Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(11); 1392-1397
doi: 10.25258/phyto.v9i11.11180
ISSN: 0975-4873

Research Article

Phenotypic and Genotypic Characterization of *Serratia marcescens* from Clinical and Environmental Sources

Gayathri N, R Subashkumar*

PG & Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore-641 029. TN, India

Received: 13th Aug, 17; Revised 4th Nov, 17, Accepted: 14th Nov, 17; Available Online:25th Nov, 17

ABSTRACT

S.marcescens is opportunistic pathogens that cause nosocomical infections. There have been many reports concerning the identification, antibiotic susceptibility, pathogenicity, epidemiological investigations and typing of this organism. Accurate identification is important in defining outbreaks. In the present study, *S. marcescens* have been selected from the natural sources and were then confirmed with 16S rRNA identification method with cultures of MTCC. To investigate these *S. marcescens* isolates, random amplification of polymorphic DNA (RAPD) was used in conjunction with the specific primers. The RAPD patterns for each isolate were identified on the basis of identical numbers and sizes of the bands. And by using dendrogram we can able to show, that the pattern of the primers to separate isolates was closely related to their sequence homology with the genome and their amount of guanine and cytosine nucleotide content. *S. marcescens* as a pathogen shows a tissue-damaging capacity. The cytotoxic activity is mainly elicited by the secreted hemolysin/cytotoxin ShlA. Here, the isolates were tested for hemolytic activity. *S. marcescens* is known to be frequently resistant to various antibiotics and our results also showed such a tendency.

Keywords: *Serratia marcescens*; 16SrRNA; RAPD; Pathogenicity; Antibiotic susceptibility; Phosphatase activity; Hemolytic analysis.

INTRODUCTION

S. marcescens is ubiquitous. It is commonly found in soil, water, plants, and animals[1]. It is widely present in non-potable water in under developed countries due to poor chlorination. Optimally, it grows at 37°C, but it can also grow in temperaturs that range from 5 to 40°C. They grow in pH levels that range from 5 to 9. *S. marcescens* is well known for the red pigmentation it produces called prodigiosin. Prodigiosin is made up of three pyrrole rings[2]. and is not produced at 37°C, but at temperatures below 30°C[3]. The red pigment production is not present in all strains but in those that it is present, it can resemble blood. It is a pathogenic microorganism; the mode of transmission is by either direct contact, or by catheters, droplets, saline irrigation solutions and other solutions that are believed to be sterile. It is resistant to many antibiotics traditionally used to treat bacterial infections. It has been implicated as an etiological agent in every conceivable kind of infection, including respiratory tract infection, urinary tract infection, septicemia, meningitis and wound infections[4,5,6,7,8]. It has been reported to cause infective endocarditis acquired in the community[9] and in hospitals[10,11,12]. Therefore, more research on this organism will be relevant for their control and management strategies. The objective of the study is to perform the phenotypic and genotypic characterization of *S. marcescens* of clinical and environmental samples.

MATERIALS AND METHODS

Collection and Isolation of Serratia sp.

Serratia sp. was isolated from water, sewage, food, soil and clinical samples. In the present study, *Serratia marcescens* was targeted by enriching on nutrient broth and selectively grown on nutrient agar medium. Phenotypic and genotypic identification methods were employed to characterize the isolated strains. Biochemical profile of the isolated strains studied by the standard methods with reference to the Berge's manual classification of Bacteria.

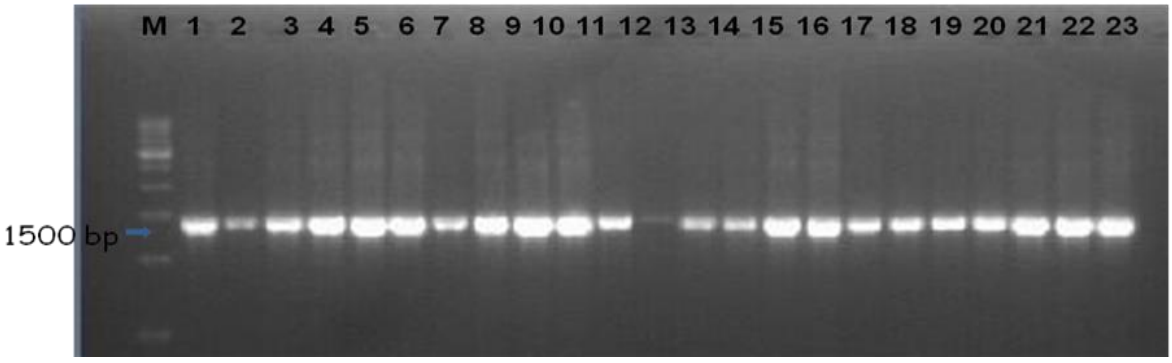
Morphological identification of Serratia sp.

Initially, the isolates were selected based on colony morphology and pigmentation. All the isolates showed convex, opaque centre effuse with almost transparent periphery and irregular with differencing pigmentation (colourless, orange and pink) was noticed. Similarly results have been reported by Tariq and Prabakaran who isolated *Serratia* from apple garden[13].

Motility assay

The motility of the organism was microscopically identified by hanging drop (wet-mount) method. Vaseline was placed on four edges of the clean coverslip. A loop full of culture broth was placed on the center of the coverslip. A clear concavity slide was placed upside down (concavity down) over the coverslip and sealed. The slide was turned over, was placed under the microscope and the edge of the drop was focused to observe the bombardment of the cells towards the edges of the drop.

*Author for Correspondence: rsubashkumar@gmail.com



Lane 1- SM01, Lane 2- SM04, Lane 3- SM05, Lane 4- SM06, Lane 5- SM07, Lane 6- SM09, Lane 7- SM10, Lane 8- SM11, Lane 9- SM15, Lane 10- SM16, Lane 11- SM17, Lane 12- SM18, Lane 13- SM21, Lane 14- SM22, Lane 15- SM24, Lane 16- SM27, Lane 17- SM31, Lane 18- SM33, Lane 19- SM36, Lane 20- SM38, Lane 21- SM40, Lane 22- SM42, Lane 23- SM43,

Figure 1: Identification of *S. marcescens* by 16S rRNA – PCR method.

Lane 1- SM01, Lane 2- SM04, Lane 3- SM05, Lane 4- SM06, Lane 5- SM07, Lane 6- SM09, Lane 7- SM10, Lane 8- SM11, Lane 9- SM15, Lane 10- SM16, Lane 11- SM17, Lane 12- SM18, Lane 13- SM21, Lane 14- SM22, Lane 15- SM24, Lane 16- SM27, Lane 17- SM31, Lane 18- SM33, Lane 19- SM36, Lane 20- SM38, Lane 21- SM40, Lane 22- SM42, Lane 23- SM43,

Figure 2: RFLP analysis of 16S rRNA product of *S. marcescens isolates.*

PCR amplification of 16S rRNA gene of S. marcescens

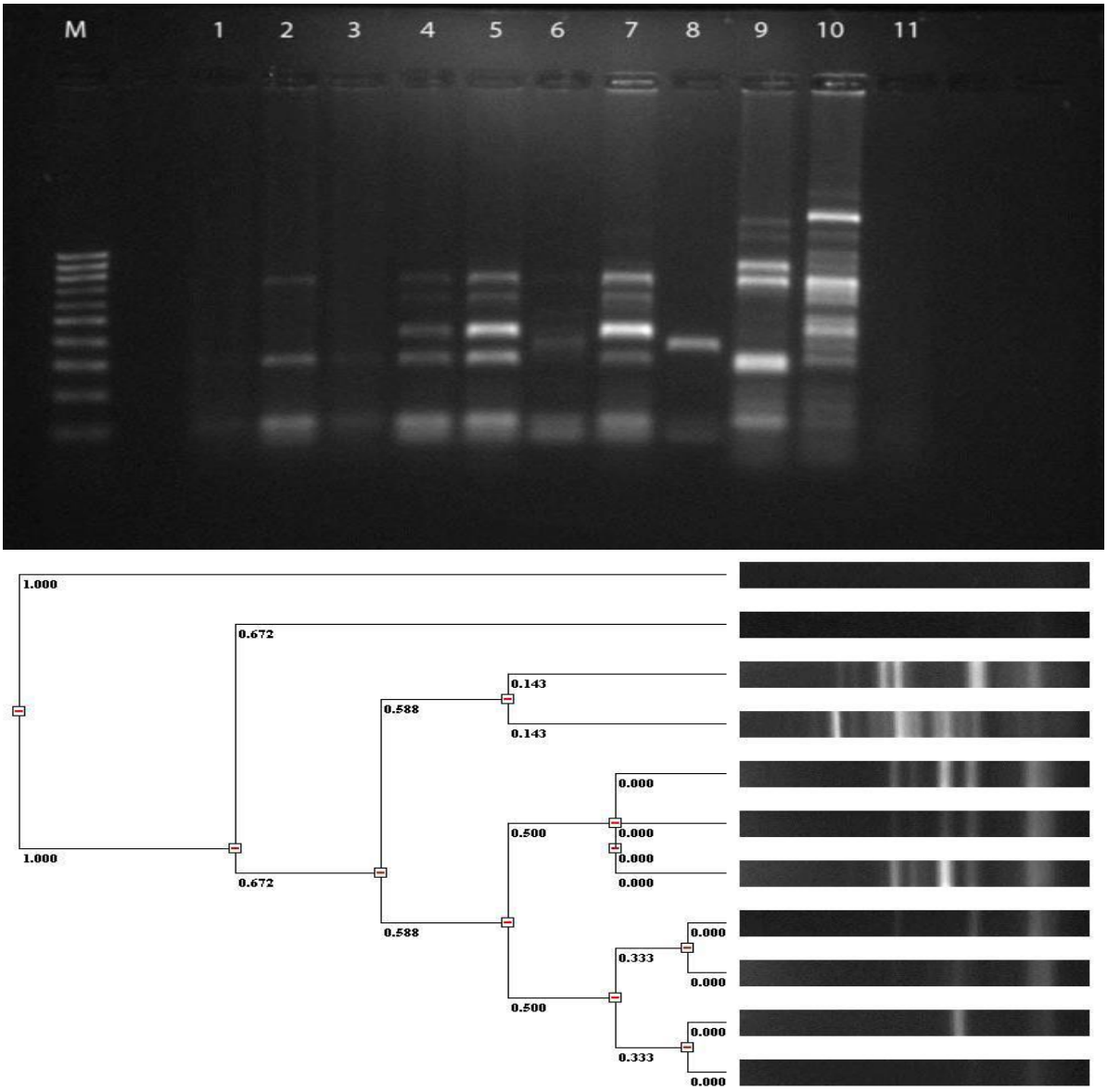
DNA isolation

For preparing genomic DNA, bacterial cultures in 1.5 mL of Nutrient broth was harvested by centrifugation at 13,000 rpm for 2 min at 4 °C using microcentrifuge tubes. The supernatant was discarded and the pellet was suspended in 400 µL of STE buffer (100mM NaCl; 100mM Tris HCl; 1mM EDTA pH 8.0), vortexed well and centrifuged at 12,000 rpm for 3 min at 4 °C. The supernatant was discarded and to the pellet 200 µL of TE buffer (10mM Tris HCl; 1mM EDTA) and 100 µL of Tris saturated phenol (Add equal volume of 1M Tris HCl pH 8.0 to liquid phenol and mix with glass rod, allowed to settle separate the phenol layer and store for further use) was added, vortexed well and the contents were centrifuged at 14,000 rpm for 5 min at 4 °C. Only 160 µL of aqueous phase was transferred to fresh microcentrifuge tube, to that 40 µL of TE buffer and 100 µL of choloroform was added, vortexed well. The contents were centrifuged at 14,000 rpm for 5 min at 4 °C. About 150 µL of aqueous phase was transferred to fresh microcentrifuge tube, to that 300 µL of ice cold isopropanol was added and incubated in room temperature for 5 min. The contents were washed with 150 µL of 70 % ethanol (70mL of ethanol and 30mL of distilled water) and contents were centrifuged at 12,000 rpm for 5 min at 4 °C. The supernatant was discarded carefully and the pellet was air dried at 37 °C for 5 min. The DNA was again dissolved in a 30 µL of TE buffer. This was stored at -20 °C for PCR analysis.

Primer designing

Unique primers were designed for the amplification of the genes coding for the 16S rRNA of *S. marcescens.* All the available partial and full length gene sequences for a given gene were determined. Primers were designed from the conserved regions. The primers were then searched for their uniqueness and specificity to the respective 16S rRNA gene from different *S. marcescens* only by using the basic local alignment search tool (BLAST). A 1700 bp fragment from the *S. marcescens* encoding 16S rRNA gene was identified by using a primer set. The forward primer is 5' – CGGACGGGTGAGTAATGT – 3' and the reverse primer was 5' – GCAGGTTCCCCTACGGTT - 3'. The PCR reaction was performed and the sizes of the amplification products were determined by comparison with 500 bp DNA ladder. Bands were scored as present or absent.

Genotypic characterization of S. marcescens



Lane 1- SM01, Lane 2- SM04, Lane 3- SM05, Lane 4- SM06, Lane 5- SM07, Lane 6- SM09, Lane 7- SM10, Lane 8- SM11, Lane 9- SM15, Lane 10- SM16,

Figure 3: RAPD-PCR profile and dendrogram analysis of *S. marcescens.*

16S rRNA-RFLP PCR profile of S. marcescens: The RFLP analysis was performed by adding EcoR I, Sma I, EcoR I specific buffer and Sma I specific buffer to 16S rRNA product. The tubes were then incubated at 37°C for 30 min. The final product was separated and visualized using agarose gel with reference to the standard DNA marker. Restriction fragments were scored based on the banding pattern.

RAPD-PCR fingerprinting analysis of S. marcescens: The RAPD – PCR analysis was performed by adding template DNA, V-RAPD primer, primer S1 5'-CCGCAGCCAA-3', S2 5' –TGCCGAGCTG-3', V1 5'-CCGCAGCCAA-3' and Y1 5'-CCGCAGCCAA-3' Y2 5'-AGTGACCCAC-3', master mix and nuclease free water for 10 µl of reaction. The PCR product was electrophoresed using 1.5% agarose gel along with DNA molecular weight marker. The sizes of the amplification products were determined by comparision with 100 bp and 500 bp DNA ladder. Bands were scored as present or absent. Based on the banding pattern dendrogram was performed.

Phylogenetic analysis of RAPD PCR profiles

The length of the both RAPD PCR gel was normalized and molecular size of the fragments were compared independently (data not shown), but data for all molecular markers were combined in a final analysis. Each amplified product revealed by electrophoresis was recorded as binary data, presence (1) and absence (0): an initial matrix of 0 and 1 was constructed. The similarity of DNA pattern was measured by simple matching and clustering was achieved by unweighed pair group method using average linkage (UPGMA). Dendrogram branches with bootstrap values higher than 50% and relationships on the right side of vertical line were considered

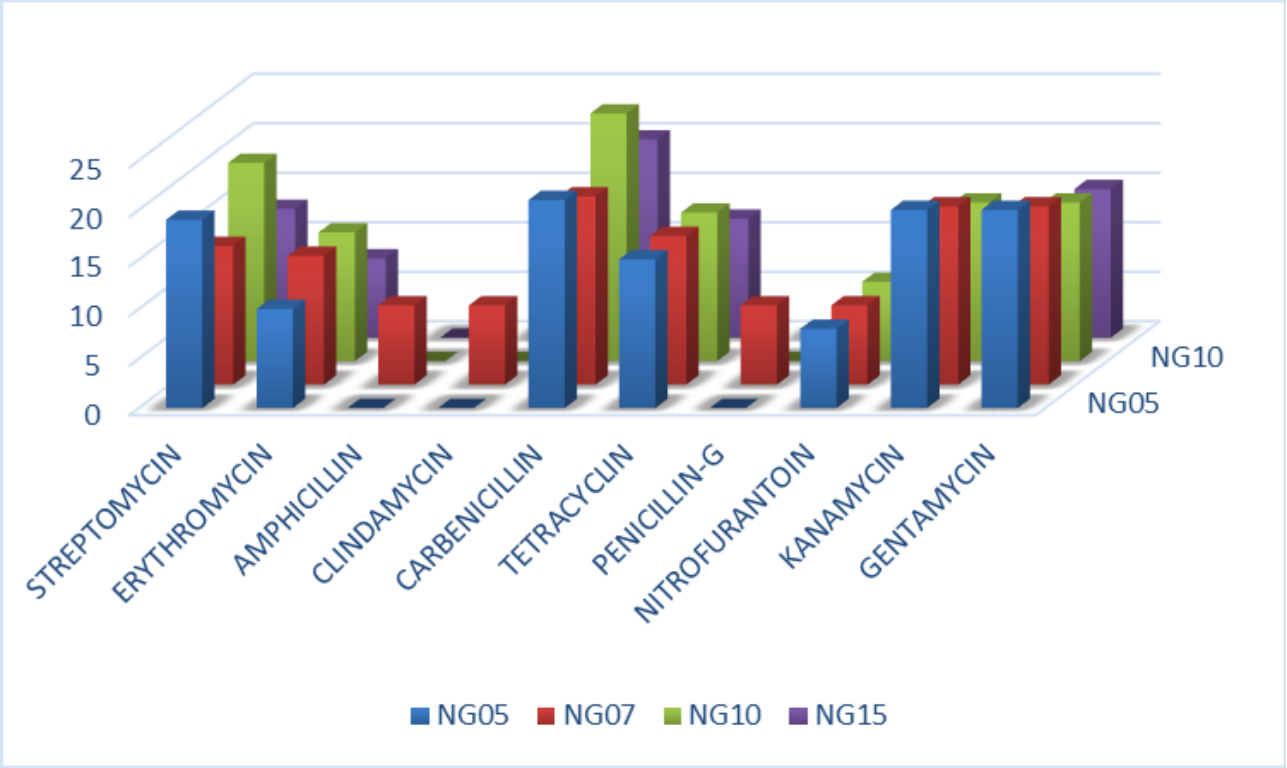


Figure 4: Antibiotic resistant pattern of the *Serratia marcescens* isolates.

significantly supported. All of these analyses were made with the NTSYS-PC software, version 2.02j and PHY-LIP version 3.6 software. The genetic diversity of all the strains was estimated in combined dendrogram of RAPD PCR.

Hemolytic analysis of S. marcescens

Blood agar plates were prepared by adding 5ml of human blood for every 100ml of nutrient agar medium. The loop full of *S. marcesccens* isolates were then streaked on the blood agar plates. The plates were then incubated overnight at room temperature. The hemolytic activity of the isolates was then recorded by measuring the lysis of RBCs on plate.

Qualitative analysis of antibiotic susceptibility of S. marcescens isolates

Disc sensitivity testing was performed on Mueller-Hinton agar with the following antibiotics: streptomycin (25 µg/disc), tetracycline (30 µg), kanamycin (30 µg), gentamycin (10 µg), nitrofurantoin (300 µg), carbenicillin (10 µg), ampicillin (2 µg), penicillin-G (10 units), erythromycin (15 µg) and clindamycin (2 µg). The antibiotic resistance of the isolates was determined by measuring the zone of inhibition formed after the period of overnight incubation at room temperature.

RESULTS AND DISCUSSION

In the present study, the various sources of *Serratia marcescens* like water, sewage, food, soil and clinical samples (n=31) were collected from various locations. The samples were processed immediately and the percentage incidences of *Serratia* and *Serratia marcescens* were recorded in each sample. 21 out of 31 isolates proved as *Serratia* and *Serratia marcescens* based on the biochemical profiles.

PCR amplification of 16S rRNA gene of S. marcescens

Out of 31 isolates 21 were found to be *S. marcescens* and this is confirmed with 16S rRNA PCR method by targeting the 16S rRNA of *S. marcescens* using the species specific primer (fig 1). Significantly most of the isolates showed positive PCR results and amplicons determines the species specificity of the targeted bacterium with 16S rRNA specific DNA is a conserved region. Amplification of this gene in PCR using *S. marcescens* specific primer indicated the presence of 1500bp and on agarose gel for the amplification of partial 16S rRNA specific DNA of the species.

16S rRNA-RFLP PCR profile

Amplified 16S rRNA gene from *Serratia* isolates was digested with 2 restriction endonucleases in order to check the presence of recognition sites. The informative restriction endonucleases used were: *Eco*RI and *Sma*I. Digestion was performed for 30 min at 37°C. The digested fragments were separated by agarose gel electrophoresis (Fig 2). The size of DNA fragments were compared to the PCR marker (100+500 bp) run on the same gel.

RAPD-PCR fingerprinting of S. marcescens

Five different primers with different sequences were tested for RAPD-PCR assay. The suitability of each primer was examined on the basis of intensities and distributions of amplified bands. All the twenty three strains SM from water, sewage, food, soil and clinical samples were analyzed by RAPD-PCR using independently prepared DNA template. The mixture of all the five primers was choosen because it generated

distinctive bands reproducibily as shown in Fig. 3. The twenty three isolates showed one common banding pattern of 500bp in size. RAPD-PCR patterns of strain SM were different to eah other. RAPD-PCR method presented in this work is useful and results indicate that all the isolates showed polymorphism with around 16 bands ranging from 100 to 10000 bp. The molecular data obtained from the RAPD method were processed with simple co-efficient. The *r* and *P* values (*CCCr = 0.70953 and P = 0.0020*) have been calculated by UPGMA method. In the RAPD analysis 81 bands have been observed. Of which, 7 were polymorphic bands. All the *S. marcescens* have formed most variable groups. However, the genetic diversity of all the strains showed many clusters. None of the strains had genetically indistinguishable similarity. The molecular data indicate highest similarity between the strains from others. However there was significant level of genetic diversity noticed among *Serratia* sp.from water, sewage, food, soil and clinical samples.

Hemolytic activity of S. marcescens

Hemolytic activity was analysed for all the 23 isolated by streaking the culture on blood agar plates and incubating it overnight at room temperature. The pathogenic strains were found by the zones formed and the degree of pathogenicity was determined by analyzing the type of zone formed. The hemolytic patterns of the isolates were given in Fig 4. Among the 23 isolates four of the isolates (NG05, NG07, NG10 and NG15) showed hemolytic activity. In that, NG05 showed gamma type of hemolytic activity. NG07 and NG10 showed beta and NG15 showed alpha hemolytic activity respectively. So, these four strains were considered to be more pathogenic and were taken for further studies.

Qualitative analysis of antibiotic susceptibility of S. marcescens isolates

Antibiotic susceptibility was tested on Muller-Hinton medium using a paper disc according to the Kirby-Bauer method[14]. With respect to the inhibition zone, most of the bacterial strains were showed susceptible to the selected antibiotics. Intermediate pattern were scored as resistant. The strain *S. marcescens* NG15, was found to be significantly higher level of resistant pattern among the strains tested (Fig 5).

Four isolates were tested with ten antibiotics for their susceptibility. Fig 6 showed that isolates were resistant to 5 of the antibiotics (erythromycin, amphicillin, clindamycin, penicillin-G and nitrofurantoin), intermediate to 2 of the antibiotics (carbenicillin and tetracylin) and sensitive to 3 of the antibiotics (streptomycin, Kanamycin and gentamycin).

CONCLUSION

Serratia marcescens is emerging as an important opportunistic pathogen, particularly for immunocompromised patients and in ocular infections. This work presents Phenotypic and Genotypic characterization of *Serratia marcescens* from clinical and environmental sources. 16S rRNA is the specific identification method to determine species of a particular bacterium and *Archeae,* and is also specific DNA for conserved regions. Amplification of this gene of *S. marcescens* in PCR is 1500 bp. RAPD – PCR fingerprinting analysis of *S. marcescens* was done, and obtained different polymorphism of around 16 bands ranging from 100-10,000bp and further dendrogram was constructed by UPGMA. It concludes that the strains of *Serratia* are heterogenic among the source of isolation. The resistance of microbes towards various types of antimicrobial agents poses a serious threat to the pharmaceutical industries. The application of findings in this study with a view to preventing such microbes in food processing environment. Further study on this includes effective control strategies to prevent infection, effective treatment strategies for eradication and complete understanding.

REFERENCES

1. Grimont, P. A. D. and Grimont, F. 1984. Genus Serratia Bizio 1823, 288AL. In krig, N. R., and Holt, J. G. (ed.), Bergey's manual of systematic bacteriology, Vol. 2. Williams and Wilkins. Baltimore, 477-484.
2. Schlegel and Hans. 1992. General Microbiology. © Georg Thieme Verlag, Stuttgart. pp. 88.
3. Tanaka, Y., Yuasa, J., Baba, M., Tanikawa, T., Nakagawa, Y. and Matsuyama, T. 2004. Temperature-dependent bacteriostatic activity of Serratia marcescens. Microbes Environ., 19:236–240.
4. Gouin, F., Papazian, L. and Martin, C., et al. 1993. A non-comparative study of the efficacy and tolerance of cefepime in combination with amikacin in the treatment of severe infections in patients in intensive care. Journal of Antimicrobial Chemotherapy, 32:205-214.
5. Cox, C. E. (1985). Aztreonam therapy for complicated urinary tract infections caused by multidrug-resistant bacteria. Rev. Infect. Dis. 7 Suppl, 4:S767-71.
6. Korner, R. J., Nicol, A., Reeves, D. S, MacGowan, A. P. and Hows, J. 1994. Ciprofloxacin resistant Serratia marcescens endocarditis as a complication of non-Hodgkin's lymphoma. J Infect., 29:73-76.
7. Salgado, C. D., Calfee, D. P. and Farr, B. M. 2003. Interventions to prevent methicillin-resistant *Staphylococcus aureus* transmission in health care facilities: what works. Clin Microbiol Newslet, 25:137-44.
8. Godwin, P. G. R., Choyce, A. and McCarthy, S. 2001. The prevalence of MRSA carriage measured over five years in a District General Hospital. J Hospital Infect, 47:73-5.
9. Mills, J. and Drew, D. 1976. Serratia marcescens endocarditis: a regional illness associated with intravenous drug abuse. Ann Intern Med., 84:29–35.
10. Hall-Stoodley L., Costerton, J. W. and Stoodley, P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol, 2:95-108.
11. Kalivoda, E. J., Stella, N. A., O'Dee, D, M., Nau, G. J. and Shanks, R. M. Q. 2008. The cyclic AMP-

dependent catabolic repression system of *Serratia marcescens* mediates biofilm formation through regulation of type 1 frimbriae. Appl Environ Microbiol, 74:3461-3470.

12. Mah, T. F. and O'Toole, G. A. 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol, 9:34-39.
13. Tariq. A. L. and Prabakaran. J. J. 2010. 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.
14. Bauer, A. W., Kirby, M. M., Sherris, J.C. and Truck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol., 45: 493-6.