African Journal of Biotechnology Vol. 5 (4), pp. 362-365, 16 February 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Toxicity test and bacteriophage typing of *Staphylococcus aureus* isolates from food contact surfaces and foods prepared by families in Zaria, Nigeria

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Accepted 4 January, 2006

Strains of *Staphylococcus aureus* isolated from foods prepared in five families in Zaria Local Government Area were screened for enterotoxin production and phage pattern. Toxigenic strains of *S. aureus* were screened by the cat toxicity (emetic response), coagulase and DNase production tests and later phage typed by group I, II, III and IV phage sets at RTD (routine test dilution). Out of 44 *S. aureus* strains tested for enterotoxin production, 10 (22.7%) were toxigenic by the cat emetic response, 30 (68.2%) were β-haemolytic, 12 (27.3%) α-haemolytic while 24 (54.5%) and 20 (45.5%) coagulated human and sheep plasma, respectively. All the 44 strains were DNase positive. Forty two (95.5%) were typable at RTD with 35 (83.3%) and 7 (16.7%) strong and weak lysis, respectively. Most (54.8%) of the typable strains were lysed by group III phages while a small portion 8 (19.1%) were lysed by Group IV phages. About 7 (16.7%) were of mixed phage group. Contamination of foods in the families by toxigenic strains could be said to be low, however, the prevalence of phage group III and α-haemolytic strains of *S. aureus* calls for concern since these groups have frequently been implicated in food borne diseases. Effective hazard analysis critical control point (HACCP) evaluation is suggested as a means of preventing contamination of products by toxigenic strains of organisms.

Key words: *Staphylococcus aureus,* enterotoxin production, phage typing, haemolysis and food poisoning.

INTRODUCTION

The high level of multiple drug resistance by *Staphylococcus aureus* is of great concern (Lowy, 2003; Chamber, 2005). The impact of such concerns has been reported in some Nigerian cities such as Jos, Abuja, and Zaria (Ehinmidi, 2003; Olayinka et al., 2004; Onanuga et al., 2005). In this vein, there is on going research in various African countries geared at discovering new antimicrobial compounds to replace the older antibiotics (Eloff et al., 2005; Karou et al., 2005). The present concern, however, revolves around the presence of this microorganism in foods, which may lead to food poisoning.

Toxigenic tests for various strains of microorganisms may be performed by using screening tests such as vascular permeability and intestinal necrosis reactions, emetic responses, phospholipase, haemolysin and mouse lethal tests, antibiotic resistance, isoenzyme comparison and phage sensitivity (Turnbull et al., 1979; Turnbull and Kramer, 1983; Willshaw et al., 2001). However, emetic responses, phage sensitivity and DNA based methods have been particularly useful (Su and Wong, 1995; Olorunfemi et al., 2005).

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

Test	No. positive N = 44	% Total no. tested
Coagulase		
Human plasma	24	54.5
Sheep plasma	20	45.5
Human and sheep plasma	6	13.6
DNase	44	100
Haemolysis		
α-haemolytic	12	27.3
β-haemolytic	30	68.2
γ-haemolyic	2	4.5
Cat emetic response		
Milk feed	6	13.6
Rice feed	2	4.5
Milk and Rice feed	2	4.5
Total*	10	22.7

* = Total enterotoxigenic strains by emetic response
n = Number of isolates tested.

Bacillus cereus and *S. aureus* have been successfully screened from food products locally obtained in Zaria (Umoh et al., 1990; 1991; Yusuf et al., 1992). It was observed that large percentages of the isolates were toxin producers. Many attempts have been made to associate enterotoxin production and pathogenic capabilities to phage types (Melconian et al., 1983). Umoh et al. (1991) found no correlation between enterotoxin production in *S. aureus* and phage pattern but Sinkovicova and Gilbert (1971) and Asheshov et al. (1976) reported contradictory results. Phage typing results have also been used in conjunction with epidemiological data to provide direct or indirect evidence for the likely route of transmission of pathogens to food, thus typing supports the implementation of measures to control the spread of foodborne pathogens. With the above facts in mind the aim of this present investigation is to screen food and food contact surfaces for *S. aureus*, phage type the isolates and suggest practical and feasible means of reducing/preventing food contamination.

MATERIALS AND METHODS

Coagulase, DNase and haemolysis test

Coagulase production was determined in 10^{-1} dilution of both human and sheep plasmas in test tubes. Result interpretation was as described by Collins and Lyne (1986) and Umoh et al. (1999) while 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), Beta (β), and Gamma (γ) representing incomplete, complete, and no haemolysis respectively.

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

This was performed using the methods of Parsons and Summers (1971) and Melling et al. (1976). Kittens 1-3 months old and weighing approximately 1.6 kg 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 that had been grown on nutrient broth (Oxoid) for a period of 8 h were prepared as a suspension in sterile normal saline. This was adjusted to a standard of 10^6 cells using standard opacity tubes. About 3 ml aliquots of this suspension were inoculated into 500 ml sterile milk and 500 g sterile rice respectively incubated for 6-8 h and fed to kittens that had been starved for 8 h. Production of emesis within 5 h from feeding indicated a positive toxicity test (Melling et al., 1976).

Phage typing

Forty-four coagulase and DNase positive *S. aureus* isolates obtained from raw foods, cooked foods, food contact surfaces, and hand and finger nails of cooks were purified by sub-culturing on nutrient agar plates (Oxoid). Pure isolates were phage typed using group I, II, III and IV phage types as described by Williams and Rippon (1962). A routine test dilution (RTD) of 10^{-2} and 10^{-3} of the phages was made in nutrient broth. Approximately 12-h broth cultures of the *S. aureus* isolates were seeded on to nutrient agar plates which were allowed to dry before being spot inoculated with the phages. Incubation was at 37ºC for 6 h at an ambient room temperature of 28ºC overnight before examination for plaque formation. The results were recorded as ++, for strong lysis; +, for weak lysis and -, for no lysis (no visible plaque formation) (Williams and Rippon, 1962).

RESULTS

A total of 44 strains *of S. aureus* isolates were subjected to toxicity tests. Twenty-four (54.5%) of the 44 *S. aureus* isolates coagulated human plasma and 20 (45.5%) coagulated sheep plasma. All the *S. aureus* isolates were DNase positive. Table 1 shows the results of the distribution of the *S. aureus* strains by toxicity tests. It reveals that 30 (68.2%) of the isolates were β-haemolytic while 12 (27.3%) were α-haemolytic. On the whole, however, only 10 (22.7%) of the *S. aureus* isolates were enterotoxigenic by emesis (cat bioassay). Table 1 also shows that milk as a medium supported toxin production more than rice.

Table 2 shows the distribution by source of the toxigenic *S. aureus* isolates. It reveals that, raw foods and food handlers had 35.3 and 11.8% toxigenic strains, respectively. All the toxigenic strains of *S. aureus* in this study were haemolytic and coagulated either human or sheep plasma or both. There was, however, no specific relationship in the pattern of distribution of the toxigenic

Table 2: Distribution of toxigenic strains (n = 10) of **S. *aureus*** by source of isolates.

Source	No of isolates tested (% total)	Cat emetic response	Coagulase			Haemolysis		DNase
			Hp	Sp	Hp + Sp	α	β	
Ready to eat food	2(4.5)	1(10.0)	-	1	-	-	1	1
Raw food	28(63.6)	6(60.0)	3	2	1	1	5	6
Food contact surface	6(13.6)	1(10.0)	-	1	-	-	1	1
Food handlers	8(18.2)	2(20.0)	2	-	-	-	2	2
Total	**44**	**10(22.7)***	**5**	**4**	**1**	**1**	**9**	**10**

- = No reaction
* = Percentage of total (n = 44)
Hp = Human plasma
Sp = Sheep plasma

strains and source of isolation, coagulase and haemolytic reactions.

Table 3 shows the frequencies of lysis of *S. aureus* stains and the different phage groups. Twenty-three (54.8%) were typable with Group III phages, 8 (19.1%) with group IV phages and 7 (16.7%) by mixed phage sets.

Analysis of phage group, source of *S. aureus,* coagulase and haemolytic pattern shows no obvious relationship. However, the presence of similar phage groups of *S. aureus* in food, food handlers and food contact surfaces was established.

DISCUSSION

All the *S. aureus* isolates were DNase and coagulase positive for both human and sheep plasma. Such results are indicators of potential pathogenicity and therefore of serious health significance (Bergdoll, 1980). Thirty (68.2%) of the isolates were β-haemolytic while 12 (27.3%) were α-haemolytic. Alpha-haemolytic strains of *S. aureus* are known to be more of human biotype and more toxigenic than β-haemolytic strains which are more of animal strains and less toxigenic (Bergdoll, 1980). The finding of this study points to contamination from both human and animal sources and also shows that β-haemolytic strains, probably of animal origin, were the major contaminants of food and food products. Only 10 (22.7%) of the *S. aureus* isolates were toxigenic by the cat emetic response. This suggests that strains of *S. aureus* may be coagulase and DNase positive and haemolytic, but not necessarily toxigenic. The percentage (22.7%) of toxigenic strains of *S. aureus* in this study could be said to be low, but still of health concern since there is the possibility that these toxigenic strains could multiply in the food if it is not consumed within a short duration of time. This is of importance since children, the elderly and compromised individuals are more prone to food poisoning, even at a low dose of enterotoxins. However, the present investigations revealed no relationship between the frequency of occurrence of toxigenic strains and positive coagulase and haemolytic reactions.

A total of 27 (64.47%) of the *S. aureus* strains were typable with group I-III phages while 8 (19.1%) were typable with group IV phages. Phage group I-III *S. aureus* are known to be more of human strains while group IV phages are more of animal strains. However, the strains of human *S. aureus* are more of β-haemolytic strains, which are known to be more of animal strains and less toxigenic (Bergdoll, 1980). The close association between man and animal in the Zaria rural community could be responsible for such transfer of animal biotypes to man (Adekeye, 1976; Umoh et al., 1991).

Aureli et al. (1984) reported that *S. aureus* isolates from mastitic milk were typable by 41% and 19% of human and animal phage sets, respectively. Umoh et al. (1991) found that *S. aureus* from fermented milk products and raw milk were of both human and animal biotype but with more from human biotype (52.4%) than animal biotype

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

Frequency of lysis	No. positive n = 44	% of total no.
Strong Lysis	35	79.5*
Weak lysis	7	15.9*
Strong + weak lysis	42	95.9*
Phage group		
I	2	4.8**
II	2	4.8**
III	23	54.8**
IV	8	19.1**
Mixed	7	16.7**

* % of total no. of strains tested n = 44
** % of total typable strains n = 42s

(39.1%). Most (54.8%) of the isolates typed in this present investigation belonged to phage group III which is in agreement with the results of some other researchers (Williams and Rippon, 1962; Aureli et al., 1984; Swartz et al., 1985; Umoh et al., 1991). The prevalence of phage groups III and IV in this study stresses the high level of contamination of food and food products by *S. aureus* strains of both human and animal sources. The findings also reveal that there is no relationship between sources of isolates, phages group and phage pattern. The presence of *S. aureus* with the same phage pattern in both raw and cooked foods, food contact surfaces, and among food handlers points to cross contamination within sources.

In conclusion, the high prevalence of phage group III strains calls for concern since phage group III *S. aureus* have frequently been implicated in food borne diseases. Constant hand washing during food preparation, adequate cleaning of food contact surfaces and effective hazard analysis critical control point (HACCP) evaluation are necessary measures to prevent post process contamination of foods by toxigenic strains of various organisms.

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