Correlation between Prodigiocin , Biofilm formation and Drug Resistant in Virulent *Serratia marcescens*

Mun'im R. Ali; Ali H. Alsakini and Sawsan M. Karim
Department of Biology, Collage of Science, Al-Mustansiryah University, Baghdad-Iraq.

Abstract

One hundred and fifty samples were collected from urine and blood, with a male to female ratio 1.45:1, culture positivity seen in the urinary tract infection was the most frequent (77%), followed by blood infection (18%). A total of 86 (57.33%) bacterial isolates were obtained. The study focused on 9 clinical isolates of *Serratia marcescens* (15.2%), differentiated into Prodigiosin producer (11.6%) and non- Prodigiosin producer (3.6%). Almost all isolates (6/9) showed visible growth at 37°C. All *S. marcescens* strains of different sources were hemolytic, whereas the formation of a pellicle was noticed among 7 isolates (77.77%) from 9 isolates. The biofilm formation performed was assessed through the crystal violet assay noticed that out of the 9 *S. marcescens* isolates 5 (55.5%) were non-adherent, 4 (44.4%) were weakly adherent, No isolate showed strongly adherent. The tube method for assay biofilm formation showed good correlation with the Tissue culture plate (TCP) method assay for biofilm forming isolates and total 2 (22.2%) isolate were picked up as strong and 5 (55.5%) were moderat biofilm producers. The tube test correlates well with the TCP test for biofilm producing isolates but it was difficult to discriminate between weak and biofilm negative isolates due to the variability in observed results by different observers. All 9 isolates were sensitive to the IPM and AZM (100%). The biofilm MICs were found to be much higher than the planktonic MICs. The medium with antibiotic used not inhibited the prodigiosin production at the MIC for planktonic cells. Therefore prodigiosin production in media with antibiotics used not marker of the growth and activity of bacterial cells. Biofilms are considerably less susceptible to antibiotics than their planktonic counterparts; biofilm production may vary largely among different strains of the same species isolated from different sites.

Keyword: *Serratia marcescens* , Prodigiosin , Biofilm , Multidrug resistance.

Introduction

Serratia marcescens is a Gram-negative, enteric bacterium that is able to inhabit a wide variety of ecological niches and cause disease in plant, vertebrate and invertebrate hosts [1]. It is an opportunistic human pathogen and is responsible for an increasing number of serious nosocomial infections, a problem exacerbated by the resistance of many strains to multiple antibiotics [2;3]. *S. marcescens* is a well-known cause of hospital-acquired infections, including nosocomial pneumonia, wound infections, urinary tract infections, and septicemia [4]. 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 cephalosporin's [5]. Treatment of these infections is often very difficult, which to a big extent is due to the widespread natural and acquired resistance of the organism to antimicrobials [6]. *S. marcescens* strains with combined resistance to expanded-spectrum ß-lactams, Aminoglycosides, Fluoroquinolones, and/or co-trimoxazole have been identified in numerous hospitals worldwide [7]. Liu *et al*. [8] referred that *S. marcescens* often develops multidrug resistance and tends to spread rapidly in the nosocomial environment, and has been implicated in outbreaks of nosocomial infection both in neonates and adults.

Serratia marcescens strains produce a range of secreted products, including proteases, nuclease, lipase, chitinases and haemolysin, many of which are likely to represent virulence factors in human infection [3]. These bacteria grow well on standard media and produce a red to dark pink pigment that aids in identification [9]. Some strains of *S. marcescens* are capable of producing a pigment called prodigiosin, which ranges in color from dark red to pale pink, depending on the age of the colonies [10]. It is a non-diffusible red pigment attached

to the inner membrane, it is a secondary metabolite formed by the condensation of 2-methyl-3-amylpyrrol and 4-methoxy-2,2-bipyrrole-5-carboxyaldehyde, leading to a tripyrrol derivative, 2-methyl-3-amyl-6-methoxyprodigiosene [11].

The abilities of *S. marcescens* to cause nosocomial infections and survive in the environment are attributed to its ability to form biofilms, its broad metabolic capacity, and its high natural resistance to antimicrobials and cleaning agents [12].One common survival strategy employed by bacteria pathogens is to form a biofilm, an amorphous and dynamic structure that is not only resistant to antibiotics, but also resistant to host immune clearance [13].

Adhesion, which is the first step of biofilm formation, is directly related to the presence of short, thin, hair like projections called fimbriae, also known as "filamentous hemagglutinins [14].

Therefore, the present study was undertaken on clinical isolates of *S. marcescens* to determine the frequency of biofilm formation by different methods in different surface and correlate biofilm formation with development of multiple antibiotic resistance and prodigiocin production.

Materials and Methods

Sample collection:

One hundred fifty samples were collected from patients (male and female) from welfare pediatric hospital and Baghdad hospital, at the period from Oct. 2009 to Aug. 2010. The samples comprised isolates from 50 bloods and 100 urine sample.

Isolation and Identification:

Samples were cultured on blood agar, MacConkey agar and Nutrient agar 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 tests:

All *S. marcescens* were grown in liquid medium as describe in [15] containing 1.0% glycerol, 0.5% ammonium citrate, 0.05% magnesium sulfate, 1.0% potassium phosphate, 0.5% sodium chloride, 0.005% ferric ammonium citrate, 0.1% yeast extract and 0.2% peptone. The tubes were incubated at 27^0C without shaking and observed for up to 7 days for pigment formation.

Pellical formation test:

This test was done according to [16]. All isolates inoculated on BHIB sterile glass tube, incubated at 37ºC for 24 hrs. The positive result defined by pellical formation on top of culture .

Haemolysis activity:

Blood agar, for detecting secreted haemolytic activity, was prepared by adding 5% washed erythrocytes to LBA, the erythrocytes were washed in cold phosphate-buffered saline [17].

Antibiotic susceptibility:

The antibiotic resistance patterns of the isolates were determined by inoculating on Oxoid-Mueller-Hinton agar plates, using the disc diffusion method. The inocula were prepared directly from an overnight agar plate adjusted to 0.5 McFarland standards. The zones of inhibition were measured and interpreted according to NCCLS (NCCLS, 2000) after incubation at 37ºC for 24 h. *Serratia* strains that showed resistance to three or more antibiotics were taken to be multi-antibiotic resistant [18].

Biofilm assay:

Tissue culture plate method (TCP):

Overnight cultures of the strains to be tested were diluted to an OD600 of 0.2 in LB, 200 µl of each diluted culture was placed in eight wells of a 96-well microtitre plate, and the plate was incubated for 5 hours at 30C. To develop the assay, culture medium was removed from the wells by aspiration and the wells were washed with water and then filled with 240 µl 0.1% crystal violet and allowed to stain for 1 h. The crystal violet was then removed and the wells washed with water to remove non-attached material. Next, 250 µl 50% ethanol was added to each well and gently agitated for several hours to allow the crystal violet to dissolve. Finally, the contents of each well derived from the same original culture were pooled and at OD 550 was measured. The following values were used to assess biofilm production: OD595 $\leq$ 0.1 (no biofilm production); 0.1<OD595 $\leq$ 0.2 (weak

biofilm production); 0.2<OD595 ≤ 0.3 (medium biofilm production), and OD595>0.3 (strong biofilm production) [19].

Tube method (TM):

A qualitative assessment of biofilm formation was determined as previously described by Christensen *et al***.** [20]. TSB (10mL) was inoculated with loopful of microorganism from overnight culture plates and incubated for 24 hrs at 37°C. The tubes were decanted and washed with PBS (pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes were washed with deionized water. Tubes were than dried in inverted position and observed for biofilm formation.Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Tubes were examined and the amount of biofilm formation was scored as 0-absent, 1-weak, 2-moderate or 3-strong Experiments were performed in triplicate and repeated three times.

MICs for planktonic cells:

The MICs of single antibiotics for planktonic cells at midlog phase were determined by standard broth micro dilution [21]. To examine the MICs of single antibiotics, one hundred microliter volumes of twofold serial dilutions of antibiotics, ranging from 1024 µg/ml to 0.5 µg/ml, in MHB were added to micro wells. Plates were incubated at 35°C aerobically for 18 hrs (for all antibiotics except).MICs for planktonic cells were defined as the lowest concentration of antibiotics that prevented the establishment of visible turbidity after overnight exposure.

MICs for biofilm-grown bacteria:

MICs using bacteria at stationary phase could not be tested by standard procedures as the maximum cell density had already been reached. The method to determine MICs for biofilm-grown bacteria was adapted from that of Ceri *et al.* [22]. Biofilms were established in 96-well microplates and exposed to 100 µL of twofold serial dilutions of antibiotics ranging from 1024 µg/ml to 0.5 µg/ml, in MHB. Microplates were then incubated at 35°C for 18 or 24 hrs. depending on the antibiotics tested. After overnight challenge, the supernatants from each well were carefully transferred to wells in a new 96-well microplate without disturbing the biofilms, and the turbidity of the contents was visually assessed. The biofilm MIC was defined as the lowest concentration of antibiotic at which no visible growth was observed. When exposed to antibiotics at or above this concentration, the planktonic bacterial population could not be established by bacterial shedding from the biofilms

Result and Discussion

One hundred and fifty samples were collected from urine and blood. The mean age of patients was (1 day-6yr). There were 89 males (59.3%) and 61 females (40.6%) with a male to female ratio (1.45:1).

Table (1)
Illustrated S. marcescens according to source of sampling, site of infection, gender and age of patients.

Bacterial strain	*Site of infection*	*Source*	*Gender*	*Age*
S_1	Urine	Preterm	Female	4 days
S_2	Urine	Intensive care unite	Female	3 years
S_3	Urine	Intensive care unite	Female	4 years
S_4	Urine	Intensive care unite	Male	4 months
S_5	Blood	Preterm	Male	6 days
S_6	Urine	Intensive care unite	Female	3 years
S_7	Urine	Intensive care unite	Male	2 year
S_8	Urine	Intensive care unite	Male	7 days
S_9	Blood	Intensive care unite	Male	2 year

Culture positivity seen in the urinary tract infection was the most frequent (77%), followed by blood infection (18%). A total of 86 (57.33%) bacterial isolates were obtained, this study focused only on 9 (15.2%) clinical isolates of *S. marcescens* as shown in Table (1) source of sampling, site of infection, gender and age of patients.

All locally isolates is differentiated into Prodigiosin producer (11.6%) and non-Prodigiosin producer (3.6%) Table (2).

Almost all isolates (6/9) showed visible growth at 37°C. The purified colony morphology appears as non-pigmented convex and relatively opaque center effuse, colorless, almost transparent periphery, and irregular crenate edge surrounded by a clear zone around the colonies were observed. The healthy human beings do not often become infected by *Serratia*, whereas, the hospitalized patients were frequently colonized or infected. *S. marcescens* were generally an opportunistic pathogen causing infection in immunodeficient patients [23]. Manikandan *et al*. [24] reported that *Escherichia coli* was the predominant uropathogen, while *S. marcescens* were the least dominant uropathogen causing UTI strains (6.47%).

While (1/9) prodigiosin producing organism. The isolated colonies were identified based on their morphology and biochemical characteristics. The isolates were confirmed as *S. marcescens*.

Williams and Quadri [25] reported that no prodigiosin was produced when cultures were incubated at 38°C; however pigment production was observed when the temperature was shifted to 27°C. A complete block in prodigiosin was observed in most of the basically used media tested at 37° C was similar to the result observed by [26]. Non-pigmented strains of *S. marcescens* may be confused with several members of the Enterobacteriaceae family. Only by routine use of a battery of bacteriological tests can these bacilli be separated from other enteric bacteria and properly identified. Colonial morphology on blood agar is quite similar to other enteric organisms, while growth on Mac- Conkey agar resembles that of the non-lactose fermenters. It is probable that cultures of nonchromogenic *S. marcescens* have been mistaken for anaerogenic Paracolobactrum species (especially the Hafnia group) or for Aerobacter cloacae, Aerobacter subgroup C (liquefaciens), and, to a lesser extent, for other members of the *Klebsiella aerogenes* group[27;28].

Table (2)
Total sample, number and percentage of S. marcescens from all positive culture samples according to gender.

Sample	*Total sampling*	*No. of positive culture*	*No. of S. marcesance*	*Prodigiosin producing*	*Gender*	
					Male	*Female*
Urine	100	77	7(7%)	1	3	4
Blood	50	9	2(2%)	2	2	-
Total	150	86	9(13.5)	3	5	4

There have been frequent reports of *S. marcescens* outbreaks in intensive care and neonatal care units [29;30].Potential virulence factors involved in this pathogenicity are proteases, a nuclease, a lecitinase, and the hemolysin, all of which are secreted by the bacterium.

All *S. marcescens* strains (total of 9) of different sources were hemolytic. Hemolysis by *Serratia marcescens* is caused by two proteins, ShlA and ShlB .The hemolysis zones formed by ShlA are very small, which may explain why the hemolytic activity of the clinically important *S. marcescens* and *S. liquefaciens* had been largely overlooked. The small hemolysis zone comes from the low diffusion rate of the large molecule and its instability [31].

Examinations for pellicle formation in aerobic static broth were made at intervals during growth for 24 hr. at 37C on the *S. marcescens* studied; the results for the strains are summarized in Table 3**.** The formation of a clump or a pellicle as a ring at the side of the

test tube was regarded as a positive result was noticed among 7isolates (77.77%) from 9 isolates. The characteristic of the cells of bacterial components of the cilia is to create thin-film on the surface of the liquid media for better ventilation is also a way to collect bacteria and characteristic of this type, so the composition of the thin film is a way to protect the bacteria from the drought and environmental conditions of other such as high temperature [32].

Serratia marcescens was isolated from clinical sample and found to grow as red-pigmented colony on nutrient agar. Results revealed that the isolate *S. marcescens* S_6 gave a high productivity of prodigiosin as compared with the amount produced by *S. marcescens* $S_{5,8}$ Prodigiocin production normally done in nutrient broth and peptone glycerol broth [33]. was found *S. marcescens* to produce more prodigiosin in maltose containing medium. *S. marcescens* was found to produce least prodigiosin in carbohydrate containing medium. This may be due to repressive effect of glucose or lactose on prodigiosin production, which was already observed by [34].

The biofilm formation performed was assessed through the crystal violet assay (Fig.(1)) to measure the degree of adherence and subsequent biofilm formation of all *S. marcescens* isolates. Out of the 9 *S. marcescens* isolates 5 (55.5%) were non-adherent, 4 (44.4%) were weakly adherent, No isolate showed strongly adherent.

Bacterial biofilm is a structured community of bacteria enclosed in a self-produced polymeric matrix and adherent to the surface of tissues and biomaterials [35]. Establishment of a biofilm is the prelude to the development of various chronic, intractable infections [36].

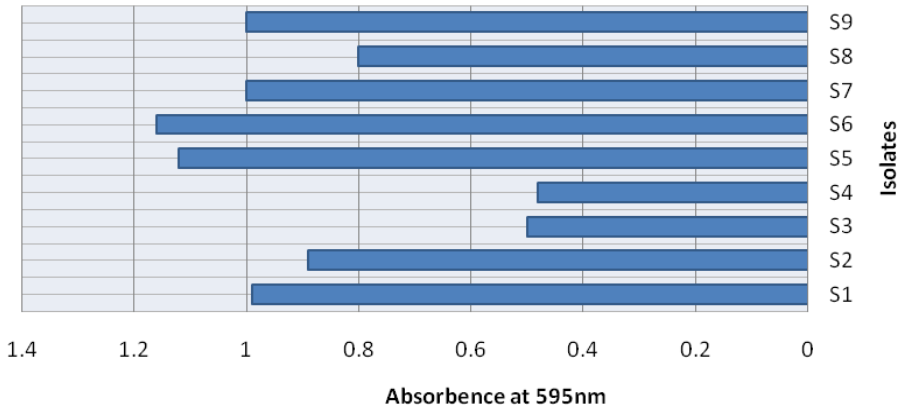

Fig.(1) Biofilm production among local S. marcescens isolates by using Tissue culture plate method (TCP).

The tube method for assay biofilm formation showed good correlation with the tissue culture plate method assay for biofilm forming isolates and total 2 (22.2%) isolate (S6 ; S5) were picked up as strong and 5 (55.5%) were moderate biofilm producers(S1,S2,S7,S8,S9). However, it was difficult to discriminate between moderate and weakly biofilm producing isolates.

The tube test correlates well with the TCP test for biofilm producing isolates but it was difficult to discriminate between weak and biofilm negative isolates due to the variability in observed results by different observers. Consequently, high variability was observed and classification in biofilm positive and negative was difficult by tube method. In agreement with the previous reports, tube test cannot be recommended as general screening test to identify biofilm producing isolate [20].

The antibiotic sensitivity test was applied on all isolated microorganisms using commercially available disks. As indicated in Fig.(2), All 9 isolates were sensitive to the new IPM and AZM, whereas all isolates where resistant to T,FOX,AMC,PRL, CX,OX,MA, CFM, DO While showed variable resistant to other antibiotics used.

Our results also showed an increased spectrum of antibiotics resistant pattern of *S. marcescens* in infected individuals. The antibiotic resistance pattern varied within the

age groups. These results were similar to [28]. The showed multiresistant against (4-8) antibiotics, all isolates were resistant by (%100) against Cefuroxime, Cefixime, Cephalothin and Penicillin, while Cephotaxime, Azteroname, Imipeneme and Ciprofloxacin were sensitive by (%100).

Our results showed high multidrug resistance to antibiotic used in this study. Among 9 isolates, 4 isolates (S7,S1,S3,S2) had high resistance to 14,15,16 and 17 antibiotic respectively. While reset showed resistant to 9 different antibiotics (Table (4)). Horcajada *et al*. [37]. Described an outbreak of multi drug resistant *S.marcescens* Infection and colonization involving adults admitted to surgical intensive care unit.

MICs and MBCs recorded for the tested antimicrobial agents against the four S. marcescens isolates showed biofilm formation. The biofilm MICs were found to be much higher than the planktonic MICs. These results are shown in Fig.(1). In case of Lomofloxacin, the biofilm MBC was 3 to 64 times higher than the planktonic MIC. Also, the biofilm MBC for Cefotaxime was 2 to 16 times higher than that of the planktonic culture, whereas, the biofilm MIC in case of amoxicillin-clavulanic acid was 1 to 4 times higher than that of the planktonic culture. Variable behavior in biofilm formation for the 4 *S. marcescens* isolates was observed in biofilm and planktonic cultures; no fixed increase was noticed for all strains with the different antibiotics used.

Table (3)

Number of multidrug resistant S. marcesans.

Bacterial strain	***No. of antibiotics resist***	***Antibiotics***
S_1	15	K,T,DO,CFM,FOX,MA,OX,CX,PRL,AMC,CRO,CTX,CAZ,CZC,FEP
S_2	17	K,T,DO,CFM,FOX,MA,OX,CX,PRL,AMC,CRO,CTX,CAZ,CZC, FEP,LOMO,LEV
S_3	16	K,T,DO,CFM,FOX,MA,OX,CX,PRL,AMC,CRO,CTX, CZC,FEP, LOMO,LEV
S_4	9	T,DO,CFM,FOX,MA,OX,CX,PRL,AMC
S_5	9	T,DO,CFM,FOX,MA,OX,CX,PRL,AMC
S_6	9	T,DO,CFM,FOX,MA,OX,CX,PRL,AMC
S_7	14	K,T,DO,CFM,FOX,MA,OX,CX,PRL,AMC,CRO,CTX, CAZ,FEP
S_8	9	T,DO,CFM,FOX,MA,OX,CX,PRL,AMC
S_9	9	T,DO,CFM,FOX,MA,OX,CX,PRL,AMC

K=Kanamycin, T=Oxytetracyclin, AZM=Azithromycin, LEV=Levofloxacin, LEM=Lomefloxacin, DO=Doxcycline, MA=Cfamandole, FEP=Cefepime, CFM=Cefixime, CX=Cloxacillin, PRL=Pipracillin, OX=Oxacillin, IPM=Imipenem, AMC=Amoxicillin+clavulanic acid, CTX=Cefotaxime, CRO=Ceftriaxone

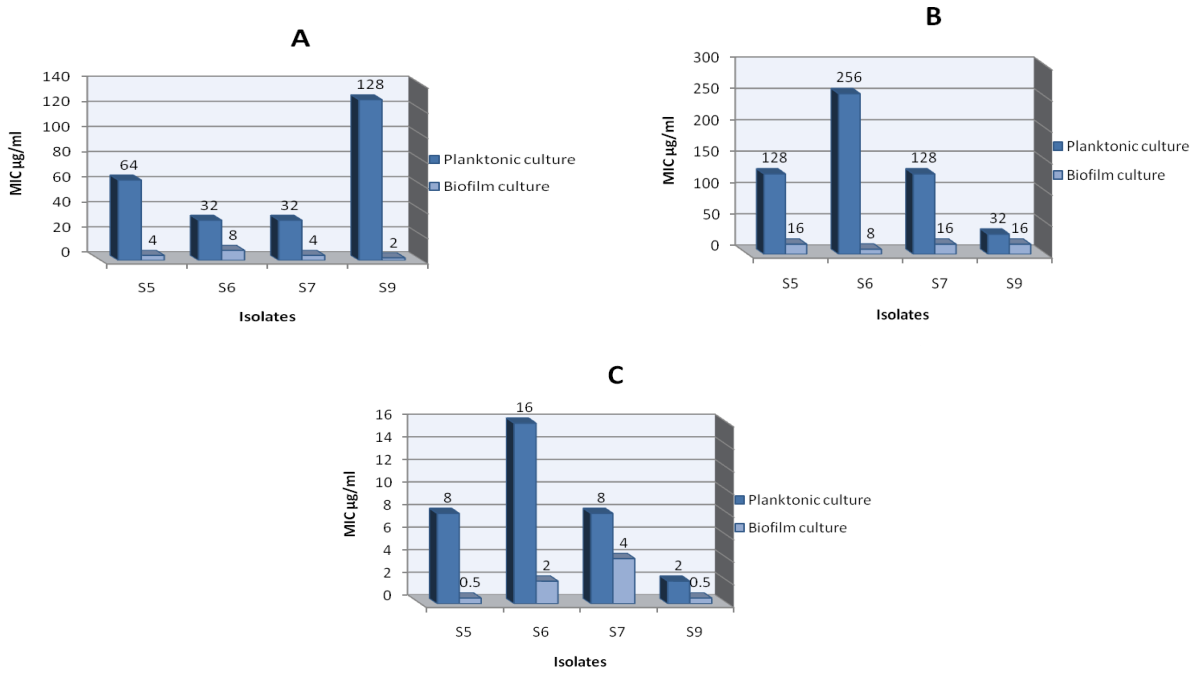

Fig. (2) Comparison between Planktonic and Biofilm MIC of Lomofloxacin (a), Cefotaxime (b), and amoxicillin-clavulanic acid (c) for the 4 S. marcescens.

In the present study, the effects of several antibiotics on *S. marcescens* adherence were tested. The studied antibiotics were chosen for several reasons. Amoxicillin-clavulanic acid and Cefotaxime was tested because it is frequently used in the therapy *S. marcescens* infections. Quinolone (Lomofloxacin) was chosen because of their interesting activity against gram-negative [38] bacterial biofilms.

At least three mechanisms have been proposed to account for recalcitrance of biofilms to antimicrobial agents: (i) failure of the antimicrobial to penetrate the biofilm, (ii) slow growth and the stress response, and (iii) induction of a biofilm phenotype [39].

In this study, cell viability was also measured by determination of biofilm MIC, which was found to be very high (64× planktonic MIC) in case of the isolates S9 when tested with Lomofloxacin. However, in other cases the difference between biofilm and planktonic MIC is much less (3 ×planktonic MIC) in case of the isolate S7 when tested with Lomofloxacin. (Fig.(2)) These results was also noticed by other *S. marcescens* in study of [40] they noticed that MIC was confirmed tobe higher for the biofim than planktonic cells .also *Pseudomonas aeruginosa* in biofilm showed a 4-fold greater resistance against ciprofloxacin and gentamycin compared with free-living forms [41]. The medium with antibiotic used not inhibited the prodigiosin production at the MIC for planktonic cells. Therefore prodigiosin production in media with antibiotics used not marker of the growth and activity of bacterial cells. Hirata *et al*. [40] showed in medium with Fosfomycin and Imipenem the prodigiosin production inhibited at a concentration much lower than the MIC for planktonic cells while in case of Ampicillin prodigiosin production inhibited at the MIC for planktonic cells.Biofilms are considerably less susceptible to antibiotics than their planktonic counterparts; biofilm production may vary largely among different strains of the same species isolated from different sites. Although the ability to form biofilm may contribute to the pathogenicity of this organism, its clinical importance in our series seems to be marginal [19].

References

[1] Grimont, PAD.; Grimont, F.; Dulong, HLC.; Rosnay, De and Sneath, PHA. "Taxonomy of the genus *Serratia*." J. Gen. Microbiol. 98: 39.1977.

[2] Hejazi, A. and Falkine, FR. "*Serratia marcescens*." J Med Microbiol, 46: 903–1012. 1997.

[3] Auken, H.M. and Pitt, T.L. "Antibiotic resistance and putitive virulence factors of *Serratia marcescens* with respect to O and K serotype." J.Med. Microb. 47(12):1105-1113. 1998.

[4] Liu, P.Y.F.; Lau, Y.J.; Hu, B.S.; Shyr, J.M.; Shir,J.M.; Cheung,M.H.; Shi, Z.Y. and Tsai, W.S. "Use of PCR To Study Epidemiology of *Serratia marcescens* Isolates in Nosocomial Infection. "J. Clin. Microbiol., 32:1935–11938.1995.

[5] Matsuo, T.; Chen, J.; Minato, Y.; Ogawa, W.; Mizushima, T.; Kuroda, T. and Tsuchiya, T. SmdAB, "A Heterodimeric ABC-Type Multidrug Efflux Pump, in *Serratia marcescens*. ″ J. of Bacteriol., 190(2):648-654. 2008.

[6] Acar,J.F. "*Serratia marcescens* infections." Infect Control., 7: 273-278.1986.

[7] Queenan, A.M.; Viera, C.T.; Gold, H.S.; Carmeli, Y. and Bush, K. "SME-type carbapenem-hydrolyzing class-A β-lactamases from *Serratia marcescens* strains." J. Antimicrob. Agent. Chemoth. ″44(11): 3035-3039. 2000.

[8] Lai, K.K.; Baker, S.P. and Fontecchio, S.A. "Rapid eradication of a cluster of *Serratia marcescens* in a neonatal intensive care unit: use of epidemiologic chromosome profiling by pulsed-field gel electrophoresis." Infect. Control Hosp. Epidemiol., 25(9):730-734.2004.

[9] Abdollahi, A.; Nasirpour, S.; Shahmohammad, N. and Zolfaghari A. "*Serratia marcescens*, an Opportunistic Gram Negative Infection in Cardiac Valve Surgery Iranian." Journal of Pathology, 3 (1), 40 – 42. 2008.

[10] Metintas, S; Akgun, Y; Durmaz, G Kalyoncu, C. "Prevalence and character-istics of nosocomial infections in a Turkish university hospital." American Journal of Infection Control, 32:409–413.(2004).

[11] Trias, J.; Vifias, M.; Guinea, J. and Loren, J. G. "Brown pigmentation in *Serratia marcescens* cultures associated with tyrosine metabolism." Can. J. Microbiol., 35:1037-1042.1989.

[12] Kalvioda, EJ.; Stella, NA. ; Aston, MA.; Fender, JE; Thompson, PP.; Kowalski, RP.; Shanks, RM. "Cyclic AMP negatively regulates prodigiosin production by *Serratia marcescens*. " Research in Microbiology. 161(2):158-67. 2010 .

[13] Chen, L. and Wen, Y.M. "The role of bacterial biofilm in persistent infections and control strategies. "Int J Oral Sci .,3: 66-73. 2011.

[14] Adegbola, R. A., and Old, D. C. "New fimbrial hemagglutinin in *Serratia* species. Infect. "Immun. 38:306–315. 1982.

[15] Carbonell, GV.; Della Colleta, HH. ; Yano, T.; Darini, AL.; Levy, CE. and Fonseca, BA. "Clinical relevance and virulence factors of pigmented *Serratia marcescens*." FEMS Immunol Med Microbiol., 28, 143–149. 2000.

[16] Snelling , A.M.; Smidet , P.G.; Hawkey , P.M.; Heritage, J.; Parnell,C.; Bodenham, A.R. ; and Inglis , T. "Validation of use of whole-cell repetitive extragenic palindromic sequence-based PCR (REP-PCR) for typing strains belonging to the *Acinetobacter calcoaceticus*-Acinetobacter baumannii complex and application of the method to the investigation of a hospital outbreak." J.Clin. Microbiol. 34 (5): 1193 1202. 1996.

[17] Coulthurst, S. J., Williamson, N. R., Harris, A. K., Spring, D. R. & Salmond, G. P. C. "Metabolic and regulatory engineering of *Serratia marcescens*: mimicking phage-mediated horizontal acquisition of antibiotic biosynthesis and quorum sensing capacities." Microbiology, 152, 1899-1911. 2006.

[18] Yah, S. C.; Eghafona, N. O. and Forbi J. C. "Plasmid-borne antibiotics resistant markers of *Serratia marcescens*: an increased prevalence in HIV/AIDS patients. "Scientific Research and Essay , 3 (1): 028-034. 2008.

[19] Pinna, A.; Usai, D.; Sechi, LA.; Molicotti, P; Zanetti, S. & Carta, A. "Detection of virulence factors in *Pseudomonas aeruginosa* strains isolated from contact "

[20] Christensen, G.D.; Simpson, W.A. Bison, A.L. and Beachey, E.H. "Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces." Infect. Immun., 37: 318-326. 1982.

[21] Clinical and Laboratory Standards Institute (CLSI): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. CLSI document M7-A7, approved standard. 7th edition. Wayne, PA; CLSI., 2004.

[22] Ceri, H.; Olson, M.; Morck, D.; Storey, D.; Read, R.; Buret, A.; Olson, B. "The MBEC Assay System: multiple equivalent biofilms for antibiotic and biocide susceptibility testing." Methods Enzymol., 337:377-385. 2001.

[23] Tariq, A. L. and Prabakaran, J. J. "Molecular Characterization of Psychrotrophic *Serratia marcescens* TS1 Isolated from Apple Garden at Badran Kashmir." Research Journal of Agriculture and Biological Sciences, 6(3): 364-369. 2010.

[24] Manikandan, S.; Ganesapandian, S.; Singh, M. and Kumaraguru, A.K. "Antimicrobial Susceptibility Pattern of Urinary Tract InfectionCausing Human Pathogenic Bacteria." Asian Journal of Medical Sciences 3(2): 56-60, 2011.

[25] Williams, RP. and Hussain Quadri, SM. "The pigment of *Serratia*. CRC press, Bocarato", USA., 31-75. 1980.

[26] Pryce, LH. and Terry, FW. "Spectrophotometric assay of gene expression: *Serratia marcescens* Pigmentation." Bioscene. 26(4):3-13. 2000.

[27] Elston, H. R. A. "bacteriological study of non-chromogenic variants of *Serratia marcescens* from human sources." J. clin. Path., 18, 618-621. 1965.

[28] Ali, M.R.; Hussin, M. S. and Kadum, M. M. " Study of prodigiosin and virulence factor producing by multi drug resistance *Serratia marcescens* isolated from some of Baghdad hospitals environment. " Al-Mustansiriya J. Sci., 19(5):1-9.2008.

[29] Assadian, O.; Berger, A.; Aspock, C.; Mustafa, S.; Kohlhauser, C.; Hirschl ,AM. "Nosocomial outbreak of Serratia marcescens in a neonatal intensive care unit." Infect Control Hosp Epidemiol., 23: 457-461. 2002.

[30] Fleisch, F.; Zimmermann-Baer, U.; Zbinden, R. et al. "Three consecutive outbreaks of Serratia marcescens in a neonatal intensive care unit." Clin Infect Dis., 34:767-773.2002.

[31] Hilger, M. & Braun, V. "Superlytic Hemolysin Mutants of Serratia marcescens." J. of Bacteriol., 177(24): 7202–7209.1995.

[32] Gohl, O.; Friedrich, A.; Hoppert, M. and Averhoff, B. "The thin pili of Acinetobacter sp. Strain." Appl. Env. Microbiol. 72(2):1394-1401. 2006.

[33] Sundaramoorthy, N.; Yogesh, P. and Dhandapani, R. "Production of prodigiosin from *Serratia marcescens* isolated from soil." Indian Journal of Science and Technology. 2 (10): 35-37. 2009.

[34] Oller, A. R. "Media effects of sugars on pigmentation and antibiotic susceptibility in *Serratia marcescens*." Sci. & Technol., Transac. of Missouri Acad. Sci. 243-246. 2005.

[35] McLaughlin-Borlace, L.; Stapleton, F. Ma; theson, M. & Dart, J. K. Bacterial biofilm on contact lenses and lens storage cases in wearers with microbial keratitis. ″ J Appl Microbiol., 84, 827–838. 1998.

[36] Bonaventura, GD.; Spedicato, I.; D.'Antonio D.; Robuffo, I.; Piccolomini, R. "Biofilm Formation by *Stenotrophomonas maltophilia*: Modulation by Quinolones, Trimethoprim-Sulfamethoxazole, and Ceftazidime. " Antimicrob. Agents Chemother., 48: 151-160. 2004.

[37] Horcajada, JP.; Martinez, JA.; Alcon, A. et al. "Acquisition of multidrug-resistant *Serratia marcescens* by critically ill patients who consumed tap water during receipt of oral medication. "Infect. Control. Hosp. Epidemiol. : 27(7):774-777. 2006.
[38] Baskin, H.; Dogan, Y.; Bahar, IH.; Yulug, N. "Effect of subminimal inhibitory concentrations of three fluoroquinolones on adherence of uropathogenic strains of *Escherichia coli*. Int." J. Antimicrob. Agents, 19: 79–82. 2002.
[39] Lai, K.K.; Baker, S.P. and Fontecchio, S.A.(2004). "Rapid eradication of a cluster of *Serratia marcescens* in a neonatal intensive c are unit: use of epidemiologic chromosome profiling by pulsed-field gel electrophoresis." Infect. Control Hosp. Epidemiol., 25(9):730-734.
[40] Hirata, T.; Kitahara,E. and Nakagawa,Y. "Novel screening of antibiotic–sensitivity biofilm-forming mutants by utilizing aproduct of *Serratia marcescens.*" Microbes. Environ.21(3):148-153.2006.
[41] Agarwal, G. ;Kapil, A.; Kabra, SK. ; Das, BK. and Dwivedi SN. "In vitro efficacy of ciprofloxacin and gentamicin against a biofilm of *Pseudomonas aeruginosa* and its free-living forms." Natl. Med. J. India, 18(4): 184-186. 2005.

الخلاصة

جمعت 150 عينة من الدم و الادرار و بمعدل 1:1.45 نسبة الذكور الى الاناث. بينت الدراسة ان نسبة العينات الموجبة للزرع كانت اكثر تردد في حالات التهاب المسالك البولية بمعدل (77%)، تبعتها حالات التهاب الدم بنسبة (18%)، من مجموع 86 (57.33%) عزلة بكتيرية تم الحصول عليها. ركزت الدراسة على 9 عزلات سريرية فقط تعود لبكتريا *Serratia marcescens* (15.2%) قسمت الى منتجة لصبغة البرودجيوسين (11.6%) و غير منتجة للصبغة (3.6%). معظم العزلات (9/6) اظهرت نمواً واضحاً عند 37°م. جميع العزلات البكترية *Serratia marcescens* ومن المصادر المختلفة كانت محللة للدم، في حين اظهرت 7 عزلات فقط (77.77%) قدرة على تكوين الغشاء الرقيق. تم قياس قدرة البكتريا على الغشاء الرقيق من خلال طريقة صبغة الكرستال فيوليت بطريقة صفيحة الزرع النسيجي اذ لوحظ من بين 9 عزلات لبكتريا 5 *Serratia marcescens*منها لم تظهر القدرة على تكوين الغشاء الحيوي، و 4 عزلات اظهرت قدرة ضعيفة على تكوينه، بينما لم تظهر اي عزلة قدرة عالية على تكوين الغشاء الحيوي. في حين اظهرت طريقة الانابيب لتكوين الغشاء الحيوي قدرة اوضح مقارنة بطريقة صفيحة الزرع النسيجي، اذ اظهرت 2 عزلة قدرة عالية و 5 عزلات قدرة متوسطة على تكوين الغشاء الحيوي. اظهرت طريقة الانابيب نتائج مقاربة مع طريق TCP للتحري عن الغشاء الحيوي ولكنها كانت صعبة في التمييز بين العزلات الضعيفة وغير المنتجة للغشاء الحيوي. جميع العزلات اظهرت حساسية لمضادات الاميبنيم و الازتريونام وبنسبة 100%. بينت النتائج ان التركيز المثبط الادنى للعزلات المكونة للغشاء الحيوي اعلى بكثير عن العزلات الخضرية. من جهة اخرى لم تؤثر الاوساط الحاوية على المضادات المختلفة قدرة تثبيط لانتاج صبغة البرودجيوسين للخلايا الخضرية، لذلك انتاج الصبغة في الاوساط الحاوية على المضادات لا يعد دليلاً على نمو وفعالية الخلايا لبكتيرية. اظهرت الخلايا المكونة للغشاء الحيوي اقل حساسية للمضادات الحيوية عنة في الخلايا الخضرية، وهذه القدرة على الانتاج كانت متغايرة بصورة كبيرة بين السلالات المختلفة لنفس الانواع المعزولة من الاماكن المختلفة.