



DETECTION OF *Staphylococcus aureus* ENTEROTOXIN IN TRADITIONAL DAIRY PRODUCTS SOLD IN SOME PARTS OF KADUNA, NIGERIA

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ABSTRACT

Staphylococcus aureus is one of the most frequent causes of food poisoning (FP). The main etiologic agents of food poisoning are staphylococcal enterotoxins (SEs). Staphylococcal enterotoxins (SEs) are superantigenic toxins. They are five major classical types; SEA, SEB, SEC, SED, SEE, and new SEs or SE-like superantigens, such as SEG to SEU. *Staphylococcus* species implicated in the contamination of milk and milk products were isolated and identified using standard biological methods. Agglutination method was used for phenotypic detection of SE serotypes using the Reversed Passive Latex Agglutination (SET-RPLA) kit. The results indicated that out of the 320 milk and milk product sampled, 56 (17.5%) were contaminated by staphylococci. 'Manshanu' (cream) were mostly contaminated 22 (6.9%) while the least frequently contaminated was 'Kindrimo' 7 (2.2%). Enterotoxin detection revealed that 20 (71.4%) of the *S.aureus* strains isolated from fresh milk and milk products were enterotoxinogenic. Enterotoxin A serotype were mostly produced by the isolates 19 (67.9%) while enterotoxin D were the least produced 5 (7.1%). The growth of these pathogenic organisms and their toxins in local dairy products indicates poor sanitary practices in the production of fresh milk and its products, posing a public health challenge. Hence milk and dairy products may serve as an important means of Staphylococcal food intoxication.

Key Words: Staphylococcal enterotoxin, enterotoxin, milk products, 'Manshanu', 'Kindrimo'.

INTRODUCTION

Food-borne infections are common and constitute an important health and economic burden globally (Nazzal *et al.*, 2012). Foods of animal origin, especially milk and dairy products, are associated with food borne diseases (Jorgensen *et al.*, 2005).

Milk can contain Pathogenic bacteria, such as *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Listeria* spp. and *Brucella* spp. (Bereda *et al.*, 2014). However, the presence of these pathogenic bacteria in milk and milk products has emerged as a major public health concern (Lingathurai and Vellathurai, 2010).

Staphylococcus aureus enterotoxins are the most frequent causes of food poisoning, with outbreaks caused by mishandling of foods after heat treatment (Soriano *et al.*, 2002). The heat destroys the vegetative bacterial microbiota in food, and the non competitive staphylococci, introduced by inadequate handling process, may grow.

Staphylococcal enterotoxins are low molecular weight proteins (MW 26.900 – 29.600 KD). These are encoded by genes embedded in mobile genetic elements such as phages, (not in plasmids) and Pathogenicity islands (Martin *et al.*, 2007). These toxins are heat resistant; an important physical and chemical property as their biological activity remains unchanged even after thermal processing of food (Mclauchlin *et al.*, 2000) hence, the ability of these toxins to cause epidemic gastroenteritis. Several studies have shown that 15 – 80% of the *S.aureus* isolated from various sources (dairy products, ice cream, meat products among others) are able to produce enterotoxin (Banja *et al.*, 2006). This research therefore focuses on ascertaining the extent of contamination of milk and milk products by *Staphylococcus* species and detects Staphylococcal enterotoxin produced. The results would be of importance not only in scientific aspects considering the scarcity of available data in this respect, but in providing practical information about food safety.

MATERIALS AND METHODS

Study area

Fresh milk and dairy samples were obtained from four (4) local government areas; Giwa, Kaduna North, Soba and Chikun in Kaduna State, Nigeria.

Sample Collection

A total of 320 Fresh milk and milk products samples; comprising of 80 samples each of Fresh milk, 'Nono', 'Manshanu' and 'Kindrimo' were collected over a one year period. Fresh milk samples were collected from farm Steads or Fulani settlements while 'Nono'samples were obtained from motor parks and markets. The samples were collected in sterile containers and placed in ice-packed coolers then taken to the Department of Microbiology laboratory, Ahmadu Bello University Zaria, for analysis.

Isolation of *Staphylococcus* species

This was conducted according to the procedure described by Imanifooladi *et al.* (2010) with slight modification. Depending on the type (solid or Liquid), each fresh milk and milk product sample was diluted in the ratio 1/100 in normal saline. From each solution produced, 1ml was transferred to 9mls cook meat media culture with 9% NaCl and incubated at 37°C for 48 hours. In the second phase, 0.1ml from each previously cultured medium was then transferred to Baird-Parker agar (BPA) and incubated for 24 hours. Black colonies with transparent zone on Baird-Parker agar were considered as presumptive *Staphylococcus* species. They were picked and stored on nutrient agar slants for further confirmation tests.

Biochemical Characterization of isolates

Typical isolates from Baird Parker agar (BPA) were Gram stained. Presumptive *Staphylococcus* species that were gram positive cocci in clusters were subjected to some biochemical tests as described by Cheesbrough (2009). These were; Coagulase, catalase, fermentation of glucose and Mannitol. The presumptive *Staphylococcus* species were further confirmed using Microgen™ STAPH- identification system (Microgen Bioproducts, United Kingdom). Single colonies of the presumptive isolates were tested with 12 standardized biochemical substrates following the manufacturer's instructions. The test organisms were identified by interpreting the permutations of metabolized substrates using the microgen identification system software (MID-60).

Assays for Enterotoxin in Culture Fluids

Production of Staphylococcal enterotoxins A,B,C and D in culture fluids of *S.aureus* isolates was evaluated using Staphylococcal Enterotoxin Test Reversed Passive Latex Agglutination (SET-RPLA) (TD900, Oxoid) as recommended by the manufacturer.

The strains to be tested were grown in 10 ml of tryptone soya broth (CM0219B, Oxoid) for 18 hours at 37°C with shaking aerobically. At the end of incubation, bacterial cells were harvested by centrifuging at 900g for 20 minutes in refrigerated centrifuge. The supernatant was tested for the presence and typing of Staphylococcal enterotoxins.

In each test well, 25µl of the latex reagents sensitized with antisera to SEA, SEB, SEC and SED and the controls were mixed with supernatant dilutions while performing a doubling dilution on each row. The content of each well was mixed by agitation by hand and covered with a lid to avoid evaporation. The plates were left undisturbed on a vibration free surface for 24 hours at room temperature. Soluble antigens of bacterial toxins were detected judging agglutination patterns of each. These were compared to the positive and negative controls.

Data Analysis

Data obtained was presented using frequency and percentages.

RESULTS

Contamination by Staphylococci: Out of the 320 milk and milk product samples, the most frequently contaminated by staphylococci was manshanu (cream) with 22 (6.9%) and the least frequently contaminated was kindrimo 7 (2.2%), as revealed in Table 1. Overall, 56 (17.5%) of the total dairy samples were contaminated.

Detection of SEs production ability of isolates: The results show that 20 (71.4%) of the *S.aureus* strains isolated from fresh milk and milk products were enterotoxinogenic as revealed in Table 2. The maximum numbers of enterotoxin producer isolates were detected in manshanu 7(25.0%) while Kindrimo had the least 3 (10.7%). The type of enterotoxin produced by the isolates is revealed in Figure 1. Enterotoxin A were mostly produced by the isolates 19 (67.9%). No isolates were able to produce all four (4) enterotoxin types simultaneously.

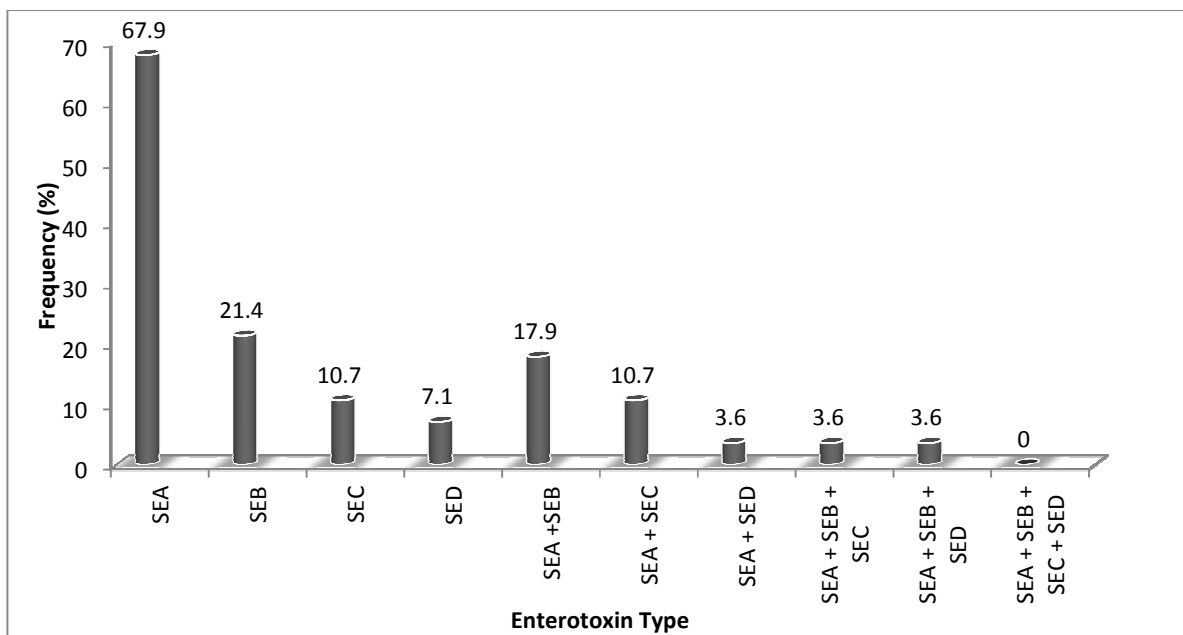
Table 1: Frequency of Staphylococci Isolated From Fresh Milk and Dairy Products.

Staphylococcus Spp.	Sample Type				
	Fresh Milk (n=80)	Nono (n=80)	Kindrimo (n=80)	Manshanu (n=80)	Total (n=320)
<i>S.aureus</i>	08(14.3)	06(10.7)	05(8.9)	09(16.1)	28(50)
<i>S.chromogens</i>	01(1.8)	0(0)	0(0)	03(5.4)	04(7.1)
<i>S.hyicus</i>	02(3.6)	01(1.8)	01(1.8)	02(3.6)	06(10.7)
<i>S.haemolyticus</i>	01(1.8)	0(0)	0(0)	02(3.6)	03(5.4)
<i>S.capitis</i>	0(0)	0(0)	0(0)	01(1.8)	01(1.8)
<i>S.xylosus</i>	07(12.5)	01(1.8)	01(1.8)	05(8.9)	14(25.0)
Total (%)	19(33.9)	8(14.3)	7(12.5)	22(39.3)	56(100)

Key: Values in parenthesis () are percentages.

Table 2: Frequency of Enterotoxin Detection in Milk and Milk products.

Sample Type	No Tested	No. Positive (%)
Fresh Milk	08	4 (14.3)
Nono	06	6 (21.43)
Kindrimo	05	3 (10.7)
Manshanu	09	7 (25.0)
Total (%)	28 (100%)	20 (71.4)



Key: n = 28

Figure 1: Frequency of Staphylococcal Enterotoxin (SE) types among isolates.

DISCUSSION

The results indicated *Staphylococcus aureus* as the most prevalent bacteria being isolated. The isolation of *Staphylococcus aureus* is of public health significance since it is said to be a commonly recovered pathogen in outbreaks of food poisoning due to milk and milk products (Junaidu *et al.*, 2011). *Staphylococcus aureus* may originate from mastitic animals or human sources (Akram *et al.*, 2013; Oliver *et al.*, 2005). Moreover, the quantities of *S.aureus* in food products are related to many factors: the number of contaminated carriers and personnel

in preparing the food ignoring the rules of hygiene in food factories, transport systems and rate of animal contamination. *Staphylococcus xylosus* were the second most frequent species isolated in this study. *Staphylococcus xylosus* had been reported to produce enterotoxin (Souza da Cunha *et al.*, 2006). This is of great importance since *S.xylosus* is used as a starter culture in fermented meat products (Montel, 2000). In this study, it was revealed that fresh milk was less contaminated than manshanu (cream).

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Human interference and the dairy food producers also determine the level of contamination of dairy products (Imanifooladi *et al.*, 2010). 'Manshanu' was more contaminated with *S.aureus* suggesting more handling. 'Manshanu' is usually dished out with bare hands by the local producers and retailers, hence the more the handling, the more contamination.

Enterotoxin A was mostly detected in this study. It is known that SEs are similar in structural and biological properties but differ in amounts produced (Klotz *et al.*, 2003). Under the best conditions, 40% - 50% of *S.aureus* isolates with the SEB gene are capable of enterotoxin production (Najero – Sanchez *et al.*, 2003). This was not in agreement with the findings of this research as lower values (21.4%) for SEB production were obtained. However, the expression of enterotoxin genes depends on factors such as the origin and identity of the bacterial isolate and the host environment of the bacteria. The host plays an important role in assisting an adaptation between the bacteria and their surrounding environment. For example most of the bacteria isolated from cows produce SEA and SED (Morandi *et al.*, 2007, Normanno *et al.*, 2005). This proves the high incidence of enterotoxin A. Bacteria isolated from skin and human wounds produce SEB (Imanifooladi *et al.*, 2007), while those from goats and sheep produce SEC (Normano *et al.*, 2005). Obviously the presence of these enterotoxin types (SEABCD) is in agreement with the findings of

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earlier researchers (Imanifooladi *et al.*, 2010, Morandi *et al.*, 2007, Normanno *et al.*, 2005).

CONCLUSION

From the study, *Staphylococcus aureus* had the highest frequency of occurrence (50%) among other Staphylococci isolated from the samples. Enterotoxin production by *S.aureus* isolates from milk and milk products were detected. The growth of these pathogenic organisms and their toxins in local dairy products indicates poor sanitary practices in the production of fresh milk and its products.

RECOMMENDATIONS

The study has provided practical information about food safety as regards to these informally hawked food products hence the following recommendations:

1. There is need to institute effective control measures by the government to protect the consumers from these food borne pathogens. These would include; mandatory milk pasteurization by traders and adequate storage thereafter, this might require a cooling system during the distribution process.
2. Also recommended is improved hygienic handling of the commodity during milking and milk being processed using potable water.
3. However there is still great need to study the enterotoxigenic potentials of other staphylococci such as Coagulase negative Staphylococci in foods.

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