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SUSCEPTIBILITY OF FOOD PRESERVATIVES ON MRSA AND MSSA ISOLATED FROM CLINICAL SAMPLES

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Abstract:

Food preservatives are those substances which prevent spoilage caused by microbial or chemical causes. These preservatives may be natural or artificial. Natural preservatives are a hundred percent safe although the effectiveness lasts for shorter duration as compared with the artificial ones.

Staphylococcus aureus was isolated from various clinical samples and confirmation was done using various biochemical tests. Antibiogram revealed the presence of 22 MRSA islolates and also the sensitivity pattern of the isolates. Susceptibility checking of various selected natural and artificial preservatives showed that natural preservatives are more efficient in controlling the pathogen Staphylococcus aureus than artificial ones and among the tested natural preservatives, honey gave the best results. Honey has been used from ancient times for various used in food , medical and cosmetic field. This study has gone a step further to say that it can act as a competitive antibiotic agent as well.

Key words: *Staphylococcus aureus, artificial preservatives and antibiotics*

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INTRODUCTION:

Staphylococcus aureus has been one of the major bacteria which has threatened mankind with ailments varying from simple skin boils to life threatening meningitis and endocarditis [1]. Although this pathogen has been around since ancient times, it is rather unfortunate that still the health industry has failed to effectively control this pathogen. Recent times have seen the evolution of this pathogen into one which is resistant to multiple drugs. Once effectively controlled by penicillin, now these have almost completely become resistant to it [2]. Due to the arrival of penicillinase producing strains, Methicillin arrived as the drug of choice. The arrival of Methicillin Resistant *Staphylococcus aureus* (MRSA) spread panic among the health care professionals sue to its non responsiveness to most commercial antibiotics Benner *et al*., 1988. The high risk group includes people with serious underlying conditions, patients under immunosuppressant therapy and patients hospitalized for a long time[3]. Hence current research is focused on finding newer compounds which are effective against such multiple drug resistant strains.

Food preservatives are those substances which prevent spoilage caused by microbial or chemical causes. These preservatives may be natural or artificial. Natural preservatives are a hundred percent safe although the effectiveness lasts for shorter duration as compared with the artificial ones. The artificial preservatives allowed in food substances are controlled by strict FDA laws as most of these have side effects.

In these times where the necessity of novel and alternative antimicrobial agents has peaked, this study aims at studying the activity of some of the food preservatives against the various *Staphylococcus aureus* isolates.

MATERIALS AND METHODS:

Sample Collection

A total of 100 clinical samples like pus, throat swabs, sputum, blood and urine were collected from various hospitals for the isolation of *Staphylococcus aureus*. These were transported to the laboratory and processed immediately.

Isolation of *Staphylococcus aureus*

The clinical samples were inoculated on Blood agar, Nutrient agar and Mannitol Salt agar and were incubated at 37^{O}C for 24 hours.

Gram's staining

The isolates from plates were taken and subjected to Gram's Staining and were observed under a microscope.

Catalase Test

From each plate, a single colony was taken and added into a drop of hydrogen peroxide placed on a clean slide and observed for effervescence [4].

Coagulase Test:

Slide coagulase test

Two suspensions of the test organisms in sterile saline was prepared and to one of this, a drop of human plasma was added and mixed gently by shaking the slide. The other suspension was placed on another slide and acted as negative control to check for auto agglutination. Clump foration within 5- 10 seconds in the first slide indicated the presence of bound coagulase.

Tube coagulation test

This test is done for the detection of free coagulase. 0.5 ml of human plasma was added to 0.5 ml of the broth culture tube and kept in the water bath at 37^{O}C for 4-6 hours. Clot formation within the incubation time indicated a positive result.

Antibiogram

Sterile Muller Hinton agar plates were prepared and the isolates were swabbed onto these plates and the antibiotics to be tested were placed as discs on the swabbed plates [5]. The antibiotics used were Penicillin, Clindamycin, Erythromycin, Amikacin, Gentamycin, Vancomycin and Cefoxitin. Cefoxitin was used to identify MRSA from the other strains [6].

Susceptibility testing of food Preservatives against MRSA by paper disc diffusion method

The natural food preservatives used were salt, vinegar and honey and the artificial preservatives used were Sodium nitrite and Sodium benzoate. These preservatives were taken in varying concentrations (0.5%, 1%, 1.5%, 2% and 2.5%) and were added to paper discs. Sterile Muller Hinton agar plates were prepared and were swabbed with 24 hour old MRSA broth culture and incubated at 37^{O}C for 24 hours[7].

RESULTS AND DISCUSSION:

Isolation and gram's staining

The isolated showed off white smooth colonies on Nutrient agar and beta hemolytic colonies on blood agar, indicated by a zone of clearance around the colonies. The isolated showed bright yellow colored colonies on mannitol salt agar due to their ability to ferment mannitol, which is an important confirmatory test for *Staphylococcus*.

On staining, the isolated showed gram positive cocci

in bunch, indicative of *Staphylococcus aureus.* Out of the 100 samples processed, 60 were identified as *Staphylococcus aureus,* most of which were isolated from pus samples. Out of these, 40 were from males and 20 were from females.

Catalase Test and Coagulase Test Results

All the 60 isolates were positive for both catalase and coagulase test. Formation of effervescence on addition of hydrogen peroxide indicated the presence of the enzyme catalase while clump formation in both the slide and test tube indicated the presence of both free and bound coagulase respectively.

Antibiogram

The percentage of resistance toward each antibiotic is shown in Table 1.

Table 1: percentage of resistance against the antibiotics

ANTIBIOTIC	PERCENTAGE OF RESISTANCE (MRSA)	PERCENTAGE OF RESISTANCE (MSSA)
Penicillin	100	36
Clindamycin	63	5
Erythromycin	81	78
Amikacin	50	97
Gentamycin	90	84
Ciprofloxacin	20	28
Vancomycin	0	0

All the isolates tested showed sensitivity to Vancomycin. Penicillin was resisted by all the MRSA isolates whereas most of the MSSA strains were inhibited effectively. Almost all MRSA isolates resistance to Clindamycin, Gentamycin and Erythromycin but minimum resistance was seen against Ciprofloxacin. Even though hals the MRSA isolates were susceptible to Amikacin, almost all the MSSA isolates were resistant. Most of the MSSA was found to be inhibited effectively by Clindamycin and Ciprofloxacin whereas Erythromycin and Gentamycin was found to be effective against minimal number of isolates.

Susceptibility testing of food Preservatives against MRSA by paper disc diffusion method

The susceptibility of the food preservatives has been tabulated in Table2 and 3.

Table 2: susceptibility testing of Natural Preservatives

Natural Food Preservatives	Concentration in %	Sensitivity (zone in mm)
Honey	0.5	8
	1	10
	1.5	12
	2	15
	2.5	15
Salt	0.5	-
	1	-
	1.5	-
	2	-
	2.5	-
Vinegar	0.5	8
	1	10
	1.5	11
	2	12
	2.5	12

Among the three natural preservatives tested, honey was found to be more effective than the other two in controlling *Staphylococcus aureus* strains. Salt was highly ineffective, which may be justified by the fact that the pathogen is halophilic. Vinegar was found to be equally effective as honey at lower concentrations even though at higher concentrations honey was more active.

Table 3: susceptibility testing of Artificial Preservatives

Chemical Food Preservatives	Concentration in %	Sensiitivity (zone in mm)
Sodium benzoate	0.5	-
	1	1
	1.5	3
	2	8
	2.5	11
Sodium nitrite	0.5	-
	1	-
	1.5	-
	2	8
	2.5	12
Potassium metabisulphate	0.5	-
	1	4
	1.5	11
	2	13
	2.5	15

Here, potassium metabisulphate was found to possess higher antibacterial activity at all concentrations. Sodium nitrte was found to be effective at higher concentrations only. The activity of Sodium benzoate was found to increase with increased concentration.

CONCLUSION:

Staphylococcus aureus was isolated from various clinical samples and confirmation was done using various biochemical tests. Antibiogram revealed the presence of 22 MRSA islolates and also the sensitivity pattern of the isolates. Susceptibility checking of various selected natural and artificial preservatives showed that natural preservatives are more efficient in controlling the pathogen *Staphylococcus aureus* than artificial ones and among the tested natural preservatives, honey gave the best results. Honey has been used from ancient times for various used in food , medical and cosmetic field. This study has gone a step further to say that it can act as a competitive antibiotic agent as well.

REFERENCES:

1.Downes F.P and K. Ito, compendium of methods for the microbiological examination of foods, American Public Health Association, Washington D.C. 4th ed.

2.Esperson. F, L. Back, P. Kjaelgaard and V.T. Rosdahl, Detection of Staphylococcal toxic shock syndrome toxin 1 by latex agglutinatin kit, Scandinavian Journal of Infectious Diseases. 2002; 21: 530- 534

3.Herold B.C, L.C. Immergluck, M.C. Maranan, D.S. Lauderdale, R.E. Gaskin, S. Boyle Vavra, C.D. Leitch and R.S. Daum, Community acquired methicillin resistant S*taphylococcus aureus* in children with ni identified predisposing risk, JAMA. 1998; 279: 593-598

4.Thaker HC, Brahmbhatt MN and Nayak JB (2013) Isolation and identification of Staphylococcus aureus from milk and milk products and their drug resistance patterns in Anand, Gujarat, Vet World 6(1):10-13.

5.Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck,M. approved the final manuscript. (1966). Antibiotic susceptibility testing by a standardized Acknowledgements single disk method. Amer. J. Clin. Pathol., 45: 493-496

6.Shopsin. B, B. Mathema,J. Martinez,E. Ha, M. L. Campo,A. Fierman,K. Krasinski and J. Kornblu, Prevalence of Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus in the Community, the Journal of Infectious Diseases. 2000; 182(1): 359-362

7.Zaidan, M.R.S.1, Noor Rain, A.1, Badrul, A.R.2, Adlin, A.2, Norazah, A.3 & Zakiah, In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method, Tropical Biomedicine. 2005; 22(2): 165–170.