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Molecular Identification of *Serratia marcescens* Isolated from Milk and Detection of Beta-Lactam Resistance Genes

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Abstract | On a global scale, milk is an essential and basic source of food nutrition. It is not only rich in high-quality protein but also an excellent source of vitamins and minerals. One of the most important causes of food poisoning is the contamination of milk with pathogenic microorganisms. *Serratia marcescens* is a significant but less studied bacteria in public health. This pathogen causes milk contamination and infects humans and animals. However, studies are less common and therefore, this study aims to investigate *Serratia marcescens* in raw, pasteurized, and mastitis milk. Additionally, to identify how resistant *Serratia* is to antibiotics, whether the isolates contain beta-lactam resistance genes, and detect the *bsmB* gene for protease and lipase production. We collected 200 milk samples from various areas of Basrah. We found and confirmed *Serratia marscecens* in 11 (5.5%) of the 200 milk samples by growing them, staining them with gram stain, and then identifying them by PCR with specific primers and sequencing. We also used the disk diffusion method to investigate the antibiotic resistance of the isolates. The results showed that the isolated strains were sensitive to gentamycin (5/11, 45.45), imipenem (11/11, 100%), and Cefotaxime (8/11, 72.72%). All isolates shared ampicillin, amoxicillin, penicillin G, oxacillin, amikacin, and neomycin as the most common resistance. Additionally, the presence of beta-lactam resistant and virulence genes was examined. 90.90% of isolates carried the *blaTEM* gene. A total of 18.18% carried the *blaSHV* gene and 27.27% carried the *blaCTX-M1* while the *bsmB* gene was detected in all isolates. These findings indicate the significance of *Serratia marcescens* in both animal health and public health.

Keywords | Milk, Serratia, Resistant, Beta-lactam, Protease, Lipase

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INTRODUCTION

Humans consume a substantial amount of milk, which is an optimal growth environment filled with nutrients for microbes when kept at the right temperature (Msalya, 2017). Therefore, it is necessary to work with the highest hygiene during the handling and production process of dairy products to prevent any external threats. It is important to handle the contamination and observe the microbial growth during product storage and transportation. Bad hygienic conditions lead to the presence of microorganisms, resulting in poor-quality products (Bauman *et al*., 2018).

Serratia species are gram-negative, rod-shaped, and opportunistic bacterias. They are classified within the recently reclassified family *Yersiniaceae* within the *Enterobacterales* order (Friman *et al*., 2019). *Serratia* species is set apart from other genera by its ability to produce three enzymes: DNase, lipase, and gelatinase (Giri *et al*., 2004).

Different environmental sources contain these organisms, like soil, water, and different types of plants, suggesting they have a widespread in the environment (Chen *et al*., 2017).

Serratia species, mainly *Serratia marcescens* and *Serratia liquefaciens*, are common environmental bacteria that can cause opportunistic diseases in humans and animals (Mahlen, 2011). Including mastitis in dairy cows (Schukken *et al*., 2012) and causing spoilage at different stages of milk processing (Decimo *et al*. 2014).

There are many virulence factors causing the high pathogenicity of *Serratia marcescens*, such as hemolysin, lipase, protease, prodigiosin, and motility (Khayyat *et al*., 2021).

Antibiotic therapy is the main method for treating most bacterial infections, including *Serratia marcescens* (Tavares-Carreon *et al*., 2023). However, as new resistant strains emerge over time, the effectiveness of this therapy has been reduced (Iguchi *et al*., 2014). As a result, treating *Serratia marcescens* infections is complicated due to the bacteria's multidrug-resistant nature (González-Juarbe *et al*., 2015).

Studies show that the commonly used beta-lactam antibiotics face an increasingly concerning resistance when used in treating *Serratia marcescens* infections (Abbas and Hegazy, 2020).

The resistance is practically due to various determinants, including encoding extended-spectrum beta-lactamase genes (*blaSHV, blaTEM*, and *blaCTX*) and carbapenemase genes (*blaOXA-48, blaOXA-1*), which contribute to beta-lactam resistance. Accordingly, this study aims to isolate *Serratia marcescens* from milk samples in Basrah, Iraq, and identify antibiotic resistance in isolates with detectable resistance and virulence genes.

MATERIALS AND METHODS

Samples collection

In our study, two hundred milk samples (Raw and mastitis milk) were collected from College of Veterinary Medicine, University of Basrah, the College of Agriculture, University of Basrah, and dairy farms in different areas of Basrah, including Abi Al-Khasib, Al-Seeba, Qurna, Dair, Faw, Shatt Al-Arab, Um Qasr, Safwan, and Zubair as shown in Table 1. A clinical examination was conducted according to (Massé *et al*., 2020). Consequently, every udder was examined closely to ensure that the quarters were symmetrical, and then each udder was thoroughly palpated to look for signs of fibrosis, inflammatory swellings, obvious damage, tick infestation, tissue atrophy, and swelling of the supra mammary lymph nodes. Additionally, each mammary quarter's milk secretion's viscosity and appearance were checked for the presence of clots, flakes, blood, and watery secretions. Along with udder and teat morphological abnormalities, the presence of clots, flakes, blood, and other consistency alterations was one of the markers for clinical mastitis (Tezera and Ali, 2021). Additionally, the California Mastitis Test (CMT) was applied to milk that appeared normal in order to identify subclinical mastitis, as described by (Michael, 2011). Before milk sample collection, the udder was cleaned with tap water and dried with a clean towel. The teat was then dipped in a 1:1000 iodine solution and allowed to dry. The teat was then dipped in 70% alcohol and allowed to dry. One or two streams of milk were sampled and discarded. The milk was collected in a sterile container (60 ml), and the samples were transferred into an ice box.

Table 1: Regions and the total number of collected samples per region.

Regions	Number of samples
Collage of agriculture	5
College of veterinary medicine	5
Abu Al-Khaseeb	10
Al Qurnah	20
Al Zubair	3
Al Dayr	50
Al Hartha	49
Al-Faw	16
Al Seeba	14
Safwan	7
Shat Al-Arab	2
Um Qasr	19

Identification of *Serratia marcescens*

Cultural characteristics

The morphology and pigmentation of the colonies were analyzed after the specimens were cultured on chrome Serratia agar (Himedia, India). The colonies that exhibited typical morphology and pigmentation were subsequently cultivated in two cultures, on MacConkey's agar and nutrient agar. We then placed each pair of colonies in incubators at temperatures of 30 and 37 °C, respectively. Furthermore, we employed Gram staining to determine whether the microorganisms were Gram-positive or Gram-negative.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of *Serratia marcescens* was assessed using the disk diffusion method on Mueller-Hinton agar to test 11 antimicrobial agents, according to the Clinical and Laboratory Standards Institute (CLSI, 2018) protocol. Antimicrobial agents examined in this study

Table 2: Primers used in this study.

S. No.	Primers	Sequence	Size of product	Reference
1	*Serratia* specific primers	F:GGTGAGCTTAATACGTTCATCAA R: AATTCCGATTAACGCTTGCAC	107bp	Bussalleu and Althouse (2018)
2	*bsmB*	F: GCGGATGTGTA TGCCTTCG R: GCCACGCATTTCTTCACTCA	200bp	Fekrirad *et al*. (2020)
3	*blaTEM*	F: TGCGGTATTATCCCGTGTTG R: TCGTCGTTTGGTATGGCTTC	296bp	Ferreira *et al*. (2020)
4	*blaSHV*	F:AGCCGCTTGAGCAAATTAAAC R:ATCCCGCAGATAAATCACCAC	712bp	Ferreira *et al*. (2020)
5	*Blaoxa-48*	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	438bp	Ferreira *et al*. (2020)
6	*Blaoxa-1*	F: GGCACCAGATTCAACTTTCAAG R: GACCCCAAGTTTCCTGTAAGTG	563bp	Ferreira *et al*. (2020)
7	*Blactx-m1*	F: ACAGCGATAACGTGGCGATG R: TCGCCCAATGCTTTACCCAG	216bp	Ferreira *et al*. (2020)
8	*16S rRNA*	F: AGAGTTTGATCCTGGCTCAG R: CGGTTACCTTGTTACGACTT	1500bp	Hasan *et al*. (2022)

include Ampicillin (10µg), Ceftriaxone (30µg), Oxacillin (1µg), Amoxicillin (10µg), Penicillin G (10µg), Tobramycin (10µg), Amikacin (10µg), Gentamycin (10µg), Neomycin (10µg), Imipenem (10µg), and Cefotaxime (30µg).

Molecular identification and detection of resistance genes

DNA extraction

Genomic DNA was extracted from overnight broth cultures using a genomic DNA extraction kit (trans/china). In summary, 180 µl of the sample suspension was mixed with 20 µl of proteinase K and 200 µl of lysis buffer and then incubated at 70 ˚C for 10 minutes. Following the incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged according to the manufacturer's instructions. Finally, the nucleic acid was eluted using 100 µl of the provided elution buffer from the kit and kept at -20 C until use.

DNA Concentration Estimation

We measured the concentration and the quality of DNA samples using a nanodrop spectrophotometer (nanodrop one C, Thermo Fisher).

Oligonucleotide Primer

Primers used were supplied by Macrogen, Korea, as listed in Table 2.

PCR amplification

In all the PCR reactions, we used the GoTaq® G2 green master mix (Promega, USA). The reagents were added to each PCR tube for the genes listed in Table 3. The thermocycling conditions for Taq polymerase PCR amplification are listed in Table 4.

Table 3: The reaction mixture (25 µl) for each gene.

S. No.	Green master mix	12.5 µl.
1	F primer (10 µl)	1.5 µl.
2	R primer (10 µl)	1.5 µl.
3	DNA Template	4 µl.
4	Nuclease free water	5.5 µl.

Analysis of the PCR Products

The PCR products were separated by electrophoresis on 1.5% agarose gel in 1x TBE buffer (Promega/USA). Ten µl of DNA and 5µl of ladder were loaded into the wells of the agarose gel. After the electrophoresis, the gels were examined under a UV transilluminator.

Sequencing and phylogenic analysis

To further confirm the isolates, the 16S RNA gene was PCR amplified using universal primers. We conducted the sequencing in Macrogen, Korea, using 16S RNA forward and reverse primers on both DNA strands. In addition, the Maximum Likelihood approach and Tamura-Nei model were used to determine the evolutionary history of the isolates and other closely related ones from the NCBI deposited sequences are listed in the supplementary material. The tree with the maximum log probability (-1753.05) was generated. This analysis included a total of 12 nucleotide sequences. Evolutionary studies were performed using the MEGA11 software (Tamura *et al*., 2021).

RESULTS AND DISCUSSION

Cultural characteristics

Out of 200 milk samples, the bacterial isolation results identified 11 samples as *Serratia marcescens*, representing 5.5%. The positive isolates showed different morphological

Table 4: PCR thermocycler conditions for each gene.

Genes	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
Serratia specific primers	95 °C 5 min	95 °C 15 sec	59.5 °C 15 sec	72 °C 20 sec	72 °C 7 min	35
bsmB	95 °C 5 min	95 °C 15 sec	57 °C 20 sec	72°C 45 sec	72 °C 7 min	35
Blashv	95 °C 10 min	95 °C 40 sec	55.5°C 40 sec	72 °C 1 min	72 °C 7 min	35
BlaTEM	95 °C 5 min	95 °C 20 sec	56 °C 15 sec	72 °C 30 sec	72 °C 10 min	35
Blaoxa-1	95 °C 5 min	95 °C 15 sec	53 °C 20 sec	72 °C 45 sec	72 °C 10 min	35
Blaoxa-48	95 °C 5 min	95 °C 15 sec	56 °C 20 sec	72 °C 45 sec	72 °C 10 min	35
Blactx-m1	95 °C 5 min	95 °C 15 sec	57 °C 20 sec	72 °C 45 sec	72 °C 10 min	35
16s rRNA	95 °C 5 min	95 °C 15 sec	49 °C 20 sec	72 °C 1.5 min	72 °C 10 min	35

and cultural characteristics. On chrome agar, MacConkey agar, and nutrient agar, isolates displayed colonies with varying colors according to the incubation temperature. On chrome agar, colonies appeared pink with a dark center at 30 °C, while others exhibited blue to purple colonies at 37 °C, as shown in Figure 1A, B. After 24 hours of incubation at 30 and 37 °C, colonies on MacConkey agar showed lactose fermentation and appeared red, as shown in Figure 1C. On nutrient agar, after 24 hours of incubation at 30 °C, colonies appeared red like blood due to *Serratia marcescens*' ability to produce pigment. This characteristic makes it simple to differentiate *Serratia marcescens* from other *Enterobacteriaceae*, as shown in Figure 1D. Microscopic examination of the isolated bacteria showed gram-negative rods, as shown in Figure 2.

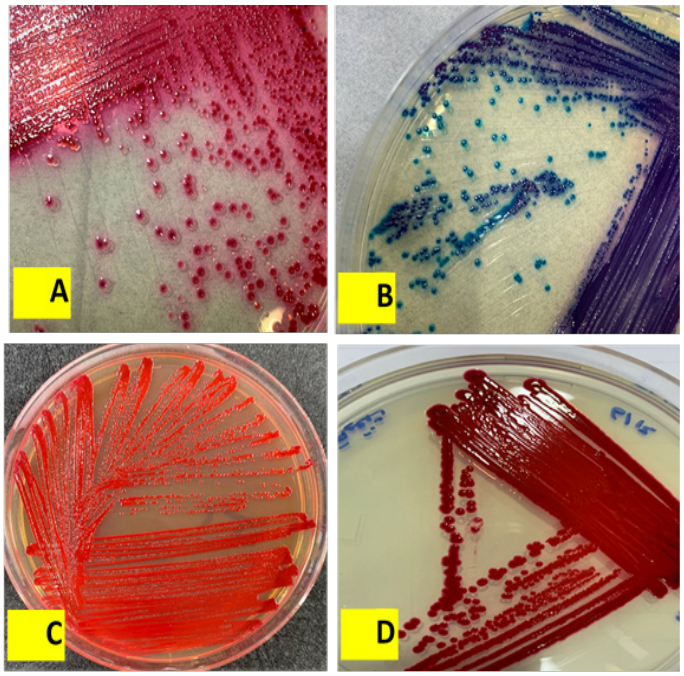

Figure 1: Cultivation of *Serratia marcescens* on differential media. A, *Serratia marcescens* on chrome agar colonies appeared pink with a dark center at 30 °C. B, on chrome agar colonies, appeared blue to purple at 37 °C. C, on MacConkey agar colonies, appeared red and exhibited lactose fermentation. D, on nutrient agar colonies at 30 °C, appeared red like blood.

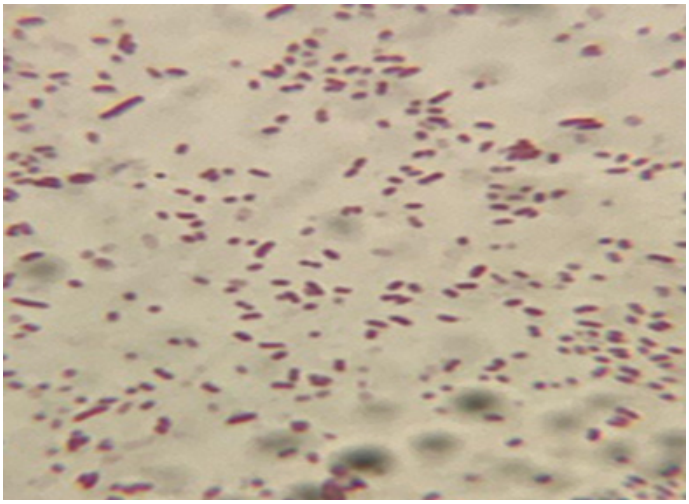

Figure 2: *Serratia marcescens* stained by gram stain.

Antibiotics susceptibility testing

Antibiotic susceptibility test showed that *Serratia marcescens* were resistant to ampicillin (11/11, 100%), amoxicillin (11/11, 100%), penicillin G (11/11, 100%), oxacillin (11/11, 100%), ceftriaxone (10/11, 90.90%), tobramycin (5/11, 45.45%), amikacin (11/11, 100%), neomycin (11/11,100%), while sensitive to gentamycin (5/11, 45.45), Imipenem (11/11, 100%) and cefotaxime (8/11, 72.72%) as shown in Table 5 and Figure 3.

Table 5: Results of antibiotic resistance expressed as numbers and present of sensitive, Intermediate and resistant antibiotic to *S. marcescens*.

Antibiotics	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ampicillin	0	0	0	0	11	100
Ceftriaxone	1	9.09	0	0	10	90.90
Oxacillin	0	0	0	0	11	100
Amoxicillin	0	0	0	0	11	100
Penicillin G	0	0	0	0	11	100
Tobramycin	3	27.27	3	27.27	5	45.45
Amikacin	0	0	0	0	11	100
Gentamycin	5	45.45	2	18.18	4	36.36
Neomycin	0	0	0	0	11	100
Imipenem	11	100	0	0	0	0
Cefotaxime	8	72.72	0	0	3	27.27

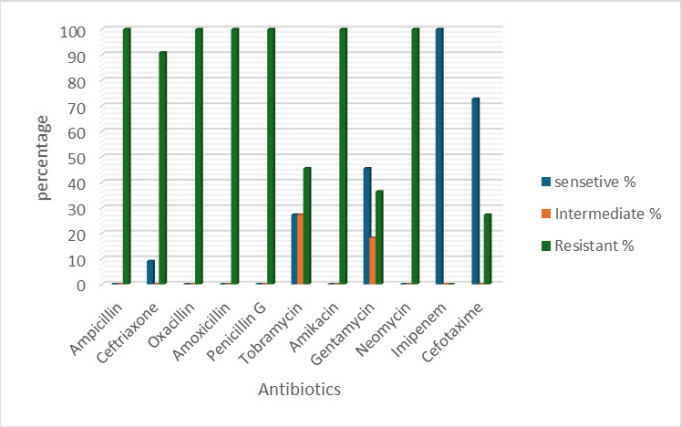

Figure 3: Results of antibiotic susceptibility test of all *Serratia marcescens*.

Serratia specific PCR

Isolates showed positive results at 107 bp using a 100bp DNA ladder marker as shown in Figure 4.

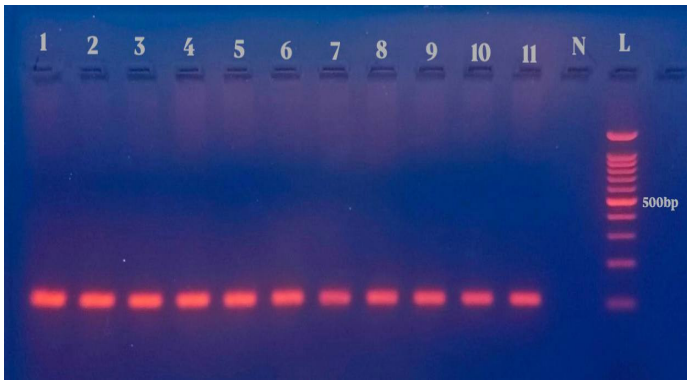

Figure 4: Results of *Serratia* specific PCR, The size of the PCR product is 107bp, L: marker DNA ladder 100bp, N: negative control. Lines (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11) represent samples.

16S rRNA gene PCR amplification

The 16S rRNA gene was amplified in all the isolates, and the PCR bands showed 1500 bp as shown in Figure 5.

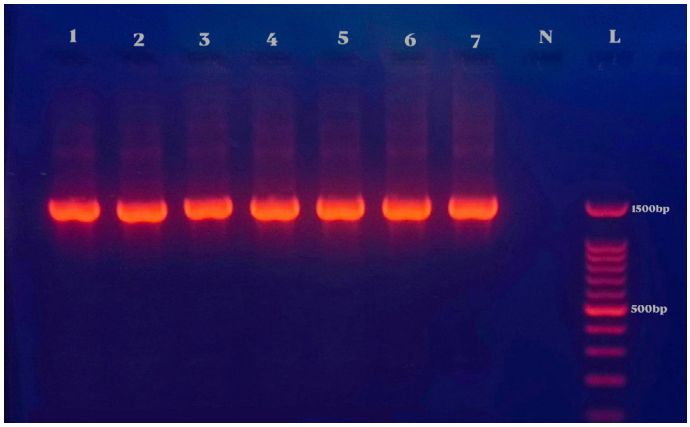

Figure 5: Results of *Serratia 16s RNA* gene, The size of the PCR product is 1500bp, L: marker DNA ladder 100bp, N: negative control. Lines (1, 2, 3, 4, 5, 6, 7) represent samples.

bsmB gene PCR

All isolates showed positive results at 200bp using a 100bp DNA ladder marker as shown in Figure 6 and 7.

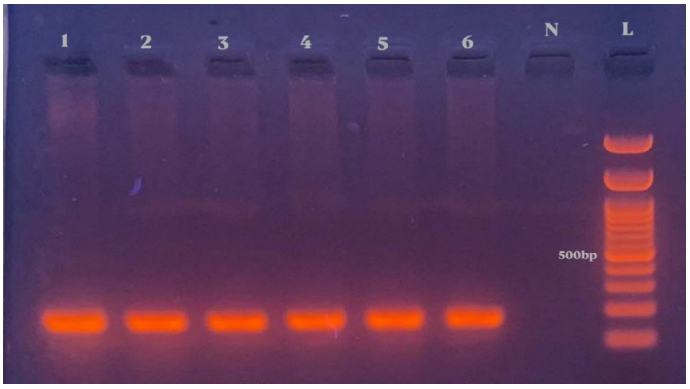

Figure 6: Results of *bsmB* gene, the size of the PCR product is 200bp, L: marker DNA ladder 100bp, N: negative control. Lines (1, 2, 3, 4, 5, 6) represent samples.

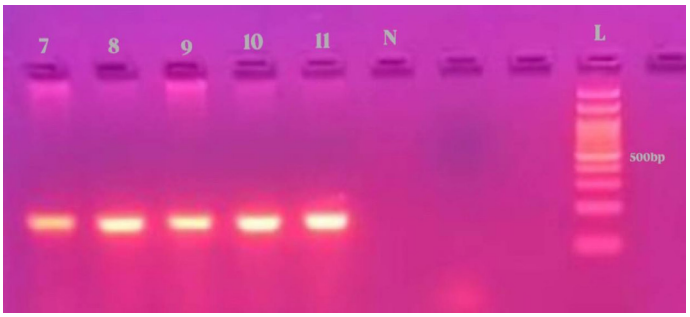

Figure 7: Results of *bsmB* gene, the size of the PCR product is 200bp, L: marker DNA ladder 100bp, N: negative control. Lines (7, 8, 9, 10, 11) represent samples.

Screening of resistance genes encoding extended-spectrum beta-lactamase

Results of resistant genes encoding extended-spectrum beta-lactamase indicate that all *Serratia marcescens* isolates carried *blaTEM* (10/11, 99.99%), *blaSHV* (2/11, 18.18%) and *blaCTX-M1* (3/11, 27.27%), as shown in Figures 8, 9, 10. *blaOXA-1, and blaOXA-48* genes were not detected in any of the isolates Table 6.

Table 6: Results of resistance genes detection.

Samples	*blaTEM*	*blaSHV*	*blaCTX-M1*	*blaOXA-1*	*blaOXA-48*
1	+	-	-	-	-
2	+	-	-	-	-
3	+	-	-	-	-
4	+	-	-	-	-
5	-	-	-	-	-
6	+	+	+	-	-
7	+	-	-	-	-
8	+	+	+	-	-
9	+	-	+	-	-
10	+	-	-	-	-
11	+	-	-	-	-

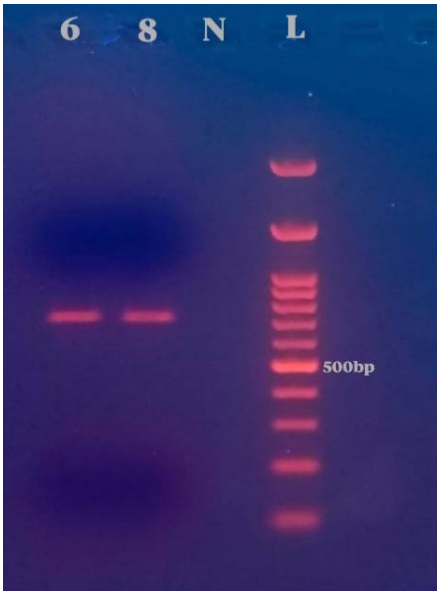

Figure 8: Results of *blaSHV* gene, the size of the PCR product is 712bp, L: marker DNA ladder 100bp, N: negative control. Lines (6, 8) represent samples.

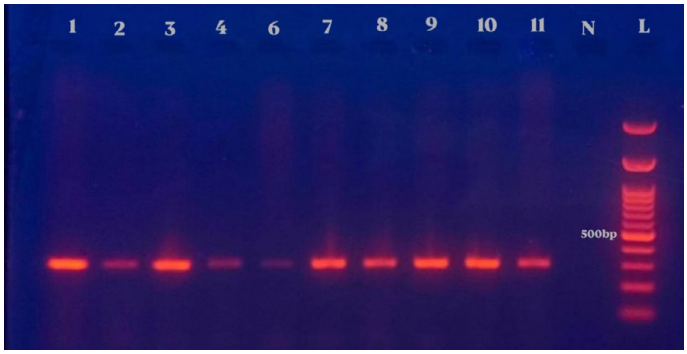

Figure 9: Results of *blaTEM* gene, the size of the PCR product is 296bp, L: marker DNA ladder 100bp, N: negative control. Lines (1, 2, 3, 4, 6, 7, 8, 9, 10, 11) represent samples.

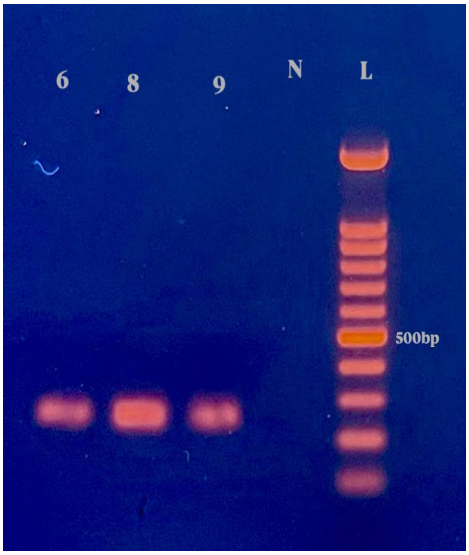

Figure 10: Results of *blaCTX-M1* gene, the size of the PCR product is 216bp, L: marker DNA ladder 100bp, N: negative control. Lines (6, 8, 9) represent samples.

Sequencing and the evolutionary analysis by maximum likelihood method

The sequence and blast analysis showed that all the sequenced isolates were *Serratia marcescens*. The sequences were deposited in the NCBI gene bank with the following accession numbers: PP856650, PP856651, PP856652, PP856653, PP856654, PP856655, and PP856656 as shown in Figure 11.

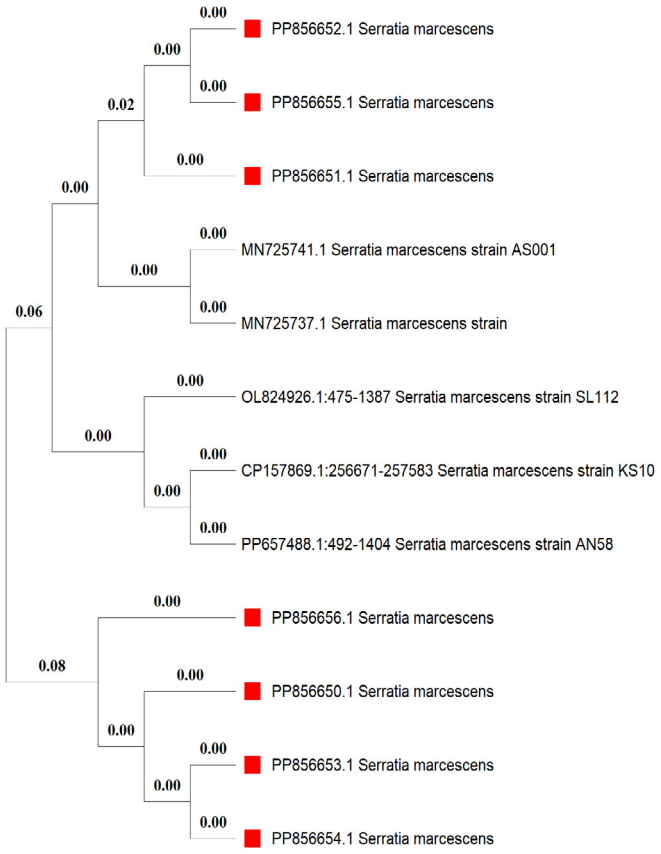

Figure 11: Phylogenetic analysis using the maximum likelihood approach of the local isolates and the closely related isolates from the NCBI database. The red square represents the local isolates.

Serratia marcescens is an important opportunistic pathogen that poses a risk to public health. The bacterial isolation results of 200 milk samples showed that 11 samples were identified as *Serratia marcescens*, accounting for 5.5% of the milk samples. This finding is higher than (Abdullah *et al*., 2017) who found that 6 out of 150 (4%) cows were affected by *Serratia marcescens* mastitis and a previous study reported in Iraq. Also, higher than Di Guardo *et al*. (1997), who revealed that 4(3%) *Serratia marcescens* isolates out of 120 reported in China. The isolates were also identified using the polymerase chain reaction of the 16S rRNA gene, *Serratia specific primers*, and sequencing.

The sample culture findings revealed distinct morphological attributes of bacteria on various media following a 24h incubation period at 30°C or 37°C. The colonies observed

on MacConkey agar exhibited lactose fermentation and appeared as red colonies. These findings are consistent with the results reported by Abdou (2003). When using Chrome agar (Himedia/India), the isolates showed colonies of different colors. Some colonies were pink with a dark center, while others were blue to purple. After 24 h of incubation at 28°C on nutrient agar, colonies of *Serratia marcescens* turned red. This red pigment production by *Serratia marcescens* allows for easy differentiation from other *Enterobacteriaceae*. These findings are consistent with the research conducted by Samrot *et al.* (2011). The isolated bacteria appeared as gram-negative rods under the light microscope.

Due to the global problem of antibiotic resistance, the effectiveness of antibacterial drugs has been diminished. The excessive and widespread use of these antimicrobials on dairy farms may be linked to increased resistance levels (Swinkels *et al.*, 2015). The commonly used aminoglycosides and penicillins groups antimicrobials in treatment were chosen for this study. *Serratia marcescens* has shown high resistance levels to antibiotics including ampicillin (11/11, 100%), amoxicillin (11/11, 100%), penicillin G (11/11, 100%), oxacillin (11/11, 100%), and ceftriaxone (10/11, 90.90%), tobramycin (5/11, 45.45%), amikacin (11/11, 100%) and neomycin (11/11, 100%). Studies by Ahmed and Shimamoto (2011), Hawkey and Choy (2015), and Yang *et al.* (2018) have consistently shown that both *Serratia marcescens* and other *Enterobacteriaceae* isolates commonly display resistance to these antimicrobials. However, the bacteria showed susceptibility to gentamycin (5/11, 45.45%), imipenem (11/11, 100%), and cefotaxime (8/11, 72.72%) as reported by Stock *et al.* (2003) and Abdullah *et al.* (2017).

The phenotypic resistance is mainly granted by the genes that encode that specific type of resistance. These can be passed to other bacteria and become a risk factor to public health through the food chain. *Serratia marcescens* harbors *blaTEM* and *blaSHV* genes, which encode conventional class A beta-lactamases located on plasmids. These genes hydrolyze penicillins and cephalosporins that belong to the first generation (Bush, 2010).

Our study observed the *blaTEM* gene in 10 isolates and the *blaSHV* gene in two isolates. The *blaCTX-M* gene is frequently present in several bacterial species, especially in members of the *Enterobacteriaceae* family. It is important to mention that this gene is transmitted through plasmids, making it highly mobile and capable of spreading among many bacteria (Sun *et al.*, 2017). This study observed the blaCTX-M1 in 3 isolates. According to (Ferreira *et al.*, 2020), *Serratia marcescens* bacteria that carry the genes *blaOXA-1* and *blaOXA-48* have been found in number of investigations conducted in Brazil and other countries. These strains may carry these genes alone or in combination with extended-spectrum beta-lactamases (ESBL) genes such as *blaTEM*, *blaSHV*, and *blaCTX-M*. While in this study *blaOXA-1*, *blaOXA-48* were not detected. To invade the host and elude its defenses and cause diseases, pathogenic bacteria normally express several virulence factors (Leitão, 2020). In this study, all *Serratia marcescens* isolates carried *bsmB* gene. The *bsmB* gene plays an important role in virulence factor production such as protease, serralysin, lipase, and S-layer protein synthesis (Liang *et al.*, 2023). Our research has revealed the presence of *Serratia marcescens* in the local environment and has mapped out the antibiotic resistance patterns in the local isolates. Furthermore, we demonstrated that *Serratia marcescens* poses a potential risk to human populations with regard to of public health.

CONCLUSIONS AND RECOMMENDATIONS

Serratia marcescens are opportunistic pathogen, that poses a risk to public health and also can lead to food quality issues. *Serratia marcescens* can harbor several types of antibiotic resistance including resistance to beta-lactam antibiotics. This study identified the presence of *Serratia marcescens* in milk samples collected from local markets and dairy farms in Basrah province. The isolation percentage is as following, 3(27.27%) in zubair, 3(27.27%) in Hartha, 1(9.09%) in college of agriculture/ university of Basrah, 3(27.27%) in Seeba and 1(9.09%) in safwan. Molecular detection was performed using PCR and sequencing, which confirmed the presence of *Serratia marcescens*. The study also involved determining the antibiotic susceptibility of the isolates, which showed a high percentage of resistance to most antibiotics. Additionally, the study identified antibiotic resistance genes in the isolates; 90.90% of the isolates had the *blaTEM* gene, 18.18% had the *blaSHV* gene and 27.27% had the *blaCTX-M1*. The study also involved the detection of the virulence gene (*bsmB* gene) and revealed its presence in all isolates. This study is the first in Basrah province and first that involved antibiotic resestance and virulence gene, which require further investigation and also should increase the focus on public health effects of *Serratia marcescens* in Basra province.

ACKNOWLEDGEMENT

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NOVELTY STATEMENT

This is the first study in Basra Governorate, south of Iraq,

as we did not find a previous study that addressed the topic of our current study, as the study addressed to investigate *Serratia marcescens* in raw, pasteurized, and mastitis milk additionally, to identify how resistant to antibiotics, whether the isolates contain beta-lactam resistance genes, and detect the bsmB gene for protease and lipase production.

AUTHOR'S CONTRIBUTION

This study was conducted with the participation of both researchers in the work. Both researchers read and approved the final manuscript .

Conflict of interest

The authors have declared no conflict of interest.

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SUPPLEMENTARY MATERIAL

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CCTTGTACACACCGCCCGTCACACCATGG-
GAGTGGGTTGCAAAAGAAGT

>CP157869.1:256671-257583 *Serratia marcescens* strain KS10 chromosome, complete genome
TCCGTGCCAGCAGCCGCGGTAATACGGAG-
GGTGCAAGCGTTAATCGGAATTACTGGGCG-
TAAAGCGCACG
CAGGCGGTTTGTTAAGTCAGATGT-
GAAATCCCCGGGCTCAACCTGGGAACTG-
CATTTGAAACTGGCAAGC
TAGAGTCTCGTAGAGGGGGGTAGAATTC-
CAGGTGTAGCGGTGAAATGCGTAGAGATCT-
GGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACGAAGACT-
GACGCTCAGGTGCGAAAGCGTGGGGAG-
CAAACAGGATTAG
ATACCCTGGTAGTCCACGCTGTAAACGAT-
GTCGATTTGGAGGTTGTGCCCTTGAGGCGT-
GGCTTCCGGAG
CTAACGCGTTAAATCGACCGCCTGGGGAG-
TACGGCCGCAAGGTTAAAACTCAAATGAATT-
GACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGT-
TTAATTCGATGCAACGCGAAGAACCT-
TACCTACTCTTGACATCCAG
AGAACTTTCCAGAGATGGATTGGTGCCT-
TCGGGAACTCTGAGACAGGTGCTGCAT-
GGCTGTCGTCAGCTC
GTGTTGTGAAATGTTGGGTTAAGTCCCG-
CAACGAGCGCAACCCTTATCCTTTGTTGC-
CAGCGGTTCGGCC
GGGAACTCAAAGGAGACTGCCAGTGA-
TAAACTGGAGGAAGGTGGGGATGACGTCAA-
GTCATCATGGCCCT
TACGAGTAGGGCTACACACGTGCTACAATGG-
CATATACAAAGAGAAGCGACCTCGCGAGAG-
CAAGCGGAC
CTCATAAAGTATGTCGTAGTCCGGATTG-
GAGTCTGCAACTCGACTCCATGAAGTCG-
GAATCGCTAGTAAT
CGTAGATCAGAATGCTACGGTGAATACGT-
TCCCGGGCCTTGTACACACCGCCCGTCACAC-
CATGGGAGTG
GGT

>PP657488.1:492-1404 *Serratia marcescens* strain AN58 16S ribosomal RNA gene, partial sequence
TCCGTGCCAGCAGCCGCGGTAATACGGAG-
GGTGCAAGCGTTAATCGGAATTACTGGGCG-
TAAAGCGCACG
CAGGCGGTTTGTTAAGTCAGATGT-
GAAATCCCCGGGCTCAACCTGGGAACTG-
CATTTGAAACTGGCAAGC
TAGAGTCTCGTAGAGGGGGGTAGAATTC-
CAGGTGTAGCGGTGAAATGCGTAGAGATCT-
GGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACGAAGACT-
GACGCTCAGGTGCGAAAGCGTGGGGAG-
CAAACAGGATTAG
ATACCCTGGTAGTCCACGCTGTAAACGAT-
GTCGATTTGGAGGTTGTGCCCTTGAGGCGT-
GGCTTCCGGAG
CTAACGCGTTAAATCGACCGCCTGGGGAG-
TACGGCCGCAAGGTTAAAACTCAAATGAATT-
GACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGT-
TTAATTCGATGCAACGCGAAGAACCT-
TACCTACTCTTGACATCCAG
AGAACTTTCCAGAGATGGATTGGTGCCT-
TCGGGAACTCTGAGACAGGTGCTGCAT-
GGCTGTCGTCAGCTC
GTGTTGTGAAATGTTGGGTTAAGTCCCG-

CAACGAGCGCAACCCTTATCCTTTGTTGC-
CAGCGGTTCGGCC
GGGAACTCAAAGGAGACTGCCAGTGA-
TAAACTGGAGGAAGGTGGGGATGACGTCAA-
GTCATCATGGCCCT
TACGAGTAGGGCTACACACGTGCTACAATGG-
CATATACAAAGAGAAGCGACCTCGCGAGAG-
CAAGCGGAC
CTCATAAAGTATGTCGTAGTCCGGATTG-
GAGTCTGCAACTCGACTCCATGAAGTCG-
GAATCGCTAGTAAT
CGTAGATCAGAATGCTACGGTGAATACGT-
TCCCGGGCCTTGTACACACCGCCCGTCACAC-
CATGGGAGTG
GGT

>OL824926.1:475-1387 *Serratia marcescens* strain SL112 16S ribosomal RNA gene, partial sequence
TCCGTGCCAGCAGCCGCGGTAATACGGAG-
GGTGCAAGCGTTAATCGGAATTACTGGGCG-
TAAAGCGCACG
CAGGCGGTTTGTTAAGTCAGATGT-
GAAATCCCCGGGCTCAACCTGGGAACTG-
CATTTGAAACTGGCAAGC
TAGAGTCTCGTAGAGGGGGGTAGAATTC-
CAGGTGTAGCGGTGAAATGCGTAGAGATCT-
GGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACGAAGACT-
GACGCTCAGGTGCGAAAGCGTGGGGAG-
CAAACAGGATTAG
ATACCCTGGTAGTCCACGCTGTAAACGAT-
GTCGATTTGGAGGTTGTGCCCTTGAGGCGT-
GGCTTCCGGAG
CTAACGCGTTAAATCGACCGCCTGGGGAG-
TACGGCCGCAAGGTTAAAACTCAAATGAATT-
GACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGT-
TTAATTCGATGCAACGCGAAGAACCT-
TACCTACTCTTGACATCCAG
AGAACTTTCCAGAGATGGATTGGTGCCT-
TCGGGAASTCTGAGACAGGTGCTGCAT-
GGCTGTCGTCAGCTC
GTGTTGTGAAATGTTGGGTTAAGTCCCG-
CAACGAGCGCAACCCTTATCCTTTGTTGC-
CAGCGGTTCGGCC
GGGAACTCAAAGGAGACTGCCAGTGA-
TAAACTGGAGGAAGGTGGGGATGACGTCAA-
GTCATCATGGCCCT
TACGAGTAGGGCTACACACGTGCTACAATGG-
CATATACAAAGAGAAGCGACCTCGCGAGAG-
CAAGCGGAC
CTCATAAAGTATGTCGTAGTCCGGATTG-
GAGTCTGCAACTCGACTCCATGAAGTCG-
GAATCGCTAGTAAT
CGTAGATCAGAATGCTACGGTGAATACGT-
TCCCGGGCCTTGTACACACCGCCCGTCACAC-
CATGGGAGTG
GGT