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CORRELATION BETWEEN ANTIBIOTIC-RESISTANCE, ENTEROTOXIGENICITY AND ENZYMATIC ACTIVITIES OF STAPHYLOCOCCUS AUREUS RECOVERED FROM FOODS

By

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INTRODUCTION

Strains of *S.aureus* implicated in staphylococcal food poisoning are mostly of human origin (Bryan, 1978; Gilbert, 1983). Coagulase positive staphylocci are frequently found on the skin (nares, hands, face, etc....) of normal population (Nobel, 1981) and food cans readly become contaminated by carrier workers during handling in food processing plants, food service establishements and homes as determined by bacteriological examination of various foods (Niazi et al., 1986).

There is no doubt that a number of antibiotics reduces the incidence of staphylococcal infections (skin infections in human and/or mastitis in dairy animals (Allenstein, 1977; MacDonald and Anderson, 1981; Stratford, 1960). Nevertheless, the wide spread use of antibiotics in therapy and prophylaxis has been criticized on the grounds of drug toxicity and possibility of acquiring drug resistance mainly due to B-lactamase production by *S. aureus* might be encourged among a variety of enterotoxigenic staphylococci. In addition, plasmids have been discovered which carry genetic determinants of both drug resistance and enterotoxin production and these

plasmids can transfer from one strain of staphylococci to another (Transduction, Richmond, 1980).

This work was made to investigate the possible correlation between enterotoxigenicity, enzymatic activity and the in vitro antibiotic sensitivity of staphylococcal strains recovered from different food sources.

MATERIAL AND METHODS

Tested cultures:

A total of 72 *S.aureus* strains was studied, of which 38 strains were of the enterotoxigenic type (8 producing enterotoxin type A, 4 type D, 6 type A+B, 10 type A+D, 6 type A+E, 2 type A+B+C, and 2 type A+C+D. The other tested strains (34) were of nontoxigenic type. Both types were isolated during routine laboratory examination in Bacterial Toxins Unit, Animal Health Research Institute. The enterotoxigenicity of the strains was tested by using a highly purified and lyopholysed enterotoxins A,B,C,D, and E and their specific antisera (Robbins et al., 1974).

Antibiotic susceptibility:

All isolates were tested for their sensitivity towards 13 antibiotics by disc diffusion method recommended by the National Committee for Clinical Laboratory Standards, (N.C.C.L.), Subcommittee on Antimicrobial Susceptibility Testing (1974) and by Barry (1976). A heart infusion agar (Oxoid) plate was inoculated with 10^4 C.F.U. (colony forming unit) of each tested Staphylococcal strain, on the surface of which a set of disc containing the following antibiotics was distributed: lincomycin (2µg), neomycin(30µg),streptomycin(10µg),tetracycline(30µg),penicillin-G(10 I.U.),chloramphenicol(30µg),gentamicin, (10µg), erythromycin (5µg), Kanamycin (30 µg), amoxycillin (25µg), ampicillin (10µg), nalidixic acid (30µg) and nitrofurantoin (200µg). The diameter of inhibition zones was measured and the isolates were classed as sensitive,

intermediate or resistant according to the interpretative table of Barry (1976).

Testing for enzymatic activity:

All the staphylococcal isolates were tested for the production of haemolysin (Elek and levy, 1950), alpha and beta haemolysins (by using 3% washed rabbit and sheep erythrocytes, respectively), DNA-hydrolysis (Barry et al. 1973), thermonuclease (Barry et al., 1973), fibrinolysin (Christie and Wilson, 1941) and coagulase with rabbit's plasma using the tube test (Cruickshank et al., 1975).

RESULTS

Sensitivity of entertoxigenic and non-toxigenic staphylococcal strains isolated from different food sources to 13 antibiotics and antibacterials was determined by disc diffusion and the results are presented in **Tables 1 and 2.**

From these tables it is clear that toxigenic and non-toxigenic strains were equally highly sensitive to erythromycin, tetracycline, chloramphenicol, amoxycillin, kanamycin and nitrofurantoin. However, it was observed that enterotoxigenic staphylococcal isolates were more likely to acquire resistance to tetracycline,erythromycin,penicillin-G gentamicin, kanamycin, amoxycillin, ampicillin, and neomycin. On the other hand, there was no marked difference between the toxigenic and non-toxigenic *S.aureus* strains in the frequency of resistance to lincomycin, streptomycin, chloramphenicol,nalidixic acid and nitrofurantoin. Moreover, a high rate of resistance of both types of tested strains was noted in case of nalidixic (65.58; 76.47%), neomycin (76.32; 52,94%) and gentamicin (68.42; 35,30 %) respectively.

Although, the toxigenic and non-toxigenic staphylococcal strains exhibited a high degree in vitro susceptibility to streptomycin (73.68 and 82.35 % respectively), this activity was mostly of intermediate type.

Table 1 : Antibiotic sensitivity of 38 strains of enterotoxigenic Staphylococcus aureus recovered from foods.

Antibiotic	Disc Potancy	Susceptibility						Resistant	
		Sensitive		Intermediate		Total			
		No.	%	No.	%	No.	%	No.	%
Lincomycin	2 µg	2	5.26	28	73.68	30	78.94	8	21.05
Neomycin	30 µg	---	----	9	23.68	9	23.68	29	76.32
Streptomycin	10 µg	4	10.53	24	63.15	28	73.68	10	26.32
Tetracycline	30 µg	25	65.79	6	15.79	31	81.58	7	18.42
Penicillin-G	10 I.U.	17	44.74	1	2.63	18	47.37	20	52.63
Chloramphenicol	30 µg	10	26.32	24	63.15	34	89.47	4	10.35
Gentamicin	10 µg	---	----	12	31.58	12	31.58	26	68.42
Erythromycin	15 µg	20	52.63	16	42.11	36	94.74	2	5.25
Kanamycin	30 µg	8	21.05	24	63.16	32	84.21	6	15.79
Amoxycillin	25 µg	19	50.00	7	18.42	26	68.42	12	31.58
Ampicillin	10 µg	8	21.05	10	26.32	18	47.37	20	52.63
Nalidixic acid	30 µg	---	----	13	34.21	13	34.21	25	65.79
Nitrofurantoin	200 µg	28	73.68	8	21.05	36	94.74	2	5.26

Table 2 : Antibiotic sensitivity of 34 strains of non-toxigenic Staphylococcus aureus recovered from foods.

Antibiotic	Disc potancy	Susceptibility						Resistant	
		Sensitive		Intermediate		Total			
		No.	%	No.	%	No	%	No	%
Lincomycin	2 µg	12	35.3	14	41.17	26	76.47	8	23.53
Neomycin	30 µg	---	----	16	47.06	16	47.06	18	52.94
Streptomycin	10 µg	6	17.65	22	64.70	28	82.35	6	17.65
Tetracycline	30 µg	22	64.70	12	35.30	34	100.00	--	-----
Penicillin-G	10 I.U.	12	35.30	12	35.30	24	70.60	10	29.40
Chloramphenicol	30 µg	26	76.47	8	23.53	34	100.00	--	-----
Gentamicin	10 µg	--	-----	22	64.70	22.	64.70	12	35.30
Erythromycin	15 µg	26	76.47	2	5.88	28	82.35	5	17.65
Kanamycin	30 µg	18	52.94	16	47.06	34	100.00	--	-----
Amoxycillin	25 µg	24	70.60	6	17.64	30	88.24	4	11.76
Ampicillin	10 µg	6	17.64	16	47.06	22	64.70	12	35.30
Nalidixic acid	10 µg	--	-----	8	23.53	8	23.53	26	76.47
Nitrofurantoin	200 µg	14	41.18	20	58.82	34	100.00	--	-----

Correlation between antibiotic-Reesistance,.....

As shown in **Tables 1 and 2,** the 3 types of penicillins tested were unequally active, amoxycillin was found to be more active against toxigenic and non-toxigenic strains than penicillin-G and ampicillin. However, the toxigenic types expressed more resistance to the tested types of penicillins than the nontoxigenic ones.

The relationship between the type of staphylococcal enterotoxin, enzymatic activities and antibiotic resistance pattern is summerized in **Table 3.** It was remarkable that cultures producing more than one type of enterotoxin showed a higher rate of resistance (polyresistance) than that producing a single type of enterotoxin. Cultures producing enterotoxins A and D together and A+C+D gave the highest rate of total resistance (51.25 and 69.20 %, respectively). In addition, *S.aureus* strains producing enterotoxins A+D showed a complete resistance to neomycin and gentamicin (100 %), a high frequency of resistance (60-80 %) to penicillin-G, kanamycin, ampicillin and nalidixic acid, but low frequency of resistenee was exhibited to tertracycline, chloramphenicol and erythromycin. In contrast, *S. aureus* cultures producing either enterotoxin type A or D alone showed a lower rate of resistance to the most tested agents than those demonstrated by strains producing both types of enterotoxins (A+D). Furthermore, *S. aureus* cultures producing enterotoxins A+C+D were completely resistant to most of the tested antibiotics and were sensitive only to lincomycin, erythromycin, kanamycin, amoxycillin and nitrofurantion. With exception of 2 strains of *S. aureus* producting enterotoxins A+E, all enterotoxigenic isolates were sensitive to nitrofurantoin.

The results in **Table 3** revealed that there was a particular association with the high rate of resistance exhibited by staphylococcal cultures producing enterotoxins A+D and A+C+D and the rate of enzymatic activity by these cultures. All strains producing enterotoxins A+D gave positive reactions with fibrinolysin and alph and beta-haemolysin, 80 % gave NDase positive, while 60 % of cultures produced thermonuclease. In addition, both tested strains producing enterotoxins A+C+D were positive to all

Table 3 : Relationship between type of enterotoxin , antibiotic-resistance pattern and enzymatic activity of Staphylococcus aureus recovered from food.

Type of enterotoxin	No. of strains	Antibiotic resistance (No. of strains) *														Enzymatic activity (positive reaction)					
		L (2µg)	NE (30µg)	ST (10µg)	T (30µg)	PEN (10 I.U)	CH (30µg)	GE (10µg)	ER (15µg)	KA (30µg)	AMO (25µg)	AMP (10µg)	NA (30µg)	NI (200µg)	total resistance (%)	DNase	Thermo-nuclase	Fibrino-lysin	coagulase	Haemolysin α	Haemolysin β
A	8	-	6	2	2	4	-	4	-	--	2	2	4	-	25	6	--	2	8	4	4
D	4	-	2	2	1	-	-	2	-	--	-	2	3	-	23.07	4	2	--	4	-	4
A + B	6	2	5	-	-	6	-	6	-	--	4	4	2	-	37.17	4	-	6	6	-	6
A + D	18	4	18	4	2	6	2	18	2	6	4	8	8	-	51.35	8	6	18	18	2	8
A + E	6	2	2	-	-	-	-	2	-	--	-	-	6	2	26.42	6	4	-	6	4	2
A + B + C	2	-	2	-	-	2	-	-	-	--	2	2	-	-	30.76	2	2	-	2	-	2
A + C + D	2	-	2	2	2	2	2	2	-	--	2	2	2	-	69.20	2	2	2	2	-	2
Total (%)	38	8 21.05	29 76.32	10 26.32	7 18.42	20 52.63	4 10.53	26 68.42	2 5.26	6 15.79	12 31.58	20 52.63	25 65.79	2 5.26		30 78.95	16 42.10	20 52.63	38 100.0	10 26.31	28 73.68
Non-Toxigenic (%)	34	8 23.53	18 52.94	6 17.65	0 0.0	10 29.40	0 0.0	12 35.30	6 17.65	0 0.0	4 11.76	12 35.30	26 76.47	0 0.0		10 29.41	2 5.88	16 47.05	28 82.35	18 52.94	16 47.06

* Lincomycin (L), Neomycin (NE), Streptomycin (ST), Tetracycline (T), Penicillin-G(PEN), Chloramphenicol (CH), Gentamicin (GE), Erythromycin (ER), Kanamycin (KA), Amoxycillin (AMO), Ampicillin (AMP), Nalidixic acid(NA), Nitrofurantoin (NI).

enzymatic reactions. In contrast, the other tested enterotoxigenic staphylococcal cultures producing single or more than one type of toxins and showed a relatively lower resistance pattern to different antibiotics, gave variable results with the enzymatic reactions.

DISCUSSION

The obtained results have demonstrated that the incidence of resistance among the enterotoxigenic staphylococcal cultures to various antibiotics was higher than among the non-toxigenic ones. Such results substantiated the findings of Mochmann et al. (1976) and Osvath-Marton et al. (1976). In contrast, Pelledo et al.(1985) reported that the incidence of resistance among the enterotoxin *S. aureus* cultures isolated from food handlers, was lower than the non-toxigenic cultures (50 and 62.2 % respectively). However, this does not apply generally to all antibiotics as both the toxigenic and non-toxigenic *S. aureus* strains were equally not only highly sensitive to erthromycin, tetracycline, chloramphenicol, amoxycillin, kanamycin, and nitrofurantoin, but also highly resistant to neomycin, gentamicin and naladixic acid. Similar findings concerning the resistance and susceptibility of *S. aureus* isolated from human and animal sources have been reported by other investigators (Plorde and Sherris, 1974; Lacy, 1975; Witte 1977, Jawetz et al., 1977, Bauer and Seeger, 1979; Linton, 1982).

The higher rate of resistance exhibited by both types of tested staphylococci to penicillin-G and ampicillin than amoxycillin was anticipated, since penicillin-G and ampicillin are considered to be highly sensitive to the destructive action of B-lactamase enzyme produced by some strains of *S. aureus* than amoxycillin (B-lactamase sensitive penicillins (Lacy, 1975; Witte, 1977). Moreover, the high incidence of resistance and/or intermedate

sensitivity among enterotoxigenic and non-toxigenic S. aureus strains to the tested aminogylocosidal antibiotics, particularly gentamicin, neomycin and streptomycin, demonstrated here, was correlated to the explanations of Plorde and Sherris (1974) and Bauer and Seeger (1979) in study of the various mehanisms of staphylococcal resistance to various chemotherapeutic agents. Theyreported that the increase in the resistance to *S. aureus* aminoglycosidal antibiotics might be referred to structural changes in specific protein attached to the antibiotic.

The only paper dealing with the correlation between the type of enterotoxin produced by staphylococci involved in human food poisoning and spectrum of antibiotic-resistance (Pelledo et al., 1985) has denoted that cultures producing enterotoxin A (alone or together with D toxin) gave the highest rate of resistance (75 %) but were not associated with any particular antibiotic. However, in our case, cultures producing enterotoxins A+D or A+C+D gave the highest rate of resistance (51.25 and 69.20 % respectively), while *S. aureus* cultures producing either A or D toxin alone showed a lower rate of resistance to most tested antibiotics (25 and 23 %, respectively). The difference between our study and that done by Pelledo et al (1985) is the limited numbers of antibiotics (penicillin,ampicillin,streptomycin , penicillin+ ampicillin) used by them. With Regard to the Pelledo et al. (1985) results the present study has demonstrated that cultures producing two toxins, namely A+D showed a complete resistance to neomycin and gentamicin (100 %), high frequency to penicillin-G, Kanamycin, ampicillin and naladixic acid (60-80 %) and low frequency of resistance to tetracycline, chloramphenicol and erythromycin. In addition, cultures producing multiple enterotoxins (A+C+D) were completely resistant to most of the tested antibiotics.

On the other hand, a high tendency in the ability of *S. aureus* to produce more than one type of enterotoxin

such as A+D or A+C+D was found to be associated with a high rate of antibiotic resistance and high rate of specific positive enzymatic reactions. In contrast, cultures producing a single type of enterotoxin gave a variable data either in antibiotic resistance or enzymatic reaction.

Based on these data, it seems that there is a particular association between enzymatic activity, enterotoxigenicity and antibiotic resistance of *S. aureus* to various antibiotics.

SUMMARY

A total of 72 staphylococcal strains recovered from different food sources, of which 38 strains were of enterotoxigenic type and 34 strains of non-toxigenic type, were tested for antibiotic resistance and enzymatic activities. The possible relationships between the enterotoxigenicity, antibiotic resistance and enzymatic activities were also investigated. Enterotoxigenic *S. aureus* isolates were more likely to acquire resistance to the majority of tested antibiotics, tetracycline, erythromycin, penicllin-G, gentamicin, kanamycin, amoxycillin, ampicillin and neomycin. However, no marked difference in the frequency of resistance between the toxigenic type and non-toxigenic ones to lincomycin, streptomycin, chloramphenicol, nitrofurantoin and nalidixic acid. Staphylococcal cultures that were producing multiple enterotoxins, particularly A+D and A+C+D, showed a high rate of total resistance (51.25 and 69.20 %, respectively) to the tested antibiotics than that producing a single type of enterotoxin (A, 23 % ; D, 25 %). In addition, a particular association between the rate of antibiotic resistance among *S. aureus* cultures producing multiple enterotoxins and the high rate of enzymatic activities by these cultures was remarked.

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