Research Article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Comparison of Antibiotic Resistance Pattern of Coagulase Positive and Coagulase Negative *Staphylococcus aureus* Isolates from Indian Sweets

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ABSTRACT

Out of 100 samples of sweets collected 80 samples showed the presence of *S. aureus*. Among them 61 (76%) isolates were positive for coagulase and 19 (24%) isolates were negative for the same. Incidences of *S. aureus* was more in Laddu (88%) and least (69%) in Mysore Pak and Gulab Jamun. Among coagulase positive isolates, isolates VO4M and GS10MP were least potent showing resistant to zero antibiotics and isolates PR7MP and SK4DP were more potent being resistant to 12 antibiotics. Whereas, among coagulase negative isolates, isolate GO1P was least potent showing resistant to zero antibiotics and isolate SO3M was more potent showing resistant to 14 antibiotics. Streptomycin and Gentamycin were found to be effective drugs and Oxacillin and Amikacin were found to be least effective drugs against coagulase positive isolates. Gentamycin, Chloramphenicol, Clindamycin, Erythromycin and Streptomycin were found to be effective drugs and Penicillin and Ampicillin were found to be least effective drugs against coagulase negative isolates.

KEY WORDS: *S. aureus*, sweets, coagulase, Antibiogram

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INTRODUCTION

S. aureus is an important pathogen due to combination of toxin mediated virulence, invasiveness and antibiotic resistance. Many types of foods have been implicated as vehicles of staphylococcal food poisoning but foods with high protein, sugar or salt serve as good medium for growth. The most frequent products contaminated with *S. aureus* are meat, sausages, poultry or their products. Food poisoning or food borne outbreaks by *S. aureus* is caused due to consumption of enterotoxins produced by the organism. *S. aureus* can produce seven types of enterotoxin like SEA, SEB, SEC 1-3, SED and SEE.

Bacterial antimicrobial resistance has become a serious problem worldwide and mechanisms of resistance have been identified and described for all known antimicrobials currently available for clinical use in human. *S. aureus* is one of the most common human pathogens, responsible for a variety of infections in all age groups. It is also challenging to treat because of its resistance to antimicrobial agents. In addition to universal β-lactamase production, *S. aureus* isolates resistant to methicillin (MRSA) and thus resistant to all β-lactam antibiotics, have spread worldwide and are responsible for nosocomial and community outbreaks of infection.

Incidences of *S. aureus* in different sweets have been studied by Soomro et al.[1] Coelho et al. conducted a study to characterize pheno-genotypically the virulence factors and resistance pattern of *S. aureus* isolates from milk samples of cow with subclinical mastitis[2]. Moon et al. determined the relationship between phenotypic antibiogram and the polymorphism of coagulase gene in *S. aureus* strains isolated from bovine mastitic milk samples[3]. The present investigation was taken up in order to study the incidences of *S. aureus* in Indian sweets sold in Bangalore and also to determine the relationship between coagulase production and antibiotic resistance pattern shown by the isolates.

MATERIALS AND METHODS

MEDIA AND ANTIBIOTICS

Dehydrated media, antibiotics and other chemicals used for the present study were procured from Hi-media Pvt. Ltd., Mumbai.

SAMPLE COLLECTION

A total of 100 samples were collected from different sweet shops in and around Bangalore. Twenty samples each of five different sweets, Mysore Pak, Dhoodpedha, Laddu, Jalebi and Gulab Jamun were collected from each shop using separate polythene bags and subjected to isolation of *S. aureus.*

ISOLATION OF *STAPHYLOCOCCUS AUREUS*

Isolation of *S. aureus* was carried out using Mannitol Salt agar medium and Baird Parker's agar medium. Small quantities of samples were used for isolation purpose. Colonies showing yellow zone on Mannitol Salt agar medium and black colored colonies with hallow zone on Baird Parker's agar medium were selected for further studies.

COAGULASE TEST

S. aureus isolates were subjected to slide coagulase test[4]. A small quantity of plasma was placed on a clean grease free slide. A loopful of each culture was mixed with plasma separately and checked for agglutination. Cultures which showed agglutination were recorded as positive for coagulase test.

ANTIBIOTIC SUSCEPTIBILITY TEST

All the isolates were subjected to antibiotic susceptibility test against fifteen antibiotics. Kirby-bauer disc diffusion method was used for the same[5]. Susceptibility or resistance pattern of the isolates were studied by measuring zone of inhibition and referring to standard chart for each antibiotics (Table 1).

RESULTS AND DISCUSSION

Out of 100 samples collected, 80 samples were positive for *S. aureus*. All 80 isolates showed colonies with yellow zone on Mannitol salt agar medium and black coloured colonies with hallow zone on Baird parker's agar medium (Fig. 1 and 2). Out of 80 *S. aureus* isolates 61 (76%) isolates were positive for coagulase test and 19 (24%) were negative.

Incidences of *S. aureus* were equal in all the five different sweets, where out of 20 samples of each sweet, 16 isolates (80%) were positive for *S. aureus*. Incidences of coagulase positive isolates were more in Laddu samples, where 14 (88%) out of 16 were positive for coagulase test. A least of 69% (11 out of 16) incidences of coagulase positive *S. aureus* was recorded from Mysore Pak and Gulab Jamun (Table 2).

When the coagulase positive isolates were subjected to antibiotic susceptibility test, the number of antibiotics against which the coagulase positive isolates showed resistance ranged between 0 to 12 and susceptibility ranged from 3 to 15. Among the coagulase positive isolates, the isolates VO4M and GS10MP were least potent showing resistance against zero antibiotic and the isolates PR7MP and SK4DP were more potent showing resistance against 12 antibiotics (Table 3).

Among the coagulase negative isolates the number of antibiotics against which they showed resistance ranged between 0 to 14 and susceptibility ranged from 1 to 14. The isolate G01P showed resistance against zero antibiotics being least potant and isolate S03M was found to be more potent showing resistance against 14 antibiotics (Table 4).

When the effect of fifteen antibiotics were checked against each isolate the number of coagulase positive isolates showing resistance to each antibiotic ranged between 4 (7%) to 52 (85%) and sensitivity ranged between 7 (11%) to 56 (92%). Streptomycin and Gentamycin were found to be effective drugs against which only 4 (7%) isolates were resistant. Oxacillin and Amikacin were also found to be effective against which 5 (8%) isolates were resistant. Penicillin was found to be least effective drug against which 52 (85%) isolates were resistant. Forty eight (79%) isolates showed resistant towards Ampicillin which is also not an effective drug (Table 5).

Similarly when effect of 15 antibiotics were checked against coagulase negative isolates the number of isolates showing resistance against each antibiotic ranged between 0 (0%) to 16 (84%) and the number of isolates showing sensitivity against each antibiotic ranged between 03 (16%) to 17 (89%). Gentamycin was found to be the most effective drugs against which 0 (0%) isolates showed resistance. Chloramphenicol, Clindamycin, Erythromycin and Streptomycin were also found to be effective against each of which 2 (11%) isolates were resistant. Penicillin was found to be least effective drug against which 16 (84%) isolates were resistant, Ampicillin being the next least effective drug against which 12 (63%) isolates were resistant (Table 6).

Though incidences of *S. aureus* are same in all the five different sweet samples collected, there is a difference in the incidences of coagulase positive *S. aureus* in different samples. Out of the total isolates obtained only 76% of the isolates showed positive result for coagulase test and 24% were negative for coagulase test. Incidences of coagulase positive isolates in sweets were more compared to that of coagulase negative isolates. But effect of antibiotics on the isolates showed that the presence or absence of coagulase has no role in giving antibiotic resistance to the isolates. Streptomycin and Gentamycin were found to be the effective drugs against both coagulase positive and coagulase negative isolates whereas Penicillin and Ampicillin were found to be least effective drugs in both the groups of isolates. But in case of coagulase negative isolates, in addition to Streptomycin and Gentamycin, Chloramphenicol, Clindamycin and erythromycin were also found to show considerable efficiencies in inhibiting the growth. Ei-Jakeel et al. have shown that a correlation exists between toxigenic isolates and coagulase production[6]. Moon et al. showed that *S. aureus* isolates harboring a predominant coa genotypes were more resistant to antibiotics compared with the isolates having rare coa types[3]. Abd Ei-Salam recorded that all toxigenic strains of *S. aureus* were coagulase positive[7]. According to Bautista et al. coagulase positive *S. aureus* isolates showed a higher incidence of enterotoxin production and significantly higher levels of enterotoxins production[8].

Table 1: List of antibiotics with diameter of zone for resistance or susceptibility

Sl. No.	Antibiotic disc	Symbol	Disk content	Diameter of zone in mm		
				R (in mm)	I (in mm)	S (in mm)
1	Amikacin	AK	30 mcg	≤19	20-26	≥27
2	Ampicillin	AMP	10 mcg	≤26	27-35	≥36
3	Chloramphenicol	C	30 mcg	≤18	19-26	≥27
4	Ciprofloxacin	CIP	5 mcg	≤21	22-30	≥31
5	Clindamycin	CD	2 mcg	≤23	24-30	≥31
6	Erythromycin	E	15 mcg	≤21	22-30	≥31
7	Gentamycin	GEN	30 mcg	≤18	19-27	≥28
8	Linezolid	LZ	30 mcg	≤24	25-32	≥33
9	Methicillin	MET	5 mcg	≤16	17-22	≥23
10	Oxacillin	OX	1 mcg	≤17	18-24	≥25
11	Penicillin G	P	10 units	≤25	26-37	≥38
12	Rifampicin	RIF	5 mcg	≤25	26-34	≥35
13	Streptomycin	S	10 mcg	≤13	14-22	≥23
14	Tetracycline	TE	30 mcg	≤23	24-30	≥31
15	Vancomycin	VA	30 mcg	≤16	17-21	≥22

‘R’=resistance, ‘I’=intermediate, ‘S’=sensitive

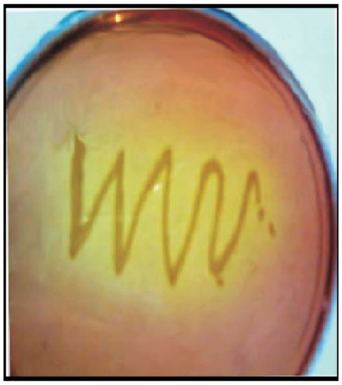

Fig. 1: Growth of *S. aureus* on Mannitol on salt agar medium

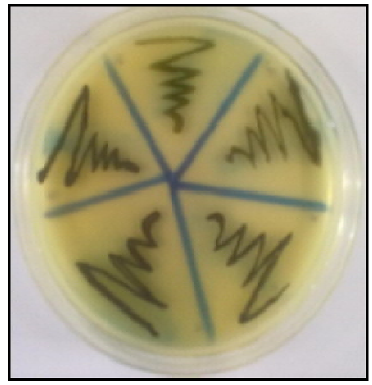

Fig. 2: Growth of *S. aureus* on Baird Parker agar medium

Table 2: Incidences of *S. aureus* and coagulase positive *S. aureus* in different sweets

Type of sweets	Total samples	Incidences of *S. aureus*	Incidences of Coagulase +ve *S. aureus*
Laddu	20	16 (80%)	14 (88%)
Mysore Pak	20	16 (80%)	11 (69%)
Doodhpedha	20	16 (80%)	12 (75%)
Gulab Jamun	20	16 (80%)	11 (69%)
Jalebi	20	16 (80%)	13 (81%)

Table 3: Antibiotic resistance pattern of coagulase positive isolates

Sl. No.	Isolate	No. of antibiotics to which isolate is resistant	No. of antibiotics to which isolate is intermediate	No. of antibiotics to which isolate is sensitive
1	UN10L	2	1	12
2	UN10M	2	2	11
3	UN10JA	5	0	10
4	KO2L	6	0	9
5	KO2P	3	2	10
6	KO2J	7	0	8
7	CPO9L	2	2	11
8	CPO9M	4	2	9
9	CPO9P	5	0	10
10	CPO9J	2	0	13

11	CPO9JA	1	2	12
12	KO5L	5	3	7
13	KO5JA	4	4	7
14	CO8L	3	0	12
15	CO8M	6	2	7
16	CO8P	4	3	8
17	CO8J	3	3	9
18	CO8JA	10	1	4
19	GO1L	4	3	8
20	GO1JA	2	0	13
21	VO4L	2	1	12
22	VO4M	0	1	14
23	VO4JA	4	1	10
24	AO7M	5	1	9
25	AO7P	3	3	9
26	AO7J	5	1	9
27	AO7JA	5	2	8
28	RO6M	6	0	9
29	RO6P	7	0	8
30	RO6J	4	0	11
31	SO3L	5	2	8
32	SO3P	2	1	12
33	SO3JA	5	0	10
34	PR7MP	12	0	3
35	PR7L	8	0	7
36	PR7DP	7	2	6
37	PR7J	9	1	5
38	PR7JA	6	2	7
39	SK4MP	7	3	5
40	SK4L	9	3	3
41	SK4DP	12	0	3
42	SK4J	5	3	7
43	SK4JA	8	3	4
44	GS10MP	0	0	15
45	GS10L	1	1	13
46	GS10DP	6	2	7
47	GS10J	3	3	9
48	GS10JA	5	2	8
49	A2MP	6	3	6
50	A2L	5	3	7
51	A2DP	4	2	9
52	A2J	3	1	11
53	A2JA	3	1	11
54	NAB8L	2	4	9

55	NAB8DP	3	3	9
56	NAB8J	2	2	11
57	V6MP	6	4	5
58	V6L	6	4	5
59	V6DP	7	5	3
60	V6J	4	2	9
61	V6JA	6	2	7

Table 4: Antibiotic resistance pattern of coagulase negative isolates

Sl. No.	Isolate	No. of antibiotics to which isolate is resistance	No. of antibiotics to which isolate is intermediate	No. of antibiotics to which isolate is sensitive
1	UN10P	3	0	12
2	UN10J	3	3	9
3	KO2M	7	1	7
4	KO2JA	5	3	7
5	KO5M	9	3	3
6	KO5P	7	1	7
7	KO5J	4	3	8
8	GO1M	4	1	10
9	GO1P	0	1	14
10	GO1J	2	1	12
11	VO4P	2	0	13
12	VO4J	2	1	12
13	AO7L	3	2	10
14	RO6L	6	3	6
15	RO6JA	1	4	10
16	SO3M	14	0	1
17	SO3J	5	1	9
18	NAB8MP	5	2	8
19	NAB8JA	6	2	7

Table 5: Effect of antibiotics on coagulase positive isolates

Sl. No.	Antibiotic	The number of isolates showing resistance	The number of isolates showing intermediate	The number of isolates showing Sensitive
1	Amikacin	05 (8%)	06 (10%)	50 (82%)
2	Ampicillin	48 (79%)	0 (0%)	13 (21%)
3	Chloramphenicol	09 (15%)	11 (18%)	41 (67%)
4	Ciprofloxacin	17 (28%)	07 (11%)	37 (61%)
5	Clindamycin	06 (10%)	08 (13%)	47 (77%)
6	Erythromycin	08 (13%)	20 (33%)	33 (54%)
7	Gentamycin	4 (7%)	02 (3%)	55 (90%)
8	Linezolid	14 (23%)	0 (0%)	47 (77%)
9	Methicillin	38 (62%)	08 (13%)	15 (25%)
10	Oxacillin	5 (8%)	09 (15%)	47 (77%)
11	Penicillin	52 (85%)	02 (3%)	07 (11%)
12	Rifampicin	26 (43%)	14 (23%)	21 (34%)
13	Streptomycin	04 (7%)	01 (2%)	56 (92%)
14	Tetracyclin	24 (39%)	21 (34%)	16 (26%)
15	Vancomycin	25 (41%)	0 (0%)	36 (59%)

Table 6: Effect of antibiotics on coagulase negative isolates

Sl. no.	Antibiotic	The number of isolates showing resistance	The number of isolates showing intermediate	The number of isolates showing Sensitive
1	Amikacin	03 (16%)	01 (5%)	15 (79%)
2	Ampicillin	12 (63%)	0 (0%)	07 (37%)
3	Chloramphenicol	02 (11%)	03 (16%)	14 (74%)
4	Ciprofloxacin	04 (21%)	03 (16%)	12 (63%)
5	Clindamycin	02 (11%)	03 (16%)	14 (74%)
6	Erythromycin	02 (10.52%)	07 (36.84%)	10 (52.63%)
7	Gentamycin	0 (0%)	02 (10.52%)	17 (89.47%)
8	Linezolid	07 (36.84%)	0 (0%)	12 (63.15%)
9	Methicillin	07 (36.84%)	03 (15.7%)	09 (47.36%)
10	Oxacillin	08 (42.10%)	01 (5.26%)	10 (52.63%)
11	Penicillin	16 (84.21%)	0 (0%)	03 (15.7%)
12	Rifampicin	07 (36.84%)	07 (36.84%)	05 (26.31%)
13	Streptomycin	02 (10.52%)	0 (0%)	17 (89.47%)
14	Tetracyclin	09 (47.36%)	02 (10.52%)	8 (42.10%)
15	Vancomycin	07 (36.84%)	0 (0%)	12 (63.15%)

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