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Phage typing and toxigenicity test of *Staphylococcus aureus strains from food contact surfaces and foods prepared in boarding schools in Zaria, Nigeria.*

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Out of 34 *Staphylococcus aureus* strains isolated from food contact surfaces and foods prepared in five schools with boarding facilities in Zaria, 7(20.6%) were toxigenic using cat toxicity test. Thirteen (38.2%) were alpha haemolytic, 16(47.1%) beta- haemolytic, 19(55.9%) coagulated sheep plasma, 15 (44.1%) coagulated human plasma while 8 (23.5%) coagulated both human and sheep plasma. All the 34 strains were DNase positive. Thirty two (94.1%) were typable at routine test dilution (RTD), 28(82.3%) showed strong lysis while 4(11.8%) showed weak lysis. Nineteen (59.4%) of the typable strains were by group IV phage set and 9(28.1%) by group III phages. The isolation of toxigenic strains of *S. aureus* and the presence of alpha –haemolytic and phage group III strains of *S. aureus* in food is of concern because alpha-haemolytic strains are known to be toxigenic and phage group III strains of *S. aureus* have been implicated frequently in food borne diseases. Hazard analysis critical control point (HACCP) of food is recommended to control and prevent presence of toxigenic strains of organisms in foods served to students in boarding schools.

Key words: *Staphylococcus aureus*, toxigenic, food-borne diseases, haemolytic, HACCP, boarding schools.

INTRODUCTION

Outbreaks of food poisoning caused by *Staphylococcus aureus* are frequently reported (Bergdol, 1989, Maguire *et al*., 1991; Su and Wong, 1995). The possibility that staphylococcal food poisoning will keep occurring is considered very high because *Staphylococcus aureus* is ubiquitous. Staphylococcal enterotoxin, the causative agent of staphylococcal food poisoning may be produced although the organisms themselves die out during processing and storage. Neither tactile, visual nor olfactory (organoleptic) changes occur in foods containing high levels of *Staphylococcus aureus* or their enterotoxin (Collins and Lyne, 1986; Bergdol, 1989).

The mechanism of pathogenicity of this organism has been investigated using different models including screening for enterotoxin production via emetic response in monkeys and cats (Su and Wong, 1995; Oranusi *et al*., 2006), haemolysin production, isoenzyme comparison and phage sensitivity (Umoh *et al*., 1991; Betley *et al*., 1992; Oranusi *et al*., 2006). However, DNA analysis has been particularly useful in recent time

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(Olorunfemi *et al.*, 2005; Willen *et al.*, 2006; Thanh *et al.*,; 2006; Elisa *et al.*, 2006).

Phage typing is an established technique in epidemiological studies of S. *aureus* infection in man. The technique has also been used on animal strains, particularly those from diary cattle. Mastitis due to this organism is a serious problem in all the major dairy producing countries (Frost, 1967). Typing provides evidence for the establishment of association with potential sources and has been used in conjunction with epidemiological data to provide direct or indirect evidence for the likely route of transmission of pathogen to food.

The identification, typing and screening for toxigenic strains of *S. aureus* throughout food processing and production chain is useful to the hazard analysis critical control point (HACCP)-based food safety plans aimed at the control and prevention of food borne pathogens. The main objectives of this study is to screen *S. aureus* strains isolated from foods and food contact surfaces for enterotoxin production using cat emetic response, coagulase and DNase tests, phage typing of *S. aureus* isolates to determine their phage types and pattern and to proffer practical solutions to prevent food contamination.

MATERIALS AND METHODS

Pure cultures of isolates dully characterized by biochemical and physiological tests using standard techniques as described by Collins and Lyne,1986 were subjected to coagulase, DNase and haemolysis tests.

Coagulase, DNase and haemolysis tests

Coagulase production was determined in 10^{-1} dilution of both human and sheep plasmas in test tubes. The results were interpreted as described by Collins and Lyne (1986) and Umoh *et al.* (1999).

DNase production was on DNase agar (Difco) reconstituted according to the manufacturer's instruction and following the procedures as described by Collins and Lyne (1986).

Haemolytic reaction was determined on 10% blood agar plates (Oxoid). Haemolytic zones were identified as alpha (α) haemolytic or beta (β) haemolytic where there is incomplete or complete haemolysis, respectively. No haemolysis was recorded as gamma (γ) haemolysis.

Cat toxicity test (emetic response) for *S. aureus*

This was performed using the methods of Persons and Summers (1971) and Melling *et al.* (1976). Kittens 1-3 months old and weighing approximately 1.6kg were acclimatized to the laboratory for two weeks during which they were fed only with milk and rice. This was done to counteract food sensitivity and thus emesis due to change in diet (Ridgway, 1973; Guilford *et al.*, 2001). *S. aureus* cultures which had been grown for 8 hours on nutrient broth (Oxoid) were prepared as a suspension in sterile normal saline. This was adjusted to 10^6 cells standard (suspension) using the standard opacity tubes. About 3ml aliquots of this suspension were inoculated into 500ml sterile milk and 500g sterile rice, respectively and incubated for 6-8 hours for toxin production. This was fed to kittens which had been starved for 8 hours. The animals were then monitored. Production of emesis within 5 hours from feeding indicated positive toxicity test (Melling *et al.*, 1976).

Phage typing

Thirty four dully characterized *S. aureus* isolates which are coagulase and DNase positive and obtained from raw

foods, cooked foods, food contact surfaces, hand and finger nails of cooks were purified by sub-culturing on nutrient agar plates (Oxoid). Pure isolates were phage typed using a set of groups I, II, III and IV type phages. Phage typing was conducted using the method of Williams and Rippon (1962). A routine test dilution (RTD) of 10^2 and 10^3 of the phages was made in nutrient broth. About 12 hours broth culture of the *S. aureus* isolates adjusted to 10^8 and 10^9 cells using the standard opacity tubes were seeded onto nutrient agar plates. The cultures were Incubated at 37°C for 6 hours at ambient room temperature of 28°C and left overnight before examination for plaque formation. The results were recorded as ++, for strong lysis; +, for weak lysis and − for no lysis (no visible plaque) (Williams and Rippon 1962).

RESULTS

Nineteen (55.9%) of the 34 *S. aureus* isolates coagulated sheep plasma, 15 (44.1%) coagulated human plasma while 8 (23.5%) coagulated both human and sheep plasmas. All the *S. aureus* strains were DNase positive. Sixteen (47.1%) were beta-haemolytic and 13 (38.2%) were alpha-haemolytic. Table 1 shows the distribution of the *S. aureus* isolates by toxicity tests. It reveals that 7(20.6%) of the isolates were toxigenic. Milk feed accounted for 8.8% of the positive response.

Table 2 shows the distribution by source of the toxigenic *S. aureus* isolates. It reveals that raw food accounted for 5(71.4%) of the toxigenic strains while food contact surfaces and food handlers had 14.3% respectively. All the toxigenic strains of *S. aureus* in this study were haemolytic and coagulated either human or sheep plasma. There was no specific relationship in pattern of distribution of the toxigenic strains to source of isolation, coagulase and haemolytic reactions.

Thirty two (94.1%) of the 34 *S. aureus* isolates were typable with one or more of the phages at RTD, 28 (82.3%) strong lysis, 4 (11.8%) weak lysis while 2 (5.9%) were untypeable strains.

Table 3 shows the frequencies of lysis and the different phage groups. It reveals that 19 (59.4%) were of phage group IV while 9 (28.1%) belong to phage group III. *S. aureus* of the same phage groups were established in food, food handlers and food contact surfaces. However, there was no relationship established within phage groups, source of isolation, coagulase and haemolytic pattern.

Table 1: Distribution of strains of *S. aureus* by toxicity test, coagulase and haemolytic pattern

Test	No. positive n = 34	% of Total no. tested
Coagulase:		
(a) Human plasma	15	44.1
(b) Sheep plasma	19	55.9
(c) Human and sheep plasma	8	23.5
DNase	34	100
Haemolysis:		
(a) α-haemolytic	13	38.2
(b) β-haemolytic	16	47.1
(c) γ-haemolytic	5	14.7
Cat Emetic Response		
(a) Milk feed	3	8.8
(b) Rice feed	1	2.9
(c) Milk and Rice feed	3	8.8
Total*	7	20.6

* = Total enterotoxigenic strain by emetic response; and n = No. of isolates tested

Table 2: Distribution of toxigenic strains (n = 7) of *S. aureus* by source of isolates

Source	No. of isolates Tested (% total)	Cat emetic response (%n)	Coagulase Hp	Sp	Hp+Sp	Haemolysis α	β	DNa
(a)Ready to eat food	6(17.7)	-	-	-	-	-	-	-
(b)Raw food	22(64.7)	5(71.4)	3	2	2	4	1	5
(c)Food contact surfaces	3(8.8)	1(14.3)	-	1	-	1	-	1
(d)Food handlers	3(8.8)	1(14.3)	1	-	-	1	-	1
Total	34(100)	7(20.6)*	4(57.1)	3(42.9)	2(28.6)	6(85.7)	1(14.3)	7(1

- = No reaction; * = percentage of total (n=34); Hp = Human plasma; and Sp = Sheep plasma

Table 3: **Distribution of strains of *S. aureus* by frequency of lysis and phage group**

Frequency of lysis	No. positive	% of Total no. tested
Strong lysis(++)	28	82.3*
Weak lysis(+)	4	11.8*
Strong+ Weak lysis	32	94.1*
Untypable	2	5.6*
Phage group:		
I	-	-
II	3	8.8**
III	9	28.1**
IV	19	59.4**
Mixed	1	3.1**

* = % Total no. of strains tested (n = 34); - = No reaction; and ** = % Total no. of typable strain n = 32

DISCUSSION

The finding in this study is that all the *S. aureus* isolates were coagulase positive for human and sheep plasmas and were either beta or alpha haemolytic. Coagulase positive staphylococci are potentially pathogenic. Alpha haemolytic strains of *S. aureus* are known to be more of human biotype and more toxigenic than beta haemolytic strains which are more of animal strains and less toxigenic (Bergdoll, 1980). This finding points to contamination from both human and animal sources and also shows that beta haemolytic strains coagulating sheep plasma and probably of animal origin were the major contaminants of food and food contact surfaces. Seven (20.6%) of the isolates were toxigenic by the cat emetic response. This tends to show that strains of *S. aureus* may be coagulase and DNase positive and haemolytic but not necessarily toxigenic. Although only 20.6% of the isolates were toxigenic, there is still need for serious health concern. This is because of the possible multiplication of these toxigenic strains in food if and when there is temperature abuse and foods are not promptly consumed within a short period of time. Similarly the immuno-compromised individuals could face problem of food poisoning even at low dose of the enterotoxin.

Of the typable strains, 59.4% were of phage group IV and 28.1% belong to phage group III. This again points to contamination from both human and animal sources because, phage group III *S. aureus* are known to be more of human strains while group IV phages are more of animal strains. It also stresses a higher level of contamination by animal strains of group IV which are often beta haemolytic and coagulate sheep plasma (Mayer, 1967; Frost, 1967; Adekeye, 1976; Umoh *et al*., 1991).

The contamination of food and food products by both human and animal strains of *S. aureus* have been reported by

other researchers (Frost, 1967; Adekeye, 1976; Umoh *et al.*, 1991). The findings of this study show that contamination was more by animal strains of *S. aureus* than by human strains. This however, could be explained by the close association between animals and man in this community. This result is not in agreement with the findings of others in which contamination of food and food products was more by human strains (Aureli *et al.*, 1984; Swartz *et al.*, 1985; Umoh *et al.*, 1991).

The findings in this study also reveal that there is no relationship between sources of isolates, phage group, coagulase and haemolytic reactions.

It could be concluded here that the *S. aureus* isolated from the food contact surfaces and foods are of both human and animal origin. Of significance is the fact that some of these strains are toxigenic. Similarly the presence of phage group III *S. aureus* calls for concern since this phage group has been implicated frequently in food poisoning outbreaks (Williams and Rippon, 1962; Simkovicova and Gilbert, 1971). To prevent contamination of foods by toxigenic strains of organisms, it is advocated that constant washing of hands during food preparation, adequate cleaning of food utensils, food contact surfaces and effective HACCP evaluation must be adhered to.

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