2004) reported that DNase production is associated to *Serratia marcescens* identification further more considered major virulence factor of these bacteria.

From the study results, *S.marcescens* showed that 59% of isolates had the ability to produce biofilms, at the same line Bronowskil *et al,* (2014) [20] showed the common strategies for bacterial survival in hard environment conditions, is the biofilm production , Amorim *et al* (2018) [21] have been indicated that (85%) of *S. marcescens* isolates produced biofilm, appearing as black colonies, while non-producing isolates have become depigmented or red.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 1, 2021, Pages. 5274 - 5285
Received 15 December 2020; Accepted 05 January 2021.

In our results noticed that *S. marcescens* isolates were isolated from patients were mixed between Prodigiosin and non Prodigiosin producer, these results came partially agree with Ali *et al* (2013) [22], which showed the *S. marcescens* frequently linked with hospitalized patients this reflects the ability as opportunistic pathogen.in this manner Alsakini *et al* (2020) [23] make out Non-pigmented *S. marcescens* is a main cause of infection in hospitals.

The antibiotics of the carbapenem group (Meropenem and imipenem) still maintain their therapeutic efficacy for *S. marcescens*, sensitivity of *S. marcescens* to Meropenem and Imipenem are 100% While the resistance to other antibiotics were high which make it usless for treating the infection figure (1). This finding is in agreement with the conclusion mentioned in studies in local, nearby and foreign countries. In study conducted in Saudi Arabia found out that all their (81) *S. marcescens* clinical isolates were susceptible to Meropenem and Imipenem (Faida *et al*.,2015) [24] in another study by Moradigaravand *et al*., (2016) [25] had found out that, their majority of multidrug resistant *S. marcescens* were susceptible to Imipenem.

charachterized by high level of resistance to beta-lactam group of antibiotics pencillins and cephalosporins, in the same manner, many research found to coniform this finding about *S. marcescens* to pencellin,Amoxcillin, Amoxcillin plus clavulenic acid, , Ceftazidime, cefixime , Cefotaxime and ceftraixone [25,26,27,28,29].
The resistance of *S. marcescens* isolates to fluoroquinolone (Ciprofloxacin and Levofloxacin) was only 8%, Our results close to the result obtained by Sethuraman *et al.*, (2011) [30] when their clinical *S. marcescens* isolates (100%) were all sensitive to ciprofloxacin and levoflxacin. The resistance to to aminoglycosid antibiotics were variable. In the same context Şimşek (2019) [31] found the resistance of *S.marcescens* isolates to Amikacin was only 6.3% and this results are close to our result, he also reported that only 17 out of 185 isolates of *S.marcescens* from various clinical specimens were resistant to Nettlmicin ,while Ogba *et al*., (2014) mentioned that only 32% of their *S. marcescens* isolates were resistant to Gentamicin.
In study by Fusté *et al*., (2012) conducted in spain indicated that all of their clinical and environmental *S. marcescens* isolates were resistant to Tetracycline, in an other study by conducted by Alexandre *et al*, (2019) revealed that there isolates of *S.marcescens* had intermediate resistance to Doxycyclin.
Bitew *et al*., (2018) revealed that their *S. marcescens* isolates from wound infection were 50% sensitive to Trimethoprim/Sulfamethoxazole, their result agree with the result obtained in our study.

All isolates of *S. marcescens* were resistant to Colistin 100%. In study conducted by Abdellatif Daoudi et al, 2019 they mentioned that all of collected clinical isolates of *S.marcescens* were resistant to Colistin .

Our study suggested that AHL signals synthesis by *S. marcescens* is not dependent

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Received 15 December 2020; Accepted 05 January 2021.

on *spnR* expression, as we noticed only one isolate produce AHLs while all study isolates were positive for *SpnI/SpnR* .in the same line Horng and his colleague(2002) [32] Find *spnI* transcripts level detected was not affected by the presence or absence of multicopy *spnR*, confirming that AHL synthesis independent of *spnR* . The information presented here suggest that SpnR is probaley to form DNA- binding dimers in the lack of AHL, that suggest that SpnR can act as an stimulator of *spnR* gene expression. Same regard Horng and his colleague(2002) predict that SpnR stimulate its own expression by reacting with this lux box- like element. lux box- like sequence is not found in spnI promoter region, so it appears reasonable that, spnI expression not regulate by SpnR . A similar case is noticed in *P. stewartii* (von Bodman *et al.*, 1998) where the expression of both esaR and cps operons repressed by EsaR, in which the elements of lux box- like are all existing at the −10 region, suppressing access for transcription [33].

The exchange of extracellular signaling molecules must be mediated by specific transporters, some involved in molecular uptake, others catalyzing efflux, TsqA is a membrane-spanning protein that is conserved in many bacteria needed for transportation of AI-2 and have great effect on Q.S process [34]. Walters *et al* (2006) [35] reported the reason for presence of *TsqA* in all isolates is the vital role that it play in signal transduction in by mediating the exportation of AI-2 molcules.

In the same contexst Herzberg *et al* (2006) [36] revealed that *TsqA* was shown to control biofilm and It influences resistance to several antimicrobials, including crystal violet and streptomycin, Deletion of *TsqA* caused 31% of the bacterial chromosome to be differentially expressed in biofilms.

The other gene determined by this study and have a big influence on survival of the bacteria in harsh environment are *RpoS* , known to play an important role in the adaptation of the cell to general stresses such as carbon starvation, high temperature and oxidative stress [37]. The gene was detected in one isolate and the reason for this result are the absence of the plasmid containing the gene since it's portable on plasmid the differences in gene sequence from the registered sequences, as well as the or the mutations which can happened in the downstream region of the gene [38].

In compatibity with this results Naba'a *et al* 2018 [14] reviwed that in pathogenic bacteria, the gene *rpoS* involved in some virulence genes controlling to enable those bacteria to resist harsh environments given by the host which lead to many genes controlling that involved in maintenance of cellular viability in stationary phase like hyper-osmolality,oxidative stress, , reduced PH and starvation.

The information presented in this study clearly indicated that different isolates of the same organism may employ distinct negative or positive Q.S circuits to regulate the different or same traits. These results reflected that *Serratia* possess complicated quorum- sensing circuits.

The most frequent genes were spnI/spnR and TsqA appeared in 12 isolates, one of thsee

isolates have the full set of genes. According to phenotypic characters, we noticed that the isolate produced pigment prodigiosin in higher level than other isolates in the study, as well as , the isolate also expressed all of virulence factors were tested for e.g. DNase, and biofilm produced in much high manner when compared to other isolates. The resistance profile of the the same isolate was distinctive, it proved that most of the tested 20 antibiotics are ineffective for its eradication, only meropeneme and imipeneme were effective to get rid of it

CONCLUSION:

The study pointed that AHL signals synthesis by *S. marcescens* is not dependent on *spnR* expression and the presence of the full set of genes is necessary for optimum operation of Q.S system in S. marcescens. These results reflected that *Serratia* possess complicated Q.S circuits, as well as, the production of prodigiosin and virulence factors are under control of mor than one system of Q.S in *S .marcescens*

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REFERENCE

[1] Mahlen, S.D. Serratia infections: From military experiments to current practice. Clan. Microbial. Rev. 2011, 24, 755–791.
[2] Linawati Hardjito, Anwar Huq , and Rita R. Colwell,The Influence of Environmental Conditions on the Production of Pigment by Serratia marcescens , Biotechnol. Bioprocess Eng. 2002, 7: 100-104.
[3] Petersen LM, Tisa LS. Friend or foe? A review of the mechanisms that drive Serratia towards diverse lifestyles. Can J Microbiol. 2013;59:627-40.
[4] Van Houdt R, Givskov M, Michiels CW. Quorum sensing in Serratia. FEMS Microbiol Rev. 2007;31:407-24.
[5] Choe HS, Son SW, Choi HA, Kim HJ, Ahn SG, Bang JH, Lee SJ, Lee JY, Cho YH, Lee SS. 2012. Analysis of the distribution of bacteria within urinary catheter biofilms using four different molecular techniques. Am J Infect Control 40:e249 – e254.
[6] Ray C, Shenoy AT, Orihuela CJ, Gonzalez-Juarbe N. 2017. Killing of Serratia marcescens biofilms with chloramphenicol. Ann Clan Microbial Antimicrob 16:19.
[7] Merkier AK, Rodríguez MC, Togneri A, Brengi S, Osuna C, Pichel M, Cassini MH; *Serratia marcescens* Argentinean Collaborative Group, Centrón D. Outbreak of a cluster with epidemic behavior due to *Serratia marcescens* after colistin administration in a hospital setting. J Clin Microbiol 2013; 51: 2295-2302.
[8] Mohammed Z. Al-Khayyat , Quorum Sensing in Microbial Virulence , Int. Biol. Biomed. J.

Summer 2018; Vol 4, No 3
[9] Zahra Fekrirad, Basira Gattali, Nasim Kashef, Quorum sensing-regulated functions of Serratia marcescens are reduced by eugenol, IRAN. J. MICROBIOL. Volume 12 Number 5 (October 2020) 451-459.
[10] Jun-Rong Wei, Yu-Huan Tsai, Yu-Tze Horng, Po-Chi Soo, Shang-Chen Hsieh, Po-Ren Hsueh, Jim-Tong Horng, Paul Williams, and Hsin-Chih La; A Mobile Quorum-Sensing System in Serratia marcescens; JOURNAL OF BACTERIOLOGY, Feb. 2006, p. 1518–1525.
[11] Cotter JJ, O'gara JP, Mack D, Casey E. Oxygen-mediated regulation of biofilm development is controlled by the alternative sigma factor σβ in Staphylococcus epidermidis. Appl Environ Microbiol 2009;75(1):261–4.
[12] Patel. J. B, Cockerill. F, Eliopoulos. G. Jenkins, S. Lewis, J. Limbago, (2017). M100 Performance Standards for Antimicrobial Susceptibility Testing, 27th Edn. Wayne, PA: CLSI
[13] Ali A Dashti , Mehrez M Jadaon , Abdulsamad M Abdulsamad , Hussein M Dashti, Heat Treatment of Bacteria: A Simple Method of DNA Extraction for Molecular Techniques ,Kuwait Medical Journal 2009; 41 (2): 117-122.
[14] Naba'a A. Muhammed, Muthana B. Farhan and Zeyad A. Shabeeb; Detection of luxS Gene in Serratia marcescens and rpoS Gene in Enterobacter cloacae Isolates Using PCR Reaction , Journal of Al-Nahrain University Vol.21 (1), March, 2018, pp.115-120 Science 115 .
[15] Ream, W., Gellar, B.,Trempy, J. and Field, K. (2013).Molecular microbiology 2 Merkier AK, Rodríguez MC, Togneri A, Brengi S, Osuna C, Pichel M, Cassini MH; *Serratia marcescens* Argentinean Collaborative Group, Centrón D. Outbreak of a cluster with epidemic behavior due to *Serratia marcescens* after colistin administration in a hospital setting. J Clin Microbiol 2013; 51: 2295-2302.
[16] Khudhair A M, Saadallah S and Al-Faham M (2011) Isolation of Multi Antibiotic Resistance Serratia marcescens and the Detection of AmpC & GESβL Genes by Polymerase Chain Reaction Technique. Al-Mustansiriyah J. Sci. 22(6), 329- 346.
[17] Srimathi R, Priya R, Nirmala M, Malarvizhi A, Isolation, Identification, Optimization of Prodigiosin Pigment Produced by Serratia Marcescens and its Applications, International Journal of Latest Engineering and Management Research (IJLEMR) ISSN: 2455-September 2017 || PP. 11-21
[18] Ali M R, Hussin M S and Kadum M M (2008) Study of prodigiosin and virulence factor producing by multi drug resistance Serratia marcescens isolated from some of Baghdad hospitals environment. Al-Mustansiriya J. Sci. 19(5), 1-9.
[19] Arivizhivendhan, K. V, Mahesh. M, Regina Mary. R, and Sekaran, G. (2015). Bioactive prodigiosin isolated from Serratia marcescens using solid state fermenter and its bactericidal activity compared with conventional antibiotics. Journal of Microbiology Biochemical and Technology, 7: 305- 312.

[20] Bronowskil. C, James. C.E. and Winstanley. C. (2014). Role of environmental survival in transmission of Campylobacter jejuni. FEMS Microbiol Lett., 356(1): 8-19.
[21] Amorim, A. M. B, Milo. D. H, Souza. B. V, Medeiros. L. M, Mattoso. J. M. V. and Nascimento. J. S, A reddish problem: Antibiotic-resistant Serratia marcescens in dairy food commercialized in Rio de Janeiro, International Food Research Journal 25(2): 880-883 (April 2018).
[22] Ali M R, Alsakini A H and Karim S M (2013) Correlation between Prodigiocin, Biofilm formation and Drug Resistant in Virulent Serratia marcescens. Al-Nahrain J. Sci. 16(4), 188-197.
[23] Ali H. Alsakini , Anfal Mohammed Khudhair, Shaymaa K. Abass , Munim R. Ali , Dunya Jawad Ridha3 , Khitam Sabbar Najm3 , Hanan Mohammed Abbas3 and Faten Mohammed Mahdi Shukur TYPING OF SERRATIA MARCESCENS ISOLATES : EVALUATION OF REPPCR AND ANTIBIOGRAM TYPING METHODS, Biochem. Cell. Arch. Vol. 20, Supplement 2.
[24] Faidah, H. S, Ashgar,.S. S, Barhameen. A. A, El-Said. H. M. and Elsawy. A. (2015). Serratia marcescens as opportunistic pathogen and the importance of continuous monitoring of nosocomial infection in Makah city, Saudi Arabia. Open Journal of Medical Microbiology, 5(03): 107
[25] Moradigaravand. D, Boinett. C. J, Martin. V, Peacock. S. J, and Parkhill. J. (2016). Recent independent emergence of multiple multidrug-resistant Serratia marcescens clones within the United Kingdom and Ireland. Genome Res. 26, 1101–1109.
[26] Munim R. Ali, Emergence And Detection Of Ampc Gene By Polymerase Chain Reaction From Sepsis Serratia Marcescens ,Al- Mustansiriyah J. Sci Vol. 23, No 1, 2012.
[27] Iguchi. A, Nagaya. Y, Pradel. E, Ooka. T, Ogura. Y, Katsura. K and Sebaihia. M. (2014). Genome evolution and plasticity of Serratia marcescens, an important multidrug-resistant nosocomial pathogen. Genome Biology and Evolution, 6(8):2096-2110.
[28] Selvi A, Banerjee M, Das N. Degradation of cefdinir from pharmaceutical wastewater using immobilized *Candida* sp. SMN04 and biofilm formed on gravels. *J App Pharm Sci.* 2015;5:73–79]
[29] Selvi A, Das D, Das N. Potentiality of yeast *Candida* sp. SMN04 for degradation of cefdinir, a cephalosporin antibiotic: kinetics, enzyme analysis and biodegradation pathway. *Environ Technol.* 2015;36:3112–3124
[30[Sethuraman, S.; Arunachalam, A.; Karthikeyan, M.; Ashhutosh Kumar, S.; Manidipa, S. and Senthilraj, R. (2011). Antimicrobial sensitivity profile of Serratia marcescens strains isolated in Governement General Hospital, Nagapattinam, Tamilnadu, India. International Journal of Preclinical and PharmaceuticalResearch,2(1):7-11.
[31] Şimşek M. Determination of the antibiotic resistance rates of Serratia marcescens isolates obtained from various clinical specimens. Niger J Clin Pract 2019;22:125-30.0
[32] Horng. Y. T, S. C. Deng, M. Daykin, P. C. Soo, J. R. Wei, K. T. Luh, S. W. Ho, S. Swift, H. C. Lai, and P. Williams. 2002. The LuxR family protein SpnR functions as a negative regulator of N-acylhomoserine lactone-dependent quorum sensing in Serratia marcescens. Mol. Microbiol.

45:1655–
[33]Beck Von Bodman, Doris R. Majerczak, and David L. Coplin A; negative regulator mediates quorum-sensing control of exopolysaccharide production in Pantoea stewartii subsp. stewartii SUSANNE; Proc. Natl. Acad. Sci. USA Vol. 95, pp. 7687–7692, June 1998 Microbiology
[34] Evans .K, Passador. L, Srikumar. R, Tsang. E, Nezezon. J, Poole. K;Influence of the MexABOprM multidrug efflux system on quorum sensing in Pseudomonas aeruginosa . J Bacteriol 1998;180:5443–5447.
[35] Walters. M, Sircili. MP, Sperandio. V: AI-3 synthesis is not dependent on luxS in Escherichia coli . J Bacteriol 2006;188:5668–5681
[36] Herzberg. M, Kaye .IK, Peti .W, Wood .TK: YdgG (TqsA) controls biofilm formation in Escherichia coli K-12 through autoinducer 2 transport. J Bacteriol 2006;188:587–598.
[37] Aurelia Battesti, Nadim Majdalani, and Susan Gottesman The RpoS-Mediated General Stress Response in Escherichia coli Annu. Rev. Microbiol. 2011. 65:189–213 First published online as a Review in Advance on June 3, 2011
[38]Martinez-Garcia Esteban, Tormo Antonio, Navarro-Liorens Juana Maria, :Further Studies on rpoS in Enterobacteria: Identification of rpoS in Enterobactor cloacae and Kluyeracryo crescens", ARCH MICROBIAL. 175, 395-404, 2001