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Prevalence and antibiotic susceptibility patterns of *Staphylococcus aureus* in locally pasteurised cow-milk sold at dutse metropolis, Jigawa state, Nigeria

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Abstract
Globally, Antibiotic resistant bacteria have remained an important public health problem *Staphylococcus aureus* is one of the leading causes of foodborne disease outbreaks due to its ability to produce staphylococcal enterotoxins. This aimed at determining the prevalence of antibiotic susceptibility patterns of *Staphylococcus aureus* in locally pasteurised cow-milk sold at Dutse metropolis. A total of **70** samples were obtained and transported to the Microbiology laboratory of Drugs Manufacturing Unit of Aminu Kano Teaching Hospital for analysis. *Staphylococcus aureus* were isolated using Mannitol salt agar followed by some biochemical test such as catalase and coagulase and API-Staph Identification. Antibiotics susceptibility patterns of *S. aureus* were determine using Kirby–Bauer disk diffusion method. In this study the total prevalence of *S. aureus* was 47.1 %. The result demonstrated the presence of *S. aureus* in all milk samples which indicated that 40.6% of the samples were positive for *S. aureus.* The study also revealed that the *Staphylococcus aureus* isolates demonstrated varying degrees of susceptibility and resistance to different antimicrobials with Penicillin recorded the highest resistance 10(30.3%), and the highest sensitive antibiotic was Cefoxitin 2(6.0%). This study showed presence of *S. aureus* in all the milk samples collected from different locations within Dutse metropolis. However, Penicillin was the most resistant antibiotic against the isolated *S. aureus* with Cefoxitin having the highest rate of sensitivity among all the antibiotics tested. The pasteurisation process of local Cow milk should be properly pasteurised to avoid contamination bacteria such as *S. aureus.* Antibiotics should be used according to the prescribed directives.

Keywords: prevalence, *S. aureus*, antibiotic susceptibility pattern, pasteurised cow milk

Introduction

Globally, Antibiotic resistant bacteria have remained an important public health problem (Normanno *et al*., 2007). (Mansori and Khaleghi, 1997; Lee, 2003; Ikeagwu *et al*., 2008). Associated with this concern, is the mutual affirmation that both medical and veterinary use of antimicrobial agents in promoting the emergence and rise in the prevalence of these resistant pathogens. Many researchers have investigated the role of animal food products such as meat (Bhargava, 2011) and milk (Strastkova *et al*., 2009; Virgin *et al*., 2009; Tǔrkyilmaz *et al*., 2010) [32, 38] as sources of resistant zoonotic bacteria. In 2015 microbial food poisoning accounted for 53.7% offood poisoning inChina, of which *Staphylococcus aureus* was an important pathogenic factor in this cases (Wu *et al*., 2018*)*.Approximately Staphylococcus aureus causes 241, 000 cases of food poisoning annually (Scallan *et al*., 2011; Kadariyat *et al*.,2014). *Staphylococcus aureus* is one of the leading causes of foodborne disease outbreaks due to its ability to produce staphylococcal enterotoxins (Tenhagen *et al*., 2014; Hennekinne *et al*., 2012) [33, 16].

The on-going explosion of antibiotic resistant infections continues to plague global healthcare (Spellberg*et al.*, 2008). Methicillin resistance *staphylococcus aureus* (MRSA) is at present the most commonly identified antibiotic resistant pathogen in many parts of the world, including Europe, the Americas, Middle East, East Asia and North Africa (Grundmann*et al.*, 2006).Cases of Livestock-associated(LA-MRSA) have been reported in humans but little or no work in cattle has been documented in Nigeria (Umaru *et al*., 2013; Mai-siyama *et al*., 2014) [37, 21]. Between 1996 and 1997, the prevalence of MRSA, determined in eight African countries, was relatively high in Nigeria, Kenya and Cameroon and low in Tunisia and Algeria (Fadeyi *et al*., 2010; Nwankwo *et al*., 2010) [11]. In Nigeria, MRSA colonization and infection have been reported in humans with varying prevalence of between 20 and 60% (Olonitola *et al*., 2007) [26]. Milk is considered as an excellent medium for growing of many microorganisms. Milk can be contaminated with several bacteria during milking process from the milking personnel, utensils used for milking (Rehman *et al*., 2014) [28]. Besides, microorganisms may enter the udder through teat canal, and the bacteria may come out through milk (Smith *et al*., 2007) [30]. *Staphylococcus aureus*is one of the two major contaminants of milk. The presence of the pathogen in milk largely depends on fecal contamination, and the presence of pathogen in faeces mainly originates from feed contamination (Aycicek *et al*.,

2005) [2]. Food borne diseases are of great concern around the world. However, this is an important issue in developing countries where poor sanitation is maintained during collection and processing of milk from cattle (Le *et al*., 2003).Staphylococcal food poisoning is a common cause of food borne illness in humans (Pillsbury *et al*., 2013) [27]. 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) [24]. In addition, the number and types of microorganisms in milk immediately after milking are affected by biotic factors like animal health and its cleanliness and abiotic factors such as air, soil, grass, faeces, season and milking equipment (Uddin *et al*., 2011) [36]. It is also hypothesized that differences in feeding and housing strategies of animals may influence the microbial quality of milk (Coorevits, 2008) [6]. Public health problems associated with the consumption of unpasteurized raw milk and its products have been well documented (De Valk *et al*., 2000; De Buyser *et al*., 2001 and Harrington *et al*., 2002) [9, 8, 15]. So, examination for the presence and number of specific microorganisms is therefore an integral part of any quality control or quality assurance plan. However, there is scarcity of data regarding the prevalence of *S. aureus* in pasteurised cow milk in Dutse metropolis. Therefore, this study intended to bridge in the gap by determining the prevalence of *S. aureus* in locally pasteurised cow milk in Dutse metropolis.

Material and Methods

Study area

The study was carried out in Dutse metropolis, Dutse local Government of Jigawa State Nigeria. Jigawa State is located at the centre of Northern Nigeria with the coordinate's 11°46′ 39'' North, 9° 20′ 3'' East. The state shares boundaries with Republic of Niger and other northern state in the in the North-West region of Nigeria. Statistically an estimated 3.8million people living in Jigawa, the state is mostly populated by Hausa and Fulani Ethnic tribes, other ethnic groups are also present like Kanuri and Fulfulde. Agriculture and Cattle rearing are the major occupation of about 80% of the people actively engaged in the 27 local governments within the State.

Sampling strategy

The Raw milk samples were selected within Dutse Metropolis using cluster sampling Strategy, Five **(5)** areas (Dangantsaure, Jigawa Tsada, State Secretariat, Yantifa, and Federal University Dutse represented as A, B, C, D and **E** respectively) were considered as clusters. In each cluster, fourteen (14) samples were selected by simple random sampling

Sample collection and processing

A total of 70 samples (14 from each location), were obtained, the Samples were collected in a sterile containers, using a standard hygienic protocols ,placed in ice - packed coolers and taken to the laboratory. Samples were analysed at Microbiology laboratory of Drugs Manufacturing Unit (DMU) of Aminu Kano Teaching Hospital (AKTH) within 6 hours of collection.

Isolation and Identification of *Staphylococcus aureus*

The isolation of *Staphylococcus aureus* were according to the procedure described by Imani fooladi *et al.* (2010).The raw milk samples were collected from different locations **(A-E)** in Dutse Metropolis. Dairy samples of raw milk were diluted in the ratio 1/100 in normal saline. From each solution produced, 10ml were pipetted and transferred to 90 mls peptone water, 9% NaCl. Were added and incubated at 37̊ C for 24h. In the second phase, 1ml from each previously cultured medium was thentransferred to Mannitol Salt Agar (MSA) and incubated for 24h. Yellow colonies with transparent yellow zone on Mannitol Salt agar were considered as presumptive *Staphylococcus aureus*. They were picked and stored on nutrient agar slants for further confirmatory tests. The isolates were identified phenotypically using catalase, coagulase and API-Staph identification.

Antibiotic Susceptibility Screening

The antibiotic susceptibility tests for *S. aureus* isolates were performed according to the Kirby- Bauer method as described by Kirby–Bauer disk diffusion method to determine the antibiotic susceptibility pattern. Diameter, evaluation and interpretation were carried out according to the Clinical and Laboratory's' standards Institutes (CLSI, 2019).The antimicrobial agents used were Erythromycin (15ug), Penicillin G (10ug), Clindamycin (2ug), Gentamicin (10ug), Ofloxacin (5ug), Tetracycline (30ug) and Cefoxitin. Isolates were incubated using Muller Hilton Agar (MHA) categorized as either susceptible or resistant according to guide lines of Clinical and Laboratory Standards Institute CLSI (2019). *Staphylococcus aureus* ATCC 25923 were used as quality control standard strain.

Data Analysis

The raw data was first entered into excel spread sheet. Prevalence and Antibiotic susceptibility patterns were generated and presented in tables and graphs.

Results and Discussion

The study was designed to determine the prevalence, antimicrobial susceptibility profile of *Staphylococcus aureuss* and MRSA isolated from locally fermented raw milk products in Dutse Metropolis.In this study the total prevalence of S. aureus is 47.1 % which is higher than the study conducted in Debrezeit, Ethiopia 29.1% (André,*et al*, 2008) [1] 27.0% (Belmamoun, *et al* 2016) [3] In the other side, the study done by Daka *et al*. (2012) [7], in Southern Ethiopia the prevalence was reported as 40.6% which is closer to the current studythe difference may come due to probability of high contamination due to poor hygienic standards. The presence of *S. aureus* tends to reduce the quality of the milk through their metabolic activities and could precipitate food poisoning due to elaboration of toxins that could lead to illnesses when consumed by humans.

There are so many results of prevalence by different researchers in different countries. For example in Morocco, it was found 40% prevalence of *S. aureus* (Akkou *et al*., 2016), 36.9% in Palestine (Nazari *et al*., 2014) [23], 75% in Bangladesh (Thaker *et al*., 2013) [34] and 61% in India (Fagundes *et al*., 2010) [12] which are higher than the result found in this study. The difference may be resulted from their sampling sites includes much number of local milk collection and distribution sites. Over the years, milk and milk products have been known as vehicles for the transmission of bacterial pathogens to man (Revathi *et al*., 2012). *S. aureus* is considered to be the most common mammary pathogen found in bovine mastitis in the whole world and is an obvious contributor to milk contamination (André,*et al* 2008) [1] This difference can be attributed to the fact that the most of these researchers indicated higher prevalence carried out their samples on farms, while our study was interested in the locally pasteurised cow milkthat underwent several means of transport and manipulations before arriving to the consumers.

In the other hand there are researches done and found to have lower prevalence than this study like 18.18% prevalence of *S. aureus* found in Turkey by Ekici *et al*. (2004) [10]. The results in those studies are lower than this study might be due to environmental contamination at milk distribution site. In addition, those studies may have a better prevention and hygienic environment than study area of this research. Mastitis presence will also be the factor as Asperger and Zangeri said about 40% of the cases would be associated with the presence of mastitis. Environmental contamination during raw milk handling also results *S. aureus* to get a chance contaminating the milk [19-21]. The result demonstrated the presence of *S. aureus* in all milk samples. Similar studies conductedin Morocco, Brazil, Ethiopia and Kenya reported prevalence of *S. aureus* to be 40%, 68%, 48.75% and 30.6% respectively (Bendahou *et al*., 2008), (de Oliveira *et al*.,2011), (Daka *et al*., 2012) [7], (Shitandi *et al*., 2004).Similar to this study, the high prevalence were from milk samples collected from sale points and markets. However, the difference in prevalence may be due to the fact that our samples were collected from a smaller geographical area within a shorter period compared to those reported elsewhere. The high levels of *S. aureus* in milk relates to poor hygiene practices and also the health status of the animals. Daka *et al*. (2012) [7] reported 40.6 % in South Ethiopia. The difference in the prevalence rate was due to variation in the sanitary condition of udder, size of sampling and geographic region (Sadashiva and Kaliwal, 2013 and Shopsin *et al*., 2000). In this study we described the isolation and antibiotic susceptibility characterization of *S. aureus* from milk obtained from five different areas in Dutse Metropolis. Our results indicate that 40.6% of the samples were positive for *S. aureus* and Several studies have been conducted in South Africa to evaluate the prevalence of *S. aureus* in milk (Shitandi, 2004; Lee, 2003; Gundogan *et al*., 2006). The results reported in our study were similarly high when compared to those studies (Shitandi, 2004; Gundogan *et al*., 2006) [29]. Although the prevalence of *S. aureus* has been reported to vary with the size and geographic region of the area sampled, a high proportion of these bacteria in milk relates to poor hygiene practices. Based on observations made during the collection of samples, we therefore report that improper hygiene and poor management practices contributed to the presence of *S. aureus* in the milk, especially in those from the Yantifaarea., also may be attributed to untreated groundwater was used to wash the containers that were used for milking. This may have contributed to the high level of *S. aureus* isolated. Improving the hygienic conditions of the milking environment and/or utensils may reduce the prevalence of *S. aureus* in milk and prevent its transmission to humans. The Study also revealed that the staphylococcus aureus isolates demonstrated varying degrees of susceptibility and resistance to different antimicrobials. Penicillin with 10(30.3%) recorded the highest resistance. Similar results have been observed for strains isolated from milk, dairy products, and dairy workers (Tan *et al*., 2014;. Visciano *et al*., 2014; Jamali *et al*., 2015; Castro *et al*., 2016; Chaalal *et al*., 2018; Papadopoulos *et al*., 2018). Davies and Davies, 2010; Gonzalez-Candelas *et al*., 2017 reported that the increasing levels of resistance may be associated with the ill use of antibiotics in various works of life. However, the isolates were also resistant to erythromycin, tetracycline, ofloxacine, gentamycine, clindamycine and cefoxitine with 2(6.0%), 3(9.0%), 4(12.1%), 4(12.1%), 5(15.1%) and 6(18.1%) respectively. According to Ojo *et al*., (2016), antimicrobial usage in food-producing animals has remained a major factor contributing to the emergence and dissemination of antimicrobial resistant bacterial strains.

Table 1: Isolation and prevalence of *Staphylococcus aureus* in raw milk sold at Dutse Metropolis

S/N	Sample source	Sample Collected	S.*species* (%)	S.*aureus* (%)
1	Dangar Tsaure	14	9(18.7)	8 (24.2)
2	Jigawa Tsada	14	10 (20.8)	4 (12.1)
3	State Secreteria	14	8 (16.6)	7 (21.2)
4	Yantifa	14	13 (27.0)	9 (27.2)
5	FUD	14	8 (16.6)	5 (15.1)
Total		70(100)	48(68.5)	33(47.1)

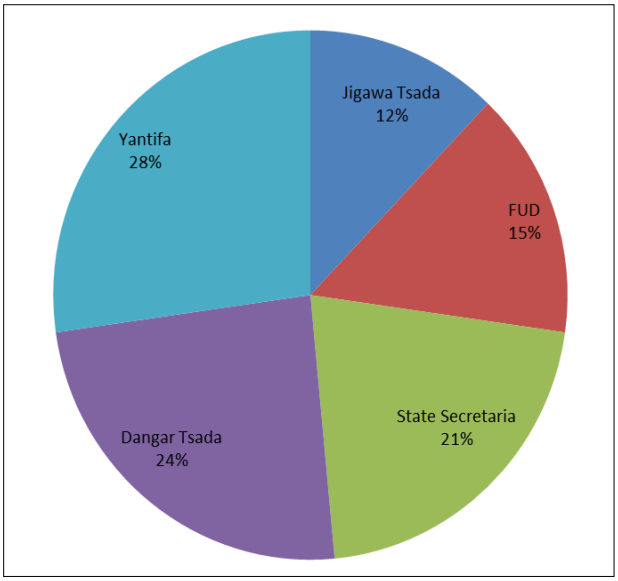

Fig 1: Shows the prevalence and distributions of S.aureus in locally pasteurized Cow milk Sold in Dutse Metropolis

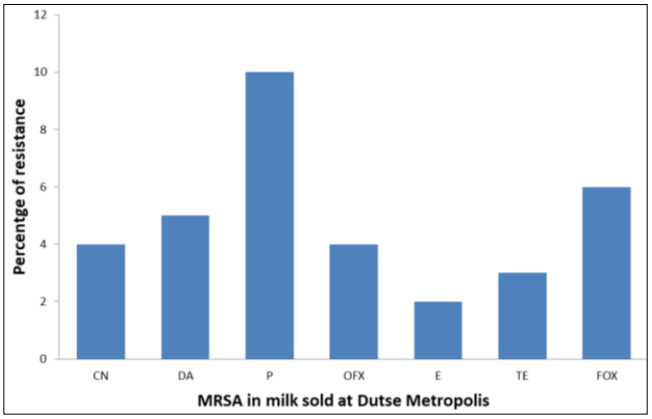

Fig 2: Antibiotics

CN=Gentamycin
DA=Clidamycin
P=Pencillin
OFX=Oflaxacin
E=Erythrumycin
TE=Teteracycline
P0X=Cepoxitin

Conflict of interest

All the authors declared no conflict of interest

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