DETECTION OF SOME VIRULENCE FACTORS IN *Serratia marcesens*[+]

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Abstract

Some virulence factors were studied for the two strains of *Serratia marcesens.* Antibiogram profile shows that both strains are resistant to Ampicillin and sensitive to Cefotaxime and Gentamicin, where as only one is resistant to Cephalexin.

It was found that both strains are able to produce colonization factor antigen type 1 (CFA/1) in the presence of mannose sugar, but none of them shows the ability to produce (CFA/3).

Colony hybridization technique reveals that none of the strains has showed hybridized signals when heat-stable enterotoxin or heat-labile enterotoxin gene are used separately as a probes.

Moreover, none of the strains is able to produce uronic acid extracellulary or hemmolysin on both rabbit blood or human blood agar at 37°C.

المستخلص

درست بعض عوامل الضراوة المنتجة من بكتريا السرتيا(*S. marcesense*) وقد أظهرت نتائج المقاومة للمضادات الحياتية ان السلالتين المستخدمتين في هذه الدراسة كانتا مقاومتين للمضاد الحيوي الامبسلين وحساستين لكل من المضاد الحيوي السيفاتاكسيم والجنتاميسين في حين كانت هناك سلالة واحدة مقاومة للمضاد الحيوي السيفالكسين.

وقد وجد ان السلالتين قادرتان على إنتاج مستضد عامل الاستعمار الأول (CFA/1) بوجود سكر المانوز في حين لم تملك أي منهما القدرة على إنتاج مستضد عامل الاستعمار الثالث(CFA/3). استخدمت تقنية تهجين المستعمرات في التحري عن وجود جينات الذيفانات المستقرة حراريا(ST) وغير المستقرة حراريا(LT) وقد أظهرت النتائج أن السلالتين لم تظهرا أي وصمة تهجين موجبة عند استخدام مجسات مماثلة لتلك الجينات.

علاوة على ذلك فان السلالتين لم تكن لهما القابلية على إنتاج حامض اليورونيك إلى خارج الخلية كما إنهما لم تمتلكا القابلية على إنتاج الهيمولايسين في أكار الدم الحاوي دم الأرنب أو دم الإنسان.

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Introduction

Serratia marcesens was formally considered nonpathogenic bacteria but in recent years, it has been increasingly recognized as a serious or deadly opportunistic pathogen especially in hospitals, and with prolonged use of antibiotics, Serratia has been found in urinary and respiratory tracts infections, meningitis, septicemia, endocarditis, and a variety of eye infections [1].

Among its known virulence factors are its ability to produce colonization factor antigens such as CFA/1, CFA/2, CFA/3 which permit specific adhesion to target cells of the host [2].

Molecular studies show that these factors are mostly carried on plasmids or transposones [3]. It was found that CFA/1 was carried on transferable plasmids in some opportunistics bacteria such as Enterobacter, Klebsiella, *Serratia,* and *Proteus* and it was observed that these bacteria in addition to their ability to produce this factor, have the ability to produce enterotoxins especially heat-labile toxin [4].

Little is known about the ability of *Serratia* to produce uronic acid which gives the bacteria mucoid phenotype. This factor is produced by some encapsulated bacteria such as *, Klebsiella* and *Enterobacter* [5].

This paper deals with studying some virulence associated factors in the local strains from the bacteria *Serratia marcecens.*

Materials and Methods

1- Strains wo of *Serratia marcecens* were used in this study, one of which was isolated from patient suffering from pneumonia [3], the other was supplied from the central health laboratory in Babyl.

2- Antibiotic resistance was studied using Kirby and Baur method. The discs were supplied by BBL, Becton Dickenson Company.

The potency of antibiotics are as followed in micrograms:- Ampicillin 10, Cephalexin 30, Cefotaxime 30, and Gentamicin 10

3- Colonization factor antigen type 1 was detected as described by the method of Heuzenroeder, et.al., [6], in the presence of mannose and a suspension of red blood cells of human group A. The strains were subcultured in the CFA agar prior to the procedure. Salt aggregation method was done to detect on CFA/3 as described by [7].

4- Colony hybridization was done on nylon membrane, heat-labile and heat-stable gene probes which were isolated from *E. coli* JMP877 and *E. coli* JMP876 respectively were used also and labeled by using Biotin-7-dATP [8]. *Serratia* strains and enterotoxigenic *E. coli* and toxin free *E. coli* HB101 were spotted on the membrane.

5- Uronic acid estimation was done by using the colorometric method [9], the standard curve of galacturonic acid was made using concentrations from 5-100 micrograms and the absorbence was read at 530nm.

6- Hemolysin detection was done on both rabbit blood agar and human blood agar. The media were incubated at 37°C for 24 hour.

Results and Discussion

Two strains of *Serratia marcecens* are used at this study to detect some virulence factors.

Effect of some antibiotics on these strains are studied (table 1). It was found that the strains are resistant to Ampicillin and sensitive to Cefotaxime and Gentamicin, where as only one is resistant to Cephalexin.

Table (1): Effect of antibiotics on *Serratia* strains

Strains No.	Antibiotics			
	Ap	**Gm**	**Cox**	**Kz**
1	+	-	-	-
2	+	-	-	-

Ap: Ampicillin, Gm: Gentamicin, Cox: Cefotaxime, Kz: Cephalexin
(+) resistante; (-) sensitive

The resistance of *Serratia* to Ampicillin or Cephalexin is of clinical importance since these bacteria spread within the hospital or among the immuno_difficient patients. It was thought that the resistance of *Serratia* strains to beta-lactam antibiotics was as a result of beta-lactamase inducibility which could destroy these antibiotics [10]. Another opinion states that the resistance to the antibiotics was also due to the repeated use of these antibiotic in the treatment of various infection in the body [3].

The two strains were tested for their ability to produce colonization factor antigen type 1 and 3 (CFA/1, CFA/3). The results showe that the strains reveal the ability to produce CFA/1 but not CFA/3. These factors are considered a primary factor which causes adhesion of bacteria to the target cell of the host, and their presence indicates that the bacteria contain cell surface fimbirial antigens, thus the presence of CFA/1 reflects the ability of *Serratia* strains to adhere to tissue of the host [2].

Colony hybridization technique was used to detect strains carriage of DNA sequences similar to DNA sequences of *E. coli* heat stable (ST) and heat labile (LT) enterotoxines encoding genes.

It was found that none of the strains reveals hybridized signals when both ST and LT genes probes are used separately.

Uronic acid production is also detected extracellulary in the culture media after removing the bacteria by filtration. The results show that none of the two strains has the ability to produce this acidic sugar, in which its production is associated with the production of mucoid phenotype. The mucoid phenotype is usually considered to be the result of capsular polysaccharide, If the bacteria lost this polysaccharide it would be less virulence [5, 11].

Moreover, none of the strains has the ability to produce hemolysin on both rabbit blood or human blood agar. As it is known that most enteric bacteria have no ability to produce hemolysin. This protein has not a direct role in their pathogenicity [12].

It can be concluded that *Serratia marcecens* has the ability to associate in some diseases to not because of the effect of virulence factors but because immuno-difficiency of the patient.

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