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SUITABILITY OF DIFFERENT PLASMAS FOR DETECTION OF FREE AND BOUND COAGULASE IN CORRELATION WITH SOME ENZYMATIC ACTIVITIES AND ENTEROTOXIGENICITY OF STAPHYLOCOCCUS AUREUS

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SUMMARY: Forty five Staphylococcus aureus isolates recovered from dairy and meat products were tested for enterotoxigenicity, haemolysin, DNase, thermonuclease, fibrinolysin and coagulase, using human, rabbit, pigeon, sheep, guinea pig and rat plasmas. 19 isolates were enterotoxigenic and 25 isolates produced B-haemolysin. 92.3% of B-haemolysin producing, enterotoxigenic isolates were DNase positive. 38.5% of the B-haemolysin producing, enterotoxigenic isolates produced thermonuclease. Fibrinolysin was detected in 41.7% of the B-haemolysin producing, enterotoxigenic isolates. With regard to coagulase test, rabbit plasma gave the best result, followed by pigeon, human sheep, rat and guinea pig plasmas. The enterotoxigenic isolates clotted the plasma earlier than the non-enterotoxigenic ones. From these results it can be concluded that none of the above - mentioned tests could be depend upon as indicative of enterotoxigenicity.

INTRODUCTION

It has been widely accepted that the positive coagulase reaction of staphylococcal strains isolated from food poisoning outbreaks is indicative of pathogenicity of such isolates supporting the oetiological role in the diseas (Casman et al., 1967; Payne and Wood, 1974; Niskanen and Korianen, 1977 and Niazi et al., 1986).

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Previous studies of the suitability of human and different animal plasmas for coagulase production either by tube or slide methods (Field and Smith, 1945 ; Orth et al., 1971 ; Hajek and Marsalek , 1976) have provided valuble informations, however, such plasmas were tested on pathogenic Staphylococcus aureus isolated form animal and human origin, but non of these papers has pointed out any relationship between the type of plasma and enterotoxigenicity. It has been reported however that all enterotoxigenic Staphylococcus aureus isolated from food poisioning outbreaks and mastitic milk were coagulase positive with rabbit plasma by the tube method (Niskanen and Korianen, 1977 ; Niazi et al., 1985).

Very little information was available concerning the comparison between coagulability of various plasmas and enterotoxin producing Staphylococcus aureus (Hirvela and Korkeala, 1982). The purpose of this work was to compare the coagulability of human and various animal plasmas with enterotoxin and non-enterotoxin producing Staphylococcus aureus isolated from dairy and meat products and to demonstrate the relationship between the type of haemolysin , fibrinolysin, DNase, thermonuclease and coagulase producton and enterotoxigenicity of Staphylococcus aureus strains.

MATERIALS AND METHODS

A total of 45 isolates of Staphylococcus aureus recovered from dairy and meat products was tested for enterotoxin production (Donnelly et al., 1967 ; Robbins et al., 1974), haemolysin production (Elek and levy, 1950 , the production of alpha and beta haemolysis were determined by using 3% washed rabbit and sheep erythrocytes respectively), DNA hydrolysis (Barry et al., 1973), thermonuclease (Barry et al., 1973), fibrinolysin production (Christie and Wilson, 1941) and coagulase production (Cruickshank et al ., 1975). Both slide and tube coagulase tests were carried out using citrated blood plasma obtained from man, rabbit, pigeon, sheep, guinea pig and rat. The coagulase reaction of the tube test was read at intervals of 1, 3, 6 hours of incubation at 37°C and 24 and 48 hours of maintenance at room temprature, while slide test was recoreded within 5 - 10 seconds.

RESULTS

1. enterotoxigenicity

Of the tested *Staphylococcus aureus* isolates, 19 were found to be enterotoxigenic, of which 4 isolates were type A, 3 type D, 3 type A + B, 3 type A + D, 4 type A + E, 1 type A + B + C and 1 type A + C + D.

2. Haemolysin

Of the 45 isolates, 25 produced B-haemolysin and 20 produced ∝ - haemolysin, 68.4% (13/19) of the enterotoxigenic isolates produced B-haemolysin in comparisoln to 46.2% (12/26) of non-enterotoxigenic ones. On the other hand. 52% (13/25) of B-haemolysin producing isolates were enterotoxigenic, while 30% (6/20) of ∝-haemolysin producing isolates produced enterotoxins **(Tables 1 and 4).**

3. Dase

As shown in **(Table 1),** the highest incidence of DNA (92.3%) was reported in B-haemolysin producing, enterotoxigenic isolates, followed by ∝ -haemolysin producing, enterotoxigenic isolates (66.7%). 21 - 25% of non-enterotoxigenic isolates were DNase positive, both in ∝ - and B-haemolysin producing isolates, respectively.

4. Thermonuclease

None of the ∝ -haemolysin producing, non-enterotoxigenic isolates produced thermonuclease, while only 2 out of 12 B-haemolysin producing, non-enterotoxigenic isolates were positive for this enzyme. The percent of positive isolates increased up to 33.3 and 38.5% among the enterotoxigenic ∝ - and B-haemolysin producing isolates, respectively. **(Table 1)** .

5. Fibrinolysin

As shown in **(Table 1),** the fibrinolysin was detected in 41.7% and 61.5% of B-haemolysin producing, enterotoxigenic and non-enterotoxigenic isolates respectively, particularly when rabbit plasma was used. The sheep plasma was unsuiltable. The fibrinolysin was rarely found in ∝ -haemolysin producing isolates.

Table 1: Production of DNase, thermonuclease, fibrinolysin and haemolysin by enterotoxigenic and nonenterotoxigenic Staphylococcus aureus isolates.

	25-β-Bhaemolysin producing strains				20 α - haemolysin producing strains			
	DNase	Thermonuclease	Fibrinolysin R	Fibrinolysin S	DNase	Theronuclease	Flbrinolysin R	Flbrinolysin S
Enterotoxigenic	12	5	8	2	4	2	1	1
13	(92.3)	(38.5)	(61.5)	(15.4)	6 (66.7)	(33.3)	(16.7)	(16.7)
Non-enterotoxigenic	3	3	5	3	3	0	3	1
12	(25.0)	(16.7)	(41.7)	(25.0)	14 (21.4)	(0)	(21.4)	(7.1)

DNase: Deoxyribonuclease Thermonuclease: Heat-stable deoxyribonuclese

R : Rabbit plasma S : Sheep plasma

() : %

Table 2: Results of tube coagulase tests of human and animal plasma by Staphylococcus sureus.

Plasma	Enterotoxigenic strains (19) Clotting time (h)							Total positive (%)	Non-enterotoxigenic strains (26) Clotting time (h)							Total positive (%)
	1	3	6	24	48	W*	N		1	3	6	24	48	W*	N	
Human	--	--	1 (5.3)	11 (57.9)	1 (5.3)	4 (21.1)	2 (10.5)	89.5	--	--	--	5 (19.2)	--	9 (34.6)	12 (46.2)	53.8
Rabbit	3 (15.8)	7 (36.8)	5 (26.3)	4 (21.1)	--	--	--	100.0	--	2 (7.7)	1 (3.8)	9 (34.6)	9 (34.9)	4 (15.4)	1 (3.8)	96.2
Pigeon	--	1 (5.3)	1 (5.3)	10 (52.6)	3 (15.8)	3 (15.8)	1 (5.3)	94.7	-- --	-- --	-- --	5 (19.2)	10 (38.5)	5 (19.2)	7 (23.1)	76.9
Sheep	--	1 (5.3)	--	3 (15.8)	10 (52.6)	2 (10.5)	3 (15.8)	84.2	--	--	--	3 (11.5)	8 (30.8)	8 (30.8)	7 (30.8)	73.1
Rat	--	--	--	4 (21.1)	9 (47.3)	3 (15.8)	3 (15.8)	84.2	--	--	--	2 (7.7)	6 (23.1)	8 (30.8)	10 (38.5)	61.5
Guinea pig	--	1 (5.3)	--	5 (26.3)	8 (42.0)	1 (5.3)	4 (21.1)	78.9	--	--	--	4 (15.4)	5 (19.5)	6 (23.1)	11 (42.3)	57.71

* : A small organized clot is considered a positive coagulase reaction (firstly recorded after 24 and 48 hours

N : Negative coagulase reaction.

( ) : %

Table 3: Results of slide coagulase test of human and animal plasma by Staphylococcus aureus

Strains	Reaction	Undiluted plasma											
		Human		Rabbit		Pigeon		G.pig		Sheep		Rat	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Enterotoxigenic (19)	+	13	68.4	15	79	5	26.3	5	26.3	12	63.2	12	63.2
	±	4	21.1	2	10.5	5	26.3	4	21.1	2	10.5	1	5.2
	a	2	10.5	2	10.5	2	10.5	2	10.5	2	10.5	2	10.5
	-	0	0	0	0	7	36.9	8	42.1	3	15.8	4	21.1
Non-enterotoxigenic (26)	+	10	38.5	6	23.1	4	15.4	2	7.7	8	30.8	8	30.8
	±	6	23.1	4	15.4	0	0	2	7.7	4	15.4	1	3.9
	a	10	38.5	10	38.5	10	38.5	10	38.5	10	38.5	10	38.5
	-	0	0	6	23.1	12	46.2	12	46.2	4	15.4	7	26.9

\+ : Positive reaction

± : Doubtful reaction

a : Autoagglutination

\- : Negative reaction

() : Number of tested strains

Table 4: Correlation between enterotoxigenicity, haemolysin, coagulase, fibrinolysin, DNase and thermonuclease of Staphylococcus aureus.

Strains	Haemolysin		Coagulase		Fibrinolysin		DNase	Thermo-nuclease
	β	α	F	B	R.	S.		
Enterotoxi-genic 19	13 (68.4)	6 (31.6)	19 (100)	15 (78.9)	9 (47.4)	3 (15.8)	16 (84.2)	7 (36.8)
Non-entero-toxigenic 26	12 (46.2)	14 (53.8)	21 (80.8)	6 (23.1)	8 (30.8)	4 (15.4)	6 (23.1)	2 (7.7)

F : Free coagulase (tube coagulase) B: Bound coagulase (clumping factor)

R : Rabbit plasma S: Sheep plasma

(): %

6. Coagulase

From the results presented in **(Table 2)**, it is clear that rabbit plasma gave the best result. All enterotoxigenic isolates were positive within 24 hours. During this period of time only 34.6% of the non-entertoxigenic isolates were positive. However, the percentage of positivity reached 96.2% when the tubes were left for 48 hours. The pigeon plasma were the second best, then followed by human, sheep, rat and g. pig plasma. It is noted that human plasma was the least sensitive in case of non-enterotoxigenic isolates of *Staphylococcus aureus*. It is also clear from the table that the enterotoxigenic isolates clotted the plasma earlier than the non-enterotoxigenic ones , particularly in case of rabbit plasma, which clotted already after one hour. The human plasma started clotting after 6 hours and rat's plasma after 24 hours. On the other hand, plasma of man and animals, whith the exception of rabbit, started to be clotted by the non-enterotoxigenic isolates after 24 hours.

The slide coagulase tes **(Table 3)** was less sensitive than the tube coagulase test. The enterotoxigenic isolates showed distinctly higher rate of positive reactions than the non-enterotoxigenic ones particularly when rabbit, human and sheep plasma were used.

The correlation between the above-mentioned tests and entertoxigenicity is presented in **(Table 4)**. From this table it is clear that not all enterotoxigenic strains were positive in these tests, inasmuch as not all non-enterotoxigenic strains were not negative.

DISCUSSION

Haemolysin, coagulase, fibrinolysin, DNase and thermostable DNase have been considered as criteria for determination of pathogenic Staphylococcus aureus (Victor et al., 1969 ; Baird-Parker, 1974 ; Lachica, 1976). With regard to food poisoning with Staphylococcus aureus only the enterotoxigenic isolates are of interest (Casman , et al., 1967 ; Bergdoll, 1970; Simikovicva and Gilbert, 1971 ; Amtsberg , 1980 ; Hobbs and Gilbert, 1982; Niazi et al., 1986) . However, the detection and identification of enterotoxins are labourious and needs well-equipped laboratories and well-trained personnel (Casman and bennett, 1963 ; Zehren and zehren, 1968 ;

Reiser, et al., 1974 ; Robbins, et al., 1974). Accordingly, the above - mentioned tests have been tested to differentiate the enterotoxigenic isolates of Staphylococcus aureus (Bugrova, 1981 ; Niskanen and Korianen, 1977 ; Barry et al., 1973 ; Victor, et al., 1969). The routine methods recommended for the isolation and identification of this organism is based on the use of blood agar, DNase, Baird Parker media or media with salts for isolation and the suspected colonies are to be tested for coagulase. From the results obtained in the present work it is clear that not all B-haemolysin procucing isolates were enterotoxigenic and 31.6% of the ∝ - haemolysin producing ones were enterotoxigenic. Therefore, it would be incorrect, if only the B-haemolytic are considered for the primary selection of the isolates. This would apply also to fibrinolysin, DNase and thermonuclease. The results of coagulase test were of particular interest as this test is considered as a key test in the routine diagnosis. The plasma of different animals and man showed variable results. This is in agreement with Hirvela and Karkeala (1982). Therefore, only one type of plasma should be agreed upon universally. It is interesting to note that, if rabbit plasma is only considered, that all enterotoxigenic strains were coagulase positive. However, 80% of the non-enterotoxigenic strains were also positive. On the base of the results of abovementioned tests it can be concluded that none of these tests is 100% indicative of enterotoxigenicity and the only reliable test for enterotoxigenicity is the detection and identification of the enterotoxins.

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