In-Vitro Antibacterial Property of Metabolites of Alkaliphilic Isolates Against Methicillin Resistant S. Aureus(MRSA)

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Abstract- *In this study antibacterial activity of metabolites of alkaliphilic isolates against clinical antibiotic resistant Methicillin resistant S. aureus (MRSA)isolate was evaluated.Metabolites of few alkaliphilic isolates showed significant activity against MRSA isolate 1 and MRSA isolate 2.Cell free extracts as well intracellular extracts, extracted with methanol were used to study antimicrobial activity of the alkaliphilic isolates. Antibacterialactivities exhibited by intracellular extracts were higher compared to cell free extracts. Plates showing antimicrobial activity were kept for few days to detect resistance. It was found that MRSA isolates were susceptible to intracellular methanolic extracts.*

Keywords- Methicillin resistant S. aureus (MRSA),Antibacterial activity, Intracellular methanolic extracts.

I. INTRODUCTION

The spread of resistance to antibiotics undermines the therapeutic utility of anti-infective drugs in current clinical use. Methicillin resistant *S. aureus* (MRSA)strains appeared in hospital environment after introduction of semi synthetic Penicillin, Methicillin. MRSA become resistant to these antibiotics by producing beta lactamase which breaks integrity of beta lactam ring present in these antibiotics.Among all known bacteria, *Staphylococcus aureus* is possibly the greatest concern of all health care associated pathogens due to its ability to cause a wide variety of life threatening infections. *S. aureus* has ability to rapidly adapt with different environmental conditions (1). MRSA is a pathogen responsible for wide variety of infections such as boils, pneumonia and bacteremia. It has developed resistance to the majority of conventional antibiotics (2). *Staphylococcus* infections are highlighted for being a major cause of systemic infections and for being the microorganism that has highest morbidity and mortality rates in hospital infections (3).
Thus, new antibiotic and therapy options are urgently needed to improve management of bacterial infections. The major challenge is to find drugs that act against Methicillin Resistant*S. aureus* (MRSA) (4).

II. MATERIAL AND METHODS

Production of antimicrobial metabolites:

The alkaliphilic microorganisms were isolated on R2A agar medium (pH-11) on the basis of colony characteristics. These isolates were inoculated into Tryptic soy broth (pH-11) and incubated for 10-14 days at 32^0C. This was further used to study antibacterial activity.

Extraction of antimicrobialmetabolites:

Cell free extracts were prepared by centrifugation at 13500 rpm for 30 minutes.Supernatant was collected and was further extracted with methanol. Cell pellets settled at the bottom of the microcentrifuge tubes were further processed for the extraction of intracellular metabolite. These cells were lysed by glass beads. These cell lysates were extracted with methanol in 1:1 proportion. Methanol was evaporated and used for antimicrobial assay.

Preparation of inoculum:

MRSA cultures (clinical MRSA isolate 1 and MRSA isolate 2) used in this study were inoculated into broth and incubated at 37^0C for 24hrs and adjusted to obtain turbidity comparable to 0.5 McFarland standards (1×10^8 cfu/ml).

Antimicrobial assay:

Antimicrobial assay was carried out by agar well diffusion method (Kirby Bauer). The 24hr old cultures of MRSA were evenly streaked on sterile Muller- Hinton (MH) agar plates with the sterile cotton swab. These plates were kept for few minutes to set the culture on Muller- Hinton (MH) agar plates. The wells were made on MH agar plates. The wells were loaded with cell free extracts, methanol extractsand

intracellular methanolic extracts. These plates were incubated at 37^0C for 48hrs.This was followed by recording diameter of zone of inhibition.

Sensitivity of the organisms for extracted metabolites:

Plates having zone of inhibition against MRSA isolates were kept for few days (20-22) and zone size was measured in order to check the sensitivity of the organism after few days, as the test organism was resistant to antibiotics.

III. RESULTS AND DISCUSSION

Zone of inhibition results by cell free extracts, Methanolic extracts and Intracellular methanolic extracts of the alkaliphilic Isolates

Table No.1

1. Results of cell free extracts

Alkaliphilic bacterial isolates	Zone of inhibition (mm)	
	MRSA-1	MRSA-2
1	13.5	0
2	14	0
3	12.5	0
4	12	0
5	0	0
6	0	0
7	0	0
8	0	0

Table No.2

2. Results of Methanol extracts

Alkaliphilic bacterial isolates	Zone of inhibition (mm)	
	MRSA-1	MRSA-2
1	12.5	0
2	14.5	10.5
3	13	0
4	13.5	12.5
5	12	10.5
6	11	10
7	13	0
8	0	0

Table No.3

3. Results of Intracellular methanolic extracts

Alkaliphilic bacterial isolates	Zone of inhibition (mm)	
	MRSA-1	MRSA-2
1	18.5	21
2	18.5	20.5
3	22.5	22.5
4	22.5	23
5	0	12
6	0	12.5
7	9.5	11
8	9.5	11.5

IMG 1 Cell free extracts
Antimicrobial activity of cell free extracts of alkaliphilic bacterial isolates against MRSA-1

IMG 2 Methanol extracts
Antimicrobial activity of methanol extracts of alkaliphilic bacterial isolates against MRSA-1

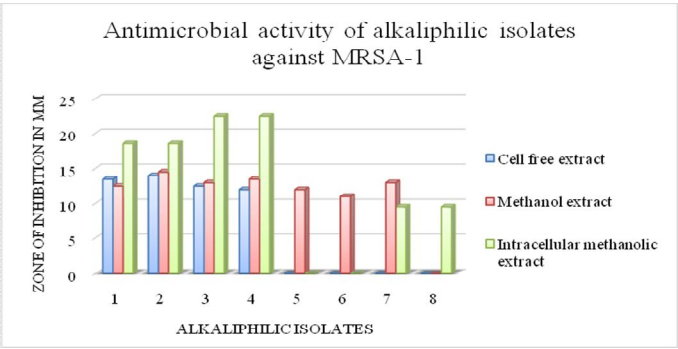
IMG 3 Intracellular methanolic extracts
Antimicrobial activity of intracellular methanolic extracts of alkaliphilic bacterial isolates 1,2, 3 and 4 against MRSA-1 after 48hrs.

IMG 4 Detection of susceptibility of MRSA isolate 1 against Intracellular methanolic extracts
No decrease in zone of inhibition size for 20 days, indicating susceptibility of MRSA-1 against intracellular methanolic extracts of isolates 1, 2, 3 and 4.

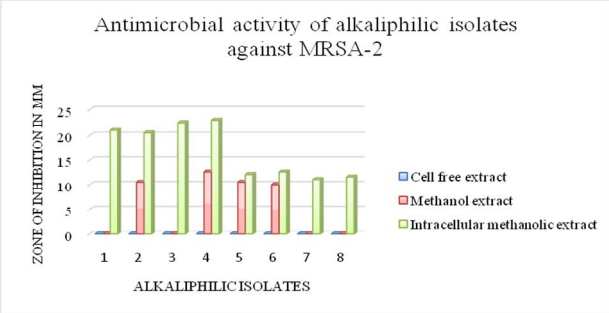
IMG 5 Intracellular methanolic extracts
Antimicrobial activity of intracellular methanolic extracts of alkaliphilic bacterial isolates 1,2, 3 and 4 against MRSA-2 after 48hrs.

IMG 6 Detection of susceptibility of MRSA isolate 2 against Intracellular methanolic extracts
No decrease in zone of inhibition size for 20 days, indicating susceptibility of MRSA-2 against intracellular methanolic extracts of isolates 1, 2, 3 and 4.

IMG 7
Antimicrobial activity of intracellular methanolic extracts of alkaliphilic bacterial isolates 5,6, 7 and 8 against MRSA-2 after 48hrs.



Graph No.1



Graph No.2

In this study cell free extractsof alkaliphilic isolates have lower antimicrobial activity than methanol extracts against MRSA isolate 1. Intracellular methanolic extracts were showing higher activity against MRSA isolate 1. Cell free extracts were not showing antimicrobial activity against MRSA isolate 2. Antimicrobial activities of intracellular methanolic extracts were higher than methanol extracts against MRSA isolate 2.

More S.M. and Shinde V.A.et al (4) screened antibiotic producing ability of *Bacillus subtilis* and activity of antimicrobial compound was determined against different microorganisms like *Staphylococcus aureus, E.coli, P. aeruginosa and Candida tropicalis.*There isolates showed higher activity against *Staphylococcus aureus* showing 30mm of diameter of zone of inhibition.

Maithilli S.S. and Senthamil M. et al (5) isolated bacteria from marine water and antimicrobial sensitivity was tested against *Staphylococcus aureus, E.coli, P. aeruginosa and Klebsiella pneumoniae.*They isolated36 bacterial strains from marine water of them twelve strains were shown sensitivity against two pathogenicbacteria *E.coli* and *Klebsiella pneumoniae.* The purified extract of fraction III shown higher zone of inhibition against *E.coli* and *Klebsiella pneumoniae.*

Asli Kousha H. and Vatankhah M.R.(6) isolated *Pseudomonas sp.* from rhizospere region and detected antibacterial activity of the isolate against Methicillin Resistant *S. aureus* (MRSA). They got higher activity against MRSA for purified proteins of *Pseudomonas sp.* ascompared to cell free supernatant.

IV. CONCLUSION

Antimicrobial activity of alkaliphilic isolates with cell free extracts, methanol extracts and intracellular methanolic extracts were evaluated against MRSA isolates.Intracellular metabolites extracted with methanol showed promising activity against MRSA isolate 1 and MRSA isolate 2 used in the study.These isolates of MRSA were found to be sensitive to intracellular methanolic extracts for few days (20), as there was no reduction observed in the zone of inhibition.Intracellular methanolic extracts needs to study in detail to unravel theseantimicrobial metabolites which may play important role in future. The active constituentscan be explored.

REFERENCES

[1] Rajan Benita Mercy and Kannabiran Krishnan (2013) Antimicrobial activity of *Streptomyces albofaciens* against Methicillin Resistant *Staphylococcus aureus* and Vancomycin Resistant *Enterococcus* Multi drug resistant species, Research Journal of Pharmaceutical, Biological and Chemical Sciences, ISSN:0975-8585Volume 4 Issue-2.

[2] Rajan Benita Mercy and Kannabiran Krishnan (2014) Extraction and identification of antibacterial secondary metabolites from marine *Streptomyces sp.* VITBRK2, IJMCM Summer 2014, Vol.3, No. 3.

[3] Joao B.A. Neto and Cecillia R da Silva et al (2015) Screening of antimicrobial metabolite of yeast isolates derived biome against pathogenic bacteria, including MRSA: Antibacterial activity and mode of action evaluated by Flow Cytometry, International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Vol.4 No.4pp.459-472.

[4] More S.M. and Shinde V.A.et al (2012) Antimicrobial activity of phospholipid compound produced by acidophilic *Bacillus subtilis* isolated from Lonar Lake, Buldhana, India, Research Journal of Recent Sciences, ISSN 2277-2502 Vol.(11), 22-26.

[5] Maithilli S.S. and Senthamil M. et al (2014) Isolation of secondary metabolite from seawater bacterial population and screening of their bioactive potential against urinary tract pathogens sourced from HIV patients, International Journal of Current Microbiology and Applied Sciences, ISSN: 2319-7706, Vol.3 No.6 pp.540-548.

[6] Asli Kousha H. and Vatankhah M.R. (2015) Antibacterial activity of *Pseudomonas sp.* isolated rhizosphere against Methicillin resistant *Staphylococcus aureus* from clinical samples, International Journal of Life Sciences Biotechnology and Pharma Research, Vol. 4, No. 2.

[7] Asha Devi N.K. and Rajendran R. et al (2011) Isolation and characterisation of bioactive compounds from marine bacteria, International Journal of Natural Products and Resources, Vol.2 pp.59-64.

[8] Farhana Alam Ripa and Farhana Nikkon et al (2010)In vitro antibacterial activity of bioactive metabolite and crude extract from a new *Streptomyces Sp.Streptomyces*

rajshahiensis,International Journal of PharmaTech Research,ISSN:0974-4304,Vol.2,No.1,pp644-648.

[9] Sharma Deepika and Kaur Talwinder et al (2011)Antimicrobial activity of Actinomycetes against multi resistant *Staphylococcus aureus, E.coli* and various other pathogens, Tropical Journal of Pharmaceutical Research,10(6):801-808.

[10] Angel Treasa Thomas and J. Venkata Rao et al (2009) Antimicrobial profile of extremophiles from aqua to terrestrial habitat, Pharmacologyonline 1: 111-126.

[11] Kerstin Engelhardt and Kristin F. Degnes (2010) Production of a new thiopeptide antibiotic TP-1161, by a marine *Nocardiopsis sp*, Applied and Environmental Microbiology.pp 4969-4976.