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Detection of *Serratia marcescens* from clinical and environmental samples with antimicrobial sensitivity test in Basrah

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ABSTRACT

Serratia marcescens is a Gram-negative bacterium which distinguished as a member of the bacterial family of Enterobacteriaceae. It is the most common species of the genus *Serratia* that cause human opportunistic pathogens with a wide host range and symbolize a rising problem for public health as a causative agent of hospital-acquired / nosocomial infection in the last three decades. In the present study out of total (185) clinical and environmental specimens were collected then processed for isolation and detection of *S.marcescens* isolates in the south of Iraq in Basrah city. The bacterial growth was seen in 85 (65.38 %) as positive cultures and 45 (34.61 %) specimens showed negative (no bacterial growth) from the totally 130 of various clinical samples. While, from the total 55 of diverse sources of environmental specimens, the positive cultures of *S.marcescens* were evident in 32 (58.18 %) and no bacterial growth in 23 (41.81 %). Also, this study revealed the strains of *S.marcescens* as a pigmented types in frequent of (69.41 %) and non-pigmented types in rates of (30.58 %), these all bacterial isolates of *S.marcescens* from clinical sources. Moreover, in our study the antibiotic sensitivity testing was done for all the (117) positive isolates of *S.marcescens* from clinical and environmental samples, among the 85 isolates of *S.marcescens* from clinical samples it's were very sensitive to Ciprofloxacin (100 %) and Cefepime (89.41 %) and were more resistant to Ampicillin (87.05 %) and Aztreonam (64.70 %). While, among the 32 isolates of *S.marcescens* from environmental samples, results showed, these isolates of *S.marcescens* it's were also susceptible to Ciprofloxacin with fully form infrequent (100 %) and susceptible to antibiotic Cefepime in rate (81.25 %) but, it is more resistant to Ampicillin infrequent (81.25 %) and Aztreonam (78.12 %).

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INTRODUCTION

Serratia marcescens is a species of rod-shaped bacteria, gram-negative, motile, aerobic, non-lactose fermenting which belongs to the family of Enterobacteriaceae, both assortment of pigmented and non-pigmented are exist which can overrun various habitats including main soil, water, plant also vertebrate and invertebrate hosts (Rjazantseva *et al.*, 1994; Grimont *et al.*, 1977). *Serratia marcescens* has been involved in Hospital-Acquired Infection (HAIs) as the cause of many hospital epidemics and it is an opportunistic human pathogen which is responsible for increasing seriously, the number of hospitalized nosocomial infections (Farmer *et al.*, 1976; Yn, 1979; Hejazi and Falkiner, 1997; Wong *et*

al., 1999). It cause a wide spectrum of clinical diseases as a secondary infections including, respiratory (pneumonia) in neonatal intensive care units, urinary, wound, central nervous system infections as meningitis, septicemia, endocarditis, conjunctivitis, sinusitis, septic arthritis and peritonitis (Echols *et al.,* 1984*;* Liu *et al.,* 1995*;* Hejazi and Falkiner, 1997*;* Eisenstein *et al.,* 2000*;* Lu *et al.,* 2012*;* Wu *et al.,* 2013). Many strains of *S.marcescens* are capable to multiply resistant to antibiotics by their ability to produce a beta-lactamase and may gain a plasmid-mediated extended spectrum B-lactamases [ESBLs] that, which afford resistance to the broad spectrum of beta-lactam antibiotics (Farmer *et al.,* 1976*;* Bennett and Chopra, 1993*;* Haddy *et al.,* 1996*;* Garner *et al.,* 1998*;* Queenan *et al.,* 2000*;* Crivaro *et al.,* 2007). Capabilities of *S.marcescens* to cause the nosocomial infections and environmental survive are imputed to its ability to form the biofilms layer, its vast metabolic capacity and its higher in natural resistance to antimicrobials and cleaning agents (Kalvioda *et al.,* 2010). The efficiency to create a red pigment in form prodigiosin is a trait of *S.marcescens* (Grimont *et al.,* 1984*;* Trias *et al.,* 1989*;* Kalvioda *et al.,* 2010).

The aim of this study was converged on the detect of *S.marcescens* isolates from clinical and environmental samples together with investigating the tendency of *S.marcescens* isolates to antimicrobial resistance in Basrah governorate.

MATERIALS AND METHODS

Specimens collection

In our study, one hundred eighty-five (185) clinical and environmental samples were collected from different sources in Basrah city. The investigation period extended from March to October 2018. A total of (130) clinical specimens were evaluated in the present study, the isolates of *Serratia marcescens* were obtained from Sadr Teaching Hospital, Basrah General Hospital, Educational Laboratory of the Dentistry College and some health care centres in Basrah city. Strains of bacteria were isolated from clinical specimens including 41 throat swabs, 21 sputum, 8 burns, 11 surgical wounds, 34 urine and 15 blood samples. While a total of 55 environmental samples were collected, consist of 21 river water (Shatt al- Arab, Al- Ashar rivers), 13 agriculture soils, 10 birds' faeces and 11 cockroaches.

Isolation and Identification

Specimens were seeded on their respective the selective and differential media including MacConkey agar, Eosin Methylene Blue Agar, Blood agar, Kligler Iron agar and Nutrient agar. The bacterial isolates were identified after incubation at 37 c0 for 24 hr by using morphological Gram reaction, characteristics culture and biochemical tests, also, the identification was definite by the API 20E test system (Bio-Merieux). Furthermore, the pigmentation test and haemolysis activity were done (Forbes *et al.,* 2007*;* Carbonell *et al.,* 2000*;* Coulthurst *et al.,* 2006).

The antibiotic susceptibility testing of S.marcescens isolates was determined by using the standard Kirby Bauer disk diffusion method on Muller Hinton agar (Bauer *et al.,* 1966). Suspension of bacterial cultures talents to 0.5 tube McFarland turbidity standards then spread on plates of M.H agar by using of sterile swabs and incubated at 37 co for 24 hrs in aerobic condition. The results of plates were read by the measure of the inhibition zones diameters around the disks of antibiotics according to the criteria which recommended by Clinical Laboratory Standards Institute of CLSI, 2012.

RESULTS

In the current study out of total 185 clinical and environmental samples were collected then processed for isolation and identification of *S.marcescens* isolates in the governorate of Basrah.

Bacterial growth was seen in 85 (65.38 %) as a positive culture and 45 (34.61 %) samples showed negative cultures from the total 130 of various clinical specimens Table (1). While, from the total 55 of diverse sources of environmental samples, the bacterial growth of *S.marcescens* was evident in 32 (58.18 %) and 23 (41.81 %) showed no growth of bacteria Table (2).

Table (3): Showed the correlation between the positive cultures of *S.marcescens* from different clinical sources with the number and percentages of isolated types (*S.marcescens* pigmented or non-pigmented), our study display the number positive of bacterial strains of *S.marcescens* was 59 in percentages of (69.41 %) as pigmented types of *S.marcescens* and 26 in percentages of (30.58 %) as non-pigmented types from bacterial isolates of *S.marcescens*.

The results of susceptibility testing are described in Tables 4, 5. Among the 85 isolates of *S.marcescens* from clinical samples it's were susceptible to Ciprofloxacin (100 %), Cefepime (89.41 %), Levofloxacin (87.05 %), Cefotaxime (85.88 %), Chloramphenicol (82.35 %) and it resistant to Ampicillin (87.05 %), Aztreonam (64.70 %), Gentamicin (58.82 %) and Amikacin (51.76 %) as shown in Table (4). While, the total number 32 of *S.marcescens* isolates from environmental samples the results showed that, *S.marcescens* was susceptible to Ciprofloxacin (100 %) and Cefepime (81.25 %)

Table 1: The number of positive cultures of *S.marcescens* from specimens correlated with clinical sources

Source	No. of Specimen	No. and (%) of +ve of *S.marcescens* culture
Throat swabs	41	29 (70.73)
Urine	34	25 (73.52)
Sputum	21	13 (61.90)
Blood	15	8 (53.33)
Surgical Wound	11	7 (63.63)
Burns	8	3 (37.5)
Total	130	85 (65.38)

Table 2: Related number of positive cultures of *S.marcescens* with samples from various environmental sources

Source	No. of Specimen	No. and (%) of +ve of *S.marcescens* culture
Rivers water	21	15 (71.42)
Agriculture soils	13	3 (23.07)
Bird faeces	10	5 (50.0)
Cockroaches	11	9 (81.81)
Total	55	32 (58.18)
Rivers water	21	15 (71.42)
Agriculture soils	13	3 (23.07)

Table 3: Association between the positive cultures of *S.marcescens* from clinical sources and number of isolated types

Sources	—	No. and (%) of +ve of *S.marcescens* with isolated types	
		Non- Pigmented	Pigmented
Throat swabs		11	18
Urine		4	21
Sputum		6	7
Blood		3	5
Surgical Wound		1	6
Burns		1	2
Total and (%)		26 (30.58)	59 (69.41)

Table 4: Antibiotics susceptibility pattern in *S.marcescens* from clinical samples

Antibiotic	—	Total No. of isolates 85	
		No. and (%) of Resistant	No. and (%) of Susceptible
Ciprofloxacin		0 (0.0)	85 (100.0)
Chloramphenicol		15 (17.64)	70 (82.35)
Ampicillin		74 (87.05)	11 (12.94)
Amikacin		44 (51.76)	41 (48.23)
Aztreonam		55 (64.70)	30 (35.29)
Gentamicin		50 (58.82)	35 (41.17)
Levofloxacin		11 (12.94)	74 (87.05)
Cefotaxime		12 (14.11)	73 (85.88)
Cefepime		17 (20.0)	76 (89.41)

Table 5: Antibiotics susceptibility pattern in *S.marcescens* from environmental samples

Antibiotic	—	Total No. of isolates 32	
		No. and (%) of Resistant	No. and (%) of Susceptible
Ciprofloxacin		0 (0.0)	32 (100.0)
Chloramphenicol		20 (62.5)	12 (37.5)
Ampicillin		26 (81.25)	6 (18.75)
Amikacin		23 (71.87)	9 (28.12)
Aztreonam		25 (78.12)	7 (21.87)
Gentamicin		24 (75.0)	8 (25.0)
Levofloxacin		24 (75.0)	8 (25.0)
Cefotaxime		18 (56.25)	14 (43.75)
Cefepime		6 (18.75)	26 (81.25)

but it was resistant to Ampicillin (81.25 %), Aztreonam (78.12 %), Gentamicin (75.0 %), Levofloxacin (75.0 %), Amikacin (71.87 %), Chloramphenicol (62.5 %), and Cefotaxime (56.25 %) as found in Table (5).

DISCUSSION

In present study the positive cultures were seen in urine samples of patient with urinary tract infection in most frequent (73.52 %), followed by throat infection (70.73 %), wounds infection (63.63 %), respiratory tract infection /sputum (61.90 %), blood infection (53.33 %) and burn infection in (37.5 %). Our findings were somewhat in accordance with the result of (Mun, Kim *et al.,* 2013 and Wolcott *et al.,* 2009) only concerning with the isolation rates of *S.marcescens* from patients with urinary tract infection. In this study among 32 positive cultures of *S.marcescens* from environmental sources were found with highly frequent (81.81 %) in cockroaches samples from the hospital environment, followed by rivers water (71.42 %), bird faeces (50.0 %) and agriculture soils (23.o7 %). These results were, to some extent, in agreement with the results of (Bahig *et al.,* 2008*;* Adeleke and Omafuvbe, 2011; Barell *et al.,* 2000).

In the current study, the positive cultures of *S.marcescens* strains from clinical sources depending on the produced pigment or not, the results were showed a total of 59 bacterial isolated of *S.marcescens* appeared pigmented strains infrequent (69.41 %) while, in 26 isolated strains of S.marcescens were seemed non pigmented with frequent (30.58 %). Our results were consisting with the results of (Puthucheary and Ngeow, 1981*;* Cooksey *et al.,* 1975*;* Miller *et al.,* 1973*;* Amit *et al.,* 2007*;* Kenai and Gupta, 2011*;* Mekhael and Yousif*,* 2009).

In this study, the antibiotic sensitivity test was performed for the all bacterial isolated of *S.marcescens*, the major isolates of *S.marcescens* from clinical specimens which identified in the present study were found to be fully sensitive to Ciprofloxacin, and this is in agreement with the study of (Sethuraman *et al.,* 2011). This result in our study seems to be confirmed the Ciprofloxacin antibiotic is an effective drug for treating those infected patients with *S.marcescens* chiefly, increasing of antibiotic resistance that used in hospitals and in the community between people at the late decade which was also monitored with respect to *S.marcescens* (Bollmann *et al.,* 1989). Also, in the present study, the totally 85 isolates of *S.marcescens* from clinical samples were highly resistance (87.05 %) to Ampicillin, followed by resistance to Aztreonam (64.70 %), Gentamicin (58.82 %) and Amikacin (51.76 %).

The antibiotic of Gentamicin is a broad- spectrum which acts against both types of bacteria include, the Gram-positive as well as Gram-negative bacteria. It's fundamentally effective against Gram-negative mostly, the enterobacteria (Livemore*,* 1995). However, other authors as well as export about the phenomenon of resistance to aminoglycoside antibiotics Gentamicin (Zhang*,* 1991). This is in accordance with the results of my research.

Results of the current study revealed that, the majority strains of totally 32 isolates of *S.marcescens* from environmental samples were confirmed a high resistance rates to Ampicillin (81.25 %), followed by Aztreonam (78.12 %), Gentamicin (75.0 %), Levofloxacin (75.0 %), Amikacin (71.87 %) and Cefotaxime (56.25 %). While, these strains of S.marcescens that's isolated from environmental samples were extremely susceptible to Ciprofloxacin with fully frequent (100.0 %), followed by Cefepime in the rate of (81.25 %). Anyhow, our results were consistent with authors (Jarvis and Marton*,* 1992) observation, regarding Gentamicin antibiotic.

The Cefotaxime antibiotic is a third generation drug which acts on to gram-negative bacteria (Choi *et al.,* 2002), this is in agreement with our results in related to strains of *S.marcescens* which, isolated from environmental samples.

Amikacin is an antibiotic with a vast spectrum of action and it's bespoke as a drug which reserve for hospital-acquired infection in related with mainly gram-negative bacillary (Tripathi*,* 2008). Also, the antibiotic of Chloramphenicol is a broad spectrum activity against bacilli, the gram-positive and gram-negative. Furthermore, its gained resistance through the transfer of plasmid between the enterobacteria and the others (Bollmann *et al.,* 1989*;* Lohr, 1994).

However, the inclusive resistance rates in the present study were in accordance with the results of (Rajaram *et al.,* 2013).

CONCLUSION

Among the total isolates of *S.marcescens* as positive cultures from clinical and environmental specimens were highly sensitive to the Ciprofloxacin and Cefepime antibiotics. Also, were more resistant to both Ampicillin and Aztreonam antibiotics in regarding with *S.marcescens* isolates from clinical specimens. While related to strains of *S.marcescens* from environmental specimens it is more resistant to Ampicillin. Furthermore, the isolates of *S.marcescens* create a different resistant spectrum to other six antibiotics that used in the current study as a result of multi-resistance. To reduce this complicated problem of bacterial multi-resistance drugs in the community especially in

hospitals, therefore, it is necessary to take most consideration in diagnostics of the microbiological and clinical performance of the results of these examinations. Also, in applied a good hand hygiene controlled by the achievement of the antimicrobial sensitivity testing to bacterial isolates, in this study, the strains were (*S.marcescens*) from clinical and environmental samples. Also, stopped the intensive use of antibiotic agents in hospitals and completed the full course of patient's treatment.

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