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Occurrence and Antimicrobial Susceptibility Profile of Coagulase Negative Staphylococci Isolated From Local Yoghurt Hawked in Gombe Metropolis, Nigeria

***[1]Hamza, A.J., [2]Onaolopo, J.A., [2]Bolaji, R.O. and [3]Ibrahim I.H.**
[1]Department of Pharmaceutical Microbiology and Biotechnology, Gombe State University
[2]Department of Pharmaceutical Microbiology, Ahmadu Bello University Zaria, Nigeria.
[3]Department of Microbiology, Gombe State University
*Correspondence to: Hamza Adamu Jabir, Department Pharmaceutical Microbiology and Biotechnology, Gombe State University.
Email: jabirgsu@gmail.com; +2347039306208

Abstract
Coagulase-negative staphylococci (CoNS) were formally believed to be non-pathogenic but recent studies have implicated them as agents of some diseases. This study was designed to investigate the occurrence and antimicrobial susceptibility profile of CoNS isolated from local yoghurt hawked in Gombe metropolis, Nigerian. A total of 177 local yoghurt samples from Gombe metropolis were investigated for the presence of CoNS using standard microbiological methods and confirm using Microgen Staph ID kit. The antibiotics susceptibility profile of the confirmed isolates was determined using disc diffusion method. Out of the 177 yoghurt samples, a total 24 (13.56%) were identified as CoNS which include: S. chromogenes 11(45.83%), S. haemolyticus 5(20.83), S. hominis 3(12.50%), S. warneri 3(12.50%), S. cohnis 1(4.17%) and S. ciuri 1(4.17%). Antimicrobial susceptibility testing revealed that the isolates were highly resistant to ampicilin (75%) and cefoxitin (54.2%) but very sensitive to gentamicin (100%), vancomycin (95%) and chloramphenicol (100%). The study reveals the presence of CoNS in the study area that are highly resistant to conventional antibiotics. Thus, to ensure public health safety and to avoid transmission of drug resistant bacteria, there is the need to educate yoghurt producers, vendors and consumers on the importance of food safety by the relevant authorities.
Keywords: *Coagulase negative staphylococci, Local yoghurt, Antimicrobial Resistance.*

INTRODUCTION

Local yoghurt also known as Kindirmo is among the various type of fermented food produced from animal products in Nigeria (Patience *et al.*, 2016). The process of its production is mainly fermentation and involve; collection of the fresh milk from the cow's udder into a clean container, allowing it to settle under the sun, followed by boiling, cooling, inoculating the milk with a starter culture and incubating it overnight for the fermentation to take place (Egwaikhide *et al.*, 2014). The production of this yoghurt in Nigeria takes place in herds and villages and usually in small scale by women of Fulani tribe (Igwe and Yakubu, 2000).

Kindirmo is greatly patronize in most cities of the Northern Nigeria being part of the staple diet. However numerous studies including those by Robert *et al.*, (2009) opined that consumption of such locally produced milk poses significant health burden in the society with increased morbidity and mortality as the milk may become contaminated with various organisms including those with multiple resistance to antibiotics in the process of production and distribution.

Antibacterial resistance is the ability of a bacteria to resist treatment with an antibiotic originally used in its treatment and it involves bacteria that cause many common to life threatening infections as there are available literature showing that much of the problem of infectious diseases worldwide result from antimicrobial-resistant bacteria and more virulent strains of such pathogenic microbes have evolved (Erb and Sturmer, 2007; Stevens and Ma, 2007).

Coagulase negative staphylococci (CoNS) are important pathogens with food safety implications as they may contaminate food of animal origin including milk (Osman *et al.*, 2016). Coagulase negative staphylococci are frequently isolated from bulk tank milk (Huber *et al.*, 2011). Coagulase negative staphylococci may also be a reservoir of different staphylococcal enterotoxins genes causing food poisoning (Rodríguez *et al.*, 2016). In addition, they may be a reservoir for multiple antimicrobial resistant genes (Szczuka *et al.*, 2016).

Coagulase negative staphylococci (CoNS) which are known to be commensals on the skin of man and animals could be introduced into food when food producers lack personal hygiene and/or there is no quality control check during food processing or production (Klibiet *et al.*, 2018). Coagulase negative staphylococci were long considered non-pathogenic and having few virulence factors however, this notion has been corrected as many studies have shown that just like the known pathogenic *S. aureus*, they also possess virulent factors and have been indicated as pathogens of diverse diseases (Akinkunmi and Lamikanra, 2012). CoNS have been recognized as etiologic agents of a wide variety of infections ranging from wound and urinary tract infection, neonatal sepsis, central nervous shunt, endocarditis etc (Patience *et al.*, 2016).

The presence of CoNS in any food could therefore raise questions about its safety for human consumption. The aim of the study was to investigate the occurrence and antibiotic susceptibility profile of CoNS isolated from local yoghurt hawked in Gombe metropolis, Nigeria.

MATERIALS AND METHODS

Sample Collection

One hundred and seventy seven (177) samples of locally fermented cow milk were collected aseptically using sterile universal bottles, from three markets in Gombe metropolis. The samples were labeled accordingly and transported in an ice pack to the Pharmaceutical Microbiology and Biotechnology Laboratory, Gombe State University for further investigation.

Isolation and Identification of Coagulase Negative Staphylococci

The collected samples were cultured on Mannitol Salt Agar (MSA) according to the method previously described by Onaolapo *et al.*, (2017), after 24 hours of incubation colonies that appeared golden yellow in colour on MSA plates with yellow surrounding (an indicative of fermentation) were considered as Staphylococci. These colonies were then sub-cultured onto a MSA again to obtain pure cultures from which stock cultures were immediately prepared. Gram-staining of isolates was carried out as described by Cheesbrough (2008). A smear of each isolate was made on a slide, the smear was heat-fixed and stained with crystal violet, and then allowed to stand for 10-60 seconds, the stain was poured off and rinsed gently with water. The smear was then mixed with Lugol's iodine solution, decolorized with ethanol and was then counterstained with safranin. The stained slide was examined under an oil-immersion lens and the isolates that appeared as violet cocci predominantly clusters were selected to be staphylococci. These isolates were then subjected to catalase test by addition of 1ml of a 3% hydrogen peroxide solution on a 24 hour nutrient agar slope culture of the isolate. This was carried out for all the suspected staphylococci isolates and evolution of gas was noted, the isolates that produced the gas were considered to be staphylococci and the isolates that were catalase negative as streptococci Cheesbrough, (2008). To differentiate CoNS isolates from *staphylococcus aureus*, the isolates were then subjected to coagulase test by dropping a physiological saline on each end of a slide and a colony of the test organism was emulsified in each drop to make two separate thick suspensions. A drop of plasma was then added to one of the suspensions and mixed gently and the ability or inability to form a clumping within 10 seconds suggested *staphylococcus aureus* or CoNS respectively (Onaolapo *et al.*, 2017). Microgen^{TM} staph ID was further used to identify CoNS to a specie level (Onaolapo *et al.*, 2017).

Antimicrobial Susceptibility Testing

The susceptibility of the isolates was tested against 10 different antimicrobials using the modified disk diffusion method as described by the clinical laboratory standard institute (CLSI, 2015). One to three (1–3) colonies were suspended in sterile saline, and the turbidity of the bacterial suspension was adjusted to a 0.5 McFarland standard. The standardized bacterial suspension was spread over Mueller Hinton agar and allowed to dry. Antimicrobial coated discs (Oxoid, Hampshire, England) that are; amoxicillin (25µg), ciprofloxacin (5µg), erythromycin (15µg), chloramphenicol (30µg), cefoxitin (30µg), sulphamethoxazole trimethoprim (25µg), doxycycline (30µg), amoxiclave (30 µg), gentamicin (10µg) and vancomycin (30 µg) were placed on the agar surface using an antibiotics dispenser, gently pressed with the aid of a sterile forceps to ensure complete contact with the agar surface. The plates were incubated aerobically at 37^0C for 18 h. Inhibition zone diameters (mm) were measured in millimeters. The isolates were classified as resistant or susceptible to an antimicrobial based on the susceptible zone diameter (mm). Isolates resistant to one or more antibiotics in three and more broad classes of antimicrobials in this study were recorded as multidrug-resistant (MDR) as described previously (Pesavento *et al.*, 2007).

RESULTS

The result of the study revealed that 26 (14.69%) and 24 (13.56%) of the isolates were *S. aureus* and CoNS respectively (Table 1).

Table 1: Occurrence of Coagulase Negative Staphylococci among the study samples

Number Examined	*Staphylococcus aureus*		Coagulase Negative Staphylococci	
	No.	%	No.	%
177	26	14.69%	24	13.56%

Table 2 revealed that among the three sampling sites, Tashan Dukku and Main Market had higher occurrence of CoNS of 15.25% each compared with Tashan Shango (13.56%). Similarly Tashan Dukku and Main Market had higher occurrence of *S. aureus* of 15.25% each compared with Tashan Shango (10.16%) (Table 2).

Table 2: Distribution of Coagulase Negative Staphylococci (CoNS) and *S. aureus* among Three Markets of Gombe Metropolis

Source		Sample size	No./Percentage of CoNS	No./Percentage of *S. aureus*
1	Tashan Shango	59	6(10.16%)	8(13.56%)
2	Tashan Dukku	59	9(15.25%)	9(15.25%)
3	Main Market	59	9(15.25%)	9(15.25%)
Total		**177**	**24(13.56%)**	**26(14.69%)**

Table 3 revealed that *S. chromogenes* and *S. haemolyticus* had the highest occurrence of 45.83% and 20.83% among the isolated CoNS, while the least was *S. cohnis* (4.17%) and *S. ciuri* (4.17%). *S. hominis* and *S. warneri* had percentage occurrence of 12.50% each (Table 3). Additionally, among the three sampling sites, Main Market had the highest number of *S. chromogenes* isolates of 5, followed by Tashan Dukku (4) and Tashan Shango (2) (Table 3). Only one isolate of *S. cohnis* was isolated from Tashan Shango and another one isolate of *S. ciuri* from Tashan Dukku (Table 3). Two isolates of *S. haemolyticus* were isolated each from Tashan Shango and Main Market, and one and two isolates of *S. warneri* from Tashan Shango and Main Market respectively (Table 3).

Table 3: Percentage Occurrence revalence of Coagulase Negative Staphylococci (CoNS)

S/N	CoNS	Distribution			Total	Percentage (%)
		A	B	C		
1	*S. chromogenes*	2	4	5	11	45.83
2	*S. haemolyticus*	2	1	2	5	20.83
3	*S. hominis*	0	3	0	3	12.50
4	*S. warneri*	1	0	2	3	12.50
5	*S. cohnis*	1	0	0	1	4.17
6	*S. ciuri*	0	1	0	1	4.17
	Total				**24**	

Key: A: Tashan Shango; B: Tashan Dukku; C: Main Market

The antibiotics susceptibility testing revealed that, the highest resistance of 75% (18/24) by the CoNS isolates was recorded against Ampicillin, followed by 54.2% against Cefoxitin and 33.3% each against Erythromycin and Trimethoprime/sulfamethaxazole (Table 4). Table 4 also revealed that the most sensitive drugs were Gentamicin (100%) and Chloroamphenicol (100%). Followed by Vancomycin (98.5%), Amoxiclave (83.3%), Ciprofloxacin (83.3%) and Doxycycline (75%).

Table 4: Antibiotics Susceptibility Profile of Coagulase Negative Staphylococci Isolates

Antibiotics	Potency	Coagulase Negative Staphylococci (n=24)	
		Sensitive	Resistant
Ampicillin	10 µg	6 (25%)	18 (75%)
Ciprofloxacin	5 µg	20(83.3%)	4(16.75)
Erythromycin	15 µg	16(66.7%)	8(33.3%)
Chloroamphenicol	30 µg	24(100%)	0(0%)
Cefoxitin	30µg	11(45.8%)	13(52.2%)
Trimethoprim/sulfamethaxazole	30 µg	16(66.7%)	8(33.3%)
Doxycycline	30µg	18(75%)	6(25%)
Amoxiclave	30 µg	20(83.3%)	4(16.7%)
Gentamicin	10 µg	24(100%)	0(0.00%)
Vancomycin	30 µg	23(95.8%)	1(4.2%)

n= number of CoNS

DISCUSSION

The result obtained from the study established that the percentage occurrence of CoNS isolated from local yoghurt samples of the study area was 13.56%. The occurrence of CoNS in the yoghurt samples might have a relation with the people preparing and serving the yoghurt since staphylococci are commensals of human body thus, they can be introduced into the yoghurt, or it could be from the cows that suffer from mastitis since the organisms are associated with the disease in cows. The result varied with the findings of Patience *et al.* (2016) who revealed a total of 79.3% CoNS in fermented food including Kindirmo, the difference observed between the two studies could be due to variation in sampling environment and sample size used in the studies.

In this study, the prevalence of CoNS varies in the three sample locations, relatively low in Tashan Shango than Tashan Dukku and Main Market; the difference may be because Tashan Shango is relatively less congested (with people) than the other markets, hence there is less tendency of contamination in the place.

Among the CoNS isolates identified in the study, *S. chromogenes* had the highest percentage of occurrence in the local yoghurt samples examined, this may be due to the frequent association of *S. chromogenes* with mastitis in cows than other species (Egwaikhide *et al.*, 2014).

The study findings reported high resistance of CoNS to Ampicillin followed by cefoxitin. High AMR to penicillin have also been reported among CoNS isolates from different animal samples; 94.2%in China, 77.8% in Pakistan (Syed *et al.*, 2018) and 70.6% in Tunisia (Klibi*et et al.*, 2018). The reasons for the widespread AMR isolates in Nigeria may be due to overuse and misuse of these antimicrobials (Yadesa *et al.*, 2015; Gebretekle and Serbessa, 2016), abuse in veterinary medicine and unregulated movement (smuggling) of the antimicrobials (Suleman *et al.*, 2016). The study also established that the CoNS isolates were completely sensitive to Chloroamphenicol and Gentamicin, followed closely by Vancomycin. The high performance of these antibiotics (chloramphenicol, vancomycin and gentamicin) against CoNS isolates could be due to their molecular sizes, a factor which enhances their solubility in diluents thus promoting their penetration power through cell wall into cytoplasm of the bacteria (Onaolapo *et al.*, 2017). Moreso, all the three antibiotics are administered using injection and not orally, hence limiting the level of their misuse in human because it requires a service of a professional for their administration, consequently reducing the level of resistance against them.

CONCLUSION

The study revealed the presence of CoNS in local yoghurt vended at Gombe municipal area with a percentage occurrence of 13.56%. The most common CoNS identified were *S. chromogenes* and *S. haemolyticus*. Most CoNS isolates were resistant to ampicillin, erythromycin and cefoxitin and were hundred percent susceptible to gentamicin and chloroamphenicol followed by vancomycin. The detection of CoNS and especially the demonstration of drug resistance in yoghurt which is being consumed widely as food not only in the study area but in Nigeria calls for the for public awareness on dangers associated with consumption of contaminated product and the various ways to avoid it by the relevant authorities.

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