Phenotypic And Molecular Characterization of Extended- Spectrum B-Lactamase Producer Serratia Marcesence Isolated from Surgical Wound Infections

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Abstract
Introduction: Serratia marcesence is a one type of Extended-Spectrum Beta-Lactamases (ESBLs) are the major cause of resistance to beta-lactam antibiotics such as amoxicillin, cefixime, cefotaxime, ceftazidime, ceftriaxone, piperacillin tetracycline and trimethoprim-sulfa. CTX-M and SHV but not TEM genes were present in Serratia marcesence resist pathogenic form. Aim: To determine the Phenotypic and molecular characterization of Extended- spectrum β-lactamase producer Serratia marcesence isolated from surgical wound infections by detection three genes resist to different types of antibiotics. Methodology: The present study was carried out from June 2020 to June 2022 in the department of pharmacy, microbiology lab, Al-noor University College. Eighty-six 86 out of 158 wound swabs samples were extended- spectrum β-lactamase producer Serratia marcesence. The isolates were collected from patients suffering from contaminated wound surgery and studied at Al-Noor University collage laboratories by using morphological, culture, manual biochemical identification method using API 20E strip test and molecular identification by PCR-based technique. Results: All patients had signs of wound contamination like redness around wound, swelling of wound area, pus and fluid drainage from the incision area after surgery. ESBL genes were detected by PCR method among eighty-six (54.4%) swabs which contain pathogenic Serratia marcescens bacteria and phenotypically confirmed ESBL producers. The rest seventy-two (45.5%) phenotypically confirmed non-ESBL producers. The genes that interested in this study were, bla TEM (0%), followed by bla SHV (10.4%), and bla CTX-M (89.5%).The patterns of antimicrobial resistance for isolates under study showed susceptible to amoxicillin and meropenem while they resist to, Ceftazidime, Ceftriaxone, and Gentamycin Conclusion: In the present study, it was concluded that antimicrobial drugs such as Amoxicillin, Amikacin and meropenem should be highly recommended for the treatment of pathogenic S. marcescens resist strains as appropriate choice; and definitive identification of ESBL genes by molecular detection is the best and rapid diagnosing technique which is the key to control and management the surgical wound infection.
Keywords: Phenotypic – Genotypic - Extended spectrum β-lactamase ESBL - Serratia marcesence

1. Introduction

Serratia species, in particular Seratia marcescens, are one of gram-negative, motile, facultative anaerobic bacteria. S. marcescens and other members of the genus was first described in 1819, they are widespread in the environment, but are not a common component of the human fecal flora. These bacteria classified in the ethic group Klebsielle and the large family Enterobacteriaceae [4]. Some strains of S. marcescens are capable of producing a pigment which is called prodigiosin, it`s ranges from dark red color to pale pink depending on the period of the colonies which aid us for easer identification. Serratia are capable of thriving in diverse environments, like water, soil, and the digestive tracts of various animals [5, 6]. The organisms in this genus, particularly S. marcescens, were long thought to be nonpathogenic but over the last 50 years, Serratia marcescens has become an important cause of nosocomial infections. Much evidence was reported to explain the ability of Serratia marcescesns to cause infection like utilize a wide range of nutrients so can survive and grow under extreme conditions, like in disinfectants, antiseptics and distilled water, also can grow at 37℃, but it can grow in temperatures that range from 5-40 ℃. Serratia marcescens grow in pH levels that range from 5 to 9[1]. Serratia species appear to be common environmental organisms, and this helps to explain the large number of nosocomial infections due to these bacteria. Since many nosocomial infections are caused by multiply antibiotic-resistant strains of S. marcescens, this increases the danger to hospitalized patients, and hospital personnel should be vigilant in preventing nosocomial outbreaks due to this organism. Additionally, Serratia species may harbor multidrug resistance mechanisms that can complicate treatment decisions [7]. S. marcescens, and probably other species in the genus, carry several antibiotic resistance determinants and are also capable of acquiring resistance genes. S. marcescens is usually identified well in the clinical laboratory by 16S rRNA gene sequencing which enable better identification

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of some of the less common Serratia species [3]. Some of the S. marcescens strains isolated from patients were an ESBL producer. ESBL producers have most commonly been documented among Klebsiella pneumoniae and E. coli strains and less commonly among other members of the family Enterobacteriaceae, such as Serratia species [12]. Wilke et al in 2005 reported that β-lactams which inhibit the cell wall biosynthesis, remained the first-line defense against bacterial infections for over 20 years, before resistant bacteria appeared in clinical practice. Resistance to this class of drugs can be the result of antibiotic target site alteration, prevention of antibiotic access by altered permeability or forced efflux, or antibiotic degradation [13]. Naturally occurring chromosomally located β-lactamases are quite common in Gram-negative bacteria; likely evolved from penicillin-binding proteins, when produced in small quantity they do not significantly contribute to antibiotic resistance. It was the appearance of the first plasmid-mediated β-lactamase TEM-1. This phenomenon war reported in 1960s. Ever since, Medeiros in 1997 improve the fact that the introduction of new natural or synthetic drugs to replace old ones in an attempt to limit the insurgence of antibiotic resistant bacteria triggered a chain reaction providing bacteria with a constant selective pressure driving the expansion of different resistance mechanisms [14,15]. The most clinically significant extended spectrum β-lactamases (ESBL) variants. i.e., CTX-M-, TEM-, and SHV- type enzymes [16].

The clinical sign of Serratia wound infection characterized by pus with drainage, bad smell coming from the wound, fever, chills, redness and pain or sore to touch. The surgeris always noticed that a surgical wound infection can be develop at any time from 2-3 days after surgery until the wound has visibly healed (need 2-3 weeks to several months after operation) Doctors call these infections surgical site infections (SSIs) because they occur on the part of the body where the surgery took place. If you have surgery, the chances of developing an SSI are about 1% to 3% [9]. Many papers improved that the most important causative agent for bacteremia among patients is S. marcescens (the antibiotic resist strains) to ampicillin, cefazolin, ephalothin and cefuroxime but sensitive to carbcnicillin, trimethoprim/sulfamethoxazole, ceftizoxime, ceftriaxone, ceftazidime, cefotetan, aztreonam, ticarcillin/clavulanate, and ciprofloxacin [10].

In this study we focused on surgical wound samples to isolates pathogenic Serratia marcescens strains collected from different infection sites of hospitalized patients in Al-Salam hospital of Mousl in Iraq.

2. Materials and Methods

2.1 Sample collection and biochemical tests

One hundred fifty-eight swabs were collected from hospitalized patients (male, female, and adults) from Al-Salam hospital in Mousl city, Iraq, at the period in 2020 to 2022. A total of 86 strains of *S. marcescens* were isolated from patient wonds after surgery. Clinical isolates were evaluated using conventional laboratory techniques for culture, isolation, purification and manual biochemical identification method using API 20E strip test to get accurate identifications based on extensive databases and are standardized, easy-to- use system (BIOMERIEUX)[8].

2.2 Molecular identification

Traditional PCR technique were used for detect the presence of *S. marcescens* from pus wound swabs. Primers for the detection of *S. marcescens* 16S rRNA were designed and specificity tested.

2.3 Antimicrobial agents

Antibiotic susceptibility profiles were done by using a standard disk diffusion method recommended by the NCCLS [11].

2.4 Molecular identification for resist genes

Extended-spectrum β-lactamases (ESβLs) including TEM, SHV, and CTX-M are the predominant types that confer resistance to beta-lactam group of antibiotics.

Genomic DNA has been isolated using a Genomic DNA purification kit (Geneaid, Thailand) according to the manufacturer's protocol. Molecular detection of ESβLs-encoding genes (*bla* $_{CTX-M}$, *bla* $_{SHV}$, *bla* $_{TEM}$) was performed using PCR based technique. The primers were used to amplify the ESβLs-encoding genes listed in Table 1.

PCR was carried out in in 50 µl PCR volumes containing 20 ng of template DNA, 0.5 mM of dNTPs, 1.25µM of each primer roe 16S rRNA, and for (SHV, TEM, and CTX-M antibiotics resist genes) and 3 µl of Taq polymerase in 1× PCR buffer. Amplification of DNA was performed in master cycle, and band for *Serratia marcesence* at 870 bp was detected with cycling parameters and primers used as described in Table 2[17, 18]. Then in 1% agarose gel containing 25µg of ethidium bromide in tris-EDTA buffer PCR products were analysed. DNA ladder 100bp was included in each run.

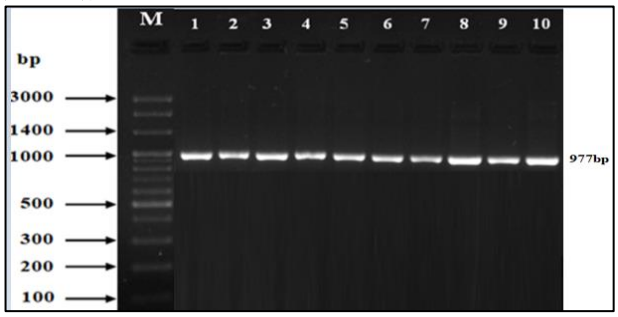
3. Results

During this study, patients with signs of wound contamination after surgery had been grouped in (A, B, and C). [Table 2]. Since no data have been documented regarding the prevalence of pathogenic ESBL Serratia marcescens in our region, this study was designed to isolate, determine and identify phenotypes and genotypes of this bacterium in our geographical region, in Mousl.

The antimicrobial susceptibilities of S. marcescens clinical isolates are characterized in Table 3. The majority of isolates were susceptible to Amikacin (100%), meropenem and gentamycin (99%), levofloxacin (95%), Aztreonam (51%). The rest of antibiotics like Amoxicillin, cefixime, cefotaxime, ceftazidime, ceftriaxone, piperacillin, tetracycline

and trimethoprim-sulfa all shows zone of inhibition on Mueller Hinton Agar media (MHA).

In this study, database showed 86 out of 158 bacterial isolates were extended- spectrum β-lactamase producer Serratia marcesence which confirmed phenotypically. ESBL genes were detected by PCR method among these eighty six (54.4%) pathogenic Serratia marcescens bacteria. The rest seventy two (45.5%) phenotypically confirmed non-ESBL producers. Out of the three beta-lactamase (bla) gene studied, bla TEM (0%), followed by bla SHV (10.4%) , and bla CTX-M (89.5%), Table 4.



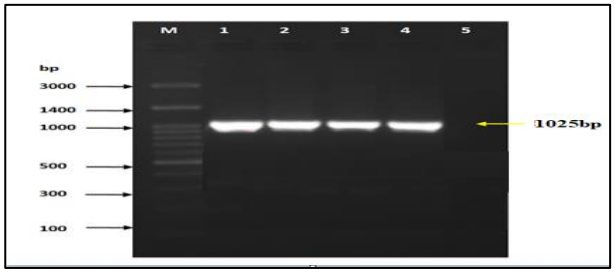


Figure 2: Gel picture of amplified products CTX-M, SHV genes of Serratia marcesence detection isolates band 1025 and 977 bps respectively M: DNA Ladder of 100 bps.

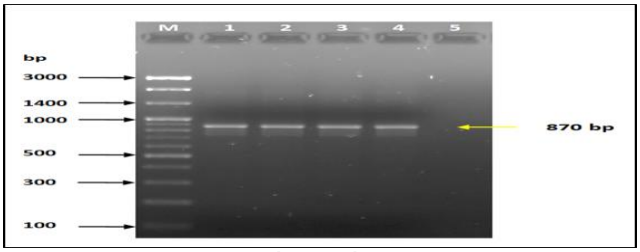


Figure 1: Gel picture of amplified products 16S rDNA Serratia marcesence detection isolates band 870 bps in 1, 2, 3, and 4. M: DNA Ladder of 100 bps.

Table 1: Cycling parameters and primers used in master cycle

Genes	Primers usedSequences(5'-------3')	Polymerase Chain Reaction Conditions	Amplicon size(bp)	References
16 S rRNA	16s rDNA-F 5'TAGGGAAGATAATGACGG 3'16s rDNA-R 5'CCTCTATCCTCTTTCCAACC3'	1min at 94℃, 30s at 55℃,1 min at 72℃ and number of denaturation cycle repeated to 30 cycles.	870	Sambrook and Russel (2007)
SHV	F- 5-GCCGGGTTATTCTTATTTGTCGC-3R-5-TCTTTCCGATGCCGCCGCCAGTCA-3	5 min at 94℃ and 32 cycles of amplification consisting of 30s at 94℃, 30s at 54℃, and 1 min at 72℃, with 10 min at 72℃ for final extension	977	Mulvey et al.,2011
TEM	F-5-CGCTCATGAGACAATAACCCTG-3R-5-GGATCTTCACCTAGATCCTT-3	5 min at 94℃ and 32 cycles of amplification consisting of 30s at 95℃, 1 min at 54℃, and 2 min at 72℃, with 5 min at 72℃ for final extension	1000	Mulvey et al.,2011
CTX-M	F-5-TCGTCTCTTCCAGAATAAGG-3R-5-AAGGAGAACCAGGAACCACG-3	5 min at 94℃ and 32 cycles of amplification consisting of 30s at 95℃, 1min at 54℃, and 2 min at 72℃, with 5 min at 72℃ for final extension	1025	Schneider et al.,2009

Table 2: Information on the antibiotics resist genes of *Serratia marcescens* isolated from 86 wound swabs

Patients	Age(years) (gender)	No. of isolates	specimens	PCR for			ESBL
				bla CTX-M	blaTEM	blaSHV	
Group A	Rang (25-50) F	39	Surgical wound	+	-	+	+
Group B	Range (32- 55) M	47	Surgical wound	+	-	+	+
Group C	Less than 15 years old	72	Surgical wound	+	-	+	-
M, male; F, female; PCR, polymerase chain reaction; CTX-M, TEM, and SHV, resist genes; bla , beta-lactam genes.							

Table 4: Distribution of ESBLs encoding genes among isolatesGene percentage	
bla SHVbla TEMbla CTX-M	9 (10.4%)0 (0%)77(89.5%)

Table 3: Antibiograms pattern of *Serratia marseciens* isolates towards antibiotics

Antibiotics	RResistance %	IIntermediate %	SSensitive %
Cephalosporins group			
Ceftazidime	72(83.7%)	4(4.6%)	10(11.6%)
Ceftriaxone	70(81.3%)	2(2.3%)	14(16.2%)
Pipracillin	2(4%)	42(48.8)	42(48.8%)
Meropenem	0(0%)	1(1.1%)	85(98.8%)
Amoxicillin	0(0%)	0(0%)	86(100%)
Aminoclycosides group			
Amikacin	3(3.4%)	0(0%)	83(96.5%)
Aztreonam	21(24.4%)	22(25%)	43(50%)
Gentamycine	24(48%)	5(5.8%)	57(66.2%)
Fluoroquinolones group			
Levofloxacin	4(4.6%)	20(23.2%)	62(72%)
Trimethoprim/sulfamethoxazole	9(10.4%)	0(0%)	77(89.5%)

4. Discussion

Serratia marcesnes belongs to family, Enterobacteriaceae [4] and is known source of Hospital acquired infection in world and also in Mousl/Iraq. The human get the infection by this type of bacteria in form of exogenous but not in form of endogenous sources. Serratia marcescens is a source of hospital acquired infection (HAI), common environmental organisms [7] infect human and colonise the wounds. Such colonized patients remain at risk because of new resist strains of Serratia marcescens that had been appeared. We here present the first study that detects Serratia marcescens resist strains from patient's wounds after surgeries in Mousl. Yadav and chauhan in 2016 improved that ESBL- producing bacteria including S. marcescens are a significant issue in management of specific bacterial diseases in hospitals if there isn't a miss use of antibiotics and there is regular use of antibiotics [19]. So, in this work we focused on this type of bacteria that produce β-lactamase enzymes that work by hydrolysing β-lactum ring of β-lactum antibiotics leading to inactivate them [20], have antimicrobial resist mechanism and cause HAI.

Findings of disk diffusion method revealed that most isolates of Serratia marcescens were highly resist to β-lactum antibiotics, especially Ceftazidime and Ceftriaxone. This type of resistance gives an indicator that these isolates can be considered as ESBL producers. Early detection of such types of these isolates in laboratories has significant importance for the proper treatment patients' wounds; minimize infection and control, especially when the facilities for molecular characterization are not available. In my city, many clinical laboratories follow CLSI guidelines and its implementations to control the spread of antimicrobials resistant isolates especially ESBL producers but it seems this protocol were not applied in hospitals for many years.

Finding of molecular PCR method revealed that there are two significant groups of Serratia marcescens, ESBL group and non ESBL group, and we found that the CTX-M with 89.5% was dominant among ESBL group resist isolate compared with SHV 10.4%, while TEM gene showed 0%. Many other studies on Serratia marcescens clinical isolates showed mixed results [21, 22]. The wound isolates in our setting, TEM which is not found among other two resist types show different results between our study and those of other authors from other countries indicated that the prevalence and type of ESBL genes may vary from one geographical region to another. Some other types of Serratia marcescens pathogenic strains lacked TEM like our study. And were highly sensitive to Meropenem 98.8%, Amoxicillin 100% and Amikacin 96.5% which can be highly dependent used in health care setting in our community. The most right explanation of our results, the expression of β-lactamase genes depends upon the environmental conditions such as presence of antibiotics, and gene presence does not necessarily indicate its expression, i.e there are some "hidden ESBL"[23,24]. Strains of Serratia marcescens that lacked TEM, SHV, and CTX-M genes may have actually been negative or might have carried "hidden gene" for ESBL production.

5. Conclusion

The results of this study highlight the value of using Meropenem, Amoxicillin and Amikacin as first choice of drug to treat Serratia pathogenic wound infection in hospital setting. Metallic surgical tools and other hospital surrounding equipment can be a good source of infection if not used aseptically "exogenous form". An appropriate antimicrobial therapy can only be started timely with the early detection of ESBLs and be useful depending on phenotypic and genotypic laboratory methods. Phenotypic methods for diagnosis of pathogenic strains are widely dependable in our clinical laboratories. The early diagnosis of ESBL producing strains will also help to establish and implement a strict infection control policy to stop the spread of ESBL *S.marcescens*, the important pathogenic bacteria that cause HAI hospital acquired infection among patients with wound infections.

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