EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
361-366
Year: 2018

SERRATIA SPECIES PIGMENT- BIO COLOUR AS MOST NOVEL ANTIBACTERIAL AGENT AGAINST *STAPHYLOCOCCUS AUREUS* ISOLATE FROM COIN

K. Aishwarya* Ayisha Begum M. Achiffa Abdul Rahiman, Aeliya Zehra and M. R. Ushasri

P.G.Dept of Applied Microbiology, JBAS College for Women.

***Corresponding Author: K. Aishwarya**
P.G.Dept of Applied Microbiology, JBAS College for Women.

Article Received on 14/06/2018 **Article Revised on 05/07/2018** **Article Accepted on 26/07/2018**

ABSTRACT

S*erratia spp* are Gram-negative, rod-shaped cell, motile by means of peritrichous flagella. Serratia species originates in widespread environment, but are common component of human fecal flora. Serratia marcescens is the primary pathogenic species of serratia and sub- species such as S. Liquefaciens, S. Rubidae, S.odoriferae S.fonticola etc. They are the species of gram negative bacteria and it can be grown either in presence or absence of oxygen, which is classified as an opportunistic pathogen belonging to the family enterobacteriaceae. The aim of this work is to evaluate anti bacterial activity of crude ethanol pigment extract of *Serratia spp* against isolates from coins isolated from crowded population. Money could also carry microbes due to unhygienic conditions there by leading to transmission of various diseases. This transmission could be prevented by improvisation of health and hygiene and promoting e- transfer to avoid much usage of papers so as to economize by bringing paper less eco friendly environment. Maximum inhibition was found to be at different concentrations were found to be 15mm, 12mm, 10mm, and 9mm respectively. The current study reported that coin plays a vital role in transmission of infections from person to person as coin is exchanged among the people in society. It was concluded that Coin transport microbes which causes infections. The pigment extract acts as a novel bio colour against *Staphylococcus .aureus* isolated from coin.

KEYWORDS: Coin, *Serratia Spp* prodigiosin, Muller Hinton agar.

INTRODUCTION

Serratia species originates in widespread environment, but are common component of human fecal flora. Serratia marcescens is the primary pathogenic species of serratia and sub- species such as S. Liquefaciens, S. Rubidae, S. odoriferae S. fonticola etc. They are the species of gram negative bacteria and it can be grown either in presence or absence of oxygen, which is classified as an opportunistic pathogen belonging to the family enterobacteriaceae. It is an opportunistic pathogen and it is resistant to many antibiotics such as pencillin, ampicillin which is used to treat many bacterial infections, such as pencillin due to its outer membrane and its ability to survive in aerobic and anaerobic conditions. Serratia species is a gram negative bacterium and made up of single layer of peptidoglycan and it is enclosed in the outer layer of the cell membrane. The outer membrane has lipopolysaccharides, which are the special kind of phospholipids and it is composed of fatty acids and which are attached to glucosamine phosphate dimer. The outer membrane serve as to regulate the uptake of nutrients and in exclusion of toxins. Members of this genus produce characteristic red pigment called prodigiosin and can be distinguished from other members of Enterobacteriaceae family by their unique production of three enzymes: DNase, lipase and gelatinase. The prodigiosin pigment is made up of three pyrrole rings and its temperature is below 30.c. The prodigiosin is an alkaloid compound produced as secondary metabolite and it shows a wide variety of activities such as anti-bacterial, anti-fungal, anti-microbial etc. The production of prodigiosin is attained by using ordinary media under aerobic conditions and they show better production on synthetic, differential and selective media. The capryllate thallous [CT] agar a selective media which contains caprylate as a carbon source for serratia species and thallous which is a salt which act as a inhibitor for the other microorganisms. The powdered peanut broth acts as a supportive for better growth of serratia and gives higher yield of prodigiosin. It grows in the crude fatty acid sources like peanut, sunflower, coconut etc and it utilizes in the form of carbon sources and shows better prodigiosin production. It shows better growth in peptone glycerol broth and the fatty acid containing seed oil broth because of the presence of readily available carbon source for pigment enhancement. The antibacterial efficacy of prodigiosin pigment is seen against the coin isolates. The prodigiosin

has their synthetic derivatives which has an effective proapopoitic properties or agents which act against various cancer cell lines with multiple cellular targets which includes multi drug resistant cell which has no toxicity towards normal cell lines.

PIGMENT PRODUCTION

Natural pigments can be obtained from two primary sources either by plants and another by micro organisms. The natural pigment from plants has several draw backs like heat, instability to light, adverse pH, low water solubility .The pigment from the microbe has a great interest due to its stability and ease of cultivating methods, yielding the pigment production from microbes is done within the laboratory with few culture Media and cost effective and easy way of attaining the pigment. Micro organisms have easy and fast growth within culture medium and unaffected by the environmental conditions and even different shades of colour was produced hence it has many advantages and used in many emerging fields and for a variety of industrial applications. Natural pigments contain pro vitamin A and many strains possess beneficial properties such as anticancer activity and have so additional valuable activities .So the pigments from the microbial source can be used as an alternative to many synthetic colorants. Prodigiosin has many characteristics like anti microbial, anti cancer, anti immune suppressive anti fungal activities. The genus serratia produce a red pigment. Prodigiosin which are secondary metabolites which is characterized with unqiue tripyrrole structure and which is responsible for many pharmacological characteristics like anti oxidant etc and it is produced by gram negative and positive bacteria.

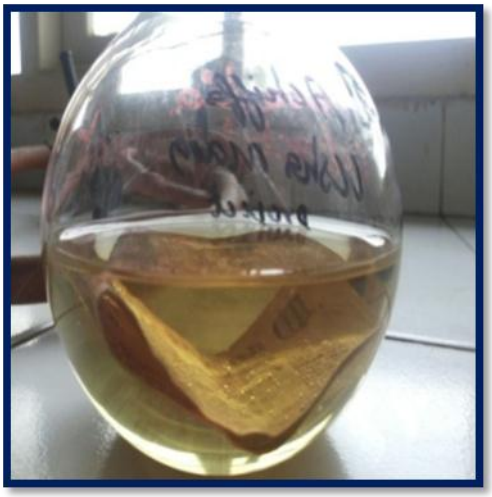
METHODOLOGY

SAMPLE COLLECTION

The currency note and coin were collected in crowded area from petty shop and transport in zip lock cover using sterile hand gloves and transferred to sterile broth for sample processing.

SAMPLE PROCESSING

The samples were then processed by inoculating into the nutrient broth and incubated for 24 hours at 37°C for determining the growth of organism followed by centrifugation. The organisms in the samples were then identified by microscopic, cultural and biochemical tests as *Staphylococcus aureus.*



Currency note in nutrient broth

Coin in nutrient broth

Identification of *Serratia species*

Serratia species was identified by Gram staining, Hanging drop, catalase, and oxidase tests. The cultural characteristics and biochemical characters were performed to identify *Serratia spec ies.*

Inoculation of Serratia species into Nutrient broth.

Incubation of pigmented broth in rotary shaker at 37◦c.

Red pigmented broth after 48 hrs.

Filtration of centrifuged supernatant broth.

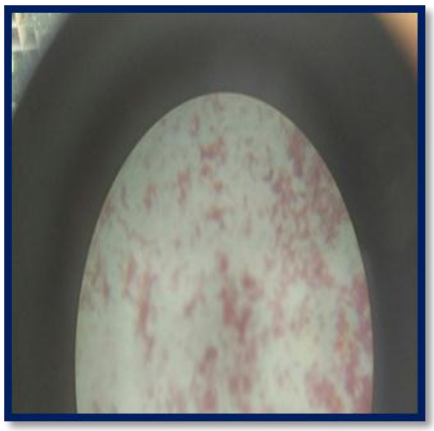
Extraction of pigment

The pigmented broth was transferred to sterile tubes and centrifuged for 30 mins at 1500 rpm. The supernatant was filtered by using sterile whats Mann filter paper. The filtrate was extracted using ethanol solvent. The filtrate was evaporated to extract crude pigment and stored in vials for antibacterial activity.

Crude ethanol pigment extract
Serratia species

MICROSCOPIC APPEARANCE

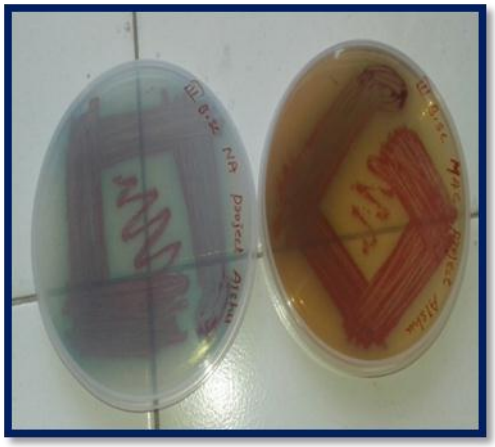
The inoculated colonies were observed for microscopic appearance and identified as motile gram negative rod bacilli.



Gram negative bacilli – serratia species

CULTURAL CHARACTERISTICS of *Serratia species*

Red pigmented colonies produced by *Serratia species* on Nutrient agar

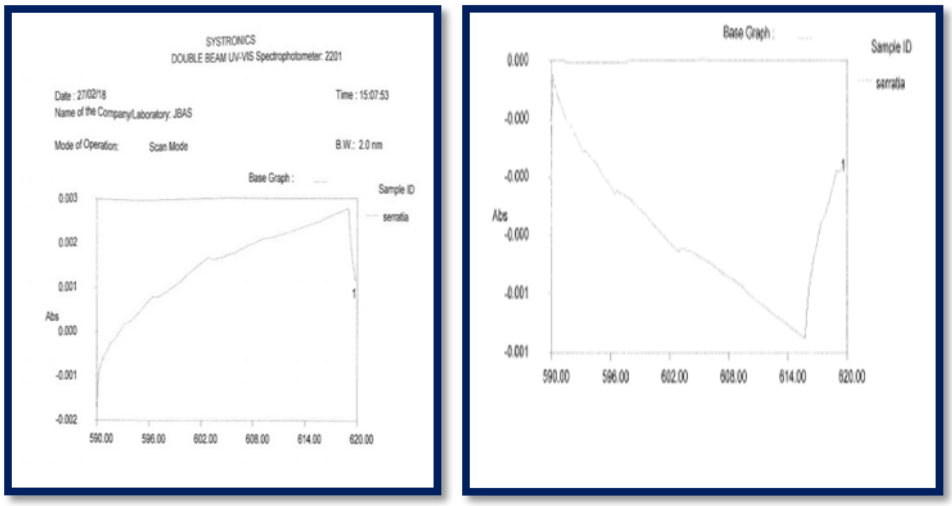


Preliminary Tests of serratia species

Sl. No	Tests	Observation
1.	Gram staining	Gram- negative bacilli
2.	Motility	Motile
3.	Catalase	Positive
4.	Oxidase	Negative

Colony characteristics

Sl.no	Colony morphology	Observation
1.	Size	2 mm
2.	Margin	Entire
3.	Shape and colour	Circular and red
4.	Opacity	Opaque
5.	Consistency	Smooth
6.	Elevation	Convex

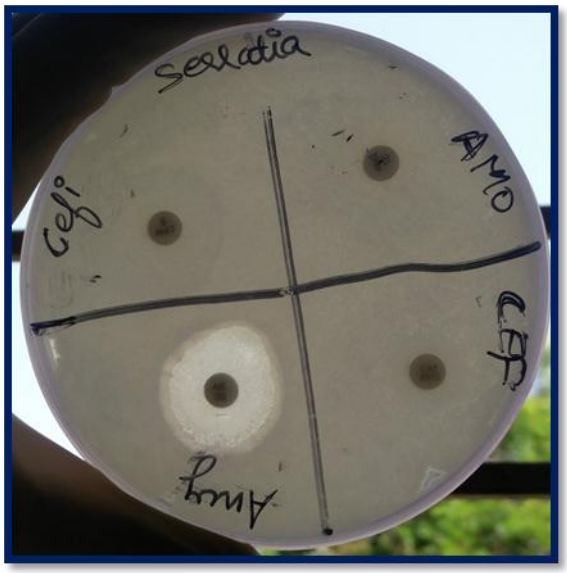


UV – VIS spectrophotometry – Serratia species

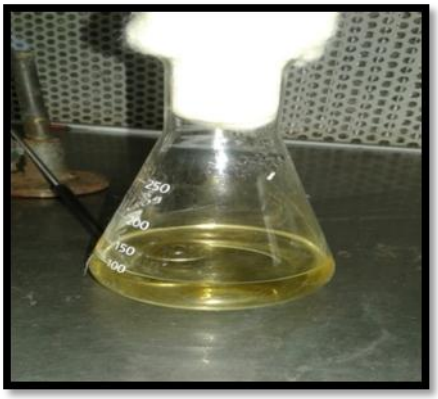
Antibiotic sensitivity test - *Serratia species*

Serratia species was found highly sensitive to Amikiacin and resistant to the antibiotics such as Ceftriaxone, Cefixime, Amorphicilin.

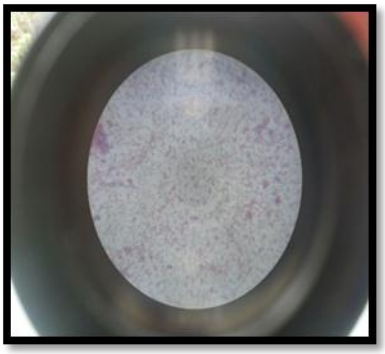
Sl.no	Antibiotics	Zone of Inhibition
1.	Amorphicilin	Nil
2.	Amikiacin	22 mm
3.	Ceftriaxone	Nil
4.	Cefixime	Nil



***S.aureus* isolate from coin**



Coin in Nutrient broth



Gram positive cocci in clusters

Yellow colonies on Mannitol salt agar

Antibiotic sensitivity test – *S.aureus*

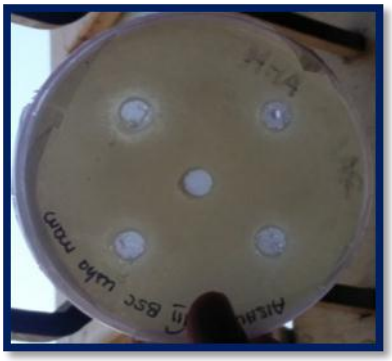
S.aureus was found highly sensitive to Amikiacin and resistant to the antibiotics such as Ceftriaxone, Cefixime, Amorphicilin.

Sl.no	Antibiotics	Zone of Inhibition
1.	Vancomycin	13 mm
2.	Erythromycin	25 mm
3.	Clindamycin	17 mm
4.	Penicillin	Resistant

Antibiotic sensitivity test for staphylococcus aurerus

ANTIBACTERIAL ACTIVITY OF CRUDE PIGMENT EXTRACT

The antibacterial activity of crude ethanol pigment of serratia species was determined by inoculating *Staphylococcus aureus* isolate in to Nutrient broth and incubated for 24 hrs at 37◦c .The turbidity of broth was compared to 0.5 N McFarland solutions. The lawn was prepared using *Staphylococcus aureus* isolate on Muller Hinton agar. The wells were cut using sterile well puncher and one milli gram of pigment extract was suspended in 100 µl of acetone and 900 µl nutrient broth. Different concentrations of pigment extracts ranging from500, 250, 125, 62.5 µl were loaded in to wells using water as control. Muller Hinton agar plate was incubated at 37 ◦c for 24 hrs and observed for Zone formation.



Well diffusion method for - Serratia species

DISCUSSION

Serratia species is a short and rod shaped bacteria and it can be grown either in presence of oxygen or in the absence of oxygen. It is commonly found in soil, water and ubiquitous in nature. It is resistant to many antibiotics which is used to treat bacterial infections, such as Pencillin due to its unique outer membrane and its ability to survive in aerobic and anaerobic condition. *Serratia species* is a gram negative bacterium and made up of single layer of peptidoglycan and it is enclosed in the outer layer of the cell membrane. The main aim of this study was to extract pigment and determine the antibacterial activity against *Staphylococcus aureus* isolated from coin exchanged among the crowded population. This study showed that crude acetone pigment extracts exhibited most potent antibacterial activity against *Staphylococcus aureus* isolate by performing well diffusion using different concentrations of crude pigment extract ranging from 500µl to 62.5 µl exhibiting inhibitory values of 15mm, 12mm, 10mm and 9mm respectively.

The isolate from coin was examined microscopically by Gram staining. Gram staining revealed that the note sample was found to contain Gram positive cocci in clusters. The sample was inoculated in to Nutrient agar and Mannitol salt agar. Golden yellow colonies and yellow colonies were observed on nutrient agar and Mannitol agar. Coagulase and DNAse test was performed and found to be positive. The antibiotic sensitivity test was performed for *Staphylococcus aureus* isolate and was found to be highly resistant to Pencillin. The isolate was found to be sensitive to Erythromycin followed by clindamycin and Vancomycin with zone formation with values of 25mm, 17mm and 11 mm. The

crude acetone pigment was used against *staphylococcus aureus* in different concentrations ranging from 500µl to 62.5 µl by well diffusion method. Maximum inhibition was found to be at different concentrations were found to be 15mm, 12 mm, 10 mm and 9 mm respectively.

SUMMARY AND CONCLUSION

Serratia species is a short and rod shaped bacteria and it can be grown either in presence of oxygen or in the absence of oxygen. It is resistant to many antibiotics which is used to treat bacterial infections, such as Pencillin due to its unique outer membrane and its ability to survive in aerobic and anaerobic condition. *Serratia species* is a gram negative bacterium and made up of single layer of peptidoglycan and it is enclosed in the outer layer of the cell membrane. The main aim of this study was to extract pigment and determine the antibacterial activity against *Staphylococcus aureus* isolated from coin exchanged among the crowded population. This study showed that crude acetone pigment extracts exhibited most potent antibacterial activity against *Staphylococcus aureus* isolate by performing well diffusion using different concentrations of crude pigment extract ranging from 500µl to 62.5 µl exhibiting inhibitory values of 15mm, 12mm, 10mm and 9mm respectively.

ACKNOWLEDGEMENTS

The Authors thank Ms Summera Rafiq, Associate Professor, Head P.G.Dept of Applied Microbiology for immense support and Ms Tharani.V. of M.Sc II nd year, Microbiology for her noble contribution in providing culture.

REFERENCES

1. Giri AV, Anandkumar N, Muthukumaran G, Pennathur G, A novel medium for the enhanced cell growth and production of prodigiosin from Serratia marcescens isolated from soil, BMC Microbiol, 4(11): 2-14 (2004).
2. Hines, D.A., P.N. Saurugger, G.M. Ihler and M.J.Benedik, Optimization study of Prodigiosin pigment, J. Bacteriol., 170: 4141 (1988).
3. Monreal, J. and E. Reese, Characterization of Pigment isolated from Serratia marcescenes, Can. J. Microbiol, 15: 689 (1969).
4. Khanafari, A., Assadi, M.M. and Fakhr, F.A., Review of prodigiosin, pigmentation in Serratia marcescens, J. Biol. Sci., 6: 1-13 (2006).
5. Pandey R, Chander R, Sainis KB., Prodigiosins A novel family of immune suppressants with anticancer activity. Ind J Biochem Biophy, 44:295–302 (2007).
6. Bollet, C., C. Gulian, H. Chaudet, B. Fichet, A. Aragon, and P. de Micco. 1988. Identification des principales especes du genre Serratia par une galerie simplifide. Ann. Inst. Pasteur (Paris) 139: 337-349.
7. Bouvet, 0. M. M., P. A. D. Grimont, C. Richard, E. Aldova, 0. Hausner, and M. Gabrhelova. 1985. Budvicia aquatica gen. nov., sp. nov.: a hydrogen sulfide-producing member of the Enterobacteriaceae. Int. J. Syst. Bacteriol. 35: 60-64. 3. Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G.
8. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner. 1985. Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. J. Clin. Microbiol. 21: 46-76. 4.
9. Gallois, A., and P. A. D. Grimont. 1985. Pyrazines responsible for the potatolike odor produced by some Serratia and Cedecea strains. Appl. Environ. Microbiol. 50: 1048-1051.
10. Gavini, F., C. Ferragut, D. Izard, P. A. Trinel, H. Leclerc, B. Lefebvre, and D. A. A. Mossel. 1979. Serratia fonticola, a new species from water. Int. J. Syst. Bacteriol. 29: 92-101
11. Grimont, P. A. D., and F. Grimont. 1984. Genus VIII. Serratia Bizio 1823, 288AL p. 477-484. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore
12. Muller, H. E., A. G. Steigerwalt, and D. J. Brenner. 1986. Isolation of Serratia fonticola from birds. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A, 261: 212- 218.