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**VIRULENCE AND TOXIGENICITY OF COAGULASE-NEGATIVE
STAPHYLOCOCCI IN NIGERIAN TRADITIONAL FERMENTED FOODS**

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Abstract

The incidence of coagulase-negative staphylococci may render food unsafe as the clinical isolates have been reported to exude virulent traits. A total of 255 CoNS isolates from six traditional fermented foods (*nono*, *kunu*, *wara*, *iru*, *ogi* and *kindirmo*) from North Central Nigeria identified as *S. epidermidis*, *S. simulans*, *S. xylosus*, *S. kloosii* and *S. caprae* were investigated for virulence traits. The stains were examined for biofilm formation, production of hyaluronidase, DNase, TNase, haemolysins and superantigenic toxins (SEA, SEB, SEC, SED and TSST-1) using standard and genotypic methods. The analysis of virulence factors revealed the production of slime in 200 (78.4%), α -haemolysin 136 (53.3%), β -haemolysin 43 (16.9%), DNase 199 (78.0%), TNase 29 (11.4%) and hyaluronidase 125 (49.0%), TSST-1 119 (46.7%) and enterotoxin producing isolates: SEA, SEB, SEC and SED (61 (23.9%), 19 (7.5%), 9 (3.5%) and 8 (3.1%) respectively). PCR analysis detected *tsst-I*, *sea*, *seb* and *sec* genes. The ability of these microorganisms to exhibit virulence evokes the potential to cause disease especially under determinate conditions or in immune-compromised patients. The occurrence of CoNS in food should not be ignored nor their pathogenic potential considered as insignificant rather safety measures should be taken to reduce or totally eliminate their occurrence in foods.

Keywords: Traditional fermented foods, Coagulase-negative staphylococci (CoNS), Biofilm, haemolysin, Exoenzyme, Enterotoxins.

Introduction

Traditional fermented foods comprise the highest proportion of people's diet in sub-Saharan Africa. Most of these foods are prepared from cereal grains (maize, sorghum, millet), root tubers (cassava), legume and other seeds (African locust bean, African oil bean, cotton and other melon seeds etc.) and to a lesser extent, animal milk. Some common Nigerian fermented foods

include *okpehe*, *nono*, *ogi*, *kunu*, *wara*, *kindirmo* and *iru*. *Nono*, *wara* and *kindirmo* are produced using raw cow milk. *Nono* is a Nigerian locally fermented milk product similar to sour yoghurt commonly prepared by nomadic Fulanis (Yahuza, 2001) while *Kindirmo* is a traditional, viscous full-fat fermented or partially skimmed cow milk product (Igwe and Yakubu 2000). *Wara* is a white, soft, un-ripened cheese made by the addition of vegetable rennet made from a native plant extract (*Calotropis procera*) or pawpaw (*Carica papaya*) to the non-pasteurized whole milk from cattle which coagulates the milk (Adeyemi and Umar 1994). *Kunu* is a non-alcoholic fermented beverage widely consumed in Northern part of Nigeria produced by fermentation of cereals such as millet, sorghum or a combination of both. *Iru* is one of the most important food condiments in Nigeria and it is prepared from the seeds of African locust beans (*Parkia biglobosa*) (Achi, 2005). These indigenous foods are locally prepared in small scale in urban, peri-urban and in the village homes and their quality depends on the skills of the household occupant as inherited over the years (Chelule *et al.* 2010). The uncontrolled, naturally initiated fermentation processes expectedly yield products of inconsistent quality attributes, and, in some instances, the fermenting microorganisms may be out-competed by other opportunistic microbes of the fermenting matrix, thereby resulting into a sub-optimal fermentation. The prevalent food-borne diseases in the sub-region could be traced to the establishment of food borne pathogens that may have gained entry as contaminants or associated microorganisms of uncontrolled fermentation processes.

Coagulase-negative staphylococci were long considered non-pathogenic and having few virulence factors however, this notion has been corrected as most studies have shown that just like the known pathogenic *S. aureus*, they also possess virulent factors and have been implicated as pathogens of diverse diseases (Akinkunmi and Lamikanra 2012). Staphylococci expresses many potential virulence factors such as surface proteins that promote colonization of host tissues, invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase), surface factors that inhibit phagocytic engulfment (capsule, Protein A), biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production), immunological disguises (Protein A, coagulase, clotting factor); and membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin, exotoxins that damage host

tissues (Proctor 2000). Slime production in CoNS has been noted to be an important factor in the pathogenesis of infections since 1972 (Huebner and Goldmann 1999; Akinkunmi and Lamikanra 2012). The slime or biofilm formation is the most important virulence factor of coagulase - negative staphylococci since it enables their attachment and persistence on foreign mater and also protects them from the action of antibiotics and the immune system (Mack *et al.* 2007). *Staphylococcus* secrete proteins or toxins such as alpha(α), beta(β), gamma(γ), and delta(δ) haemolysins, leukocidin, exfoliative toxins A and B, toxic shock syndrome toxin-1 (TSST-1) and the staphylococcal enterotoxins (SE) (Ayepola 2012). Staphylococcal toxins can be divided into two groups according to their ability to lyse cells and they are hemolysins or cytotoxins and superantigenic toxins (Freer and Arbuthnott 1983, Ayepola 2012). Staphylococci produce a wide variety of exoenzymes most of them during the post exponential growth phase. These proteins degrade the host tissue to nutrients required for the growth of bacteria, and/or allow the bacteria to penetrate deeper into the host tissue or hydrolyze nucleic acids such as hyaluronidase, thermonuclease (TNase) and deoxyribonuclease (DNase). Enterotoxins are also considered to be the major cause of food poisoning. They are associated with a form of gastroenteritis manifesting clinically with vomiting or emesis, with or without diarrhea, resulting from the ingestion of one or more staphylococcal enterotoxins contained in food that has been contaminated with these bacteria. Toxic Shock Syndrome Toxin -1 (TSST-1) is an extracellular superantigen toxin produced by some isolates of *Staphylococcus*. TSST-1 is one of the causes of Toxic Shock Syndrome (TSS). Toxic Shock Syndrome is rare but can be a serious consequence of a burn wound infection and other staphylococcal infections. It is a disease that affects multiple organs and may be fatal (Edwards-Jones *et al.* 2000). It is characterised by rapid fever, arterial hypotension, diffuse cutaneous rash, circulatory failure, vomiting, diarrhoea, myalgia, epidermis scaling, hypo-albuminemia and organ failure (multiple organ dysfunction syndrome – MODS) (Javid Khojasteh and Foster 2011). If the symptoms or infection are not properly treated soon after the onset of symptoms, a fatal shock may develop within 24 h. This study is therefore aimed at determining the virulence potential of CoNS associated with Nigerian traditional fermented foods.

Materials and Methods

Test Isolates

A total of 255 CoNS species comprising of *S. epidermidis* (128), *S. simulans* (50), *S. xylosus* (36), *S. kloosii* (28) and *S. caprae* (13) isolated from 324 samples consisting of six (6) traditional fermented foods (*kunu, nono, wara, kindirmo, ogi and iru*) from North Central Nigeria were used for this study. The CoNS species were phenotypically characterised using the identification keys by Kloos and Schleifer (1986) and molecularly characterised employing 16S rRNA sequencing according to the methods of Kim *et al.* (2008).

Slime or Biofilm Formation in Test Isolates

The Congo Red Agar (CRA) method developed by Freeman (1989) was used. The Congo red stain was prepared as a concentrated aqueous solution and autoclaved separately at 121°C for 15 min. The agar was weighed and dissolved in 1000 ml of sterile distilled water and sterilized at 121°C for 15 min. The agar was allowed to cool to 55°C and mixed with the sterile Congo red stain. The agar was poured into plates, allowed to solidify and then inoculated with the test isolate and incubated aerobically at 37°C for 24 h.

Isolates which produced black colonies with dry crystalline consistency were regarded as slime positive, whereas those showing pink colonies were labelled as slime negative.

Thermonuclease (TNase) Test

The ability to produce TNase was determined using the method described by Hawkey and Lewis (1989). For the detection of TNase activity, thermonuclease agar (Thermo Scientific, Milan, Italy) was prepared and poured into plastic Petri dishes. The test isolates were streaked on the agar and incubated at 100°C for 10 min. The formation of pink colonies indicates the presence of thermostable nuclease.

DNase test

The method of Deighton *et al.* (1988) was employed. This test was carried out by using commercially available DNase agar (Oxoid, Wesel, Germany). Spot inoculation with the test

isolates was done on the DNase agar and incubated at 37°C for 4 days. After incubation, 1N HCl was poured on the agar. A zone of clearance around the bacterial growth was interpreted as a positive result.

Haemolysin Production in Test Isolates

The method of Akinjogunla *et al.* (2014) was employed. The haemolytic activities of the isolates were identified by the presence of diffuse (α -haemolysis) or clear (β -haemolysis) halos around the colonies. A colony of each of the bacterial isolates was sub-cultured onto freshly prepared blood agar (nutrient agar containing human blood) plates incubated at 37°C for 24 h, after which the colonies were examined for haemolytic activity.

Hyaluronidase Production in Test Isolates

The presence of hyaluronidase was evaluated on Brain Heart Infusion agar (BHI, Oxoid, Wesel, Germany) containing hyaluronic acid; 0.4 mg/ml (Thermoscientific, Italy) as described by Makris *et al.* (2004). The hyaluronidase agar was inoculated with the test isolate and incubated at 37°C for 24 h, after which the plates were covered with cetylpyridinium chloride and a positive result was indicated by the formation of a transparent halo around the colonies.

Detection of Enterotoxigenic CoNS Species from Fermented Food Samples

Phenotypic Detection of Toxic Shock Syndrome Toxin (TSST-1)

The method of Wongboot *et al.* (2013) was employed. Toxic shock syndrome toxin 1 (TSST-1) was detected by the reversed passive latex agglutination (RPLA) assay as described by Shingaki *et al.* (1981). The TSST-1 superantigenic toxins were detected using commercial standardized RPLA test kit (SET- TST-RPLA-TD0940) (Oxoid, Wesel, Germany) according to the manufacturer's instructions. The test isolates were grown in brain heart infusion broth and incubated at 37°C for 18 - 24 h in an incubator shaker. The overnight culture was centrifuged at 900 x g for 20 min at 4°C. Then, a 25 μ l aliquot of supernatant was mixed with 25 μ l of the latex suspension of each superantigenic toxin control in a V-well microtiter plate. The presence of agglutination resulting in the formation of a lattice structure indicates a positive result.

Phenotypic Detection of Enterotoxigenic (SE) CoNS Isolates

The method of Shingaki *et al.* (1981) was employed using SET – RPLA toxin detection kit (TD0900) (Oxoid, Wesel, Germany). The test isolate was inoculated into tryptone soya broth and incubated at 37°C for 18 – 24 h in an incubator shaker. The culture broth was centrifuged at 900 x g for 20 min in a cold centrifuge at 4°C. The supernatant was filtered with a 0.45 µm membrane filter paper. The filtrate was used for the qualitative detection of the staphylococcal enterotoxins (SE). V-shaped microplate wells were inoculated with 25 µl of the filtrate and 25 µl of thoroughly shaken latex sensitized with anti-enterotoxins A, B, C and D (provided by the manufacturer). Standard toxins (provided by manufacturer in the kit from Oxoid diagnostics reagents) were used as positive controls and the occurrence of non-specific reactions was tested by the addition of 25 µl of the supernatant to 25 µl of the control latex. The plates were covered with a lid and placed on black cellophane, left undisturbed on a vibration free surface and incubated for 20 - 24 h at 37°C. The results were interpreted according to the agglutination pattern described by the manufacturer. Positive reactions were classified as (+), (++) and (+++) while the formation of a pink bud was interpreted as a negative result.

Detection of *tsst-1* Gene and Staphylococcal Enterotoxin Genes (*sea*, *seb*, *sec*, *sed*)

in Test Isolates

The method by Johnson *et al.* (1991) was employed. Genomic DNA was isolated from 5 ml of an 18 h broth culture from all the bacterial isolates. Cells were pelleted from the cultures by centrifugation at 1,000 x g for 10 min and re-suspended in phosphate buffered saline with 100 µg of lysostaphin (Sigma) per ml, and incubated at 37°C for 0.5 h or until viscous. Template DNA from all preparations were subsequently extracted with phenol chloroform and precipitated with ethanol. Template DNA samples were dissolved in TE buffer (10 mM Tris chloride-1 mM EDTA, pH 8.0) and adjusted to a final concentration of 2 µg/ml with TE buffer according to A₂₆₀ values. PCR was carried out in 0.5 ml microcentrifuge tubes to which 20 pmol of each primer was added as shown in Table 1 together with 2.5 U Taq DNA polymerase (Promega), 200 µM dNTPs, 20 mM Tris-HCl, pH 8.4, 0.75 mM MgCl₂, and 5 µL DNA (total volume of 50

μL). The negative control used was water which was run in parallel with that of the DNA. Amplification was performed with an MJ Research PTC-100 thermocycler as described by Johnson *et al.* (1991) with some modification as proposed by Cunha *et al.* (2006), which consisted of a first cycle at 94°C for 4 min, denaturation at 94°C for 2 min followed by annealing at 55°C for 1 min and 30 s, and extension at 72°C for 1 min and 30 s, followed by a second cycle of denaturation at 94°C for 2 min, annealing at 53°C for 1 min and 30 s, and extension at 72°C for 1 min and 30 s. In the third cycle, the annealing temperature was reduced to 51°C, followed by additional 37 cycles at 94°C for 2 min, 51°C for 1 min and 30 s and 72°C for 1 min and 30 s. At the end of the 40 cycles, the tubes were incubated at 72°C for 7 min and stored at 4°C. The amplified products were run on 2% agarose gels in 1X TBE buffer stained with ethidium bromide. The sizes of the amplified products were compared with 50- and 100-kb standards and the gels photographed under UV transillumination.

Result

The virulent attributes of the CoNS species were assessed. Biofilm formation was expressed in 78.4% of all the CoNS species. *S. xylosus* had the highest percentage of slime producers (88.9%) from *nono* samples. *S. epidermidis* (75.8%) was mostly isolated from *iru* and *kindirmo* samples. *S. kloosii*, *S. simulans* and *S. caprae* produced slime with the percentage occurrences of 86%, 67.9% and 69.2% respectively in *kindirmo* samples as shown in Table 2.

The CoNS species (11.4%) produced thermonuclease (TNase) enzyme. *S. simulans* (22.0%) had the highest frequency of isolates producing thermonuclease enzyme (TNase) with 6.0% each incidence in *kindirmo*, *nono* and *wara*. 7.8% of *S. epidermidis*, 7% of *S. kloosii*, 11.2% of *S. xylosus* and 15.0% of *S. caprae* produced TNase enzyme with their highest occurrence in *wara* and *kindirmo* as shown in Table 3.

Table 4 shows that *S. caprae* had the highest percentage (92.3%) of deoxyribonuclease (DNase) producers with the highest occurrence in *nono*, *kindirmo* and *wara*. 75.8% *S. epidermidis* isolates, 82.1% *S. kloosii*, 77.8% *S. xylosus* and 78.0% *S. simulans* isolates were

found to be producers of DNase with the highest incidence observed in *iru*, *kindirmo*, *wara* and *kindirmo* food samples respectively.

Table 5 shows the haemolytic attributes of the CoNS species. 53.3% of the isolates were α -haemolysin producers while 16.9% of them produced β -haemolysin. All the species produced haemolysins however some isolates from *ogi*, *kunu*, *kindirmo*, *wara* and *iru* did not produce haemolysins. Majority of α -haemolysin producers belonged to *S. epidermidis* (47.7%), *S. kloosii* (57.1%) and *S. xylosus* (69.4%). *S. xylosus* had the highest isolates (25.0%) producing β -haemolysin followed by *S. epidermidis* (21.9%), *S. caprae* (23.1%), *S. simulans* (3.6%).

The ability of CoNS to produce hyaluronidase was examined. 62.0% of *S. simulans* isolates produced hyaluronidase and they were the highest producers. The other species had the following occurrence; *S. epidermidis* (43.8%), *S. kloosii* (57.1%), *S. xylosus* (41.7%) and *S. caprae* (53.8%) as shown in Table 6. The occurrence of these microorganisms was more from foods isolated from *iru*, *kindirmo* and *nono*.

Toxic shock syndrome toxin (TSST-1) production by CoNS species is shown in Table 7. *S. epidermidis*, *S. simulans* and *S. xylosus* (42.2%, 42.0% and 41.7% respectively) were the predominant producers of the toxin. The occurrence of the toxin producers was remarkably high in *kindirmo*, *wara* and *nono* samples. The presence of the *tsst-1* gene in the CoNS isolates was confirmed by PCR analysis in the same organisms as detected phenotypically. PCR analysis revealed that forty seven percent (47.0%) of the CoNS isolates had *tsst-1* gene.

The production of staphylococcal enterotoxin A, B, C and D (SEA, SEB, SEC and SED) by the CoNS species revealed that 23.9% of all the CoNS species were producers of SEA. SEA was the highest occurring toxin produced as compared to SEB (7.5%), SEC (3.5%) and SED (3.1%). *S. xylosus* (86.0%) was the predominant enterotoxin producers as shown in Table 8.

PCR analysis detected *sea*, *seb* and *sec* genes but *sed* gene was not detected among the CoNS species. *Kindirmo*, *wara* and *nono* samples had the highest incidence of enterotoxigenic CoNS species as shown in Fig.1.

Discussion

A large percentage of the CoNS species were slime or biofilm producers. Biofilms are very fine extracellular polymeric fibrils attached to bacteria and they are a survival strategy used by bacteria in natural and industrial systems (Oliveira and Cunha, 2008). The ability of the organisms to produce biofilm is a useful marker in the pathogenicity of CoNS (Turkyilmaz and Kaya 2006; Landeta *et al.* 2013). Slime production may also reflect the microorganism's ability to adhere to specific host tissues and thereby produce invasive microcolonies (Gundogan and Ataol 2013; Priya *et al.* 2014). They can persist on metal devices, milking equipment as well as on the milker's hands which serve as a major source of staphylococcal spread (Pilipcincova *et al.* 2010). The production of biofilm reduces the organism's susceptibility to antimicrobials (John and Harvin 2007).

A relatively small proportion (11.4%) of the species produced thermonuclease (TNase). TNase hydrolyses single and double-stranded RNA and DNA at the 5' position of phosphodiester bonds by a calcium-dependent mechanism. This enzyme is known to break down or destroy nucleic acids and this also contributes to the virulence of these organisms.

Deoxyribonuclease (DNase) was produced by a large percentage of the CoNS species isolated in this study. Lambe *et al.* (1990) reported that *S. epidermidis* could produce DNase. The production of DNase may be responsible for tissue degradation and spreading of an infection caused by these bacteria (Stepanovic *et al.* 2001).

Hyaluronidase production by the CoNS species was observed in this study. Hyaluronidase is an enzyme that catalyzes the hydrolysis of hyaluronic acid which cements or binds connective tissues. They are also known as spreading factor because once the tissues are broken they can easily spread in tissues and cause infection and this function also contributes to the virulent attributes of CoNS.

The predominant α -haemolysin producers were *S. epidermidis*, *S. simulans*, *S. kloosii* and *S. xylosus* and this conforms to the work of (Azih and Enabulele 2013). The β -haemolysins were predominantly produced by *S. epidermidis*, *S. xylosus* and *S. caprae*. Damage to host cells is mediated by staphylococcal haemolysins which contributes significantly to their virulence (Turkyilmaz and Kaya 2006).

Majority of the CoNS species produced SEA toxin than the other enterotoxins. *S. epidermidis* and *S. caprae* were the highest SEA toxin producers while *S. xylosus* and *S. caprae* were the predominant producers of the enterotoxin SEB, SEC and SED. SEA is known to be the most potent of the isolates and this also lends credence to their virulence (Bergdoll and Crass 1986). *Nono*, *Kindirmo* and *Wara* samples had the highest numbers of staphylococcal enterotoxin producers and this may be attributed to the use of unpasteurized milk for their production coupled with the unhygienic practices of the Fulanis especially in the rural areas. Staphylococcal food-borne diseases acquired from eating enterotoxin-contaminated food are the second most commonly reported types of food-borne diseases (Reyes *et al.* 2010).

Conclusion

The virulence of CoNS isolated from food has demonstrated how these organisms may represent hazard and behave as true pathogens in determinate conditions in particular, when the host is immune-compromised. It is therefore recommended that these organisms should not be dismissed as contaminants but rather awareness about the pathogenicity of the organism be spread. Their occurrence in food may also signal caution as regards to food safety.

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Table 1: Primers used in this Study

Primers	5' to 3' nucleotide sequence	Target	Amplicon Size (bp)
<i>tsst-1f</i>	ATG GCA GCA TCA GCT TGA TA	TSST-1	350
<i>tsst-1r</i>	TTT CCA ATA ACC ACC CGT TT	TSST-1	350
<i>sea1</i>	TTG GAA ACG GTT AAA ACG AA	Enterotoxin A	120
<i>sea2</i>	GAA CCT TCC CAT CAA AAA CA	Enterotoxin A	120
<i>seb1</i>	TCG CAT CAA ACT GAC AAA CG	Enterotoxin B	478
<i>seb2</i>	GCA GGT ACT CTA TAA GTG CC	Enterotoxin B	478
<i>sec1</i>	GAC ATA AAA GCT AGG AAT TT	Enterotoxin C	257
<i>sec2</i>	AAA TCG GAT TAA CAT TAT CC	Enterotoxin C	257
<i>sed1</i>	CTA GTT TGG TAA TAT CTC CT	Enterotoxin D	317
<i>sed2</i>	TAA TGC TAT ATC TTA TAG GG	Enterotoxin D	317

Source: Johnson *et al.*, 1991

Table 2: Frequency of Slime Producing Coagulase-Negative Staphylococci in Fermented Food Samples

Food Samples	CoNS Species				
	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus kloosii</i>	<i>Staphylococcus xylosus</i>	<i>Staphylococcus simulans</i>	<i>Staphylococcus caprae</i>
	128 ^a (%)	28 ^a (%)	36 ^a (%)	50 ^a (%)	13 ^a (%)
<i>Nono</i>	10 (7.8)	4 (14.3)	10 (27.8)	10 (20.0)	3 (23.1)
<i>Iru</i>	25 (19.5)	5 (17.9)	2 (5.6)	7 (14.0)	0 (0)
<i>Ogi</i>	18 (14.1)	0 (0)	0 (0)	6 (12.0)	0 (0)
<i>Kunu</i>	15 (11.7)	1 (3.6)	0 (0)	2 (4)	0 (0)
<i>Kindirmo</i>	20 (15.6)	7 (25.0)	9 (25.0)	16 (32)	4 (30.8)
<i>Wura</i>	9 (7.0)	2 (7.1)	11 (30.6)	2 (4.0)	2 (15.4)
Total	97 (75.8)	19 (67.9)	32 (88.9)	43 (86.0)	9 (69.2)

^a: total number of CoNS species

Table 3: Frequency of TNase producing Coagulase-Negative Staphylococci in Fermented Food Samples

Food Samples	CoNS Species				
	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus kloosii</i>	<i>Staphylococcus xylosus</i>	<i>Staphylococcus simulans</i>	<i>Staphylococcus caprae</i>
	128 ^a (%)	28 ^a (%)	36 ^a (%)	50 ^a (%)	13 ^a (%)
<i>Nono</i>	2 (1.6)	1(3.6)	1(2.8)	3 (6.0)	1(7.7)
<i>Iru</i>	1 (0.8)	0 (0)	0 (0)	1 (2.0)	0 (0)
<i>Ogi</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Kunu</i>	0 (0)	0 (0)	0 (0)	1 (2.0)	0 (0)
<i>Kindirmo</i>	3 (2.3)	0 (0)	2(5.6)	3(6.0)	1(7.7)
<i>Wara</i>	4 (3.1)	1(3.6)	1(2.8)	3 (6.0)	0 (0)
Total	10 (7.8)	2 (7.1)	4 (11.2)	11(22.0)	2 (15.4)

^a: total number of CoNS species

Table 4: Frequency of DNase Producing Coagulase- Negative Staphylococci Species from Fermented Food Samples

Food Samples	CoNS Species				
	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus kloosii</i>	<i>Staphylococcus xylosus</i>	<i>Staphylococcus simulans</i>	<i>Staphylococcus caprae</i>
	128 ^a (%)	28 ^a (%)	36 ^a (%)	50 ^a (%)	13 ^a (%)
<i>Nono</i>	10 (7.8)	5 (17.9)	6 (16.7)	8 (16.0)	4 (30.8)
<i>Iru</i>	30 (23.4)	6 (21.4)	2 (5.6)	8 (16.0)	0 (0)
<i>Ogi</i>	16 (12.5)	0 (0)	0 (0)	4 (8.0)	0 (0)
<i>Kunu</i>	19 (14.8)	2 (7.1)	0 (0)	2 (4.0)	0 (0)
<i>Kindirmo</i>	12 (9.4)	8 (28.6)	10 (27.8)	12 (24.0)	4 (30.8)
<i>Wara</i>	10 (7.8)	2 (7.1)	10 (27.8)	5 (8.0)	4 (30.8)
Total	97 (75.8)	23 (82.1)	28 (77.8)	39 (78.0)	12 (92.3)

^a: total number of CoNS species

Table 5: Frequency of Haemolysin Producing Coagulase-Negative Staphylococci in Fermented Food Samples

Food Samples	<i>Staphylococcus epidermidis</i>		<i>Staphylococcus kloosii</i>		<i>Staphylococcus xylosus</i>		<i>Staphylococcus simulans</i>		<i>Staphylococcus caprae</i>	
	128 ^a (%)		28 ^a (%)		36 ^a (%)		50 ^a (%)		13 ^a (%)	
	α	β	α	β	α	β	α	β	α	β
<i>Nono</i>	5 (3.9)	1 (0.8)	5 (17.9)	0 (0)	11(30.6)	4(11.1)	4 (8.0)	0 (0)	3 (23.1)	0 (0)
<i>Iru</i>	11 (8.6)	6 (4.7)	3 (10.7)	1 (3.6)	2 (5.6)	0 (0)	5(10.0)	1 (2)	0 (0)	0 (0)
<i>Ogi</i>	13 (10.2)	8 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	3 (6.0)	0 (0)	0 (0)	0 (0)
<i>Kunu</i>	12 (9.4)	4 (3.1)	1 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Kindirmo</i>	13 (10.2)	6 (4.7)	6 (21.4)	0 (0)	7 (19.4)	2 (5.6)	10(20.0)	1 (2.0)	2 (15.4)	1 (7.7)
<i>Wara</i>	7 (5.5)	3 (2.3)	1 (3.6)	0 (0)	5 (13.9)	3 (8.3)	5(10.0)	0 (0)	2 (15.4)	2 (15.4)
Total	61(47.7)	28(21.9)	16 (57.1)	1 (3.6)	25 (69.4)	9(25.0)	27(54.0)	2 (4.0)	7 (53.8)	3 (23.1)

^a: total number of each CoNS species; α - alpha haemolysin; β - beta haemolysin

Table 6: Distribution of Hyaluronidase Producing Coagulase-Negative Staphylococci from Fermented Food Samples

Food Samples	CoNS Species				
	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus kloosii</i>	<i>Staphylococcus xylosus</i>	<i>Staphylococcus simulans</i>	<i>Staphylococcus caprae</i>
	128 ^a (%)	28 ^a (%)	36 ^a (%)	50 ^a (%)	13 ^a (%)
<i>Nono</i>	9 (7.0)	4 (14.3)	0 (0)	8 (16.0)	3 (23.1)
<i>Iru</i>	21 (16.4)	1 (3.6)	0 (0)	6 (12.0)	0 (0)
<i>Ogi</i>	5 (3.9)	0 (0)	0 (0)	2 (4.0)	0 (0)
<i>Kunu</i>	4 (3.1)	2 (7.1)	0 (0)	2 (4.0)	0 (0)
<i>Kindirmo</i>	12 (9.4)	8 (28.6)	9 (25)	8 (16.0)	2 (15.4)
<i>Wara</i>	5 (3.9)	1 (3.6)	6 (16.7)	5 (10.0)	2 (15.4)
Total	56 (43.8)	16 (57.1)	15 (41.7)	31 (62.0)	7 (53.8)

^a: total number of each CoNS species

Table 7: Frequency of TSST-1 Phenotype Producing Coagulase-Negative Staphylococci in Fermented Food Samples

Food Samples	CoNS Species				
	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus kloosii</i>	<i>Staphylococcus xylosus</i>	<i>Staphylococcus simulans</i>	<i>Staphylococcus caprae</i>
	128 ^a (%)	28 ^a (%)	36 ^a (%)	50 ^a (%)	13 ^a (%)
<i>Nono</i>	7 (5.5)	2 (7.1)	4 (11.1)	8 (16.0)	2 (15.4)
<i>Iru</i>	10 (7.8)	1 (3.6)	1 (2.8)	4 (8.0)	0 (0)
<i>Ogi</i>	8 (6.3)	0 (0)	0 (0)	1 (2.0)	0 (0)
<i>Kunu</i>	5 (3.9)	1 (3.6)	0 (0)	0 (0)	0 (0)
<i>Kindirmo</i>	14 (10.9)	5 (17.9)	10 (27.8)	8 (16)	2 (15.4)
Total	54 (42.2)	11 (39.3)	15 (41.7)	21 (42.0)	4 (30.8)

^a: total number of each CoNS species

Table 8: Frequency of Enterotoxin Producing CoNS Species

Staphylococcal		CoNS Species			
Enterotoxins	<i>S. epidermidis</i>	<i>S. kloosii</i>	<i>S. xylosus</i>	<i>S. simulans</i>	<i>S. caprae</i>
(SEs)	n = 128 (%)	n = 28 (%)	n = 36 (%)	n = 50 (%)	n =13(%)
SEA	28 (21.9)	3 (10.7)	16 (44.4)	10 (20.0)	4 (30.8)
SEB	2 (1.6)	5 (17.9)	8 (22.2)	4 (8.0)	0 (0)
SEC	4 (3.1)	0 (0)	3 (8.3)	1 (2.0)	1 (7.7)
SED	2 (1.6)	0 (0)	4 (11.1)	2 (4.0)	0 (0)
Total	36 (28.1)	8 (28.6)	31 (86.1)	17 (34.0)	5 (38.5)

n= total number of each CoNS species

Fig. 1: Number of Enterotoxigenic CoNS Isolates in Fermented Food Samples