



OCCURRENCE AND ANTIBIOGRAM OF *Staphylococcus aureus* IN SOME DAIRY PRODUCTS SOLD IN PARTS OF KADUNA STATE, NIGERIA

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Abstract

Milk and dairy products are considered nutritious as they contain several important nutrients including proteins and vitamins. Conversely, they can be vehicle for several pathogenic bacteria such as *Staphylococcus aureus*. This study was conducted to determine the occurrence of *Staphylococcus aureus* in two indigenous dairy products. The antimicrobial resistance pattern of the isolates was also investigated. A total of 160 samples were examined using standard bacteriological procedures. The isolation frequency ranged from 0 - 17.50%, consisting of *Kindrimo* 5 (6.25%) and *Manshanu* 9 (11.25%). All *S. aureus* isolates were 14(100%) susceptible to Ciprofloxacin, Chloramphenicol and Gentamicin. There was 50% resistance to tetracycline, a commonly used antibiotic. Multiple Antibiotics Resistance Index (MARI) ranged from 0.3 - 0.7. The presence of *S. aureus* in the dairy products poses serious health risk and therefore calls for public health awareness.

Key words: *Staphylococcus aureus*, dairy products, *Kindrimo*, *Manshanu*.

INTRODUCTION

Staphylococcal food poisoning is a common cause of food borne illness in humans (Pillsbury *et al.*, 2013). This occurs following ingestion of staphylococcal enterotoxins which are heat resistant and are produced in food following contamination by staphylococci, typically *Staphylococcus aureus*. Milk is a good substrate for *S. aureus* growth and among the foods implicated in staphylococcal food poisoning (SFP), milk and dairy products play an important role, since enterotoxigenic strains of *S. aureus* have been frequently isolated in them (Normanno *et al.*, 2007). Fresh milk and other dairy products such as *Kindrimo* and *Manshanu* (fat from milk) are often commonly hawked and consumed on many Northern Nigeria streets. In developing countries, especially in hot tropics high quality and safe products are desired but are not easily achieved (Asaminew and Eyassu, 2011; Bereda *et al.*, 2014). These dairy products (*Kindrimo* and *Manshanu*) due to their processing and distribution practices often are exposed to conditions which may permit growth of contaminating organisms and possible toxin producers (Ezeonu, and Ezurike, 2007). *Staphylococcus aureus* had been reported in fermented milk products (*Fura* and *Manshanu*) Umoh *et al.*, 1990 and in *Fura da Nono*'; a cereal based food prepared from sorghum or millet (Umoh, 1989). Cow milk products pose

serious health concern as such they can no longer be ignored as they are among the main entry routes of microbial contamination into the human dietary system in Africa (Okeke *et al.*, 2012). This research therefore estimates the health risk associated with the consumption of such informally marketed dairy products.

MATERIALS AND METHODS

Study area

The study area included four (4) local government areas in Kaduna state, proximal to Zaria. These included; Giwa, Kaduna North, Soba and Chikun.

Sample collection

A total of 160 samples; comprising of 80 samples each of *Kindrimo* and *Manshanu* (20 from each local government area), were obtained from motor parks and markets. Samples were collected in sterile containers and placed in ice - packed coolers and taken to the laboratory. Samples were analyzed at department of Microbiology laboratory, Ahmadu Bello University Zaria within 6 hours of collection.

Isolation of *Staphylococcus aureus*

The isolation of *Staphylococcus aureus* was according to the procedure described by Imanifooladi *et al.* (2010). Dairy product samples (*Kindrimo*) were diluted in the ratio 1/100 in normal saline.

From each solution produced, 10ml was transferred to 90 mls cook meat media culture with 9% NaCl. For *Manshanu* samples, each was placed in water bath set at 45°C and 10ml of each melted sample was also transferred to 90mls cook meat media culture with 9% NaCl. All were incubated at 37°C for 48 hr. In the second phase, 1ml from each previously cultured medium was then transferred to Baird-Parker agar (BPA) and incubated for 24hr. 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

Presumptive *Staphylococcus* species that were gram positive cocci in clusters were subjected to some biochemical tests as described by Cheesbrough (2012). These included; coagulase, catalase, fermentation of glucose and Mannitol. These were further confirmed using Microgen™ STAPH- identification system (Microgen Bioproducts, United Kingdom).

Antibiogram Testing of *Staphylococcus aureus*

Antibiotic susceptibility tests for *Staphylococcus aureus* isolates was performed according to the Kirby- Bauer method as described by Bauer *et al.* (1966) and the evaluation methods of the Clinical and Laboratory's standards Institutes (CLSI)(CLSI,2014). Isolates grown on nutrient agar overnight were suspended in 2ml sterile normal saline (0.9% sodium chloride solution). A turbidity equivalent was prepared by comparing with a 0.5 MacFarland standards. Bacterial suspensions of 0.1ml were spread on plates of sterile Mueller-Hinton agar with the help of a sterile cotton swab to form a smooth bacterial lawn. The inocula were allowed to dry for 5 minutes. Commercially prepared standard susceptibility test discs impregnated with known agent and strength were then dispensed on the agar surface. Within 15 minutes of application of the disc, plates were incubated overnight at 37°C. *S.aureus* ATCC 6538 was used as quality control organism in the antimicrobial susceptibility determination.Characterization of strains as

susceptible, intermediate or resistant was based on the size of inhibition zone around the disc compared with the interpretation standards provided by the manufacturers. The following antibiotic disks were used; Tetracycline (30µg), Trimethoprim/Sulfamethoxazole (25µg), chloramphenicol (30µg), erythromycin (15µg), gentamicin (10µg), Amoxicillin/Clavulanic acid (30µg), Cefoxitin (30µg), Ciprofloxacin (5µg) and Vancomycin (30µg).

Multiple Antibiotic Resistance (MAR) and Multiple Antibiotic Resistance Index (MARI)

Multiple antibiotic resistance (MAR) for this study is defined as resistance of isolate to three or more antibiotics (Lambert, 2003 and Osundiya *et al.*, 2013).

Multiple antibiotic resistance index (MARI) was calculated according to Furtula *et al.* (2013) as the ratio of number of resistance antibiotics to which an organism is resistant, to the total number of antibiotics to which an organism is exposed to.

RESULTS

The recovery frequency of *Staphylococcus aureus* from *Kindrimo* and *Manshanu* in various sampling locations is presented in Table 1. Out of the total of 160 samples examined, 14 *S.aureus* strains were isolated from the two products. *Manshanu* had a higher frequency of occurrence 9 (11.25%) of *S.aureus* while *Kindrimo* had a frequency of 5(6.25%). Giwa had the highest occurrence of *S.aureus* 7 (8.25%) while none was isolated from Soba. The antibiotics susceptibility profile of *S.aureus* isolates from *Kindrimo* and *Manshanu* is displayed in Table 2. Isolates showed 100% susceptibility to Gentamicin (10µg), Ciprofloxacin (5µg) and Chloramphenicol (30µg). High resistance (80%) was observed in isolates from *Kindrimo* to tetracycline and those from *Manshanu* to Gentamicin, as revealed in Figure 1. A total of 8(57.14%) were resistant to multiple antibiotics (Table 3). Multiple antibiotics resistance index (MARI) ranged from 1 (0.7) to 5 (0.4).

Table 1: Recovery frequency of *S.aureus* from samples in various sampling locations.

Sample	Location				Total(%)
	Giwa	Chikun Number	Soba Positive (%)	Kaduna North	
<i>Kindrimo</i>	1(1.25)	2(2.5)	0(0)	2(2.5)	5(6.25)
<i>Manshanu</i>	6(7.5)	0(0)	0(0)	3(3.75)	9 (11.25)
Total (%)	7(8.25)	2(2.5)	0(0)	5(6.25)	14(17.50)

Key: n = 160

Table 2: Antibiotics susceptibility profile of *S.aureus* isolates from *Kindrimo* and *Manshanu*.

Antibiotics	Conc. (µg)	Susceptibility		
		No. Res (%)	No.Sus (%)	No.Interm (%)
Amoxicillin/Clavulanic acid (Aug)	30	5(35.71)	9(64.23)	-
Cefoxitin (Fox)	30	10 (71.43)	4 (28.57)	-
Ciprofloxacin (Cip)	5	0 (0)	14 (100)	-
Erythromycin (E)	15	10 (71.43)	4 (28.57)	-
Gentamicin (CN)	10	0 (0)	14 (100)	-
Tetracyclin (TE)	30	7 (50.0)	4 (28.57)	3(21.43)
Chloramphenicol (C)	30	0 (0)	14 (100)	-
Trimethoprim/Sulfamethoxazole (SXT)	25	2 (14.29)	12 (85.71)	-
Vancomycin	30	10 (71.43)	4 (28.57)	-

Key: n = 14, Conc = Concentration, Res = Resistant, Sus = Susceptible, Interm = Intermediate.

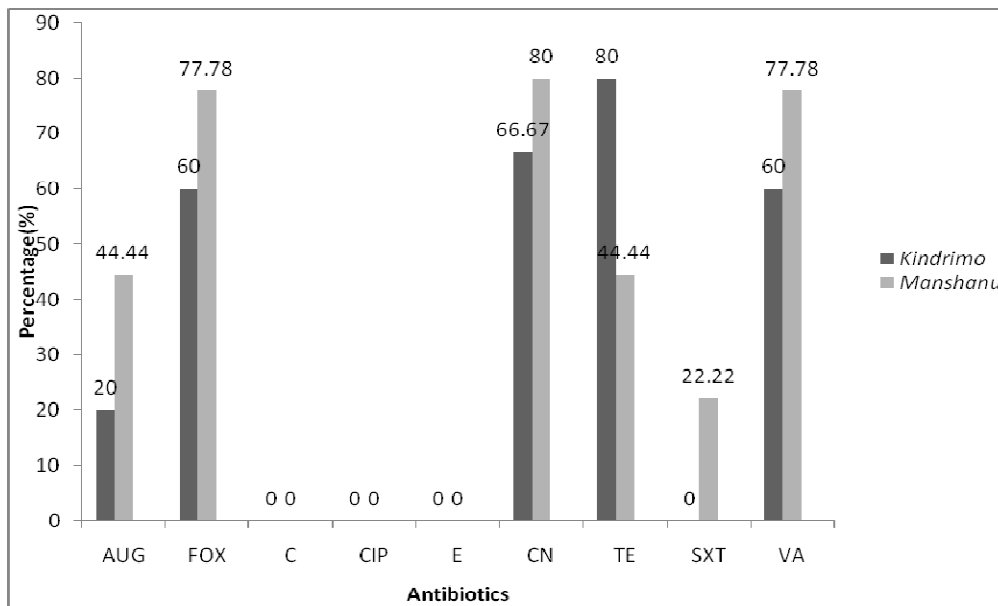


Figure 1: Percentage antibiotics resistance of isolates from samples

Table 3: Multiple Antibiotic Resistance Pattern of *S.aureus* isolates from *Kindrimo* and *Manshanu*.

No. of Antibiotics	Resistance Pattern	No.(%) of <i>S.aureus</i> isolate	MARI
3	VA,E,FOX;E,AUG,FOX	2(14.29)	0.3
4	VA,TE,E,FOX;VA,TE,E,FOX;VA,TE,AUG,FOX; VA,TE,E,FOX;VA,TE,E,FOX.	5(35.71)	0.4
6	VA,TE,E,FOX,SXT,AUG	1(7.14)	0.7
Total (%)		8(57.14)	

Key: Aug= Amoxicillin-Clavulanic acid; Fox= Cefoxitin; CN= Gentamicin; TE= Tetracyclin; Cip= Ciprofloxacin; SXT= Sulfonamides and Trimethoprim; C= Chloramphenicol; VA= Vancomycin; E= Erythromycin. Percentage = %, where n=14(Total number of *S.aureus* isolates), MARI= multiple antibiotic resistance index.

DISCUSSION

The prevalence of 14 (17.50%) of *S.aureus* in these two dairy product is of public health importance. A similar trend had been reported by other workers (Abdullahi *et al.*, 2001; Okeke

et al., 2014). *S.aureus* is said to be a commonly recovered pathogen in outbreaks of food poisoning due to milk and dairy products (Junaidu *et al.*, 2011).

This organism probably got into the milk through the crude methods of milking the cow. *Manshanu* was more frequently contaminated 9 (11.25%) than *Kindrimo* 5 (6.25%) probably due to more handling. Since human interference, the dairy food producers among others are some of the factors that determine the level of contamination of dairy products (ImaniFooladi, 2010).

Isolates demonstrated high level of susceptibility to Gentamycin, Ciprofloxacin and Chloramphenicol. This may suggest effective local and probably antibiotics resistance control programs. The high performance of some of these antibiotics (Gentamicin, Ciprofloxacin and Chloramphenicol) could also be due to their molecular sizes, a factor which enhances their solubility in diluents thus promoting their penetration power through cell wall into the cytoplasm of the target microorganism. This finding is in line with the report of Maillard (2002) and Poole (2002) who respectively opined that the high efficacy of antibiotics may be traced to their molecular sizes. *Manshanu* had higher percentage occurrence of MAR than *Kindrimo* obviously because the product had the higher number of *S.aureus* occurrence. The high percentage occurrence of multiple antibiotics resistance (MAR) index (57.14%) among the *S.aureus* isolates in this study might have arisen due to common practices such as the use of antimicrobials for growth promotion and for prophylaxis (Achi and Ugbogu, 2006). Veterinary drugs are still administered by non registered veterinarians and farmers are often seen purchasing and administering drugs by themselves. Antibiotics are widely available over the counter and

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mostly used without prescription. In addition to these is continued administration of antibiotics repeatedly against infections that appear non-responsive to the normal dose given earlier for farm animals (Ezenduka *et al.*, 2011). The residues of antibiotics have been reported in tissues of food animals and their products (Kabir *et al.* 2004; Adesokan *et al.* 2013). Another probable reason for high percentage occurrence of multiple antibiotics resistance (MAR) in *S.aureus* isolates could be as opined by Gundogan *et al.* (2006) that *S. aureus* strains are known to be frequently resistant to antibiotic therapy due to their capacity to produce an exopolysaccharide barrier and because of their location within microabscesses, which limit the action of drugs.

Conclusion

The occurrence of *S. aureus* (6.25%) in *Kindrimo* and *Manshanu* (11.25%) consumed in these locations poses a serious public health threat for consumers of these local dairy products particularly as the transfer of pathogens via such food chain is very well documented. Antibiogram of *S.aureus* isolates revealed high performance of some of the antibiotics used such as gentamicin, ciprofloxacin and chloramphenicol while a high resistance (50.0%) was observed for tetracycline a commonly used antibiotic thereby creating a public health concern.

Recommendation

There is therefore need to institute effective control measures to protect the consumers from these food borne pathogens. This would include mandatory milk pasteurization by traders and improved hygienic handling of dairy products during processing and marketing.

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