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Staphylococcus Aureus Pigment-Bio Colour as a Novel Antibacterial Agent Against Staphylococcus Aureus Isolate from Coins

Ayisha Begum, M. K. Aishwarya, Aeliya Zehra. M, Achiffa Abdul Rahiman, R. Ushasri
P.G. Department of Applied Microbiology, JBAS College for Women, Chennai, Tamil Nadu, India

ABSTRACT
The pathogen Staphylococcus aureus is Gram-positive cocci in clusters which forms golden colony on Nutrient agar. The most important characteristic feature of Staphylococcus aureus is its yellow to orange colour due to production of Staph ylox an thin. Staphyloxanthin is membrane bound Carotenoid and plays an important role in antimicrobial activity. The aim of the present study was to detect the role of Staphyloxanthin pigment production from S. aureus isolates against Staphylococcus aureus isolate from coin proving antagonistic property. The crude ethanol pigment was used against staphylococcus aureus in different concentrations ranging from 500µl to 62.5 µl by well diffusion method. Minimum inhibition was found to be 11mm at concentration range of 500. µl. The current study reported that coin plays an important role s transmitting infections

Keywords: *Staphyloxanthin, Carotenoid, MHA, MSA*

INTRODUCTION
The pathogen Staphylococcus au re us is Gram-positive cocci in clusters which forms golden colony on Nutrient agar. The most important characteristic feature of Staphylococcus au re us is its yellow to orange colour due to production of Staphyloxanthin. Staphyloxanthin is membrane bound carotenoid and plays an important role in antimicrobial activity. Membrane pigments acts as virulence factors in S. aureus. Staph yloxan thin is a secondary metabolite produced during stationary phase which has a chemical formula of (C51H78O8). Staphyloxanthin is a neutral molecule. The aim of the present study was to detect the role of Staphyloxanthin pigment production from S. aureus isolates against Staphylococcus aureus isolate from coin proving antagonistic property. Nutrient agar, Brain Heart infusion agar , Milk agar medium , Pea nut seed medium, Sunflowers seeds medium , Sesame seed medium , Trypticase yeast agar medium, , Carotenoid expression agar medium were used for production of staphyloxanthin. The pigment of Staphylococcus aureus (STX) was extracted by using Ethanol .Bacterial cells were recovered from the growth on Nutrient agar plate at 37oC. The bacterial cells were centrifuged at 2000rpm for 15 min. Staphyloxanthin from the pellet containing the bacterial cells was collected. The pellet was mixed with 8 ml of 99.9% Ethanol wrapped with aluminum foil to avoid light exposure and kept in oven for 72 hrs to obtain crude extract.. Carotenoids were estimated quantitatively by measuring the absorbance of the solution at 450 nm. The crude pigment extracts were evaluated for antibacterial activity against staphylococcus aureus isolate from coins collected from crowded area.

METHODOLOGY
Sample Collection
The 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.

Coin in nutrient broth

Filtration of centrifuged supernatant broth

Identification of Staphylococcus

Staphylococcus was identified by Gram staining, Hanging drop, catalase, and oxidase tests. The cultural characteristics and biochemical characters were performed to identify Staphylococcus.

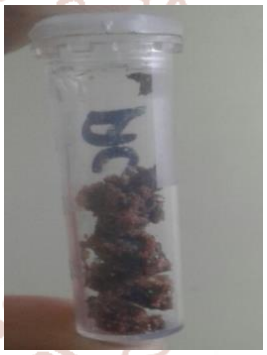
Inoculation of staphylococcus aureus
In to nutrient broth

Incubation of pigmented broth in rotary shaker at 37◦c

Red pigmented broth after 48 hrs.

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.

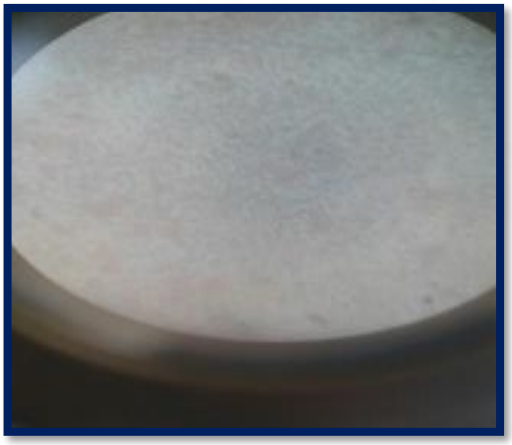


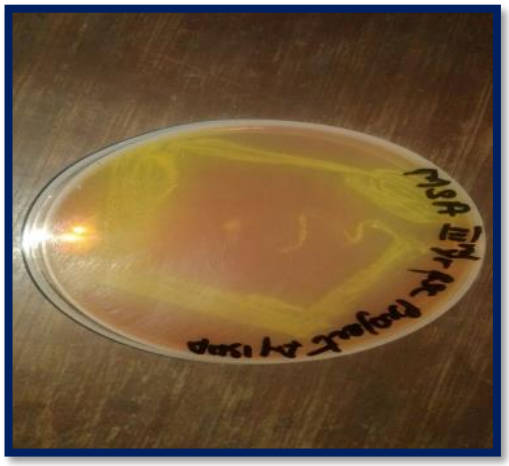
Crude ethanol pigment extract

MICROSCOPIC APPEARANCE

The inoculated colonies were observed for microscopic appearance and identified as motile gram positive cocci in clusters.

Gram positive cocci in clusters





Yellow colonies on Mannitol salt agar

Coagulase positive – S.aureus

Preliminary Tests of Staphylococcus Aureus

Sl. no	Tests	Observation
1.	Gram staining	Gram-Positive cocci in clusters
2.	Motility	Non motile
3.	Catalase	Postive
4.	Oxidase	Negative

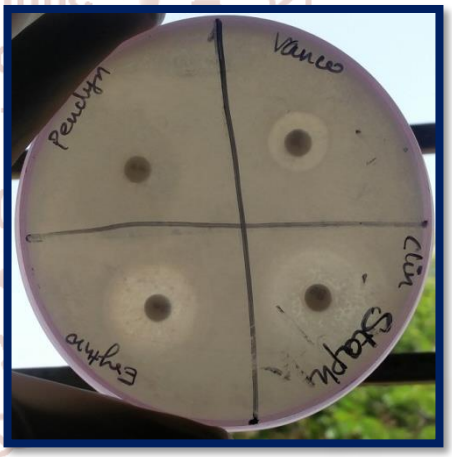
Antibiotic sensitivity test for the S. aureus

S.aureus was found highly sensitive to Erythromycin followed by sensitive to clindamycin and vancomycin and resistant to the antibiotic Penicillin.

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

Cultural Characters of Staphylococcus Aureus

S. No	Colony morphology	Inference
1.	Size and colour	1-2 mm and yellow colonies
2.	Margin	Entire
3.	Shape	Circular
4.	Opacity	Opaque
5.	Consistency	Smooth
6.	Elevation	Convex

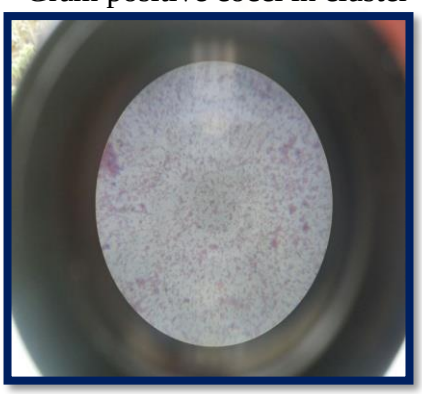


Isolation and identification of Staphylococcus aureus from coin

Biochemical Characters of Staphylococcus Aureus

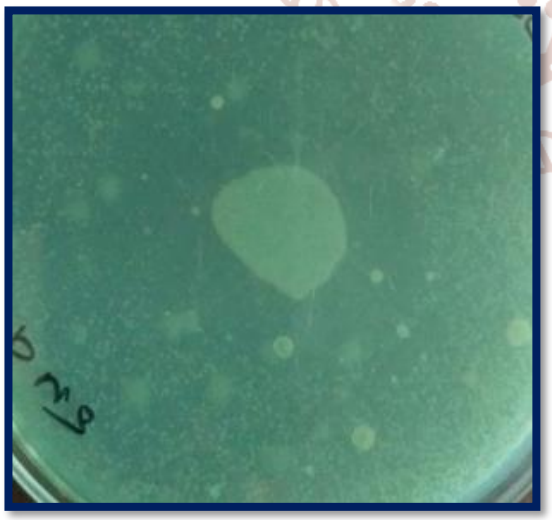
DNAse positive - S.aureus

Gram positive cocci in cluster



Yellow colonies on Mann itol salt agar

Coagula se test – Positive for isolate from coin



DNAse test – Positive for isolate Staphylococcus aureus from coin

Antibiotic sensitivity test for S.aureus isolate from coin

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

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

Antibacterial Activity of crude pigment extract

The antibacterial activity of crude ethanol pigment of Staphylococcus aureus 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- staphylococcus species

DISCUSSION

The pathogen Staphylococcus aureus is Gram-positive cocci in clusters which forms golden colony on Nutrient agar. The most important characteristic feature of Staphylococcus aureus is its yellow to orange colour due to production of Staphyloxanthin. Staphyloxanthin is membrane bound carotenoid and plays an important role in antimicrobial activity 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. The UV Visible

spectro photometric studies showed highest peak indicating estimation of pigment 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 11mm The isolate was found to be resistant to pigment extracts at concentration range of 250 µl 125 µl and 62.5 µl. Minimum bactericidal concentrations was performed and found to be turbid by showing cloudy appearance.

Summary and Conclusion

Staphyloxanthin is a secondary metabolite produced by Staphylococcus aureus during stationary phase which has a chemical formula of (C51H78O8). Staphyloxanthin is a neutral molecule. The aim of the present study was to detect the role of Staphyloxanthin pigment production from S. aureus isolates against Staphylococcus aureus isolate from coin proving low antagonistic property. Staphylococcus aureus was sub cultured and inoculated in to Nutrient broth followed by incubation at 37◦c for 48 hrs in rotary shaker for production of pigment. The pigmented broth was centrifuged at 1500 rpm for 30 mins. The supernatant was filtered using sterile what Mann filter paper. The filtrate was mixed with ethanol and kept in oven overnight to obtain crude extract. The crude extract was stored in sterile storage vials for antibacterial study. The coin was collected from public transport in crowded area and transferred to sterile Zip lock cover using sterile hand gloves. The coin was transferred to nutrient broth in flask and incubated at 37◦c. The turbidity was observed and microscopic examination was done by Gram staining technique and found to be Gram positive cocci in clusters. The broth culture was inoculated in to Nutrient agar and Mann itol salt agar and incubated for 24 hrs at 37◦c. Coagula se and DNAse tests were performed to differentiate Staphylococcus spp. Antibiotic sensitivity tests was done to find out sensitivity of Staphylococcus .aureus isolate to antibiotics. The isolate was found to be highly resistant to Pencillin and sensitive to Erythromycin. The crude ethanol 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 11mm at concentration range of 500. µl .The current study reported that coin plays an important role s transmitting infections .It was concluded that coin acts as one of the factors for spread of infections. The Staphy lox an thin acts as an important antagonist and is a novel bio colour against Staphylococcus aureus is olate from coins. This study was done for the first time to the best of our Knowledge.

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