Brief Communication

Antibiomicrobial Susceptibility Testing of Serratia Species Isolated from Hospitalized Patients in Two Hospitals in Al-Mosul, Iraq

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Abstract

A total of 150 samples (20 blood, 10 throat swabs, 20 sputum, 50 urine and 50 wounds) were collected from patients admitted Al- Salam and Al- Zahrawi hospitals in the city of Mosul, Iraq and examined for the presence of bacteria Serratia species.

By using morphological, cultural and biochemical it was possible to identify 25 Serratia isolates among the 150 samples. The identifications was confirmed by the API 20E system.

The 25 isolates were identified as *Serratia marcescens* (12) (both pigmented and non pigmented), *S. odorifera1*(10), *S. odorifera 2*(2), and 1 isolate *S. plymuthica. Serratia marcescens* isolates were highly sensitive to Cephtriaxone and Ciprofloxacin, and 100% resistant to Ampicillin, Nitrofuration, Refampicin and Tetracycline, but expressed variable degree of resistance to other antibiotics. S. *odorifera 1* isolates, on the other hand, showed high sensitivity to Cephtriaxone, Chloramphenicol, Ciproflaxocin, Cotrimoxazol and Nalidixix acid, but high resistance to Ampicillin, Cephalexin, and Refampicin, and varied in its response to other antibiotic. *S. ordoifera 2* isolates were highly sensitive to Cephalexin, Cephtraizcxone, Chloramphenicol, Ciprofloxacin, Cotrimoxazol and Nalidixic acid, but highly resistant to Ampicillin, Refampicin. *S. plymuthica* isolate was sensitive to Cephtraiacxone, Chloramphenicol, Ciprofloxacin, Cotrimoxazol, Gentamycin and Nalidixic acid, but resistant to Ampicillin, Cephlaexin, Refampicin and Tetracycline.

Keywords: *Serratia* spp., Isolation, Identification, Pigment production, Antibiotic sensitivity.

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Introduction

Serratia spp. are gram-negative rods that belong to Family Enterobacteriaceae.

Morphologically, Serratia is one of the easiest genera to identify among Enterobiacteriaceae.

Unlike other enterobacteria, strains of Serratia, usually, produce DNase, gelatinase and lipase and a resistant to the antibiotics Colistin and Cephalothin. [1] Serratia spp. are frequently identified using the Analytical Profile Index (API 20E). [2]

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Species in this genus can cause disease to plants and to a wide range of animals (both vertebrates and invertebrates). [3] Serratia spp. are opportunistic human pathogen, but the last three decades have witnessed a steady increase in life-threatening nosocomial infections caused by species of this genus. [2] Serratia spp. are sometimes associated with severe hospital-acquired infections like pneumonia and infection of lower respiratory tract, urinary tract, blood stream and wounds. [4, 5, 6] While natural *S. marcescens* strains are often red, due to the production of prodigiosin, strains associated with hospital outbreaks are mostly non-pigmented. [1]

Many *S. marcescens* strains are multiply resistant to antibiotics; some of the strains causing nosocomial infections are capable of a producing plasmid-mediated β-lactamases against a wide-spectrum cephalosporins. [7]

We are reporting here in the isolation and identification of Serratia spp. from various clinical cases and their susceptibility to a number of antibiotics.

Materials and Methods

Sample collection

One hundred fifty samples were collected from patients (male and female) with nosocomial infections from Al-Salam and Al-Zahrawi hospitals in Mosul city, Iraq, at the period in Oct. 2004 to Oct. 2005. The samples comprised isolates from 20 blood, 10 throat swabs, 20 sputum, 50 urine and 50 wounds.

Isolation and Identification

The samples were cultured on blood agar, MacConkey agar and Nutrient agar (Oxoide) and incubated overnight at 37°C. Bacterial isolates were identified by using morphological, cultural and biochemical tests. The identifications were confirmed by the API 20E test system (Bio-Merieux).

Pigmentation test

Liquid Pigmentation Medium

All *Serratia marcescens* were grown in a liquid media, [8] containing 1% glycerol, 0.5% ammonium citrate, 0.05% magnesium sulfate, 1% potassium phosphate, 0.5% sodium chloride, 0.05% ferric ammonium citrate, 0.1% yeast extract and 0.2% peptone. The tubes were incubated still at 27 °C and daily checked for pigment formation and observation continued for 7 days after incubation.

Solid Pigmentation Medium

Bacteria were grown on peptone/glycerol agar (Bacto-peptone 5 gm, glycerol 10 ml, agar 20 gm, distilled water 1 liter), incubated at 37° C for 24 hrs. and pigment formation was checked. [8]

Antibiotic Susceptibility

Susceptibility to antimicrobial agents was tested by the disk diffusion method on Mueller-Hinton agar as described by Vandepitte *et al.* (1991) [9] The antibiotics tested were Ampicillin (AMP 10 μg/ml), Carbencillin (PY 100 μg/ml), Cephalexin (CF 30 μg/ml), Cephtriaxone (CTX 30 μg/ml), Chloramphenicol (C 30/μg/ml), Ciprofloxancin (CIP 5 μg/ml), Co-trimaxazol (SXT 25 μg/ml), Gentamycin (GN 10 μg/ml), Nalidixic acid (Nal 30 μg/ml), Nitrofuration (FT 300μg/ml), Rifampicin (RA μg/ml), Tetracycline (TE 30 μg/ml) and Tobramycin (TOB 10 μg/ml).

Results

Table (1) illustrates bacteria frequencies isolated from 150 samples of human different cases on the bases of morphological, cultural and biochemical tests.

Table 1: Bacteria frequencies isolated from human different cases.

Type of bacteria	*Blood (20)**		*Throat (10)*		*Sputum (20)*		*Urine (50)*		*Wounds (50)*	
	*No.***	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Staphylococcus aureus	2	(10)	6	(60)	3	(15)	0	(0)	5	(10)
Streptococcus pyogenes	2	(10)	4	(40)	6	(30)	0	(0)	0	(0)
Escherichia coli	7	(35)	0	(0)	0	(0)	25	(50)	6	(12)
Pseudomonas aeruginosa	1	(5)	0	(0)	0	(0)	3	(6)	15	(30)
Proteus mirabilis	0	(0)	0	(0)	0	(0)	4	(8)	0	(0)
Klebsiella spp.	0	(0)	0	(0)	2	(10)	6	(12)	0	(0)

**** = Number of samples.***
***** = Number of positive isolates.***

Table 2: Serratia spp. identified from various clinical specimens.

Clinical samples	*No. of samples*	*No. of isolates*	*Serratia spp.*	*%**
Blood	*20*	*3*	*S. marcescens*	*15*
Throat	*10*	*2*	*S. marcescens*	*20*
Sputum	*20*	*3*	*S. odorifera 1*	*15*
Urine	*50*	*3*	*S. marcescens*	*6*
		7	*S. odorifera 1*	*14*
		2	*S. odorifera 2*	*4*
Wounds	*50*	*4*	*S. marcescens*	*8*
		1	*S. plymuthica*	*2*

**** represented the percentage of respective samples from which the species was isolated.***

Twelve *S. marcescens* [3 from blood (15%), 2 from throat swabs (20%), 3 from urine (6%), 4 from wounds (8%)] were identified. Ten *S. odorifera* biogroup 1 [3 from sputum (15%), 7 from urine (14%)] and two *S. odorifera* biogroup 2 [2 from urine (4%)]. One *S. plymuthica* (1 from wounds (2%)] were also diagnosed. Details of these isolates are given in Table (2).

Isolation and Identification

Serratia species are unique among the Enterobacteriaceae in that they produce three hydrolytic enzymes. These are lipase, gelatinase and DNase. Grouping of the isolates on that basis and other differentiating features is given in Table (3).

Table 3: Differentiation of species within the genus Serratia.

Properties	*S. marcescens*	*S. odorifera Biogroup*		*S. plymuthica*
		1	*2*	
	*(12)**	*(10)*	*(2)*	*(1)*
DNase	+	+	+	+
Lipase	+	*V*	+	+
Gelatinase	+	+	+	+
Odor of potatoes	-	+	+	-
Pigment	*V*	-	-	-

**** No. of isolates ; + positive ; - negative ; V variable.***

Bacterial stains were identified by using biochemical tests and the results were confirmed by API 20E.

Pigmentation Test

The 12 *Serratia marcescens* isolates separated into three red-pigmented (prodigiosin), one pink-pigmented and eight non-pigmented in liquid and solid media.

Antibiotic Sensitivity

All twenty five isolates were tested against the fourteen antibiotics. Their effect ranged from resistance to intermediate susceptibility to full sensitivity (Tables 4, 5).

Table 4: Patterns of antibiotic sensitivity test (%) of pigmented and non-pigmented Serratia marcescens.

Antibiotics	*Antibiotic concentration μg/ml*	*Sensitive isolates No. (%)*	*Intermediate isolates No. (%)*	*Resistant isolates No. (%)*
Ampicillin	*10*	*0 (0.0)*	*0 (0.0)*	*12 (100)*
Carbencillin	*100*	*0 (0.0)*	*4 (33.3)*	*8 (66.6)*
Cephalexin	*30*	*2 (16.6)*	*0 (0.0)*	*10 (83.3)*
Cephtriaxone	*30*	*12 (100)*	*0 (0.0)*	*0 (0.0)*
Chloramphemicol	*30*	*8 (66.6)*	*0 (0.0)*	*4 (33.3)*
Ciprofloxacin	*5*	*12 (100)*	*0 (0.0)*	*0 (0.0)*
Co-trimoxazol	*25*	*11 (91.6)*	*0 (0.0)*	*1 (0.33)*
Gentamycin	*10*	*0 (0.0)*	*2 (16.6)*	*10 (83.3)*
Nalidixic acid	*30*	*4 (33.3)*	*0 (0.0)*	*8 (66.6)*
Nitrofuration	*300*	*0 (0.0)*	*0 (0.0)*	*12 (100)*
Refampicin	*30*	*0 (0.0)*	*0 (0.0)*	*12 (100)*
Tetracycline	*30*	*0 (0.0)*	*0 (0.0)*	*12 (100)*
Tobramycin	*10*	*0 (0.0)*	*7 (58.3)*	*5 (41.6)*

Table 5: Percentage of antibiotic resistance of pigmented and non-pigmented Serratia marcescens isolates.

Antibiotics	*Pigmented isolates No. (%)*	*Non-pigmented isolates No. (%)*
Ampicillin	*4 (33.3)*	*8 (66.6)*
Carbencillin	*4 (33.3)*	*4 (33.3)*
Cephalexin	*4 (33.3)*	*8 (66.6)*
Cephtriacxone	*0 (0.0)*	*0 (0.0)*
Chloramphemicol	*0 (0.0)*	*4 (33.3)*
Ciprofloxacin	*0 (0.0)*	*0 (0.0)*
Co-trimoxazol	*0 (0.0)*	*1 (8.33)*
Gentamycin	*4 (33.3)*	*6 (50.0)*
Nalidixic acid	*0 (0.0)*	*8 (66.6)*
Nitrofuration	*4 (33.3)*	*8 (66.6)*
Refampicin	*4 (33.3)*	*8 (66.6)*
Tetracycline	*4 (33.3)*	*8 (66.6)*
Tobramycin	*0 (0.0)*	*5 (41.6)*

Discussion

Results shown in Table (1) are in accordance with those of Al-Daoodi (2002) [10] who isolated *S. marcescens* from wound infections of patients who were subjected to invasive instrumentation in the surgery units in Mosul city. The results are in accordance also with published works. [11- 14]

These authors isolated Serratia spp. from various cases at various percentages, and concluded that Serratia spp. are important nosocomial pathogens and a frequent source of outbreak of hospital infections.

The wide spread of Serratia spp. among patients with various clinical cases, regardless of the source reservoir, and the predominant mode of spread of *S. marcescens* infection, is thought to be the hand-to-hand transmission by hospital personnel. [15] Factors such as debilitating clinical conditions, length of ward-stay and frequent exposure to medical intervention predisposing patients to infection, most likely, act by necessitating increased frequency and intensity of direct contact with staff hands. [16]

Some strains of *S. marcescens* produce a diffusible red pigment called prodigiosin, (2-methyl-3-amyl-6-metho-oxyprodigiosin). This pigment is common in strains isolated from soil and water, but very rare in clinical samples. [17] The highest increase in biomass and maximal pigmentation was found in cultures grown in glycerol medium, where growing cells contain both mono and dimmer forms of pridigiosin in glycerol with light influencing these pigments. [2] Pigmented biotypes of *S. marcescens* (mostly recovered from natural environments) are rarely responsible for outbreak, whereas the non-pigmented biotypes are prevalent in the hospital. [1]

The present results showed Serratia spp. to be multiply resistant to antibiotics which is in agreement with those of [18] who observed that the nosocomially derived Serratia strains are often resistant to multiple antibiotics. These authors also noticed that some strains of *S. marcescens* will display a red pigmentation phenotype, and are less likely to be resistant to antibiotics. Non-pigmented *S. marcescens* are more resistant to antibiotics (most plasmid encoded) than pigmented isolates. [1]

Pigmented strains exhibited a similar pattern of resistance to antibiotics and all were resistant to ampicillin, and tetracycline. This observation was in accordance with those of Carbonell *et al.*, (2000). [1]

The results showed that all *S. marcescens* strains were resistant (100%) to ampicillin, nitrofuration, refampicin and tetracycline but all sensitive to cephtriaxone, ciprofloxacin and varied in their response to other antibiotics used in this study. Similar results were obtained by Al-Daoodi, (2002) [10] and Stock *et al.*, (2003) [19] who noticed that all isolates of *S. marcescens* were resistant to ampicillin, nitrofuration, refampicin, tetracyclin and gentamycin.

All Serratia strains appeared resistant to ampicillin (100%). This resistance is many attributed to the expression of plasmid encoded β-lactamases such as TEM1, which are common among all Enerobacteriaceae. They also express chromosomal class C, inducible Amp C β-lactamase combined with a substantially decreased outer-membrane permeability (Hejazi and Falkiner, 1997). [2]

Serratia marcescens isolates in this study are highly resistant to gentamycin (83.3%). *S. odorifera 1*isolates are also highly resistant to this antibiotic (80%). These results are in accordance with those of Edson and Terrell (1999) [20] who noticed that *S. marcescens* strains have a wide spread resistance to gentamycin.

Aminoglycoside antibiotics are widely used in clinical setting, especially for treatment of life-threatening infections caused by gram-negative bacteria. The most frequently encountered mechanism of resistance to aminoglycoside is their structural modification by specific enzymes produced by the resistant strain. [21] Other mechanisms of resistance include ribosomal alterations, efflux of the agents by extrusion pump or altered permeability leading to reduced uptake. [22]

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اختبار الحساسية للمضادات الحيوية لانواع السراشيا المعزولة من المرضى الراقدين في مستشفيين في مدينة الموصل، العراق

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الملخص

جرى جمع 150 عينة من حالات مرضية مختلفة في الانسان (20 من الدم ، 10 من اللوزتين ، 20 من القشع ، 50 من اصابات القناة البولية و 50 من الجروح) من مستشفيي السلام والزهراوي في مدينة الموصل–العراق . وجرى التحري عن جراثيم السراشيا باستخدام الصفات الشكلية والزرعية والكيموحيوية ، وتأكيد ذلك باستخدام نظام API 20E . وقد تم تشخيص 25 عزلة من جراثيم السراشيا وتوزعت بواقع 12 عزلة من النوع *Serratia marcescens* بنمطيه المنتج للصبغة وغير المنتج لها و 10 عزلات من النوع *Serratia odorifera 1* وعزلتين من النوع *Serratia odorifera 2* وعزلة واحدة من النوع *Serratia plymuthica* . اظهرت عزلات *Serratia marcescens* حساسية مطلقة تجاه المضادين Cepthriaxone و Ciprofloxacin وكانت مقاومة 100% للمضادات Ampicillin ، Nitrofuration ، Refampicin و Tetracycline في حين تباينت استجابتها للأنواع الاخرى من المضادات . واظهر النوع *S. odorifera 1* حساسية مطلقة تجاه Cephtriaxone ، Chroamphenicol ، Ciprofloxacin ، Cotrimoxazol و Nalidixic acid ، ومقاومة 100% تجاه Ampicillin ، Cephlexin و Refampicin وتغايرت للأنواع الاخرى . اظهر النوع *Serratia odorifera 2* حساسية مطلقة تجاه Cephalxin ، Cephtriaxone، Chloramphenicol ، Ciprofloxacin ، Cotrimoxazol وNalidixic acid ومقاومة 100% تجاه Ampicillin و Refampicin . اما النوع *S. plymuthica* فاظهر حساسية 100% تجاه Cephtriaxone ، Chloramphenicol، Ciprofloxacin، Cotrimoxazol، Gentamycin و Nalidixic acid في حين اظهر مقاومة مطلقة تجاه Ampicillin ، Cephalexin ، Refampicin و Tetracycline.

الكلمات الدالة: Serratia spp. ، العزل ، التشخيص ، انتاج الصبغة ، الحساسية للمضادات الحيوية.