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Transfer of Antibiotic Resistance from Clinical Isolates of Serratia spp. to E.coli

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ABSTRACT

Fifty clinical samples (20 Urinary Tract Infections, 20 wounds and 10 septicemia) were collected from Al-Salam general hospital in Mosul/ Iraq, and examined for Serratia spp. Diagnosis was done by conventional biochemical tests and confirmed by the API20E system (BioMeurix). Seven S. odorifera1 and three S. marcescens were identified. All ten strains were resistant to Ampicillin, Cefalexin, Chloramphenicol, Gentamycin and Tertracycline. Plasmid DNA was purified from five Serratia isolates and used to transform the Ampicillin - sensitive E. coli JM83 to Ampicillin resistant. Transformants were obtained at a frequency of $(0.2\text{-}0.5)x10^{-4}$. Three S. marcescens and two S. odorifera1 were used to donate the antibiotic resistance to E. coli JM83 and E. coli JMP294. The frequency of conjugants ranged between $(7\text{-}30)x\ 10^{-6}$. This indicates that resistance can transfer from pathogenic to non-pathogenic strains. This could constitute a serious heath problem to the public health.

نقل المقاومة للمضادات الحيوية من عزلات سريرية من انواع السراشيا الى الاشريكيا القولونية

الملخص

لقد تم جمع خمسين عينة (20 من حالات التهاب المجاري البولية ، 20 من الجروح و 10 من حالات التسمم الدموي) من مستشفى السلام العام في مدينة موصل / العراق . وقد جرى تشخيص هذه العينات بأستخدام الاختبارات الكيموحيوية وجرى تأكيدها بأستخدام نظام API20E . تم تشخيص (10) عزلات من Serratia (7 عزلات S.odorifera1 و 3 عزلات S. marcescens) ، وقد كانت جميع العزلات

العشر مقاومة للمضادات الحيوية Ampicillin ، Cefalexin ، Chloramphenicol ، Gentamycin و Tetracycline .

وقد امكن عزل وتنقية الدنا البلازميدي من خمس من هذه العزلات واستخدم لتحويل سلالات حساسة من E.coli الى مقاومة للامبسلين . وكان مدى التحول هو $(0.2\text{-}0.5)\times10^{-4}$. كما جرى استخدام ثلاث عزلات من S.marcescens وعزلتين من S.odorifera1 للاقتران ونقل المقاومة للمضادات الحيوية الى السلالتين E.coli JM83 و E.coli JMP294 وكان تردد المقترنات بين $10^{-6} \times$ (7-30) وهذا يشير الى امكانية انتقال المقاومة للمضادات الحيوية من سلالات ممرضة ومقاومة الى سلالات غير ممرضة مما يشكل خطورة على صحة المجتمع .

Keywords: Serratia marcescens, S. odorifera 1, Antibiotic resistance, Transformation, Conjugation.

INTRODUCTION

Serratia marcescens is one of eight species that belong to the genus Serratia of the family Enterobacteriaceae. Serratia liquefaciens, S. marinorubra and S. odorifera are other three pathogenic species of the genus (Farmer et al., 1995). S. marcescens is an opportunistic pathogen with a clinical significance that has been appreciated only in the last three decades (Okuda et al., 1984). S. marcescens is a rare cause of community-acquired infections. It has emerged as an important nosocomial pathogen and a frequent source of outbreaks of hospital infection (Goullet and Picard 1997). The infections include urinary tract, bloodstream, lower respiratory tract and wounds (Osornio et al., 1986;Coderch et al., 2004). The morbidity and mortality of these infections might be the result of the patients having other serious diseases in addition to the multiple antibiotic resistance of the infecting strains. Some of the strains causing nosocomial infections have been found to produce plasmid-mediated β-lactamases (Jacoby and Medeiros 1991).

Aminoglycoside antibiotics are widely used in clinical settings, especially for treatment of life–threatening infections caused by gram-negative bacteria. S. marecescens exhibits an unusually high degree of resistance to aminoglycosides. This resistance was found to be mediated by a large plasmid (Doi et al., 2004).

The present study was aimed at checking the ability of Serratia spp. strains to transfer their antibiotic resistance through transformation and conjugation to standard E. coli strains that are sensitive to these antibiotics. Such transfer could support the plasmid nature of this resistance.

MATERIALS AND METHODS

Samples collection and bacterial identification

Twenty urinary tract-infection samples, twenty wound infections and ten septicaemia samples were collected from patients (males and females) of Al-Salam hospital, Mosul city, Iraq. Bacterial strains were identified by using morphological, cultural and biochemical characteristics. Results were confirmed by using API20E system (Baron and Finegold 1990 ; Collee et al.,1996).

Reference bacterial strains

Two reference strains, E. coli K12 JM83 and E. coli JMP294 were used for genetic experiments conducted in this work. The genotypes and origins of the two reference strains are given in Table 1.

Table 1: Genotypes and origin of reference strains.

Strains	Genotype	Source
E. coli K12 JM83	ara, Δ (lac^- pro A,B),	George M.Weinstock, Department of Biochemistry
	rpsl,Ø80,lacz ΔM15 K^{r+}, K^{m+}	and Molecular Biology, University of Texas, U.S.A
E. coli JMP294	lac^+, p^+(3.45kb)	Medical Central laboratory, Baghdad

Δ: Deletion ; Δ lac: Deletion of the gene responsible of lactose metabolism.
Δ pro: Deletion of the gene for the proline synthesis ; K^{m+}: DNA methylation positive.
K^{r+} : Ability to restrict foreign DNA; Ø80: Having the Ø80 specifying integration site.
Lac^+ : Ability to utilize lactose. ; p^+ : Harboring a 3.45 kb plasmid

Sensitivity test of the isolates

The isolates were tested for their susceptibility to the antibiotics Ampicillin (AP 50 µg/ml), Amoxicillin (AX50 µg/ml), Cefalexin (CF 30 µg/ml), Chloramphenicol (CM 10 µg/ml), Gentamycin (GM 30 µg/ml), Nalidixic acid (Nal 30 µg/ml), Streptomycin (Sm 25 µg/ml), Tetracycline (Tc 15 µg/ml), Trimethoprim (Tm 10 µg/ml). The tests were done using the agar diffusion technique on Muller –Henton agar as described by Bauer et al., (1966).

Pharmaceutical samples (SDI, Iraq) of six antibiotics were used to prepare stock solutions that were employed in genetic experiments. Preparations were done according to Ahmed (1989). and sterilization was by filtration. The solutions were kept at, - 20°C before use. The antibiotics, solvents and the final concentrations of the stock solutions are shown in Table-2. The antibiotics were added to nutrient agar medium after cooling to (40 - 45)°C according to Grant and Pittrand (1974).

Table 2: Antibiotic stock solutions and their final concentrations in growth media.

Antibiotics	Stock solution concentration (mg/ml)	Final concentration in to the media (µg/ml)	solvent
Ampicillin	25	50	Ethanol (70%)
Cephalexin	10	30	Strile distilled water
Nalidixic acid	20	30	Ethanol (70%)
Streptomycin	25	25	Strile distilled water
Teracycline	12.56	15	Ethanol (70%)
Trimethoprim	20	10	Strile distilled water

Preparation of plasmid DNA

Plasmid DNA was prepared from three S. odorifera1 isolates and two. S. marcescens isolates, using the alkaline lysis procedure as described by Birnboim and Doly (1979), and modified by Kado and Liu (1981). The concentration of plasmid DNA in each samples was determined according to Brown (1995). The plasmid DNA concentration was calculated as:

O.D. at 260nm x Dilution factor x 50μg/ml = ( ) μg/ml

Preparation of competent cells and transformation

Competent cells of E. coli JM83 were prepared using the $CaCl_2$ methods of (Mandel and Higa1970). These cells were used to receive plasmid DNA from Serratia donors. The transformation frequency was calculated as:

$$\textbf{Transformation frequency} = \frac{\textbf{Number of transformant colonies per 1}\mu\textbf{g of plasmid DNA}}{\textbf{Total number of viable colonies}}$$

Conjugation

One S. marcescens and one S. odorifera1 were used to donate their plasmids to each of the two recipients , E. coli JM83 and E. coli JMP294. Conjugation were performed on filter paper using the method described by Casen etal.(2000).

The frequency of conjugation was calculated as:

$$\textbf{Conjugation frequency} = \frac{\textbf{Number of transconjugant cells in 1ml of conjugation mixture}}{\textbf{Total number of recipient cells in 1ml of the bacterial culture}}$$

RESULTS AND DISCUSSION

Fifty clinical samples (20 UTI, 10 septicaemia and 20 from wounds) were collected and examined for the presence of the bacterium Serratia. Seven S. odorifera 1 (4 from UTI and 3 from wounds) and three S. marcescens (2 from septicaemia and 1 from wounds) were diagnosed. Details of these isolates are given in Table 3 These results are in accordance with published works(Leranoz etal. 1997; Passaro et al., 1997), associating S. marcescens and other species of this genus with these sites of infections. In fact it has been suggested.(Hejzai and Falkiner1997), that S. marcescens is becoming a common nosocomial infections.

Antibiotic Resistance

All ten isolates were tested against the nine antibiotics. Some of them were resistant, some were intermediate and some were sensitive. Five isolates, 2, 5, 6, 7 and 8 (Table 3) were chosen for further genetical analysis. Their patterns of antibiotic resistance are given in Table 4 . These were chosen on being similar in there API code number (Table 3) and different in at least two resistance markers compared with the two standard E. coli strains (Table 4).

Table 3: Clinical samples, species and code numbers of API20E of Serratia spp. identified .

Clinical samples	No. of samples	Isolates No.	Species	Code No.	% Identification
Urinary tract infection	20	1	S. odorifera 1	4346773	99.9
		2	S. odorifera 1	4346773	99.9
		3	S. odorifera 1	4347773	99.9
		4	S. odorifera 1	4347773	99.9
Septicaemia	10	5	S. marcescens	4317761	99.8
		6	S. marcescens	4317721	99.9
Wounds	20	7	S. marcescens	5316561	99.6
		8	S. odorifera 1	4346773	99.9
		9	S. odorifera 1	4346773	99.9
		10	S. odorifera 1	4347773	99.9

% Identification = % purity of isolation

Table 4: Sources and antibiotic resistance profile of Serratia isolates used in genetic experiments

Isolate No.	Source	Antibiotic resistance								
		AP	AX	CF	CM	GN	Nal	Sm	Te	Tm
1	Septicaemia	R	R	R	R	R	R	S	R	S
2	Septicaemia	R	S	R	R	R	R	S	R	S
3	Wound	R	S	R	R	R	R	S	R	S
4	Urinary tract	R	R	R	R	R	R	S	R	S
5	Urinary tract	R	R	R	R	R	S	R	R	S
E. coli JM83	Standard	S	S	S	S	R	S	R	S	S
E.coli JMP294	Standard	S	S	R	R	R	S	R	R	R

R: resistant ; S: susceptible

Similar results were obtained by Hejazi and Falkiner (1997) . Nass etal. (1994). found it difficult to treat infections by S. marcescens using Ampicillin or the first or the second generations of Cephalosporins because these strains might have the β-lactamase. As the killing effect of β-lactam antibiotics is time dependent, the longer the bacteria are exposed to antibiotics the more probable they would acquire resistance. The β-lactam resistance may arise from a high level production of chromosomal AmpC Cephalosporinase combined with a decreased membrane permeability to the antibiotics and an increase in the β-lactamase synthesis (Nass et al., 1994).

The isolates were all resistant to Gentamycin (Table 4). This observation is in accordance with that of Dio et al., (2004) who noticed, among isolates of this genus, a widespread resistance to a variety of aminoglycosides including Gentamycin.

The concentrations of plasmid DNA extracted from the five isolates of Serratia spp. are shown in Table - 5. They ranged between 1 and 1.6 μg/ml.

Table 5: Plasmid DNA concentration extracted from isolates of Serratia bacterium.

Isolates No.	Plasmid DNA concentration (μg/ml)
1	1.6
2	1.3
3	1.1
4	1.2
5	1

Transformation

The frequencies of transformation of E. coli JM83 with Serratia plasmid DNA are shown in Table - 6. They ranged between $0.2x10^{-4}$ and $0.5x10^{-4}$.

Table 6: The transformation of E. coli JM83 with S. marcescens and S. odorifera1 plasmid DNA .

plasmid DNA donor of Serratia	Transformation frequency
1	$0.2\ x10^{-4}$
2	$0.4\ x10^{-4}$
3	$0.5\ x10^{-4}$
4	$0.3\ x10^{-4}$
5	$0.5\ x10^{-4}$

Results shown in Table - 6 indicate that our isolates harbour plasmids at various concentrations. Similar observations were previously reported by Carbonell et al., (2000). who noticed that all their S. marcescens isolates harboured plasmids with molecular weights varying from 38 to 76MD.

Transformation in the present experiments could be the result of direct plasmid penetration to the recipient cells or to the penetration of plasmid fragments. Transformation frequency, however, is lower than that observed by Matsumura et al., (1989).who obtained a high degree of transformation between S. marcescens and E. coli JM83. This difference could be attributed to the fact that we have used the plasmid DNA directly to transform E. coli JM83, while these authors restricted their plasmid DNA making a recombinant plasmid and using the BamHI-restricted plasmid which could be more efficient in transformation than the natural plasmid. Doi et al., (2004), also succeeded in transferring Gentamycin resistance from S. marcescens to E. coli and found that resistance was mediated by a large plasmid inhabiting S. marcescens. Ito et al., (1995) has also successfully used transformation to transfer Imipenem resistance from S. marcescens to E. coli HB101. The size of their plasmid was 25 Kb and it was possible to isolate it back from each transformant. Therefore it appear that transfer of plasmid DNA through transformation from S. marcescens to other bacterial strains is feasible.

Conjugation

Conjugation experiments were done using five donors of Serratia to the two recipient E. coli. The results are shown in Table - 7.

Table 7:Genotypes and frequencies of conjugants recovered from mating E. coli JM83 and E. coli JMP294 to various Serratia donors.

Isolate No.	Mated strains	Conjugant genotypes	Frequency of conjugants ($x10^{-6}$)
1	S.marcescens Sm^S CF^R E.coliJM83 Sm^R CF^S	Sm^R CF^R	7
2	S.marcescens Sm^S Nal^R E.coliJM83 Sm^R Nal^S	Sm^R Nal^R	9
3	S.marcescens Sm^S Te^R E.coliJM83 Sm^R Te^S	Sm^R Te^R	10
4	S.odorifera1 Tm^S Nal^R E.coliJMP294 Tm^R Nal^S	Tm^R Nal^R	20
5	S.odorifera1 Tm^S AP^R E.coliJMP294 Tm^R AP^S	Tm^R AP^R	30

The frequency of conjugants ranged between $7x10^{-6}$ and 30×10^{-6}. These frequencies are comparable to the frequency of 10^{-5} for Imipenem obtained from conjugants Serratia donors and E. coli recipients (Ito et al., 1995). The results indicate the the possibility of plasmid transfer through conjugation between S. marcescens and E. coli. This might suggest that intergeneric conjugation can take place between two bacterial genera if they co-inhabited the same environmental niche. This is especially important if we know that Serratia spp. are widely distributed in the hospital environments resulting in various nosocomial infections. This is an alarming observation as S. marcescens harbours a large R-plasmid (41 megadalton) for Gentamycin and Tobramycin that remained stable in hospital strains for more than six years (John and MeNeill 1981).

Moreover, Okuda et al., (1984), have shown that many clinical isolates of S. marcescens were highly resistant to current antibiotics and they attributed this resistance to R-plasmid that was transferable from Serratia spp. to E. coli recipients. They also noticed that decline in S. marcescens susceptibility might be in part due to conjugational transfer of resistance between various bacterial species.

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